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Cannabinoids and Sleep

Molecular, Functional and Clinical Aspects

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"Cannabis is the most versatile herbal remedy, and the most useful plant on Earth. No other single plant contains as wide a range of medically active herbal constituents."

—Dr. Ethan Russo
Cannabinoid Research Institute

"The illegality of cannabis is outrageous, an impediment to full utilization of a drug which helps produce the serenity and insight, sensitivity and fellowship so desperately needed in this increasingly mad and dangerous world."

—Carl Sagan

This volume is dedicated to our respective families.

Foreword

For most researchers, and certainly for the general population, "cannabis" relates to the plant and its constituents alone. However, since the mid-1980s and early 1990s, research has expanded our knowledge. Today, the cannabinoid field of science covers the cannabinoid receptors, the endocannabinoids (particularly anandamide and 2-AG), their synthetic and degradation pathways, and endogenous anandamide-like compounds, which are fatty acid amides with amino acids or ethanolamines. All these entities are parts of a major new physiological system—the endocannabinoid one. Most probably, the field will expand further.

Plant Cannabinoids While many dozens of plant cannabinoids are known today, most research and acquired knowledge are on Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). CBD was isolated in the 1930s in the labs of Adams in the USA and Todd in the UK, but its structure was elucidated many years later—in 1963; THC was isolated in pure form, and its structure was elucidated only in 1964. These chemical advances were made many decades after the isolation of morphine and cocaine—the two other major illicit plant constituents. The reason for this discrepancy seems to be the technical difficulty in isolating the plant cannabinoids in pure form, due to the stupendous mixture of this family of compounds produced by the plant. Modern methods for separation and purification not available previously were needed.

In addition to THC and CBD, there are indications that cannabigerol (CBG) and possibly cannabichromene (CBC) are likewise of medicinal interest. Very little is known about the rest of the plant cannabinoids, except on the cannabinoid acids, which are the precursors of the neutral cannabinoids. These acids are not stable, which seems to be the main reason why their biological properties were not well investigated. However, recently CBD acid was stabilized (by esterification to its methyl ester). It seems to parallel CBD in its actions. We already know that it is a potent anti-nociceptive, antiemetic, and anxiolytic compound. Shall we see it in the market, like CBD, in all kinds of industrial-prepared foods and beauty lotions? I hope not.

Most "medical cannabis" sold today is in the form of mixtures in which the amount of specific cannabinoids varies. Can we guess where we shall be with such mixtures or pure cannabinoids in about a decade from now? Given the huge market today, the mixtures, as well as pure CBD, will probably be still around. However, we can expect to have better-defined mixtures, as well as

semi-synthetic CBD and CBG derivatives, as drugs in many areas. Numerous pharmaceutical companies have cannabinoid programs. In addition to the pain and anxiety mentioned above, we shall probably see synthetic and semi-synthetic cannabinoids in additional areas of psychiatry and neurology as well as, presumably, in gastroenterology and immunology.

Endogenous Cannabinoids The discovery of a receptor in the 1980s led to the isolation of endogenous cannabinoids (endocannabinoids) in the 1990s. Two of these, anandamide and 2-AG, have been the topic of thousands of publications. We have learned much about their chemistry, including the synthesis and degradation of these molecules in the animal body, as well as their bioactivities. The endocannabinoid system has turned out to be a central one in animal physiology. Indeed in a recent review, it was stated that "...modulating endocannabinoid activity may have therapeutic potential in almost all diseases affecting humans." Even the dopaminergic or cholinergic systems have not been so described.

What are the research pathways ahead of us in this area?

A. Will the endocannabinoids be investigated in humans? More than 25 years after their discovery human studies are almost unavailable!
B. Shall we see additional endocannabinoids, which have not been isolated so far? They may differ in their activity from anandamide and 2-AG.
C. Has the activity of endocannabinoids been looked into in all animal biochemical systems?
D. Do we know enough about the role of the endocannabinoids in our emotions and personality?
E. Can we expect to see endocannabinoid derivatives as drugs?

Anandamide-like Endogenous Molecules The biosynthesis of anandamide is based on fatty acid (arachidonic acid) and amino acid derivatives (an ethanolamine). The animal body has numerous fatty acids and amino acids, and indeed, it uses the established biosynthetic pathway of anandamide for the synthesis of many additional, chemically related molecules, most of which do not bind to the cannabinoid receptors. Over the last two decades, several groups have investigated these anandamide-like endogenous molecules. A few examples of such compounds (tested only in mice and rats so far) are as follows:

Arachidonoyl serine is neuroprotective after brain trauma. It causes vasodilation, thus allowing better blood flow into damaged areas.

Oleoyl serine acts on osteoblasts and prevents bone loss in osteoporosis by increasing bone formation and restraining bone resorption.

Oleoyl glycine has powerful anti-nicotine addiction properties. It blocks the establishment of nicotine place preference—a test for addiction formation—and reduces withdrawal responses in nicotine-dependent mice. In morphine-dependent rats, it was also found to reduce withdrawal responses but did not affect morphine addiction, thus demonstrating selectivity.

These are just a few examples. Many other anandamide-like compounds are present in the animal body and act in numerous biological processes. Indeed, it has been speculated that the huge number of such compounds—the concentration levels of which may differ from person to person—may be involved in the personality differences.

I would like to end with a look at the future of cannabinoid drugs—as seen from afar. At present, most patients who use cannabinoid-based drugs are prescribed "medical marijuana"—a term that from a medical point of view is unacceptable. "Medical marijuana" reaching the public has to be better defined as regards constituents, whose levels in many cases are not even mentioned. The level of constituents in cannabis depends to a large degree not only on the genetics of the plant but also on the conditions under which it was grown. Hence, today the consumption of "medical marijuana" is to a large extent a medical gamble. As mentioned above, I believe that in most countries, within the next few years, strict regulations will be enacted, so that patients will always be able to get the same material as regards constituents.

A second point—many of the drugs we use today are derivatives of natural products. Thus, we have not prescribed cortisone (an important hormone) but derivatives of cortisone. Such derivatives are better suited to be used as drugs than natural constituents are. It seems reasonable to expect that within a decade pharmaceutical companies will develop derivatives of CBD and THC, and possibly CBG, which will be used as novel drugs. We may also have synthetic drugs, unrelated to the plant cannabinoids, which bind to the cannabinoid receptor, particularly to the CB2 receptor, whose activation does not lead to marijuana-like activity.

In summary, I assume that within a decade we shall have both new cannabinoid drugs and well-defined extracts, used in parallel. Let us hope so.

Hebrew University, Medical Faculty, Pharmacy School,
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Raphael Mechoulam, PhD

Preface

The editors are pleased to present the first edition of *Cannabinoids and Neuropsychiatric Disorders*, which has been included in the prestigious *Advances in Experimental Medicine and Biology* series (volume 1264). As editors, we are very happy about this decision as our volume fits perfectly in this landmark biomedicine and the life sciences series.

The plant *Cannabis sativa* has been used both recreationally and medicinally for thousands of years. It was only in 1964 that chemists Yehiel Gaoni and Raphael Mechoulam at the Hebrew University of Jerusalem identified and isolated the psychoactive components in cannabis, Δ 9-tetrahydrocannabinol (Δ 38 9-THC; Gaoni and Mechoulam 1964).

To give an overview on this subject, we have included 13 chapters. The topics covered include *Cannabis sativa* and its constituents, its natural and synthetic derivatives, clinical pharmacokinetics and potential drug–drug interactions, receptor and ligands, mechanisms, regulation of serotonergic and noradrenergic systems by cannabinoids, cannabinoids and sleep/wake control both in animals and humans, cannabis and restless legs syndrome, addiction, and sleep disorders.

We are privileged to have compiled this volume. During the course of our assignment, we learned much in the process of editing this important volume. We sincerely hope that the readers will find this volume uniquely valuable as a research and clinical resource. We sincerely hope that our volume will be useful to researchers and practicing clinicians.

Montevideo, Uruguay — Jaime M. Monti
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Reference

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Cannabinoids and Neuropsychiatric Disorders provides scientific and medical information on cannabis to all healthcare workers interested in basic, translational, and clinical medicine. It is our pleasure to acknowledge the contributions of those who were instrumental in the production of this book.

Our sincere appreciation goes to Prof. Raphael Mechoulam at the Hebrew University of Jerusalem who identified and isolated the psychoactive components in cannabis, Δ 9-tetrahydrocannabinol (Δ 38 9-THC), who agreed to write the foreword. We wish to express our appreciation for his contribution.

We would like to express our deep appreciation to all the contributors for their scholarly contributions that facilitated the development of this volume. These authors have done a superb job of producing authoritative chapters that synthesize vast amounts of scientific and clinical data to create informative chapters. The expertise of contributors to *Cannabinoids and Neuropsychiatric Disorders* reflects the broad diversity and knowledge concerning cannabis research, which has continued to grow over the last several decades. These authors represent the cutting edge of basic and applied research and provide the most recent information regarding how such knowledge can be utilized in clinical settings. Their informed opinions and insights have significantly contributed to our scientific understanding of cannabinoids and have provided important interpretations regarding future research directions.

The highly talented people of Springer USA made this project an especially pleasurable one. We were delighted to have the professional and highly enthusiastic support of Dr. Beatrice Menz, Senior Editor, Springer Nature, Switzerland AG. Without her continuous and unstinting support, this volume would not have been possible.

It was a pleasure to work with the entire production team of Springer. Their guidance, technical expertise, and commitment to excellence were invaluable. We wish to acknowledge the help of Amrei Strehl, Senior Editor, Springer Vienna, Austria; Coral Zhou, Project coordinator, Springer Nature, Beijing City, China; Bibhuti Bhusan Sharma, Project coordinator (Books), Springer Nature, India; and Chitra Sundarajan of SPi Global, Chennai, India.

Finally, and most importantly, we want to thank our spouses and families for their support and understanding during the development of this book.

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Constituents of *Cannabis sativa*

1

Carolina Echeverry, Miguel Reyes-Parada, and Cecilia Scorza

Abstract

Cannabis sativa L. is a psychoactive plant that contains more than 500 chemical components. Even though the consumption (in the form of marijuana, hashish, or hashish oil) for recreational purposes, is the most popular way of using the plant, the knowledge of its components has also led to classify *Cannabis sativa* L. is a plant with medicinal or therapeutical use. Several comprehensive reviews have already been published focused on the chemical composition of *Cannabis sativa*. In this chapter, we will summarize relevant information about those components, which may help to understand its biological actions that will be described in the following chapters.

Keywords

Cannabis · Constituents · Chemical analysis · Non-cannabinoids

1.1 Introduction

Cannabis sativa L., better known as marijuana or hemp, is a plant native from Asia that belongs to the family *Cannabaceae.* This plant has been cultivated worldwide as a resource of a psychoactive drug (marijuana) and its use in the production of fibers, and the manufacturing of several textile products dates from 4000 BC. This use contrasts with palliative and medicinal applications that date from 2700 BC. According to the popular knowledge, several effects such as analgesic, muscle relaxant, antidepressant, hypnotic, immunosuppressive, anti-inflammatory, anxiolytic, and bronchodilator, among others, have been attributed to *Cannabis sativa* L.. Despite these varied beneficial properties, its medicinal use has been seriously restricted because of illicit cultivation, the very well known psychoactive properties and the potential dependence elicited after marijuana chronic consumption, as well as the variable content of the active chemical components.

Cannabis has very complex chemistry due to the vast number of constituents and the interaction between them; actually, these features convert it into one of the most versatile plants in

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nature. Studies indicate that the earliest compound isolated from the *Cannabis* plant in a pure state was cannabinol (Wood et al. 1899). It was initially wrongly assumed that cannabinol was the main active compound of the plant, responsible for its psychoactive effects (Mechoulam and Hanus 2000). The next identified compound as cannabidiol (CBD) which was found and reported by Mechoulam and Shvo in 1963 (Mechoulam et al. 1963). One year later, Gaoni and Mechoulam isolated the main psychoactive compound, Δ9-tetrahydrocannabinol (Δ9-THC), whose active properties depend on the interaction with cannabinoid receptors (endocannabinoids) in specific brain regions (Gaoni and Mechoulam 1964; Pertwee 1997).

Although cannabinoids, terpenes, phenolic compounds, and alkaloids are the most frequently reported components in *Cannabis* plants (Flores-Sanchez and Verpoorte 2008), the chemical composition of *Cannabis sativa* L. is continuously changing. New non-cannabinoid and cannabinoid constituents are frequently discovered. Further attention is now drawn towards non-THC *Cannabis* active components, which may act synergistically and contribute to the pharmacological power and entourage effects of medicinal-based *Cannabis* extracts (Russo 2011).

1.2 Phytocannabinoids

Phytocannabinoids (i.e. a term introduced to emphasize the botanical origin of cannabinoids) or cannabinoids represent the most studied group of compounds of *Cannabis*, mainly due to their wide range of pharmaceutical effects in humans, including psychotropic activities. According to their chemical structure, phytocannabinoids represent a group of C21 or C22 terpenophenolic compounds (from the carboxylated forms) predominantly produced in *Cannabis* (Fig. 1.1). Although these compounds have also been reported in other plants of the *Radula* and *Helichrysum* genera (Appendino et al. 2008), the characterization of a non-*Cannabis* source of cannabinoids is quite recent (Gertsch et al. 2010).

According to its core structure, cannabinoids can be classified in several and different types: (1) CBG type, like cannabigerol; (2) CBC type, like cannabichromene; (3) CBD type, like cannabidiol; (4) THC type, like Δ9-tetrahydrocannabinol; (5) Δ8-THC type, like cannabicyclol; (6) CBL type, like cannabielsoin; (7) CBE types, like cannabinol and cannabinodiol; (8) CBND, CBN and CBT types, like cannabitriol and (9) the miscellaneous cannabinoids (Mechoulam et al. 1963; Brenneisen 2007). Besides, it is important to mention that carboxylated (or acid) is the most abundant form of cannabinoids found in the plant. Among them, the most predominant compounds are tetrahydrocannabinolic acid (Δ9-THCA), cannabidiolic acid (CBDA) and cannabinolic acid (CBNA), followed by cannabigerolic acid (CBGA), cannabichromenic acid (CBCA) and cannabinodiolic acid (CBNDA; ElSohly and Slade 2005).

While Δ9-THCA is the major cannabinoid found in drug-type *Cannabis*, CBDA is highly concentrated in fiber-type hemp. CBCA has been reported to be concentrated in the cannabinoid fraction of young plants and tends to decline along with plant maturation (Meijer et al. 2009). All phytocannabinoids are accumulated in the secretory cavity of the glandular trichomes, which frequently occur in female flowers and most of the aerial parts of the plant. They also can be detected in lower quantities in other parts of the plant including seeds (Ross et al. 2000), roots (Stout et al. 2012) as well as in the pollen (Ross et al. 2005). It is important to remark that the psychoactive compounds are the decarboxylated form of the varieties, i.e., THC.

The biosynthetic pathway of the most abundant cannabinoids has been reported by Andre et al. 2016. A schematic draw of this pathway is shown in Fig. 1.2. Briefly, precursors of cannabinoids are originated from two distinct biosynthetic pathways: (1) the plastidic 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, leading to the synthesis of geranyl diphosphate (GPP), which utilizes pyruvate as precursor; and (2) the polyketide pathway, producing the olivetolic acid (OLA). OLA is synthesized from hexanoyl-CoA, derived from the short-chain fatty

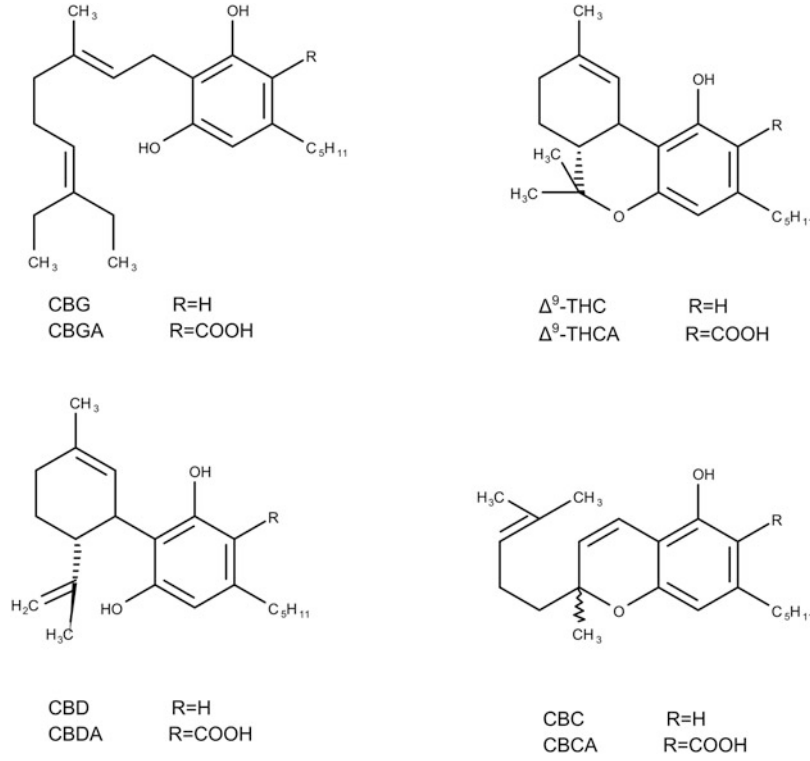

Fig. 1.1 Chemical structures of some of the main phytocannabinoids found in *Cannabis* in its carboxylated and decarboxylated forms

acid hexanoate, by aldol condensation with three molecules of malonyl-CoA (Sirikantaramas et al. 2007; Stout et al. 2012; Gagne et al. 2012). Through different catalytic enzymes, both pathways collaborate with the formation of CBGA, the central precursor of several cannabinoids, such as CBD, CBC, Δ9-THC, among others (Fellermeier and Zenk 1998). Moreover, three oxidocyclases are responsible for the great diversity of cannabinoids: the THCA synthase (THCAS) converts CBGA to THCA, CBDA synthase (CBDAS) forms CBDA while CBCA synthase (CBCAS) produces CBCA (Sirikantaramas et al. 2004, 2005; Taura et al. 2007). Other compounds, defined as propyl cannabinoids (cannabinoids with a C3 side-chain instead of a C5 side-chain), such as tetrahydrocannabivarinic acid (THCVA), which can be synthesized from the divarinolic acid precursor, have also been identified (Flores-Sanchez and Verpoorte 2008). The phytocannabinoid acids are non-enzymatically decarboxylated into their corresponding neutral forms, which occur both within the plant and, to a much larger extent, upon heating after harvesting (Flores-Sanchez and Verpoorte 2008).

Δ9-THC and CBD contents could change depending on breeding conditions and different plant strains. Over the past decade, dramatic increases in Δ9-THC content were observed, ranging from ~3% to 12–16% or even higher (w/w or percent THC weight/per dry weight of *Cannabis*), although this content can differ between different countries (Radwan et al. 2008; Niesink et al. 2015; Swift et al. 2013). Additionally, in some *Cannabis* preparations, Δ9-THC levels can significantly increase by using a concentrating process that implies butane hash oil, which can reach levels of about 80% (Stogner and Miller 2015). In a non-regulated environment, other factors such as soil quality or availability, bacterial, viral and/or fungal contamination, the use of herbicides, pesticides and/or insecticides, water, light, temperature,

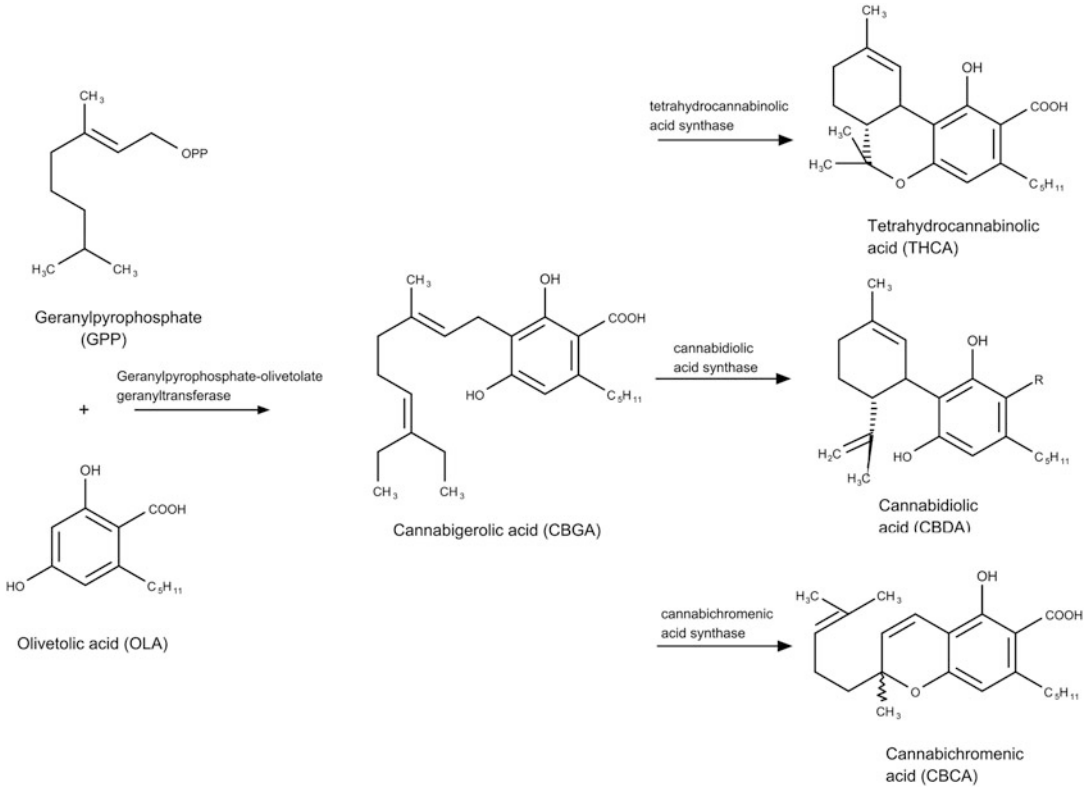

Fig. 1.2 Biosynthetic pathway of the most abundant cannabinoid constituents of *Cannabis* (THCA, CBDA and CBCA). Modified from Sirikantaramas et al. 2007

animal waste, insects, toxic chemicals, active compounds, heavy metals, etc., can produce robust variations in *Cannabis* quality.

The pharmacology of phytocannabinoids has been extensively reviewed (Pacher et al. 2006). Most of the biological properties related to cannabinoids rely on their interactions with the endocannabinoid system in humans (Russo 2011; Hill et al. 2012; Giacoppo et al. 2014; Burstein 2015). For many years, it was assumed that the beneficial effects of the cannabinoids were mediated by the cannabinoid CB1 and CB2 receptors. However, we now know that the picture is much more complex since multiple targets have been identified for the same phytocannabinoid. Thus, over the last two decades, molecular targets outside the endocannabinoid system have been identified for certain cannabinoids. Even though nowadays the rule is that phytocannabinoids show different affinities for both CB1 and CB2 receptors, it has been shown that these compounds can also interact with other G-protein coupled receptors (GPCRs) such as the putative cannabinoid receptors GPR55 or GPR18, and other well-known GPCRs such as opioid or serotonin receptors (e.g., 5-HT_{1A}). Besides, several studies have reported the ability of certain phytocannabinoids to modulate nuclear receptors, ligand-gated ion channels or transient receptor potential (TRP) channels, among others (Vara et al. 2013; De Petrocellis et al. 2012; Morales et al. 2017).

1.3 Non-cannabinoid Constituents

In addition to the aforementioned cannabinoids, other less studied components like terpenes (e.g.,

limonene), phenolic compounds such as flavonoids (e.g., quercetine), stilbenes (e.g., dihidroresveratrol), lignans (e.g. syringaresinol) and alkaloids (e.g., muscarine), among others, have been identified in the *Cannabis* plant.

1.3.1 Terpenoids

At least 200 structurally different terpenoids have been isolated and characterized from *Cannabis sativa* flowers (Ross and ElSohly 1996), roots (Slatkin et al. 1971), leaves (Hendriks et al. 1975) and trichomes (Kim and Mahlberg 2003). The structure of several terpenoid compounds is shown in Fig. 1.3. Terpenes are responsible for the odor and flavor of the different *Cannabis* strains and they have contributed to the selection of *Cannabis* narcotic strains under human domestication (Small 2015). The most abundant and representative terpenoids are β-myrcene, trans-caryophyllene, α-pinene, trans-ocimene, and α-terpinolene (Malingré et al. 1975). Specifically, in those *Cannabis* strains destined to psychoactive drug consumption, the most prominent and unique terpenoids identified were β-caryophyllene-epoxide (in fact, this is the compound sensed by dogs trained to detect drugs) and m-mentha-1,8(9)-dien-5-ol (Russo 2011). As in the case of other constituents, the level of terpenoids in the plants depends on the cultivation and breeding methods as well as the harvest time and the mode of processing (Brenneisen 2007; Fischedick et al. 2010). Terpenes, along with cannabinoids, have been successfully used as chemotaxonomic markers in *Cannabis*, as they are both considered as the main physiologically active secondary metabolites (Fischedick et al. 2010; Elzinga et al. 2015). Indeed, when plants are grown in standardized conditions, a significant and positive correlation was found between the level of terpenes and cannabinoids (Fischedick et al. 2010). This may be explained by the fact that mono- and sesqui-terpenes are synthesized in the same glandular trichomes where cannabinoids are also produced (Meier and Mediavilla 1998). This association could not be confirmed even after the analysis of a large panel of samples coming from different origins (Elzinga et al. 2015). It is important to remark that terpenes are lipophilic compounds that easily cross membranes and particularly the blood-brain barrier (Fukumoto et al. 2006), indicating that they could have different actions on the central nervous system. In this regard, several current reviews describe a wide array of pharmacological properties of these compounds (Russo 2011; Singh and Sharma 2015).

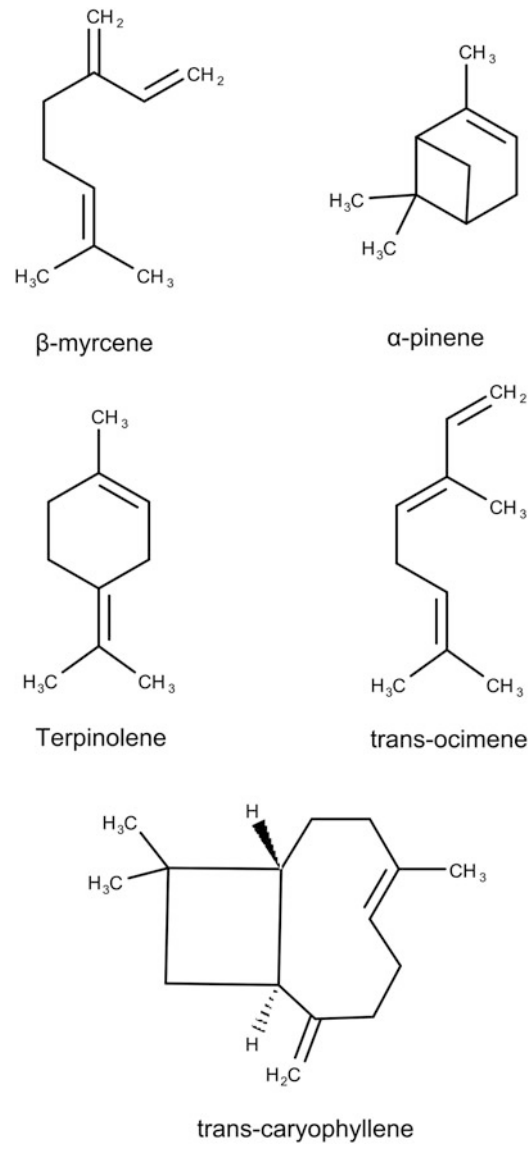

Fig. 1.3 Structure of most abundant terpenes in *Cannabis sativa*

1.3.2 Phenolic Compounds

Phenolic compounds, also known as phenylpropanoids, constitute one of the most widely distributed groups of secondary metabolites in the plant kingdom. They present more than 10.000 different structures, including phenolic acids, such as benzoic and hydroxycinnamic acids, flavonoids such as flavonols and flavones, stilbenes and lignans (Andre et al. 2010; Fig. 1.4). In *Cannabis*, about 20 flavonoids have been identified, mainly belonging to the flavone and flavonol subclasses (Flores-Sanchez and Verpoorte 2008).

The flavonoids of *Cannabis* can be classified into three categories: (1) *O*-glycosides of apigenin, luteolin, quercetin and kaempferol, described by Mc Partland and Mediavilla 2002; (2) *C*-glycosides of orientin and vitexin (Vanhoenacker et al. 2002) and, 3) prenylated flavonoids of cannaflavin A and B (Barrett et al. 1986). The lignanamides including cannabisin-like compounds (A-, B-, C-, D-, E-, F-, and G types; Flores-Sanchez and Verpoorte 2008) and lignans also were found in the *Cannabis sativa*.

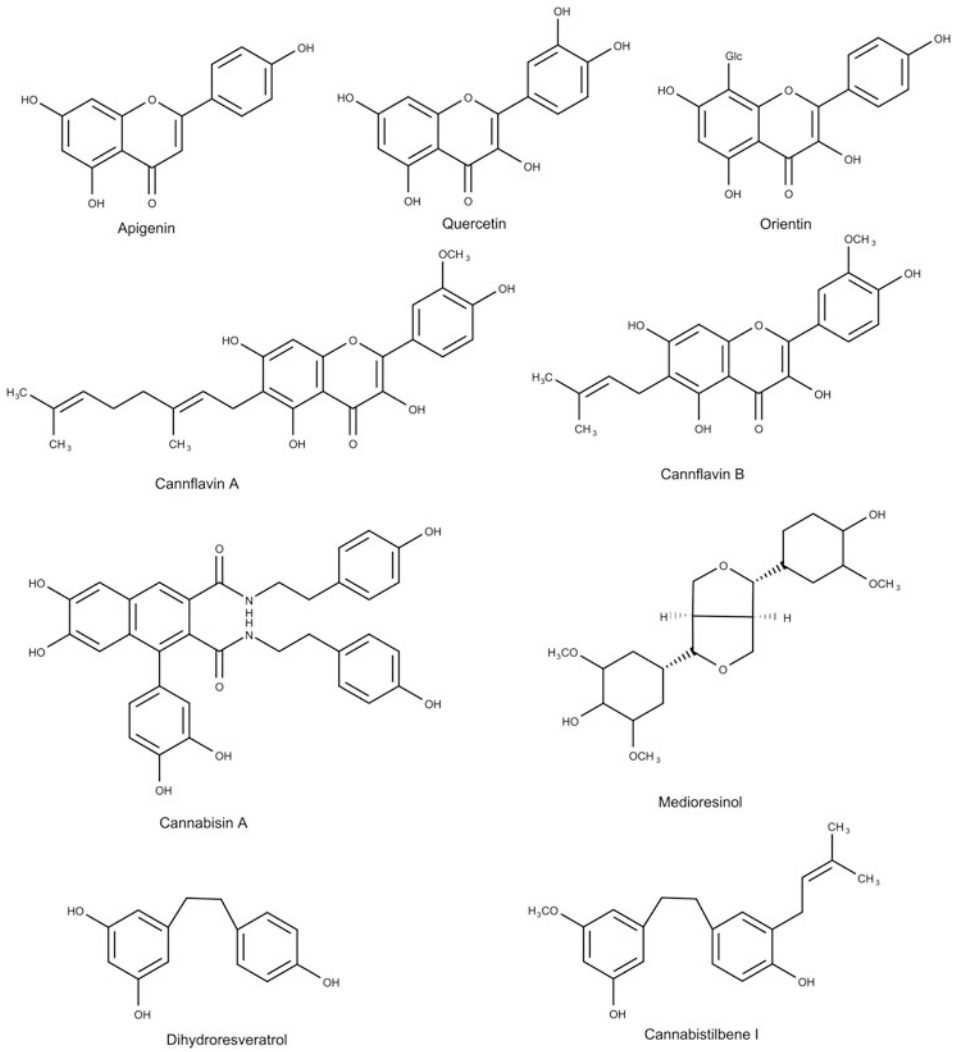

Fig. 1.4 Structure of most abundant phenolic compounds in *Cannabis sativa*

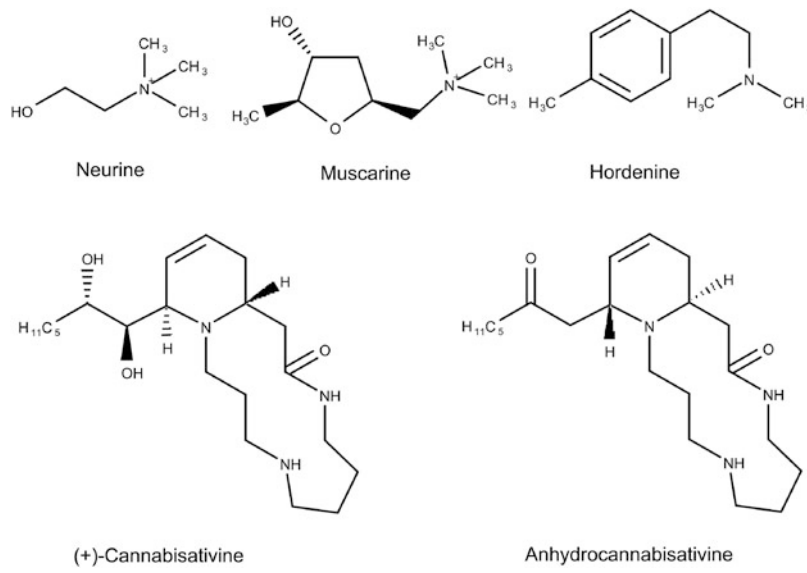

Fig. 1.5 Structure of most abundant alkaloids in *Cannabis sativa*

The lignan profile in hemp seeds was shown to be mainly determined by the presence of syringaresinol and medioresinol (Fig. 1.4) and also by secoisolariciresinol, lariciresinol, and pinoresinol (Smeds et al. 2012). Additionally, nineteen stilbenes have been isolated in *Cannabis* with a prototypical structural backbone such as spirans, phenanthrenes, and bibenzyls (Flores-Sanchez and Verpoorte 2008). They include molecules such as cannabistilbene I, IIa, and IIb, as well as dihydroresveratrol (Fig. 1.4).

Phenolic compounds may act as antioxidants and, thereby, protecting plants against oxidative stress. In humans, it has been shown that there is a good correlation between the intake of dietary phenolic compounds and the reduced incidence of chronic illnesses such as cancer, cardiovascular and neurodegenerative diseases (Arts and Hollman 2005). However, it is relevant to take into account that these positive health effects may not be entirely explained by the phenolic antioxidant properties, because they are poorly bioavailable. In particular, *in vivo* conditions, phenolic compounds may induce the up-regulation of endogenous antioxidant enzymes, due to their ability to act as pro-oxidants (Dajas et al. 2013; Echeverry et al. 2015).

Flavones and flavonols found in *Cannabis* seem to exert a wide range of biological effects, including those properties shared by terpenes and cannabinoids. They can elicit anti-inflammatory, anti-cancer, and neuroprotective effects as reviewed by Andre and collaborators (2010)

1.3.3 Alkaloids

Nitrogen-containing compounds of *Cannabis sativa* have been investigated and only a small number of alkaloids have been identified (Fig. 1.5). Some pseudoalkaloids and related precursors, such as choline, trigonelline (a pyridine), muscarine (a protoalkaloid), isoleucine betaine, and neurine (Turner et al. 1980) have been identified. All these components have been isolated from *Cannabis* leaves, stems, pollen, roots, and seeds (ElSohly et al. 1978; Mechoulam 1988).

1.4 Conclusions

Given the botanical and chemical features of *Cannabis sativa*, this is considered one of the most important plants in the plant kingdom, with a great potential utility for the treatment of several diseases. It contains a great variety of cannabinoids, single and exclusive metabolites

of this species, making it a unique species. Due to great variability in its chemical composition, it is mandatory to have *Cannabis sativa* plants or extracts properly standardized. This procedure guarantees that pharmacological evaluations can be reliable and allows a correct indication and dosage for human therapy. The current growing knowledge about the key molecular components of *Cannabis* as well as their diverse phytochemical pathways may allow increasing the production of cannabinoids, terpenes, or phenolic compounds with more specific therapeutical effects.

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Synthetic and Natural Derivatives of Cannabidiol

2

Paula Morales and Nadine Jagerovic

Abstract

The non-psychoactive component of *Cannabis Sativa*, cannabidiol (CBD), has centered the attention of a large body of research in the last years. Recent clinical trials have led to the FDA approval of CBD for the treatment of children with drug-resistant epilepsy. Even though it is not yet in clinical phases, its use in sleep-wake pathological alterations has been widely demonstrated.

Despite the outstanding current knowledge on CBD therapeutic effects in numerous *in vitro* and *in vivo* disease models, diverse questions still arise from its molecular pharmacology. CBD has been shown to modulate a wide variety of targets including the cannabinoid receptors, orphan GPCRs such as GPR55 and GPR18, serotonin, adenosine, and opioid receptors as well as ligand-gated ion channels among others. Its pharmacology is rather puzzling and needs to be further explored in the disease context.

Also, the metabolism and interactions of this phytocannabinoid with other commercialized drugs need to be further considered to elucidate its clinical potential for the treatment of specific pathologies.

Besides CBD, natural and synthetic derivatives of this chemotype have also been reported exhibiting diverse functional profiles and providing a deeper understanding of the potential of this scaffold.

In this chapter, we analyze the knowledge gained so far on CBD and its analogs specially focusing on its molecular targets and metabolic implications. Phytogenic and synthetic CBD derivatives may provide novel approaches to improve the therapeutic prospects offered by this promising chemotype.

Keywords

Cannabinoid · Cannabidiol · Synthetic cannabidiol · Cannabidiol derivative · Cannabidiol analog · Sleep

2.1 Introduction

In June 2018, the FDA approved a purified form of CBD (Epidiolex®) to treat seizures associated with two rare, severe forms of childhood epilepsies, Lennox-Gastaut and Dravet syndromes (Gupta and Santhakumar 2018; Hausman-Kedem et al. 2018; Neubauer et al. 2018). Lennox-Gastaut is severe pediatric epilepsy that generates slow mental development characterized by multiple seizure types including tonic, atypical absences, and drop attacks. A dravet syndrome is also a severe form of epilepsy that appears during the first year of life. The

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condition involves frequent febrile seizures but also myoclonus and status epilepticus. In most cases, this syndrome is due to a mutation in the *SCN1A* (sodium voltage-gated channel alpha subunit 1) gene. In both syndrome conditions, the seizures are hard to control with first-line antiepileptic drugs such as valproic acid and clobazam. The long-term prognosis is poor in most cases and epilepsy will need life-long treatment. Components of the *Cannabis* plant demonstrate efficacy for epilepsy. However, while tetrahydrocannabinol (THC) shows anticonvulsant effects in some animal models and proconvulsant in others, (Reddy and Golub 2016) much interest has been shown for the non-psychotropic component CBD. The antiepileptic properties of CBD allow for translation research from bench to bedside (Anderson et al. 2017).

CBD is also clinically used in a cannabis-based preparation (Sativex®) containing THC to treat multiple sclerosis symptoms. In its own right, CBD has generated much interest during these last years for a diversity of therapeutic applications such as neuroprotection (Fernandez-Ruiz et al. 2013), newborn hypoxic-ischemic brain damage (Ceprián et al. 2017), complex motor disorders (Libzon et al. 2018), anxiolytic (Fogaça et al. 2018), antipsychotic (Mandolini et al. 2018), and antitumor agents among others (Morales et al. 2017) (Pisanti et al. 2017),

Somnolence has been reported as one of the described adverse events of CBD treatment. However, few studies have addressed the effects of CBD, in its own right, on sleep either in human or in animal models (Murillo-Rodríguez et al. 2006, 2014, 2018; Russo et al. 2007; Chagas et al. 2014; Gates et al. 2014). A review covering clinical trials of *Cannabis*-based medicines analyses sleep parameters as a secondary outcome of neuropathic pain, symptoms of multiple sclerosis and rheumatoid arthritis treatments (Russo et al. 2007). The overall data indicate a mild activating effect with CBD- and slight sedation with THC- predominant extracts. Clinical trials with CBD in healthy volunteers and insomniacs realized between 1972 and 1981 seemed to improve sleep and reduce episodic wakening of insomniacs (Carlini and Cunha 1981). The volunteers also reported significantly less dream recall. Recently, the effect of a clinically anxiolytic dose of CBD on the sleep-wake cycle of healthy subjects has been evaluated (Linares et al. 2018). In this study, CBD does not appear to interfere with the sleep cycle of healthy subjects compared to placebo using polysomnography recordings and cognitive measurements. In other studies, CBD increased wakefulness (Nicholson et al. 2004; Murillo-Rodríguez et al. 2014).

There are contradictory data related to the effects of CBD on sleep. The available evidence to date would suggest that CBD improves sleep in patients with neurogenic symptoms including multiple sclerosis and chronic pain. In concomitant administration with THC, CBD seems to increase in awaken activity counteracting the sedative activity of THC. However, in what concerns the therapeutic potential of CBD on patients with sleep disorders, there is a clear need for large clinical trials and exploration of its mechanism of action at the molecular level.

Various molecular targets have shown to be involved in the diverse therapeutic effects of CBD. In this chapter, we analyze the knowledge gained so far on CBD and its analogs especially focusing on the biological targets related directly or indirectly to sleep events. Due to the increasing interest in CBD, the therapeutic potential of several natural and synthetic CBD derivatives with a resorcinol-based structure, have been studied. These CBD analogs could help to fine-tune the properties of CBD. Further progress is more likely to come from rational drug design, an approach based on understanding molecular target-interactions of CBD and how CBD modulates neurotransmitter synthesis, release, reuptake, and degradation.

2.2 Molecular Targets of CBD

Despite the extensive knowledge gained on its therapeutic effects, the molecular pharmacology of CBD is still intricate. Up to now, numerous research efforts are focused on its molecular

understanding trying to fully determine its mechanism of action under different physiopathological processes. Diverse receptors related or not to the endocannabinoid system have been revealed as targets for CBD.

In competitive binding assays, CBD was reported to have a very low affinity for the cannabinoid receptors type 1 (CB1) and type 2 (CB2) whereas most phytocannabinoids such as Δ^9-THC show affinity (McPartland et al. 2007). Despite this low affinity, functionality data of CBD at CB1 and CB2 have been reported. *In vitro* studies performed by diverse research groups, CBD displayed antagonist properties at CB1 and CB2 (Pertwee et al. 2002; Thomas et al. 2007). It has been recently suggested that this antagonist profile could be explained by modulation of these receptors through allosteric mechanisms of action (Laprairie et al. 2015; Morales et al. 2016; Martínez-Pinilla et al. 2017; Tham et al. 2018). Allosteric compounds modulate affinity and/or efficacy of specific orthosteric ligands. This cooperativity can be positive; in this case, the allosteric will be classified as PAM, or negative for NAM compounds. Laprairie and coworkers firstly evidenced that CBD behaves as CB1 negative allosteric modulator (NAM) of Δ^9-THC and 2-AG (Laprairie et al. 2015). The potential allosteric properties of CBD at CB2 have been reported by Franco and collaborators (Martínez-Pinilla et al. 2017). A later report from Lapraire's group suggests that CBD is a partial agonist at CB2 (Tham et al. 2018).

There is no doubt that the functionality of CBD at the cannabinoid receptors is complex and intrigued. It has also been recently suggested that biased functionality could be involved in the mechanism of action of CBD at these receptors (Navarro et al. 2018). Coupling of a receptor to various signaling effectors, such as different types of G proteins or β-arrestins, is a concept that has been emerging in GPCRs literature (Smith et al. 2018). This is a much more recent approach in the cannabinoid field (Ibsen et al. 2017). Biased agonists can activate G-protein signaling or β-arrestin-dependent signaling by tuning the receptor to obtain a selective receptor response. In this context, the biased signaling of CBD at CB1 and CB2 has been assessed using four different functional outcomes (Navarro et al. 2018). In addition to its functional selectivity, CBD seems to be able to allosterically modulate the effect of other cannabinoids such as Δ^9-THC and the synthetic cannabinoid ACEA (arachidonyl-2′-chloroethylamide). It is also noteworthy commenting that modulatory effects were observed for CBD at CB1-CB2 heteromers (Navarro et al. 2018). Effectively, CBD altered the signaling of Δ^9-THC acting on CB1-CB2 heteromers.

Modulatory divergences among reports may arise from the use of different experimental endpoints and functional outcomes, or to very complex functionality that has not be fully elucidated.

The basal endocannabinoid system tone can also be directly affected by CBD. This phytocannabinoid can potently inhibit the cellular uptake of anandamide, an endogenous ligand, blocking the enzymatic hydrolysis of FAAH (fatty acid amide hydrolase) (De Petrocellis et al. 2011; Leweke et al. 2012). It has been suggested that this effect might be due to CBD's ability to bind to fatty acid-binding proteins (FABPs) (Elmes et al. 2015). These proteins mediate endocannabinoids' transport to its catabolic enzymes.

CBD is further involved in the modulation of orphan GPCRs that have been postulated as potential members of the endocannabinoid system (Morales and Reggio 2017). For instance, CBD has been reported to antagonize the putative cannabinoid receptors GPR55 (Ryberg et al. 2007; Whyte et al. 2009; Ford et al. 2010; Morales and Jagerovic 2016) and GPR18 (McHugh et al. 2012, 2014). Moreover, the highly constitutively active receptors GPR3, GPR6, and GPR12, phylogenetically related to CB1 and CB2, have been recently identified as potential targets of CBD (Table 2.1) (Laun and Song 2017; Brown et al. 2017; Morales et al. 2018).

Besides its still puzzling cannabinoid characterization, several reports have ascertained the ability of CBD to modulate targets that are not part of the endocannabinoid system including

Table 2.1 Summary of the molecular targets of CBD

Molecular Targets of CBD			
Receptor type	Target	Activity	References
GPCRs	CB1	Antagonist	(Pertwee et al. 2002; Thomas et al. 2007)
		NAM	(Laprairie et al. 2015; Tham et al. 2018)
	CB2	Antagonist	(Thomas et al. 2007)
		NAM	(Martínez-Pinilla et al. 2017)
	GPR55	Antagonist	(Ryberg et al. 2007; Whyte et al. 2009)
	GPR18	Antagonist	(McHugh et al. 2012, 2014)
	GPR3	Inverse agonist[a]	(Laun and Song 2017)
	GPR6	Inverse agonist[a]	(Laun and Song 2017)
	GPR12	Inverse agonist[a]	(Brown et al. 2017)
	5-HT_{1A}	Agonist	(Russo et al. 2005; Rock et al. 2012)
	5-HT_{2A}	Partial agonist[a]	(Russo et al. 2005)
	A_{1A}	Agonist	(Gonca and Darıcı 2014)
	μ-OPR	NAM	(Kathmann et al. 2006; Bartuzi et al. 2015)
	δ-OPR	NAM	(Kathmann et al. 2006; Bartuzi et al. 2015)
Nuclear receptors	PPARγ	Agonist	(O'Sullivan et al. 2009; Esposito et al. 2011; Scuderi et al. 2014)
Ligand-gated ion channels	GlyR α_1	PAM	(Ahrens et al. 2009)
	GlyR α_2	ND	–
	GlyR α_3	PAM	(Xiong et al. 2012)
	5-HT_{3A}	Antagonist	(Yang et al. 2010)
	α7-nACh	Antagonist	(Mahgoub et al. 2013)
	$GABA_A$	PAM	(Bakas et al. 2017)
	Nav	Antagonist	(Ghovanloo et al. 2018)
TRP channels	TRPV1	Agonist	(De Petrocellis et al. 2011)
	TRPV2	Agonist	(De Petrocellis et al. 2011)
	TRPV3	Agonist	(De Petrocellis et al. 2012)
	TRPA1	Agonist	(De Petrocellis et al. 2011)
	TRPM8	Antagonist[a]	(De Petrocellis et al. 2008)
Enzymes	FAAH	Inhibitor	(De Petrocellis et al. 2011)
	CYP1A1	Inhibitor	(Yamaori et al. 2010, 2013, 2014)
	CYP2C19	Inhibitor	(Jiang et al. 2013)
	CYP2D6	Inhibitor	(Yamaori et al. 2011)
	CYP3A4	Inhibitor	(Yamaori et al. 2011)
	CYP3A5	Inhibitor	(Yamaori et al. 2011)

ND Not determined
[a]Weak modulation

non-cannabinoid related GPCRs, nuclear, and ionotropic receptors among others (Table 2.1).

GPCRs, from well-established families, such as the serotonin, the adenosine, or the opioid receptors are involved in the therapeutic effects of CBD. Full agonism and weak partial agonism of CBD were observed at $5HT_{1A}$ and $5HT_{2A}$, respectively (Russo et al. 2005; Rock et al. 2012). Additionally, CBD exhibited the ability to activate the A_{1A} adenosine receptors (Gonca and Darıcı 2014) and allosterically modulate the μ- and δ-opioid receptors (Kathmann et al. 2006; Bartuzi et al. 2015) (Table 2.1).

Nuclear receptors such as PPARγ (O'Sullivan et al. 2009; Esposito et al. 2011; Scuderi et al. 2014), ligand-gated ion channels such as glycine (GlyR) (Ahrens et al. 2009; Xiong et al. 2012), nicotinic acetylcholine (nACh) (Mahgoub et al. 2013), sodium channels (Nav) (Ghovanloo et al. 2018), and $GABA_A$ receptors (Bakas et al. 2016), and transient receptor potential (TRP) channels (De Petrocellis et al. 2008, 2011, 2012) are also targets for CBD. The functional activity of this phytocannabinoid at the aforementioned receptors in the experimental conditions described in the indicated references is resumed in Table 2.1.

The specific molecular targets involved in CBD's therapeutic effects in sleep/wake functions are not fully determined yet. CB1 might be among the plausible receptors through which it exerts these effects. The role of this cannabinoid receptor in the regulation of sleep has been studied by Murillo-Rodríguez and coworkers (Murillo-Rodríguez 2008; Murillo-Rodríguez et al. 2008). Systems involved in the neurochemical maintenance of the waking state such as acetylcholine, dopamine, or serotonin may play a role in CBD's wakefulness increase (Murillo-Rodríguez 2008). Moreover, the GABAergic and the adenosine systems, associated to sleep induction (Gottesmann 2002; Blanco-Centurion et al. 2006), may also be altered upon CBD's treatment.

As analyzed herein, the complex molecular pharmacology of this phytocannabinoid is delaying the elucidation of its mechanism of action on sleep modulation as well as in other pathologies. Extensive *in vivo* studies are essential to provide further insights on this topic.

2.3 Metabolism and Interaction with Other Drugs

As mentioned above, CBD shows an intricate pharmacological profile that is a main cause of concern when treating sleeps disorders and trying to understand the related mechanism of action. Another factor that needs to be taken into account and that have a significant impact on CBD for clinical use is the metabolic and the pharmacokinetic processes. Effectively, the metabolism of CBD deserves a special emphasis since it may determine its therapeutic potential under particular physiopathological conditions. CBD has been reported to interact with several metabolic enzymes that define its pharmacokinetics and its possible interaction with other drugs (Zendulka et al. 2016).

CBD is metabolized in the liver by several cytochrome P450 (CYP450) isoenzymes. The main isoforms involved in its metabolism are CYP2C19 and CYP3A4 being 7-OH-CBD its primary metabolite (Fig. 2.1) (Jiang et al. 2011; Stout and Cimino 2014; Zendulka et al. 2016). To a lesser extent, enzymes such as CYP2D6, CYP1A1, or CYP3A5 also participate in this process (Yamaori et al. 2011, b, 2013, 2014). Depending on the administered dose (Zendulka et al. 2016), CBD can function as a potent competitive inhibitor of all of these CYP450 isoenzymes.

Structural determinants of CBD's interaction with specific CYP450 isoforms have been explored. For instance, the potent inhibitory capacity of CBD at CYP1A1 has been reported to be due to direct interactions of the enzyme with the resorcinol moiety of CBD (Yamaori et al. 2013, 2014). Docking studies of CBD in the CYP1A1 enzyme model propose that the phenyl ring forms a stacking interaction while the two hydroxyl groups induce hydrogen bond with residues at the active site. These molecular insights may also explain the reduced inhibitory potential exhibited by mono and di-methoxylated CBD derivatives (CBDM and CBDD, Fig. 2.1). Likewise, the resorcinol moiety showed to be required for potent inhibition at CYP3A (Yamaori et al. 2011) and CYP2C19 (Jiang et al. 2013). In the latest enzyme, only one of the hydroxyl groups seems to be necessary for the activity (Jiang et al. 2013).

These interactions are very relevant not only for its metabolism but also in what concerns possible drug-drug interactions. Even though CBD inhibition of CYP could trigger treatment failure or drug toxicity in specific cases, it has been demonstrated that CBD can potentiate the

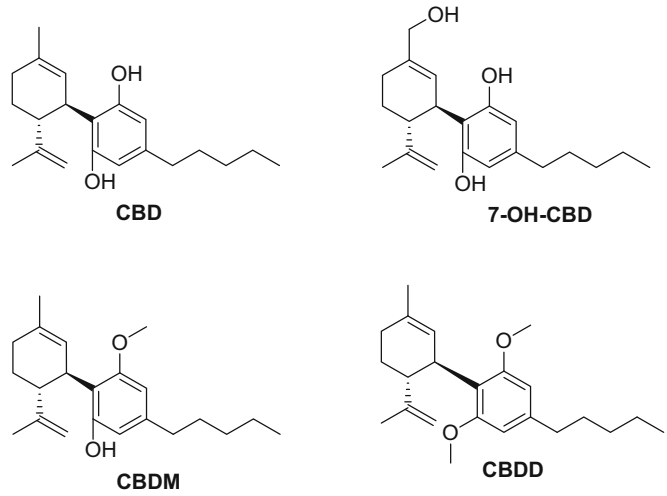

Fig. 2.1 Structure of CBD, its metabolite 7-OH-CBD (7-hydroxycannabidiol) and the methoxylated CBD derivatives CBDM (2-methoxycannabidiol); CBDD (2,6-dimethoxycannabidiol)

therapeutic properties and reduces the side effects of other phytocannabinoids such as Δ^9-THC (Varvel et al. 2006; Klein et al. 2011; Britch et al. 2017). In fact, by inhibiting these metabolic enzymes, CBD may enhance the half-life of other cannabinoids or specific drugs. Therefore, CBD can provide beneficial synergistic effects if combined with certain molecules at appropriate doses. However, the clinical relevance of CBD's interaction with other drugs has yet to be evaluated in-depth attending to specific pathologies and treatments.

2.4 CBD Derivatives

Classification of CBD derivatives following structural criteria and associating their biological targets has been extensively reviewed elsewhere (Morales et al. 2017). Here, CBD derivatives, natural or synthetic, are reported regarding the possible benefit or disadvantages over CBD from a therapeutic perspective.

Diverse molecular targets have been suggested for CBD in an attempt to elucidate mechanisms of action engaged in its therapeutic activities. This interest has been focused this last decade on CBD because it is the second most abundant phytocannabinoid present in *Cannabis*. However, other natural CBD derivatives have been isolated from the plant. So far, they have been less explored than CBD for their potential therapeutic applications (Morales et al. 2017). Among them, CBDA, CBDV, and CBD-C1 (O-1602) have been the most studied preclinically to our knowledge (Fig. 2.2). Reviewing their reported action on certain targets may help to understand the differences with CBD. Even though CBDA, CBDV, and O-1602 are natural CBD analogs, their properties may be very unlike.

The differences in activity between CBD and its analogs are common. Whereas CBD has been reported to antagonize the activity of GPR55, its short-alkyl chain analog O-1602 activates GPR55 boosting the production of the inflammatory markers, the endogenous interleukin IL-12 and the tumor necrosis factor α (TNF-α) (Chiurchiù et al. 2015). Concerning the degradation of endocannabinoids, the inhibition of DAGLα by CBDA, and its lack of activity on FAAH are opposite to the effects of CBD on these enzymes (De Petrocellis et al. 2011). The enzymatic activity of human calpain-1 is activated by CBD, but not by CBDA (Takeda et al. 2017). In other cases, this is the potency of natural CBD analogs that differs from CBD. On TPR channels, CBD is

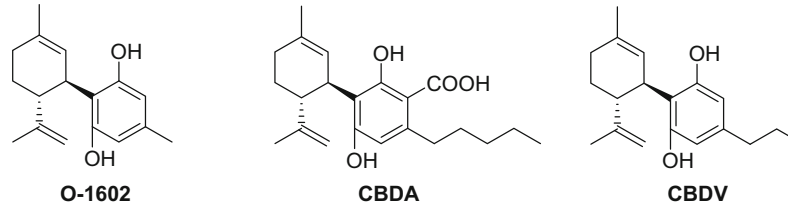

Fig. 2.2 Natural CBD analogs: cannabidiorcol (CBD-C1; O-1602), cannabidiolic acid (CBDA) and cannabidivarin (CBDV)

much more potent than CBDA. At GPR55, CBDA and CBDV significantly inhibit LPI-induced ERK1/2 phosphorylation as does CBD (Anavi-Goffer et al. 2012). The vasodilator effect of O-1602 is well reported even though its mechanism of action is not well understood. Nevertheless, there is evidence that O-1602-induced-vasorelaxation is not mediated by TRPV1 and by the release of nitrogen oxide (NO), but either by inhibition of Ca^{2+} sensitive K^{+} channels (KCa) (Al Suleimani et al. 2015). CBD showed a similar effect on arteries in a rat model of type2 diabetes, with vasorelaxant responses that have been shown to involve CB2 receptors, COX, and the superoxide dismutase SOD enzymes (Wheal et al. 2014). Concerning the antiepileptic properties, as already commented in this chapter, CBD has been recently approved for Dravet and Lennox conditions. One of its natural analog, CBDV, has reached clinical phases under the name GWP42006 in patients with inadequately controlled focal seizures (https://clinicaltrials.gov/ct2/show/NCT02365610?term=GWP42006&rank=1). However, to our knowledge, no result on these clinic assays has been published so far.

Among synthetic CBD derivatives, the most explored have been HU-331, HU-320, HUF-101, VCE-004.8, HU-308, Abn-CBD, and hydrogenated CBD (Fig. 2.3). One of them, HU-331, also named CBD-hydroxyquinone, has been proposed as a metabolite of CBD formed under a catalytic process involving CYP3A11 (Bornheim and Grillo 1998). This reactive quinone has potent antitumor activity by targeting DNA topoisomerase II (Wilson et al. 2018). VCE-004.8, a synthetic derivative obtained by treatment of HU-331 with benzylamine, is a dual agonist of PPARγ and CB2 receptors with anti-fibrotic efficacy *in vivo* in a murine model of dermal fibrosis induced by bleomycin (Del Río et al. 2016). Its neuroinflammatory properties have also been highlighted in two murine models of multiple sclerosis, the autoimmune encephalomyelitis (EAE) and the Theiler's virus-induced encephalopathy (TMEV) models (Navarrete et al. 2018). Interestingly, fluorination of CBD aromatic ring (HUF-101) potentiates the anxiolytic, antidepressant, antipsychotic, and anticompulsive properties of CBD in rodent models (Breuer et al. 2016), as well as its antinociceptive effects in different animal models (Silva et al. 2017). This increase in potency may be due to better pharmacokinetic parameters of HUF-101 over CBD. Therefore, HUF-101 represents an interesting candidate as a therapeutic drug. The results of earlier studies realized with halogenated CBD analogs such as 3-Cl-CBD (Fig. 2.4) for barbiturate-induced sleep prolongation indicate that the monohalogenated derivatives (3-Cl, 3-I) prolong the sleeping time as does CBD, whereas dihalogenated derivatives did not exhibit any time prolongation (Usami et al. 1999).

CBD as a phenolic compound is very lipophilic. Thus, its glycosylation can significantly improve its solubility and stability. Incubation of CBD with tissue cultures from *Pinellia ternata* and *Datura inoxia*, yields CBD-6'--O-β-D-glucopyranoside and CBD-(2′,6′)-O-β-D-diglucopyranoside (Tanaka et al. 1996). The resulting glycoside compounds are prodrugs that may be the target- or tissue-specific for delivery. Thus, they should release CBD or CBDV and simple glucose sugars upon prodrug decoupling. More recently, CBD and CBDV have been successfully glycosylated by UGT76G1, an enzyme from *Stevia rebaudiana* (Hardman et al. 2017).

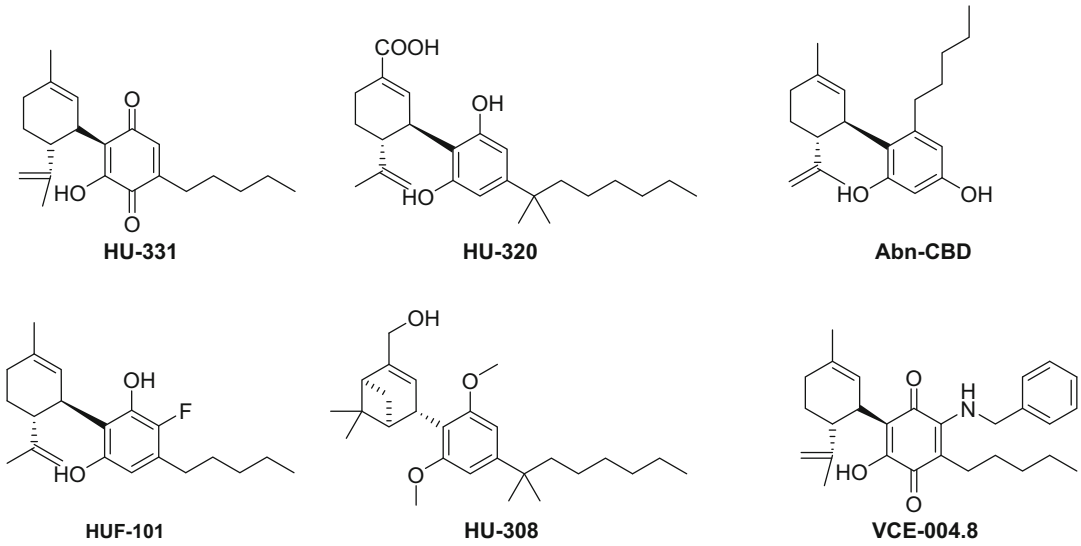

Fig. 2.3 CBD analogs. HU-311, HU-320, Abn-CBD, HUF-101, HU-308, and VCE-004.8

As CBD, its dimethoxy analog CBDD (Fig. 2.1) has no orthosteric binding affinity for the CB1 and CB2 receptors. Against hepatic CYP-mediated metabolism, CBDD has been reported to be more resistant than CBD (Yamaori et al. 2013). Effectively, CBD is converted into cannabielsoin when incubated with guinea pig liver microsomes. Therefore, the resistance of CBDD against liver microsome-mediated metabolism offers an interesting safety profile compared to CBD. There is a significant activity difference on food intake regulation and energy balance between CBD and CBDD. In general, CBD produces a significant decrease in body weight gain in rats (Ignatowska-Jankowska et al. 2011). It has been reported that CBDD enhances body weight gain in ApoE-deficient mice selectively between 10 and 20 weeks of age (Takeda et al. 2015). However, both, CBD and CBDD, have a similar profile as potential therapeutic agents for the treatment of atherosclerosis. The CBDD is a potent and selective inhibitor of oxidized low-density lipoprotein (ox-LDL) formation that plays an important role in the atherosclerosis disease process (Takeda et al. 2011).

Based on reported anti-inflammatory properties of CBD, numerous CBD synthetic derivatives have been evaluated in inflammatory processes. For instance, HU-320, the dimethyl heptyl CBD analog, possess similar anti-inflammatory and immunosuppressive properties than CBD in murine collagen-induced arthritis (Sumariwalla et al. 2004). Hydrogenated CBD, hydrogenated HU-320 analogs and the own CBD parent showed different pharmacological profiles. For instance, H2-CBD and H4-CBD (Fig. 2.4) follow the CBD pattern with a suppressive effect of inflammatory mediators such as reactive oxygen intermediates (ROI), nitric oxide (NO), and tumor necrosis factor (TNF-R) in murine macrophages. Nevertheless, H2-HU-320 and H4-HU-320 (Fig. 2.4) show an opposite effect against these inflammatory markers (Ben-Shabat et al. 2006). Moreover, it is worth pointing out the possible psychoactive effects of H4-CBD, H4-HU-320, and H2-HU-320 that bind CB1 receptor with affinity constant values in the nanomolar range, whereas H2-CBD, as CBD, binds weakly CB1 orthosteric binding site. The allosterism at CB1 and CB2 have not yet explored for these hydrogenated CBD and HU-320. It would have been interesting to explore this pharmacologic aspect due to the differences observed for the parent compounds. As previously mentioned, CBD behaves as CB2 partial agonist, whereas its dimethyl heptyl derivative (HU-308)

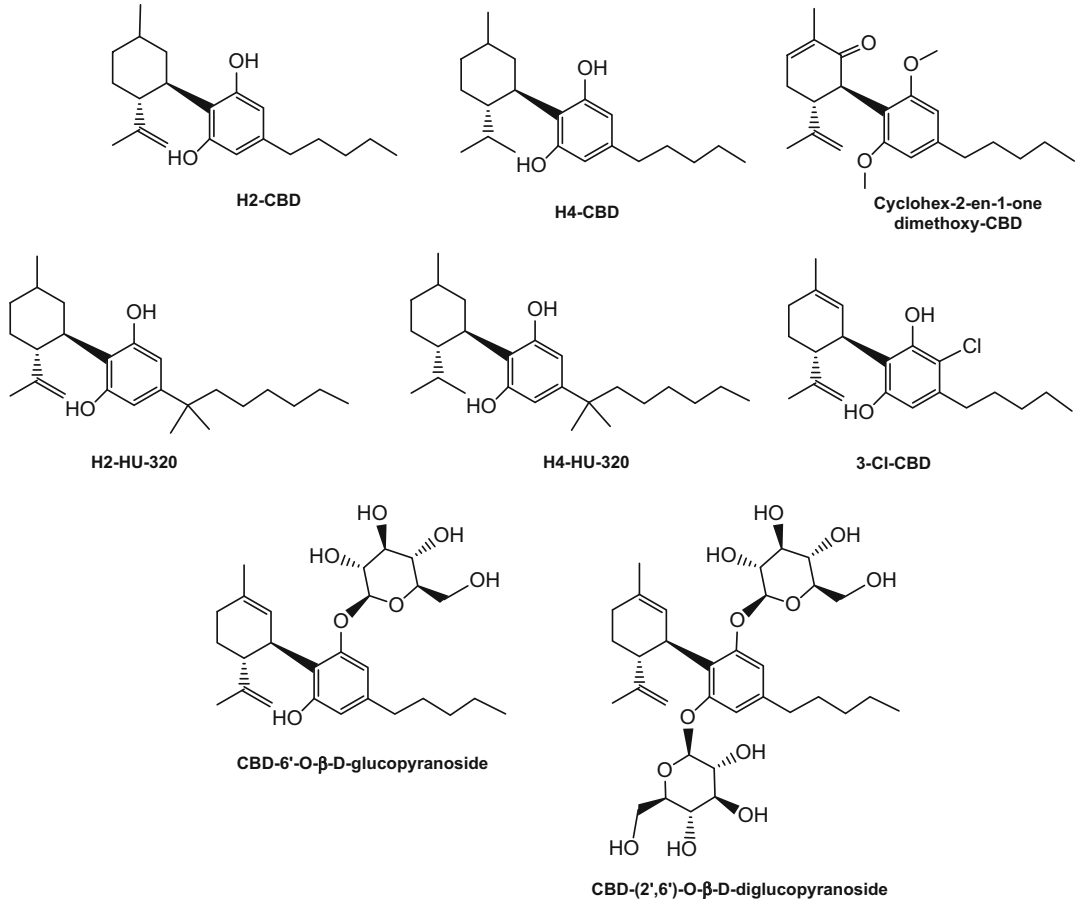

Fig. 2.4 CBD analogs. Cyclohex-2-en-1-one dimethoxy-CBD analog, CBD-6'-O-β-D-glucopyranoside, CBD-(2′,6′)-O-β-D-diglucopyranoside, H2-HU-320, H2-CBD, H4-CBD, and H4-HU-320

behaves as a PAM of cAMP modulation, but a NAM of β-arrestin1 recruitment (Tham et al. 2018).

We cannot report on CBD derivatives without mentioning HU-308, a pinene CBD derivative. HU-308 has been used as a pharmacological tool in a large number of assays involving the CB2 receptor, to which it selectively binds with high affinity. The anti-inflammatory properties of HU-308 have been widely studied as CB2 agonists, e.g. (Gómez-Gálvez et al. 2016). Due to its activity at the CB2 receptor, HU-308 certainly modulates the ECS with mechanisms of action quite different from CBD.

Abn-CBD is a regioisomer CBD derivative that conserves the non-psychoactive property of CBD. Its potential as a therapeutic agent has been shown for various pathology conditions. There is increasing evidence indicating that Abn-CBD is a potent GPR55 and GPR18 agonist (Johns et al. 2007; Console-Bram et al. 2014). It has been suggested that the most prominent actions of O-1602 and Abn-CBD in native systems are thought to be via GPR18 (Irving et al. 2017).

Exploring a novel synthetic methodology to access new CBD derivatives from (−)-carvone, new dimethoxy-CBD derivatives have been reported such as the cyclohex-2-en-1-one dimethoxy-CBD analogue which structure is described in Fig. 2.4 (Bailey et al. 2018). The therapeutic application of these new compounds has not been explored yet.

The fact that compounds are named CBD derivatives does not mean that they have similar activity to CBD. So far, the structure-activity relationship cannot be ruled out for CBD derivatives. A minimum structural change may be detrimental or beneficial for a specific target, as it is reflected, for instance, for CBD and O-1602 that have opposite activity at GPR55 with anti- or- pro-inflammatory processes respectively. To our knowledge, modulation of sleep and wakefulness has not been studied with any of these derivatives so far. Most of them have been studied in anti-inflammatory, anti-tumor, or metabolism assays.

2.5 Perspectives

Among the over 120 photogenic cannabinoids discovered so far, CBD stands out because of its promising therapeutic properties in a wide variety of diseases including the regulation of sleep. Besides, the lack of psychoactive effects exerted by this molecule provides to CBD a special relevance in the drug development scenario.

Many drugs acting on the brain may alter sleep. CBD crosses the blood-brain-barrier and could be one of them. The effects on sleep may involve the sleep onset, the quality and/or duration of sleep. These effects may be detrimental or beneficial depending on whether it is a normal or pathological physiological state. Neurotransmitters play a major role in modulating sleep and wakefulness. For instance, enhancing GABAergic transmission increases wakefulness, whereas activation of adenosine receptors A1 and A2A are involved in sleep promotion (Roehrs 2010). $GABA_A$ receptor is, for example, the therapeutic target of most sedative-hypnotic drugs. In this context, CBD may play an interesting but complex role in the neuropharmacology of sleep. As previously mentioned, CBD has been shown to interfere with GABAergic, adenosine, acetylcholine, dopamine, serotonin, and opioid systems. Therefore, it may be an interesting candidate for some sleep disorders treatments. For instance, it has been already reported that CBD potential in sleep/wake functions resides on its ability to regulate REM sleep behavior disorder and excessive daytime sleepiness. Thus, there is evidence for a potentially therapeutic application. However, characterization of the molecular targets involved in the sleep conditions and the corresponding related properties of CBD remains incomplete. Advances in this field will contribute to the development of CBD-based drugs.

Rational drug strategy for the development of CBD analogs as sedative-hypnotic drugs remains challenging. The CBD scaffold could be fine-tuned regarding the physiopathological conditions and their administration-distribution-metabolism-excretion (ADME) processes. Nevertheless, a deeper understanding of molecular interaction with specific targets may shed light on the design of CBD derivatives with improved pharmacokinetic and pharmacological profiles.

Concerning the action of CBD at the CB1 receptor, further studies to understand the role of CB1 in sleep functions upon CBD treatment may involve the use of diverse potential orthosteric ligands that may help to elucidate if CBD is acting as an allosteric modulator in these conditions.

Another concern that needs to be addressed for therapeutic purposes is the metabolism of CBD and its analogs. Even if more data are needed for deeper mechanistic insights, solid evidence suggests that the resorcinol moiety plays an important role in CYP450 inhibition.

In this chapter, CBD and its analogs have been treated as single entities. However, understanding potential interactions between CBD and other phytocannabinoids and endocannabinoids may also have relevant implications for understanding the long-term consequences of chronic cannabis use.

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3 Clinical Pharmacokinetics of Cannabinoids and Potential Drug-Drug Interactions

Marta Vázquez, Carlos García-Carnelli, Cecilia Maldonado, and Pietro Fagiolino

Abstract

Over the past few years, considerable attention has focused on cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC), the two major constituents of *Cannabis sativa*, mainly due to the promising potential medical uses they have shown. However, more information on the fate of these cannabinoids in human subjects is still needed and there is limited research on the pharmacokinetic drug-drug interactions that can occur in the clinical setting and their prevalence. As the use of cannabinoids is substantially increasing for many indications and they are not the first-line therapy in any treatment, health care professionals must be aware of drug-drug interactions during their use as serious adverse events can happen related with toxic or ineffective outcomes. The present chapter overview summarizes our current knowledge on the pharmacokinetics and metabolic fate of CBD and THC in humans and discusses relevant drug-drug interactions, giving a plausible explanation to facilitate further research in the area.

Keywords

Δ^9-tetrahydrocannabinol · Cannabidiol · Pharmacokinetics · Drug-drug interactions

3.1 Introduction

Cannabis (*Cannabis sativa* L.) is an annual herbaceous plant originated from Central-West Asia and widely distributed in the world (Andre et al. 2016). It has been used for millennia in folk medicine and to produce fiber. Due to the psychoactive properties of the plant, it has also been used in religious rituals or for recreational purposes (Wills 1998). The vegetable extracts reached maximum popularity in the western world at the end of the nineteenth century, mainly in the form of tinctures or fluid-extracts (Fankhauser 2002). Subsequently, different reasons led to its prescription abandonment, falling into gradual disuse during the first half of the last century. In this way, this plant of ancient culture and a great use for human beings became well-known in the last decades of the previous century almost exclusively for its psychoactive properties and non-medicinal purposes. Thus, cannabis became the most consumed illicit drug in the world, and despite its potential therapeutic applications, there

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was an obvious delay in basic and applied research on this plant concerning other drugs of vegetable origin.

The Cannabis plant (also referred to in many texts as Indian hemp, and commonly known as marijuana) belongs to the Cannabaceae family. The number of species within the Cannabis genus has been the product of a long controversy. Some authors recognize two or three species: *Cannabis sativa* L., *Cannabis indica* Lam., *and Cannabis ruderalis* Janisch., which can be distinguished by their way of growth, the characteristics of their fruits and the structure of their fibers (ElSohly et al. 1983). However, at present, it is considered as monospecific (*Cannabis sativa* L.), and it is classified into different varieties (Pollio 2016; Small 2017). These varieties often show clear morphological differences among themselves, but also remarkable phytochemical dissimilarities. Beyond that, different genotypes can be established (Hillig 2005) as well as different cannabis chemotypes according to the content of cannabinoids (de Meijer 2014) and terpenes (Fischedick 2017). The type and quantity of secondary metabolites not only vary according to the genotype but also to the plant organ, the age of the plant and the growing conditions (Hillig and Mahlberg 2004; Aizpurua-Olaizola et al. 2016). In this way, the effects on the biological systems of a cannabis extract will be potentially different depending on the factors mentioned above. Under controlled culture conditions, the pharmacological activity will depend on the chemotype used (Lewis et al. 2018).

Cannabis is predominantly dioecious (male and female flowers occur on separate plants). The sex of the plant is anatomically indistinguishable before the maturation and flowering phase. Virtually all aerial parts of the cannabis plant are covered with trichomes. But it is in the bracts of the female inflorescence where the highest density of glandular trichomes (rich in cannabinoids) is found (Potter 2014). Cannabinoids represent a group of secondary metabolic substances isolated only from the Cannabis plant. More than 120 different molecules of cannabinoids have been described so far (ElSohly et al. 2017). The main cannabinoids in the Cannabis plant include Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN) and cannabigerol (CBG). The molecular structures are shown in Fig. 3.1. THC, found at higher concentrations than CBD in the psychotropic ("drug-type") varieties for *Cannabis Sativa*, is the primary psychoactive compound, with CBD, a non-psychoactive

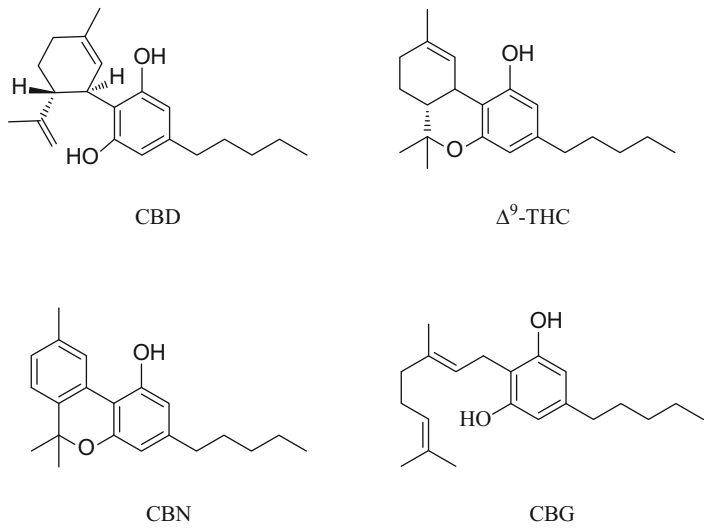

Fig. 3.1 Molecular structure of the main cannabinoids present in the cannabis resin

compound, ranking second. But the plant chemistry is far more complex than that of cannabinoids and different effects may be expected due to the presence of other chemicals. The number of other known compounds in the plant increased from approximately 400 to 650 (ElSohly and Gul 2014; Radwan et al. 2015,) so the chemical composition of *Cannabis sativa* is constantly changing. Apart from cannabinoids, the plant produces a large number of secondary metabolites such as terpenoids and flavonoids (ElSohly and Slade 2005). In recent years, terpenes have also been of interest, which, in addition to contributing to the organoleptic properties of the plant, they contribute to the pharmacological activity by modulating the effects of cannabinoids (Thomas and ElSohly 2016).

As a consequence of the development of synthetic cannabinoids and the discovery of endogenous ligands of the cannabinoid receptor (endocannabinoids) chemically different from plant cannabinoids, the term phytocannabinoids was proposed for these components of cannabis in particular (Russo 2007).

Phytocannabinoids in the plant are almost exclusively present as monocarboxylic acids and are practically not found as their neutral compounds (Flemming et al. 2007; Flores-Sanchez and Verpoorte 2008) but are formed upon decarboxylation of the acids (Fig. 3.2). The carboxyl group is not very stable and phytocannabinoids undergo spontaneous loss of this carboxyl moiety when subject to high temperature or direct sunlight. This occurs mainly in the harvest and post-harvest processes that is, when drying, heating, and storing are taking place, but also while smoking or in a hot oven when foods are prepared (Agurell and Leander 1971; Brenneisen 1984; De Backer et al. 2009).

Although the endocannabinoid system has been widely investigated and is considered an important neurotransmitter system, further investigation is needed to fully understand how this system works. Since the discovery of anandamide and 2-arachidonoylglycerol at the beginning of 1990, new molecular targets and biosynthetic and catabolic enzymes have been identified (Di Marzo 2018) and new research is being carried out as the complex functions of this novel system have created multiple new targets for drug action (Kerbrat et al. 2016; Navarro et al. 2016; van Esbroeck et al. 2017).

Cannabis and cannabinoid drugs have been increasingly accepted to treat several diseases or alleviate symptoms. Research into the medical use of cannabis and cannabinoids is constantly evolving but with differences in the available supporting data (Ware et al. 2010; Portenoy et al. 2012; Deshpande et al. 2015; Whiting et al. 2015; Devinsky et al. 2017; Meng et al. 2017; Nugent et al. 2017; Stevens and Higgins 2017; Thiele et al. 2018). An important fact to bear in mind is that the majority of the studies conclude on the use of cannabis as a third- or fourth-line therapy and the use of cannabis as monotherapy or first-line therapy is not supported for any indication. Mainly two major cannabinoids, CBD and THC have become the focus of clinical research. However, only THC and its metabolites: 11-hydroxytetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxytetrahydrocannabinol (THC-COOH) have been investigated more thoroughly.

A very important concept in pharmacology is pharmacokinetics. Pharmacokinetics, to put in simple words, is what the body does to a drug. A bit more technically, pharmacokinetics can be defined as the study of the time course of the

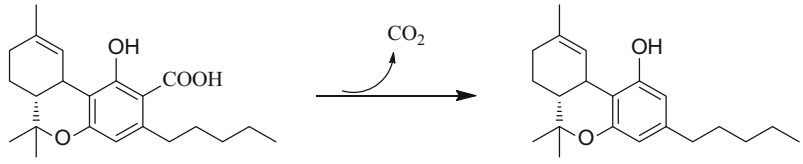

Fig. 3.2 Decarboxylation of delta-9-THC acid by heat action

absorption, distribution, metabolism, and excretion (ADME) of a drug. Dissolution is the first step in the oral absorption of solid drugs. The long and complex process of absorption culminates when the drug reaches the arteries of the great circulation. The stages of release and absorption are sequential, ie if the drug is not previously released from the pharmaceutical form, it cannot be absorbed. However, the distribution, metabolism, and excretion processes are simultaneous, and they are subsequent to the absorption. Once the drug reaches the aorta artery, it is available to be distributed to the different tissues and to undergo elimination (metabolism and/or excretion). Understanding the pharmacokinetics of a drug is essential to know the onset, magnitude, and duration of its pharmacodynamic responses and is critical in defining conditions for safe and effective use in patients.

The renewed interest in the therapeutic effects of cannabis makes cannabis available as a medicine to patients with a variety of conditions. As cannabinoids do not constitute first-line therapies as previously mentioned, health care professionals must be aware of drug-drug interactions during their use as sometimes serious or even fatal adverse events can happen either related to toxic or ineffective outcomes.

Little information on the pharmacokinetics of THC and CBD in humans and pharmacokinetic interactions of these cannabinoids with conventional medicine is available in the literature so this chapter will mainly focus on these issues. To facilitate further research in the clinical area, possible drug-drug pharmacokinetic interaction mechanisms will also be proposed and discussed in this chapter.

3.2 Pharmacokinetics of Cannabinoids

Extravascular cannabinoid pharmacokinetics encompasses absorption after diverse routes of administration and from different drug formulations, drug distribution throughout the body, and elimination (mostly by metabolism through the liver and extra-hepatic tissues, negligible by excretion). Studying the evolution and the fate of cannabinoids in the body has been a challenging task so far as the absorption and disposition of these compounds vary as a function of the route of administration. The most common methods of administration of cannabinoids are inhalation (smoking/vaporization), sublingual, and oral ingestion. Cannabinoids may be also taken by rectal administration, via transdermal delivery, eye drops, and aerosols. However, studies about the pharmacokinetics using these routes are scarce.

3.2.1 Absorption

3.2.1.1 Inhalation/Smoking

When smoked, the rapid delivery of cannabinoids compounds from the lungs to the central nervous system is observed. For this reason, smoking cannabis has high addictive potential (Grotenhermen 2007; Borgelt et al. 2013). Several studies revealed that plasma THC concentrations following inhalation are similar to those obtained after intravenous administration (Ohlsson et al. 1980). In these studies, high plasma concentrations were obtained within minutes and then concentrations dropped quickly. Due in part to intra- and inter-subject variability in smoking dynamics (number, duration, and spacing of puffs, inhalation volume, holding of breath after inhalation, etc), bioavailability following this route is highly variable: 2–56%. This is why this route contributes to uncertainty in dose delivery (Agurell and Leander 1971; Ohlsson et al. 1980; Ohlsson et al. 1982; Perez-Reyes et al. 1982; Ohlsson et al. 1985; Agurell et al. 1986).

Vaporization of cannabis has been proposed to avoid the formation of hazardous combustion products (tar, polycyclic aromatic hydrocarbons, carbon monoxide, and others) derived from smoked cannabis (Gieringer 2001; Gieringer et al. 2004; Hazekamp et al. 2006; Abrams et al. 2007). Vaporizers decarboxylate cannabinoid acids at about 200 °C and release the volatile cannabinoids entering, in this way, into the systemic circulation via pulmonary absorption from

the vapor. The onset of action is rapid but concentrations decrease very quickly and bioavailability is variable (Huestis 2007). Studies have been carried out (Abrams et al. 2007; Eisenberg et al. 2014; Lanz et al. 2016) to demonstrate the efficient administration of medicinal cannabis and cannabinoids using different vaporizers.

3.2.1.2 Oral

In comparison to the inhalation route, the onset of effects is delayed after oral ingestion. THC and CBD peak concentrations are lower attributed to the important first-pass metabolism of cannabinoids but the duration of the effect is prolonged: 4–12 hours (Huestis 2007; Borgelt et al. 2013). Bioavailability is low (approximately 5%) (Grotenhermen 2003).

First-pass metabolism is responsible for incomplete and variable bioavailability of an orally administered drug. THC and CBD do not escape from this and both of them are extensively metabolized in the intestine. The drug-metabolizing enzyme CYP3A4 is often implicated in this process as it is the most abundant P450 subfamily expressed in the small intestine and it functions there as a barrier against xenobiotics (Paine et al. 2006; Thummel 2007).

ABC transporters are a family of drug efflux pumps that utilize ATP hydrolysis to transport substrates across biological membranes. Apart from regulating drug absorption, they also play an important role in the disposition of many drugs in tissues because they are located in excretory organs such as the liver, intestine, and the blood-brain barrier (Fromm 2003). P-glycoprotein (Pgp), an ATP-dependent drug efflux transporter, plays a significant role in the absorption and disposition of many compounds (Fromm 2002). The Pgp is expressed in the apical membrane of the enterocyte and works in cooperation with intracellular enzymes such as CYP3A4, an enzyme with the highest content in the intestine as expressed before. Drug extrusion by Pgp is a way of improving enzymatic efficiency at the enterocyte and avoiding enzymatic saturation by high drug concentrations that reach the intestine from the stomach. This synergistic interaction between CYP3A4 and Pgp may enhance the first-pass loss of a drug as it was observed in many studies (Lown et al. 1997; Wacher et al. 1998).

The knowledge of this system working cooperatively was used in antiretroviral therapy combining two protease inhibitors: lopinavir and ritonavir. Ritonavir is a great inhibitor of both CYP3A4 and/or Pgp (Drewe et al. 1999) and, consequently, increases the coadministered protease inhibitor lopinavir (Boffito et al. 2004).

The enhancement of the expression of enzymes and efflux transporters leads to diminished oral drug bioavailability. An increase of Pgp expression, produced by inducers, reduces the amount of drug in the enterocyte effectively pumping drugs out of the gut wall and back into the intestinal lumen. Conversely, the inhibition of this coordinated system could improve drug bioavailability.

As both molecules, THC, and CBD, are poorly water-soluble and subjected to extensive first-pass metabolism in the gastrointestinal tract, leading to a limited oral bioavailability, several efforts have been made to avoid the loss of cannabinoids when given orally. Some researchers (Cherniakov et al. 2017) developed an advanced self-emulsifying oral drug delivery system with a natural absorption enhancer (piperine) and subsequently tested in rats. Several authors (Bhardwaj et al. 2002) showed that piperine inhibits both the drug transporter Pgp and the major drug-metabolizing enzyme CYP3A4. Because both proteins are expressed in enterocytes and hepatocytes and contribute to a major extent to the first-pass elimination of many drugs, their data indicate that dietary piperine could affect plasma concentrations of Pgp and CYP3A4 substrates in humans, in particular, if these drugs are administered orally. The results were promising as an increase in CBD and THC bioavailability was observed.

The same investigators (Atsmon et al. 2018; Atsmon et al. 2018) tested the new formulation based on pro-nano dispersion technology in healthy volunteers and compared it with similar doses from a marketed oromucosal spray. The new delivery system provided faster absorption

and improved bioavailability, compared to the oromucosal spray. Further, larger-scale clinical studies with this formulation are needed.

3.2.1.3 Sublingual

This route of administration bypasses the first-pass metabolism and goes directly into the bloodstream via the mouth. As there is a salivary gland under the tongue, sublingual formulations may stimulate the flow of saliva and it is difficult for patients to avoid swallowing leading this to a decreased bioavailability similar to that of oral delivery (Dev et al. 2016). After smoking/vaporizing, the sublingual method is the second fastest delivery method.

3.2.1.4 Buccal/Oromucosal

The medicine can be placed inside the cheeks or on the gums. The advantage over the sublingual application is that avoids the reflex of swallowing. Using this administration, a greater percentage of the active cannabinoids is absorbed compared to the sublingual method (Huestis 2007). Bioavailability following application on oral mucous membranes is around 13% (Karschner et al. 2011).

3.2.1.5 Rectal

When placed correctly (which means avoiding the superior rectal vein), the rectal route prevents first-pass metabolism (van Hoogdalem et al. 1991; Mattes et al. 1993). Unfortunately, there are very few scientific studies on the bioavailability of rectal administration. A study conducted with two patients (Brenneisen et al. 1996) deduced that the bioavailability was approximately twice that of oral ingestion.

3.2.1.6 Transcutaneous

This is another route of improving cannabinoid exposure as it avoids the first-pass metabolism. Some authors have demonstrated that CBD is more permeable than THC (Stinchcomb et al. 2004). The delivery to the brain when administered transcutaneous is much slower compared to smoking. Steady-state plasma concentrations were found to be maintained for at least 48 hours (Huestis 2007; Oberbarnscheidt and Miller 2017).

3.2.2 Distribution

The distribution of THC is governed by the high lipophilicity of this substance. The compound rapidly penetrates highly vascularized tissues resulting in a quick decrease in plasma concentrations. Subsequently, accumulation occurs in less vascularized tissues and finally in body fat, being the latter a long-term storage site. With prolonged drug exposure, for example, THC can be retained in body fat for extended periods (Huestis 2007). The steady-state volume of distribution is about 10 L/kg.

CBD is rapidly distributed into the tissues with also a high volume of distribution (more than 10 L/kg). Like THC, CBD may preferentially accumulate in adipose tissues due to its high lipophilicity (Fasinu et al. 2016).

Experimental data show that THC rapidly crosses the placenta, although concentrations are lower in fetal blood and tissues than in maternal plasma and tissues (Huestis 2007). THC also concentrates on breast milk from maternal plasma due to its high lipophilicity. Due to similar lipophilicity, CBD could follow the same behavior (Grant et al. 2018).

THC and CBD are highly protein-bound in blood and as stated in the literature (Wahlqvist et al. 1970; Widman et al. 1974; Klausner et al. 1975; Hunt and Jones 1980; Devinsky et al. 2014) are mainly bound to low-density lipoproteins, with up to 10% present in red blood cells and only 2–3% as free drug.

3.2.3 Elimination

Elimination of THC and CBD is mainly by metabolism in the liver and the intestine. To a much lesser degree, other extrahepatic organs and/or tissues such as the heart, the brain, and the lungs also contribute to the metabolism of cannabinoids (Krishna and Klotz 1994; Huestis 2007; Stout and Cimino 2014).

Cannabinoids are substrates of cytochrome P450 monooxygenases (CYP450). The enzymes CYP2C9, CYP2C19, and CYP3A4 catalyze the majority of hydroxylations that take place in their metabolism. *In vitro* data showed that hepatic isoenzymes 2C9 and 3A4 play a significant role in the primary metabolism of THC (Yamamoto et al. 2003), whereas 2C19 and 3A4 may be responsible for the metabolism of CBD (Stout and Cimino 2014). Other CYP enzymes may be involved in CBD metabolisms such as CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 (Jiang et al. 2011). One metabolite of THC: 11-hydroxy-THC (11-OH-THC) exhibits similar activity and disposition to THC (Grotenhermen 2003).

The implication of CYP3A4 and CYP2C19 was evaluated in human beings. Increases in THC, CBD and 11-OH-THC concentrations seen when ketoconazole, a well-known CYP3A4 inhibitor (Greenblatt et al. 2011), or decreases of these compounds when rifampicin, a well-recognized inducer (Mahatthanatrakul et al. 2007), were coadministered with oromucosal cannabis extract support the implication of CYP3A4 as an important contributor to the metabolism of these compounds (Stott et al. 2013). The significance of CYP2C19 contribution to CBD metabolism, in contrast, was not supported by the clinical study with omeprazole, a CYP2C19 inhibitor (Shirasaka et al. 2013). So this enzyme perhaps is not so involved in *in vivo* metabolism of CBD, THC, and 11-OH-THC at the dose of THC/CBD spray investigated or plays a less significant role in CBD metabolism. Pharmacogenetic data support CYP2C9 as a significant contributor to THC metabolism (Sachse-Seeboth et al. 2009).

Cannabinoids are the subject of UDP-glucuronosyltransferase (UGT)-dependent glucuronidation. UGTs have been identified as capable of catalyzing both primary (CBD and CBN) and secondary (Δ^{9} THC) cannabinoids metabolism (Stout and Cimino 2014).

The true elimination half-lives of THC and CBD are difficult to calculate from plasma as the equilibrium plasma/fatty tissue is slowly reached. So highly variable elimination half-lives are reported in the literature for THC, ranging from 20–30 hours for THC (Lemberger et al. 1971) to 4–6 days when plasma levels were determined for 2 weeks and 9–13 days when they were followed-up for 1 month (Johansson et al. 1989).

3.3 Pharmacokinetic Interactions

Potential drug-drug interactions are preventable and are common causes of adverse drug effects (Namazi et al. 2014). Since CBD and/or THC are often administered concomitantly with other medicines as add-on therapy, drug-drug interactions should be taken into account**.** Many studies have demonstrated (Hawksworth and McArdle 2004; Yamaori et al. 2011; Yamaori et al. 2011; Jiang et al. 2013; Zendulka et al. 2016) that CBD is not only a substrate but also an inhibitor of CYP450 enzymes, and thus, it could interfere with the metabolism of other xenobiotics. All these studies were *in vitro.* Furthermore, *in vitro* studies have shown that CBD and THC interact in some way with ABC transporters. CBD inhibits the ABC transporters Pgp (Zhu et al. 2006) and Bcrp (Breast Cancer Resistance Protein) (Spiro et al. 2012; Feinshtein et al. 2013), and thus, it may affect the pharmacokinetics of many drugs that are substrates of these transporters. Interestingly, it was proved by some authors (Brzozowska et al. 2016) that CBD was a not a substrate of efflux transporter but an inhibitor of these proteins The inhibition CBD exerts on ABC transporters might be of importance on THC pharmacokinetic as THC is a dual Pgp and Bcrp substrate (Todd and Arnold 2016), and CBD can potentiate some of THC effects via increasing its brain concentrations.

Moreover, it was demonstrated that THC exposure increased Pgp expression in various important brain regions (Brzozowska et al. 2017). This could affect many drugs that are substrates of these transporters.

So a summary of possible interactions with relevance in the clinical use of CBD and/or THC is going to be exposed so that the reader can have tools to act in the clinical setting.

3.3.1 Cannabinoids-Statins

Statins exert a competitive inhibition of the 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase to impair endogenous cholesterol synthesis. This decrease in intracellular cholesterol concentration induces the upregulation of low-density lipoprotein (LDL) receptor expression on the hepatocyte cell surface, which increases LDL-cholesterol extraction from the blood and thus decreased levels of circulation LDL-cholesterol (Schachter 2005).

As previously mentioned, both CBD and THC primarily bind to lipoproteins, mainly LDL. The unbound fractions of THC and CBD in plasma are low. Therefore, the disposition of these two compounds depends not only on physicochemical characteristics but also on lipoproteins. CBD-LDL and THC-LDL can reach the intracellular space of the hepatocyte via the LDL membrane receptor as cholesterol does. When cannabinoids are coadministered with statins, an increase in the clearance of the former may be observed as statins reduce plasma LDL and in this way, they increase the free fraction of CBD and THC. Moreover, statins upregulate LDL receptors in the liver, so THC and CBD biotransformation increases. Both factors might cause a decrease in free and total THC and CBD plasma concentrations. So cannabinoids could become less effective in patients taking statins (Fig. 3.3).

A case was reported by our research group (Eiraldi et al. 2004) with cyclosporine (CYA) and atorvastatin. CYA exhibits the same binding characteristics as cannabinoids, primarily binding to LDL. In the interaction reported, the combination of the two drugs resulted in an acute rejection episode as CYA clearance increases yielding lower concentrations.

Another factor must be taken into consideration; some statins (simvastatin and atorvastatin) are metabolized by CYP3A4 and are substrates of

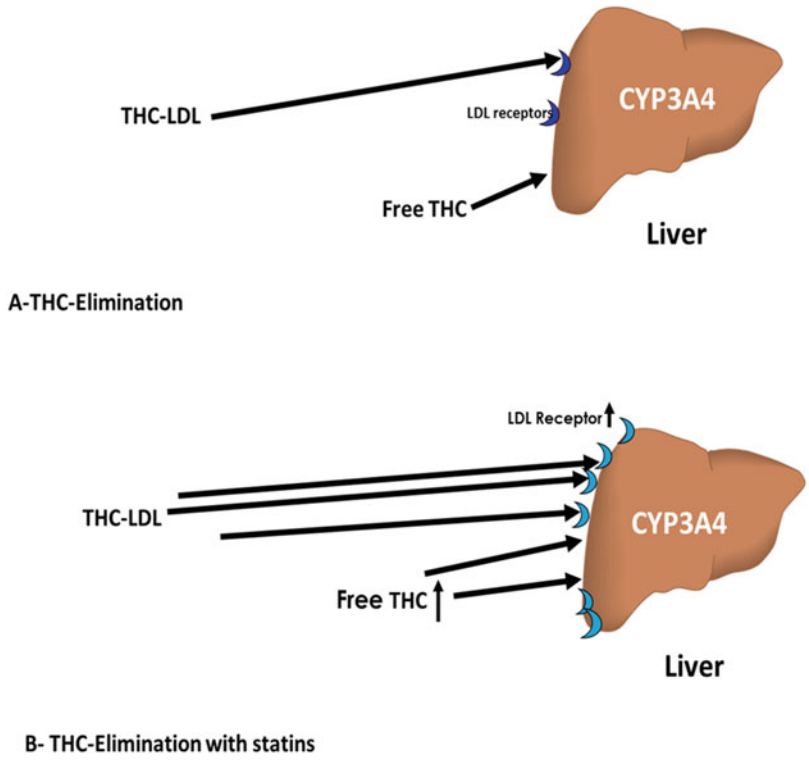

Fig. 3.3 Illustration of THC elimination given alone (**a**) or in combination with statins (**b**)

Pgp (Holtzman et al. 2006; Neuvonen et al. 2006). CYP3A4 and/or Pgp inhibitors such as CBD may increase the plasma concentration of these statins, increasing the risk of adverse reactions such as myopathy and/or rhabdomyolysis. However, as the same authors stated, the role of Pgp in these specific drug interactions remains unclear so further studies are necessary to conclude on this issue. If the interaction were important, on the one hand, CBD would increase simvastatin and atorvastatin levels inhibiting CYP3A4 and/or Pgp, but on the other hand, statins would increase cannabinoids clearance decreasing their effects. In the end, no clinical effect would be observed.

3.3.2 Cannabinoids-Warfarin

Warfarin is one of the most widely used oral anticoagulant (Pengo et al. 2006). It is administered as a racemic mixture of the R- and S- stereoisomers, being S-warfarin 3–5 times more potent than R-warfarin. S-warfarin is metabolized predominantly via CYP2C9 whereas the R-stereoisomer is utilizing the CYP3A4 isoenzyme (Ansell et al. 2008). Frequent monitoring of the INR is required to both achieve and subsequently maintain appropriate anticoagulant effects; this is mainly due to the narrow therapeutic index warfarin has. Concomitant medications, diet, alcohol intake, and genetic polymorphisms in the genes encoding CYP2C9, must also be taken into consideration. Drugs that inhibit the isoenzymes implied in warfarin metabolism may increase its plasma concentrations and INR, and thus potentially increase the risk of bleeding. CBD has been demonstrated to act as a potent competitive inhibitor of CYP enzymes, mainly CYP2C9 and CYP3A4 to a lesser extent and as such could further impair the degradation of warfarin (Yamaori et al. 2011; Yamaori et al. 2012). Some researchers (Grayson et al. 2017) observed a rise in INR values with increasing CBD doses suggesting an interaction between warfarin and cannabidiol. The situation was reversed once the warfarin dose was decreased.

3.3.3 Cannabinoids-Anticonvulsants

About one-third of patients with epilepsy suffer from drug-resistant disease and the efficacy of the medication available in the market is limited (Kwan and Brodie 2000; Kwan et al. 2010). So interest has arisen to develop new medications with anticonvulsant properties acting on novel receptors. CBD has been studied for a long time to be effective in animal models of epilepsy (Carlini et al. 1973; Rosenberg et al. 2017). The use of CBD in the treatment of refractory epilepsy in children has been increasing but only in the last few years, data from randomized trials with CBD is available (Devinsky et al. 2016; Devinsky et al. 2017; O'Connell et al. 2017; Thiele et al. 2018). These trials as well as studying safety, have also explored the potential efficacy of CBD use in children with Dravet and Lennox-Gastaut syndromes.

As CBD is used as an add-on therapy to other antiepileptic drugs (AEDs), it is important to understand how CBD can interact with them to predict or prevent drug-drug interactions.

Based on what is known about CBD metabolism and the metabolism of other AEDs, one could speculate that there could be many interactions given the involvement of CYP enzymes in the metabolism of AEDs and the inhibition or induction several of these drugs exhibit. To date, there are few data on CBD interactions with other AEDs and the studies found in the literature (Geffrey et al. 2015; Gaston et al. 2017; Perucca 2017) focus on what CBD can do to other plasma AEDs concentrations but the information is lacking about the influence of concomitant AEDs on plasma CBD levels.

3.3.3.1 With Clobazam, Clonazepam

Clobazam is a benzodiazepine that has been approved for use in the treatment of Lennox-Gastaut Syndrome and other epileptic syndromes and anxiety (Giarratano et al. 2012). The main enzyme involved in the process of N-demethylation of clobazam to form norclobazam (an active metabolite) is CYP3A4 and to a lesser extent CYP2C19 and CYP2B6.

Norclobazam itself is also metabolized via hydroxylation, primarily by CYP2C19 (Walzer et al. 2012).

The interaction with clobazam and its metabolite has been reported (Geffrey et al. 2015; Gaston et al. 2017). Some authors (Geffrey et al. 2015) reported elevated clobazam and norclobazam levels in children with refractory epilepsy by 60 and 500% respectively when CBD was introduced in their therapy. Side effects were reported in 10 of the 13 children, but once clobazam dose was reduced, side effects also decreased. The interaction appears to be more important between CBD and norclobazam than with clobazam. This is due, perhaps, to more potent inhibition of CYP2C19 by CBD than that of the isoenzyme CYP3A4. Some pharmacokinetic studies (Kosel et al. 2002) suggest a low clinical impact of CBD on the CYP3A4 function. CBD's ability to inhibit ABC transporters alters the pharmacokinetics of co-administered drugs that are ABC transporter substrates. Interestingly, clobazam is a P-gp/Bcrp substrate, opening the possibility that these transporters may contribute to this drug interaction (Nakanishi et al. 2013). If norclobazam is an efflux transporter substrate is still unknown.

Of interest is the finding that clonazepam, mainly metabolized by CYP3A4 (Anderson and Miller 2002) did not show an interaction with CBD (Gaston et al. 2017). Moreover, this drug might not be an efflux transporter substrate.

So, the dose of clobazam may need to be adjusted when starting CBD as high levels of the metabolite were associated with increases in sedation or clonazepam can be used instead.

3.3.3.2 With Valproic Acid

Valproic acid (VPA), a branched short-chain fatty acid is mainly metabolized by three routes: glucuronidation, β-oxidation in the mitochondria (major routes accounting for 50% and 40% of dose respectively) and ω-oxidation (considered a minor route, 10%), resulting, the latter, in the formation of a hepatotoxic metabolite (4-en-VPA) (Siemes et al. 1993; Ghodke-Puranik et al. 2013). It is frequently used in the management of epilepsy and bipolar disorder. Other indications include neuropathic pain and prophylactic treatment of migraine headaches (Loscher 1999).

Glucuronidation is also involved in CBD metabolism (Mazur et al. 2009; Ujváry and Hanuš 2016). Some authors (Al Saabi et al. 2013) have revealed that CBD significantly inhibited ethanol glucuronidation in a non-competitive manner. If CBD also impairs VPA glucuronidation, more formation of 4-en VPA can be the cause of elevated liver function test results observed by some researchers (Gaston et al. 2017) when VPA was coadministered with CBD. These liver abnormalities were not seen in patients taking CBD and other anticonvulsants indicating that perhaps CBD enhances the negative effects of VPA on liver functions. Once CBD and VPA were discontinued, liver enzymes levels normalized quickly. Interestingly, the patients were rechallenged on CBD alone and did not experience these abnormalities again.

Moreover, if VPA clearance is reduced by CBD administration, higher levels of VPA will be found resulting in an increase in seizures due to hyperammonemia formation (Vázquez et al. 2013; Vázquez et al. 2014; Maldonado et al. 2016).

3.3.4 Cannabinoids and Substrates of Efflux Transporters

The distribution across the blood-brain barrier of the antipsychotic drug risperidone and its active metabolite 9-hydroxy-risperidone is profoundly limited by Pgp. Some animal studies carried out by some authors (Holthoewer et al. 2010; Schmitt et al. 2016) revealed that induction of Pgp affects the disposition of such drugs increasing blood levels and decreasing brain concentrations. THC and CBD can have opposite effects on risperidone and its metabolite in the brain as it was previously mentioned, THC can upregulate those transporters and CBD may inhibit them.

According to *in vitro* studies (Boulton et al. 2002), clozapine (a weak Pgp substrate antipsychotic drug) may be a better first-line treatment for patients with schizophrenia and with a history

of cannabis use. Quetiapine as well as risperidone are good Pgp substrates and olanzapine showed intermediate affinity. *In vivo* studies are needed to confirm these findings.

Similarly, many anticonvulsant drugs such as phenytoin, phenobarbital, and clobazam are also ABC transporter substrates and subject to poor brain uptake (Nakanishi et al. 2013).

3.3.5 Drug Effects on Cannabinoids Levels

CBD and THC are metabolized, among others, via the CYP3A4 enzyme. Various drugs such as ketoconazole, itraconazole, ritonavir, and clarithromycin inhibit this enzyme resulting in higher CBD and THC concentrations because of increased bioavailability and/or reduced clearance (Stott et al. 2013). On the other hand, phenobarbital, rifampicin, carbamazepine, and phenytoin induce CYP3A4, leading to decreased levels of cannabinoids (Flockhart 2007; Stott et al. 2013).

3.4 Conclusions

Considering the absorption of cannabinoids, several efforts have been made to compensate for the disadvantages of oral use and inhalation. On the one hand, research is focusing on increasing the low bioavailability of THC and CBD after oral ingestion; on the other hand, several vaporizers are being tested to avoid the harm that combustion products can provoke. Sublingual administration of cannabis-based medicines is used nowadays to accelerate the onset of action which is slow and erratic after oral ingestion. Other alternatives such as rectal and transdermal administration are promising as they can increase either bioavailability or duration of action.

Cannabinoids are metabolized by enzymes of the cytochrome P-450 and either increasing or decreasing the activities of these enzymes could result in a lack or exacerbation of their effects respectively. Furthermore, efflux transporters can affect both absorption and disposition of THC and CBD and these transporters are plausible for being involved in interactions.

Despite the discussions of the potential drug-drug interactions studied in this chapter when drugs were administered concomitantly with THC and/or CBD, the full understanding of their relevance awaits further investigation.

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4 Cannabinoid Receptors and Ligands: Lessons from CNS Disorders and the Quest for Novel Treatment Venues

Clara M. Vecchini Rodríguez, Yma Escalona Meléndez, and Jacqueline Flores-Otero

Abstract

The potential use of cannabinoids for therapeutic purposes is at the forefront of cannabinoid research which aims to develop innovative strategies to prevent, manage and treat a broad spectrum of human diseases. This chapter briefly reviews the pivotal role of the endocannabinoid system in modulating the central nervous system and its roles on neurodegenerative diseases and brain disorders. Ligand-induced modulation of cannabinoid 1 and 2 receptors to modulate immune response, decrease neurodegeneration and pain are aspects that are also discussed.

Keywords

Cannabinoid receptors · Endocannabinoid system · Non-cannabinoid receptors · CB1R · CB2R · TRVP1 · Endogenous ligands · Cannabinoid ligands · Neurodegenerative diseases · CNS disorders

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Abbreviations

2-AG	2-arachidonoyl glycerol
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
AEA	Arachidonoyl ethanolamide
ASD	Autism spectrum disorder
Aβ	Amyloid-β
cAMP	Cyclic adenosine monophosphate
CB1R	Cannabinoid receptor 1
CB2R	Cannabinoid receptor 2
CBD	Cannabidiol
CBRs	Cannabinoid receptors
CBs	Cannabinoid's
CNS	Central nervous system
DAG	Diacylglycerol
eCB	Endocannabinoid
Epi	Epilepsy
ERK	Extracellular signal–regulated kinases
FAAH	Fatty acid amide hydrolase
FDA	Food and Drug Administration
GAB	Gamma–aminobutyric acid
GPCRs	G protein-coupled receptors
GRK	G protein-coupled receptor kinase
JNK	c-jun-N-terminal Kinase
MAGL	Monoacylglycerol lipase
MAPK	Mitogen-activated protein kinases
MDA	Maximal dentate activation
MS	Multiple sclerosis
PD	Parkinson's disease
SCZ	Schizophrenia

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TBI Traumatic brain injury
THC Δ^9-tetrahydrocannabinol

4.1 Introduction

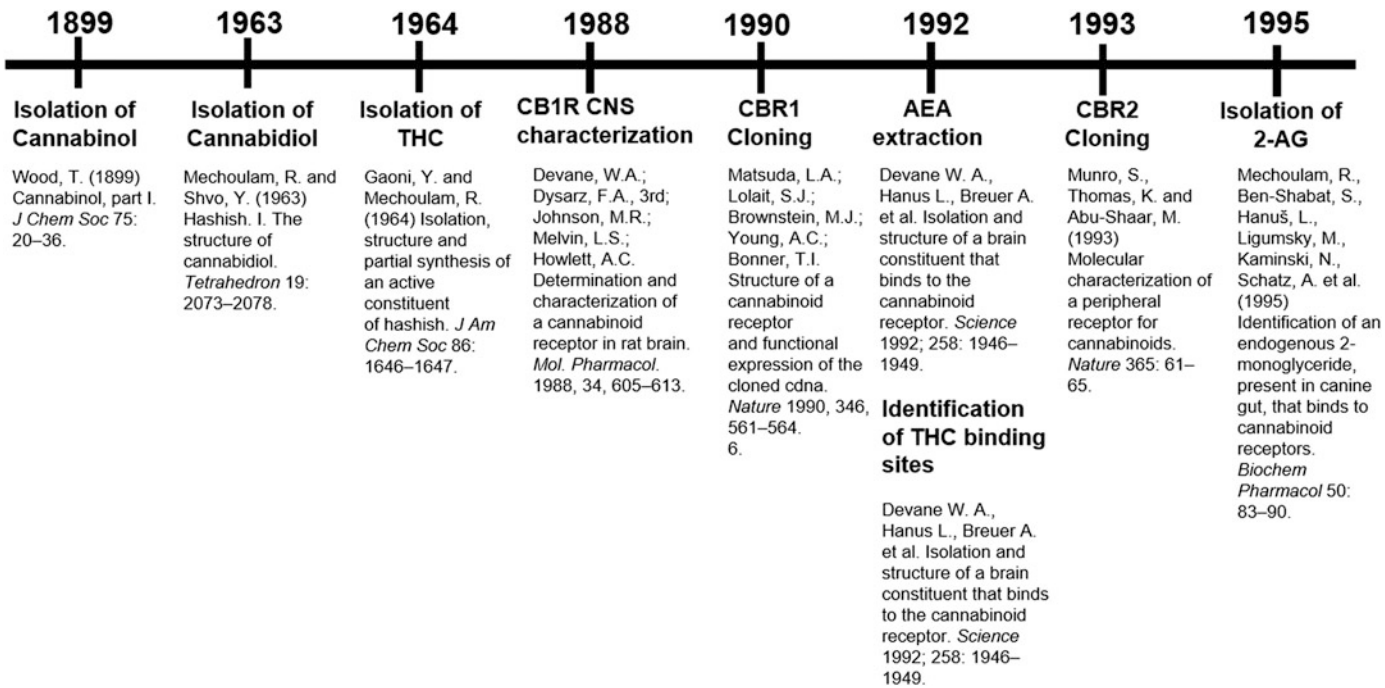
To fully appreciate the broad spectrum of benefits that the endocannabinoid (eCB) system offers as potential treatments for various neuropathologies, we need to delineate how its expression profile is systematically orchestrated throughout the life span. Research scientists take advantage of well-established discoveries that have been made using a myriad of animal models and human clinical trials. However, an emergent scenario that invites us to look at innovative ways of understanding the mechanisms behind the cannabinoid's (CBs) optimal effects is the presumptive association of the eCBs with distinct brain pathologies that arise during human brain development.

Since the discovery of the medicinal properties of the plant *Cannabis sativa* to alleviate pain, inflammation, and appetite disorders (Baron 2015; Zou and Kumar 2018) its consideration as a treatment to manage numerous diseases continue to evolve. Its potential clinical application is susceptible to the recreational and illicit use of marijuana. Over 60 phytocannabinoids, naturally synthesized molecules from the cannabis plant, have been identified (Aizpurua-Olaizola et al. 2016; Zou and Kumar 2018) with Δ^9-tetrahydrocannabinol (THC) being the main psychoactive component (Cohen and Weinstein 2018), yet one that has clinically served as an anti-emetic agent or appetite stimulator (Darmani 2010; Di Marzo and Matias 2005) after its discovery in 1964 (Gaoni and Mechoulam 1964; Pertwee and Ross 2002). In contrast, two other compounds that were isolated and found to be nonpsychoactive are Cannabinol (Wood et al. 1899) and Cannabidiol (CBD) (R. Mechoulam and Shvo 1963) (Table 4.1). CBD is currently approved as Nabiximol (Sativex) in Canada as a 1:1 ratio after combined with THC for pain management in patients with multiple sclerosis and cancer (Howard et al. 2013). The broad effects that these and other phytocannabinoids have in humans rely on their action on two cannabinoid receptors (CBRs), the cannabinoid receptor 1 (CB1R) and the cannabinoid receptor 2 (CB2R) which were characterized and cloned shortly after the identification of the THC binding sites in the brain (Table 4.1). CB1Rs and CB2Rs are members of the large family of G protein-coupled receptors (GPCRs) and the couple to $G\alpha_{i/o}$ proteins. In association with endogenous ligands that bind to these CBRs, 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (AEA: anandamide), and their synthetic enzymatic machinery, these CBRs are key constituents of the eCB system (Lu and MacKie 2016; Pertwee and Ross 2002).

Pharmacological modulation of the eCB system provides unique opportunities to control the symptomatology and advancement of multiple diseases ranging from cancer and neuropathic pain to disorders of the central nervous system (CNS) (Kendall and Yudowski 2017; Robson 1996). These benefits, however, can be masked by the complexity of the properties that distinguish the eCB system. For example, the polypharmacology of these ligands, the ability to activate multiple receptors, with non-specific binding to other GPCRs, ion channels and nuclear receptors (Glass and Northup 1999; McAllister and Glass 2002) expands the effects that CBR ligands have in a system to the point of augmenting unwanted adverse effects or perhaps inducing metabolic disorders (Silvestri and Di Marzo 2013). Besides, CBRs can form heterodimers and although these tend to enhance signaling outcomes, this benefit is compromised by the lack of knowledge of which receptors dimerize in tissue areas, thereby limiting the development of selective CBR-based treatments that target specific signaling pathways (Atakan 2012; Hudson et al. 2010).

This chapter reviews the clinical relevance of the eCB system, comprised of the CBRs, lipid-based endogenous CBs, and the enzymes responsible for their synthesis and hydrolysis. We will discuss the role of the CBRs in the physiology and pathology of the CNS. Particularly, in accordance with previous findings that show that the eCB system systematically expresses from early stages of development through adulthood in

Table 4.1 Discoveries of the eCB System



Abbreviations: *THC*, Δ^9-tetrahydrocannabinol; *CB1R*, cannabinoid 1 receptor; *CNS*, central nervous system; *AEA*, arachidonoyl ethanolamide; *CB2R*, cannabinoid 2 receptor; *2-AG*, 2-arachidonoyl glycerol

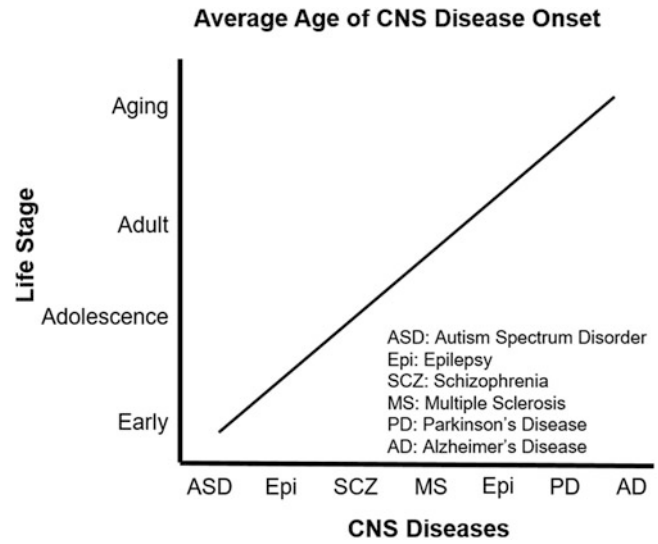


Fig. 4.1 Schematic relationship between human life stages and CNS diseases. While average age of ASD and Epi onset is during early stages of development, disorders like SCZ begin during late teen years. MS and Epi manifest during adult stages, followed by PD and AD which affect people over 60

different cells of the CNS (Zurolo et al. 2010), we postulate that the aberrant alteration of this expression leads to the emergence of distinct neurological disorders discussed herein (Fig. 4.1).

4.2 Cannabinoid Receptors, CB1Rs and CB2Rs

Following the discovery of Cannabinol, CBD and THC (Table 4.1), the identification of CBR1 as the binding site of THC led to the cloning of the CB1Rs and later CB2Rs (Matsuda et al. 1990; Munro et al. 1993). CB1Rs and CB2Rs are two members of the large family of GPCRs. These receptors have seven transmembrane spanning domains accompanied by a large extracellular N-terminal domain and an intracellular C-terminal tail. Despite displaying a 44% amino acid sequence homology at the amino acid sequence level (Zou and Kumar 2018), CBRs display distinct characteristics, from tissue expression levels to their signaling and function.

CB1Rs was first described in the brain (Devane et al. 1988), being most abundant in the cerebellum, substancia nigra pars reticulata, globus pallidus, neocortex, basal ganglia, hippocampus and at very low levels in the brainstem (Herkenham et al. 1990; Herkenham et al. 1991; Mackie 2005; Van Sickle et al. 2005). Their expression has also been detected to a lesser extent in the periphery, particularly in the testis (Howlett et al. 2002), the liver (Osei-Hyiaman et al. 2005), and other areas throughout the human body. While in humans CBRs consist of 472 amino acids, in rodents they consist of 473 amino acids (Zou and Kumar 2018). Considered to be key constituents of retrograde signaling, CB1Rs locate at presynaptic sites of GABAergic and glutamatergic neurons (Lu and MacKie 2016).

Upon synthesis of endogenous eCBs by fatty acid amide hydrolase (FAAH) and diacylglycerol (DAG), CBRs become activated and lead to the inhibition of presynaptic Ca^{2+} influx via voltage-gated Ca^{2+} channels. Activation of CB1Rs is also accompanied by the inhibition of adenylyl cyclase, which in turn results in a drastic reduction of the levels of cyclic adenosine monophosphate (cAMP) and activation of mitogen-activated protein kinases (MAPK) and of extracellular signal–regulated kinases (ERK) signaling pathways, all of which regulate the expression of genes critical for cellular function (Benovic 2004; Howlett 1987; McAllister and Glass 2002). These signaling events converge in the inhibition of neurotransmitter release, hence reducing normally-induced depolarization of

postsynaptic neurons (Bauer and Göthert 1999; Gessa et al. 1997; I. Katona et al. 1999; Shen et al. 1996).

Although the function of CB1Rs is mainly confined to regulate CNS synaptic plasticity and neurotransmitter release (Mechoulam and Parker 2013), studies in these past decades have elucidated a myriad of functions in distinct populations of CNS cells that include cognition, learning, memory, pain perception and motor activities (Howlett et al. 2002). This is not surprising, considering the widespread CB1R expression in the brain and in glial cells, which play a role in regulating CNS homeostasis and neuronal activity (Scheller and Kirchhoff 2016).

CB2Rs are prominently expressed in the immune system, specifically in B and T lymphocytes, natural killer cells, polymorphonuclear cells, and monocytes/macrophages (Basu and Dittel 2011; Tanasescu and Constantinescu 2010). They have also been described at lower levels than the CB1R, in microglial brain cells and against the past view, in hippocampal neurons (Li and Kim 2017; Mackie 2005). Comprised by 360 amino acids in humans (Zou and Kumar 2018), given their high expression profile in the immune system, CB2Rs are thought to play a modulatory role in cytokine release during inflammation, especially resulting from tissue injury (Malfitano et al. 2014). Although in the brain the function of CB2R appears to be unclear (Pertwee and Ross 2002), recent studies support a possible role in synaptic plasticity, addiction and diseases such as schizophrenia (SCZ) which tend to develop during late teen ages the to early 30s (Bonomo et al. 2018; Cohen and Weinstein 2018; Gomes et al. 2015; Stempel et al. 2016; Xi et al. 2012).

4.3 The Endocannabinoid System in the Cells of the CNS

The endogenous ligands of the CBRs are referred to as "endocannabinoids" (eCBs). Out of the three eCBs known, AEA, 2-AG, and 2-arachidonylglycerol ether, AEA and 2-AG secondly, are best characterized (Devane et al. 1992; Hanus et al. 2001; Sugiura et al. 1995). Both endogenous ligands, AEA, and 2-AG, are derivatives from arachidonic acid and are considered fatty acid-derived neuronal neurotransmitters (Pertwee and Ross 2002). The classical view is that AEA and 2-AG which predominate in the brain (Stella et al. 1997), are deactivated after hydrolysis by FAAH and/or the monoacylglycerol lipase (MAGL) (Di Marzo 1999; Howlett et al. 2002, 2004; Ulugöl 2014). ECBs are released to the synaptic cleft on-demand following increase synaptic activity (Zou and Kumar 2018). Despite the long-held belief that establishes eCB's function as retrograde messengers of presynaptic CB1Rs and mediators of synaptic plasticity inhibition (Lu and MacKie 2016), evidence also shows an unexpected postsynaptic neuronal localization of CB1Rs (Netzeband et al. 1999; Salio et al. 2002). Further research should investigate whether this postsynaptic expression of receptors translate into presynaptically localized eCBs or perhaps glia-derived eCBs (Romero-Sandoval et al. 2009; Walter et al. 2003).

In a recent study, the association of the CBRs with glial cells in the CNS was examined (Scheller and Kirchhoff 2016). Astroglia cells mainly express CB1Rs at perisynaptic processes and perivascular endfeet, and CB2Rs in response to inflammation during neurodegenerative disorders (Benito et al. 2003). Microglial, on the other hand, express CB2Rs (Li and Kim 2017; Maresz et al. 2005; Stempel et al. 2016; Walter et al. 2003; Zhang et al. 2003). While in astrocytes activation of the CB1Rs seem to play a role in regulating synaptic plasticity and modulating CNS blood flow during energy demands (Stella 2010), microglia cells activation of the CB2Rs induces neuroprotection during CNS inflammatory processes or brain injury (Massi et al. 2008) and it regulates microglia migration and proliferation (Carrier et al. 2004; Walter et al. 2003). Surprisingly, neuronal roles for the CB2Rs have also been described in the literature (Van Sickle et al. 2005), mainly as a cell-type-specific modulator of neuronal plasticity, memory, anxiety and of drug-seeking behaviors (Chen et al. 2017; Morgan et al. 2009;

Quraishi and Paladini 2016; Stempel et al. 2016; Zhang et al. 2014). According to these and other studies, CB2Rs can no longer be excluded from the repertoire of potential therapeutic targets that improve the management of CNS pathologies, especially when their immunomodulation might open new avenues for the development of agents that treat inflammatory neuropathologies like Alzheimer's disease (AD) and ischemia (Aso and Ferrer 2016; Zhang 2007). Whether targeting the prominently expressed CB1Rs or the CB2Rs, the goal is to manipulate the eCB system such that the cells of the CNS can selectively activate or deactivate targeted CBR pathways without promoting unwanted side effects.

4.4 General Downstream Signaling Mediated by CB1Rs and CB2Rs

Following ligand-induced receptor activation, is the stimulation of heterotrimeric $G\alpha_{i/o}$ proteins, adenylyl cyclase, and ion channels, among others (Howlett 1987). Downstream signaling, however, can be differentially defined by multiple factors, all of which are part of the three different waves that mediate diverse activation of intracellular pathways (Gong et al. 2006; Lohse and Calebiro 2013; Nogueras-Ortiz and Yudowski 2016). The first wave is initiated at the plasma membrane by stimulation of immediate effectors such as G proteins. Although $G\alpha_{i/o}$ mainly drive the first wave of intracellular signaling, this may vary according to the receptor and the cell type. For example, activated CBR1 has been shown to stimulate G_s (Glass and Northup 1999). The second wave is initiated by CBR desensitization resulting from phosphorylation at the CBRs C-terminal loop and subsequent recruitment of different isoforms of the β-arrestin scaffold protein (Flores-Otero et al. 2014; Carlos Nogueras-Ortiz et al. 2017), which reinforces receptor desensitization and promotes internalization via clathrin-coated pits and β-arrestin mediated signaling (Flores-Otero et al. 2014; Carlos Nogueras-Ortiz et al. 2017; Priestley et al. 2017). The third and final wave entails the activation of downstream pathways from CBRs that are located at endosomal compartments (Nogueras-Ortiz et al. 2017; Thibault et al. 2013; Tsvetanova et al. 2015).

Whether CB1R activation stimulates one wave over another, will strongly depend on the pharmacology of the ligand, its bias towards unique receptor conformations, and selected signaling pathways activated downstream (Kendall and Yudowski 2017). Emerging research has expanded our knowledge concerning how CBR ligands can induce these pathways for example by inducing unique receptor phosphorylation "barcodes" (Delgado-Peraza et al. 2016) and arrestins (Benovic 2004). A comprehensive study demonstrated that mutation of the CB1R at the G protein-coupled receptor kinase (GRK) phosphorylation sites, S426/S430, to alanine residues, not only decreased receptor internalization, but also reduced receptor desensitization via β-arrestin 1-dependent ERK1/2 activation (Ahn et al. 2013; Delgado-Peraza et al. 2016). These findings argue for a more elaborated, but targeted development of therapeutic compounds that are purposely prone to prevent CBR desensitization, which is responsible for the development of CB tolerance (Rubino et al. 2006).

Downstream signaling of CBRs is also reliable on the receptor's activation by endogenous or synthetic ligands. While one particular ligand may selectively activate both CBRs, the "dwell time" of the receptor (i.e. the time during which β-arrestin coupled-CBRs are clustered in endocytic coated pits before internalization) may also vary (Flores-Otero et al. 2014; Glass and Northup 1999). Literature findings demonstrate that 2-AG exposure to hippocampal neurons induces prolonged CB1R dwell times accompanied by sustained β-arrestin signaling, while WIN 55,212–2 exposure induced shorter CB1R dwell times accompanied by G-protein signaling (Flores-Otero et al. 2014). These findings present the first level of complexity of ligand-induced CBR1 functional selectivity. Moreover, they indicate a mechanism through which β-arrestin and G-protein signaling can be modulated by CB1R endogeneous and synthetic CBs to control downstream pathways critical for neuronal activity, such as the ERK1/2 pathway

(Rubino et al. 2006). Additional downstream cascades regulated by CBR activation include pathways associated with cellular growth, proliferation, migration, and cell death. These are the MAPK including the previously mentioned ERK family, p38-MAPK, and the c-jun-N-terminal Kinase (JNK) (McAllister and Glass 2002; Stella et al. 1997).

4.5 The Endocannabinoid System and CNS Disorders: Traumatic Brain Injury, Multiple Sclerosis, Alzheimer's Disease, Parkinson's Disease, Epilepsy, and Autism

4.5.1 Cannabinoids and Traumatic Brain Injury

Traumatic brain injury (TBI) is a debilitating disease caused by blows or damage in the head that result in brain impairment. The depth of the impairment largely depends on the intensity of the mechanical force inducing the TBI, the brain area affected, and the duration of the impact (Dinsmore 2013). Symptoms that are aimed during treatments are determined by the location and duration of brain damage, and these include thinking impairment, loss of consciousness, movement problems, sensory problems (hearing and visual), amnesia, dizziness, headaches, depression, personality changes, seizures and others (Corrigan et al. 2010; Roozenbeek et al. 2013).

Given the nature of TBI and the complexity of the mechanisms that abnormally alter after physical brain damage, the therapeutic approaches intend to minimize secondary brain injuries and manage the disease. Patients with mild or moderate TBI are therapeutically treated with medications that improve neurobehavioral, motor and cognitive functions, or surgery, while patients with severe TBI which are often paralyzed, receive neurocritical care.

Neither of these treatments is effective, and many TBI events are accompanied by internal secondary brain injuries such as cerebral neurochemical and metabolic changes, inflammation resulting from increases in intracranial pressure, hypoxia, neuronal and glial cell dysfunction, aberrant brain homeostasis, vasculature problems or even loss of brain tissue (Kinoshita 2016; Madikians and Giza 2006; Park et al. 2008; Prins et al. 2013). Lack of therapies that prevent these internal manifestations in TBI patients contributes to disease progression. For instance, following brain necrosis, the release of pro-inflammatory cytokines by polarized M1 macrophages results in a harmful cerebral inflammatory response which in turn increases the intracranial pressure (Braun et al. 2018). Patients whose cerebral edema is not controlled within a few hours of the brain trauma, are highly predisposed to suffer secondary neurological outcomes that may extend to permanent loss of brain function and therefore, physical disability (Madikians and Giza 2006; Prins et al. 2013).

The role of the eCBs as neuroinflammatory and neuroprotective therapeutic sources have attracted great attention to the scientific and clinical community (Mechoulam et al. 2002; Shohami et al. 2011). TBI-induced edema is not only characterized by the transition of anti-inflammatory M2 macrophages to pro-inflammatory M1 macrophages (Braun et al. 2018), but also by an exacerbation of the eCB system's constituents after trauma (I. Katona et al. 1999; Panikashvili et al. 2001; Tchantchou et al. 2014). In a recent study, Braun M. and colleagues confirmed upregulation of the CB2R in a cortical impact TBI model (Braun et al. 2018) as previously shown (Donat et al. 2014). Over 50% of the CB2R expression was detected in macrophages in the peri-contusional cortex. Interestingly, CB2R activation with a selective agonist, GP1a, not only provoked a transition of pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages, but brain infiltration of peripheral macrophages and cerebral inflammation was decreased. These effects were counteracted by inhibition of the CB2R with the antagonist AM630, therefore suggesting this receptor as a potential target to prevent pro-inflammatory macrophage-associated cytokine/chemokine release at sites

of brain injury. In contrast to this study where no changes of CB1R expression were observed post-TBI, a different group demonstrated a neuroprotective and anti-inflammatory role for CB1R after its modulation by the eCB 2-AG (Lopez-Rodriguez et al. 2016; Panikashvili et al. 2005; Panikashvili et al. 2006).

CBR activation shows promise as a potential TBI therapeutic target due to its anti-inflammatory and neurodegenerative actions. Although neurodegeneration has been linked to CB1R increases in oxidative stress (Mukhopadhyay et al. 2010), an increase of the neuroinflammatory response following TBI seems to be the leading reason for this cellular death (Cruz-Haces et al. 2017; DeKosky and Asken 2017; Faden and Loane 2015). Ligand-induced activation of CB2Rs, as well as the intracellular accumulation of endogenous ligands, attenuated blood-brain barrier (BBB) disruption and decreased neurodegeneration (Amenta et al. 2012). However, a limiting factor when considering therapies that accumulate endogenous ligands is that even when intracellular increases of eCBs ameliorate TBI outcomes, inhibiting CBR-ligand hydrolysis to achieve this increase could make patients more prone to suffer from CB-related adverse effects (Tchantchou and Zhang 2013).

Notably, the activation of the CBRs or an augment of their endogenous ligands by strategic inhibition of their metabolizing enzymes could be an effective therapeutic approach to prevent CNS deterioration after TBI. However, future studies need to address the mechanisms through which components of the eCB system alter during the development of TBI as patients transition from a mild to a severe state. In addition to differences in the expression levels of the CBRs following brain trauma, variations in AEA and 2-AG eCBs have been documented after TBI (Panikashvili et al. 2006; Tchantchou et al. 2014). This is critical given that the eCB's expression varies even under basal conditions in different brain areas (Mackie 2005), and the extensive physiological and pathological roles of the whole eCB machinery are still being revealed. Considering the findings discussed, CB2Rs hold promise as an alternative approach to manage and prevent the progression of TBI. An advantage that exists for the CB2R is its non-psychoactive properties that could serve patients with a better life quality as brain functioning is preserved after a traumatic impact.

4.6 Cannabinoids and Multiple Sclerosis

Multiple sclerosis (MS), is a chronic disease of the CNS characterized by the loss of motor and sensory function, due to an immune-mediated inflammation, demyelination resulting from focal lymphocytic filtration, and further axonal damage of neurons located in the brain and the spinal cord (Boyko and Boyko 2018; Hemmer et al. 2015; Milo and Miller 2014; Przybek et al. 2015; Sand 2015; Trapp and Nave 2008).

Current treatments for MS are tailored to speed a patient's recovery from attacks, delay disease progression, and relieve the symptoms which may vary among patients. However, there is currently no cure and given the lack of effectiveness of current treatments, many patients continue to present relapses and symptoms that put them at risk of increased disability (Rice et al. 2018), hence highlighting the need for novel treatments.

The use of CBs to alleviate MS symptoms has obtained attention over the past years (Colfiel et al. 2017; Rice et al. 2018). In a placebo-controlled clinical trial led by John Zajicek and colleagues, although spasticity was not improved after patient treatment with THC, patients reported an improvement in pain relief (Zajicek et al. 2003). Later in 2012, the clinical trial of Multiple Sclerosis and Extract of Cannabis aimed to investigate a standardized oral cannabis extract for the relief of muscle stiffness and pain (Zajicek et al. 2012). Their results showed that patients that received the cannabis extract presented a significant relief in muscle stiffness in comparison with patients in the placebo group (Zajicek et al. 2012). Although it is not clear how CBs relief pain in MS patients it has been suggested that these positive effects are mediated by the brain's CB1Rs (Rice et al. 2018). To support

this view, a study conducted using a mouse model of MS demonstrated that CBRs antagonism worsens the spasticity and muscle stiffness, while CB agonist inhibits spasticity (Baker et al. 2001). In addition, this study suggests that the equilibrium of CBs is affected during spasticity in response to defective neuronal signaling (Baker et al. 2001).

Not surprisingly, the use of CBs in MS is considered from the perspective of an anti-inflammatory and neuroprotective approach. (Tanasescu and Constantinescu 2010). Despite the predominance of CB2Rs in the immune system, the immunosuppressive actions of CBs which decreases T cell proliferation (Katona et al. 2005), inhibits pro-inflammatory cytokines (Jean-Gilles et al. 2010), and induces apoptosis of T-cells (Rieder et al. 2009), appear to be mediated by the independent endogenous activation of CB2Rs expressed in immune cells and activation of CB1Rs in neurons (Croxford et al. 2008; Maresz et al. 2007). Hence, the idea is to develop a combination of distinct therapeutic modalities for MS, one that augments that endogenous activation of CB2Rs to counteract the pro-inflammatory aspects of the disease and another that activates the CB1Rs to delay disease progression by reducing the speeding process of the symptoms as patients deteriorate. The anti-inflammatory effect is one that will attenuate pain, while the neuroprotective approach mediated mainly by CB1R activation will prevent neurodegeneration (Baker and Pryce 2008; Pryce et al. 2003).

One challenge in MS is dose-escalation, possibly making patients more vulnerable to psychiatric side effects (Semple et al. 2005) or cognitive impairment (Pavisian et al. 2014; Rice et al. 2018). Future work should focus in defining the dose-dependent effects that CBs exert in the different types of MS, given that the expression of the CB1Rs and the CB2Rs, and perhaps their endogenous ligands, may vary according to the stage of the disease. Should there be a correlation between the expression of the eCB system components and the different stages of MS, patients could receive a targeted and more personalized treatment that improves motor and sensory functions, hence preventing MS progression.

4.7 Cannabinoids and Alzheimer's Disease

Alzheimer's disease (AD) is a chronic neurodegenerative illness considered the most common cause of dementia (Korolev 2014). It affects brain areas including the hippocampus, the neocortex, the limbic system, the frontal, and the temporal lobes (Masters et al. 2015). AD is distinguished by the lack of amyloid-β (Aβ) clearance from the CNS, the presence of abnormally folded tau and Aβ-associated plaques and tangles, hyperphosphorylated tau, oxidative stress, increase in cerebrospinal fluid Aβ oligomers and release of pro-inflammatory cytokines by microglia and astrocytes (Hölttä et al. 2013; Karran and De Strooper 2016; Lopategui Cabezas et al. 2014; Maccioni et al. 2018; Wang et al. 2017). Presentation of AD symptoms vary according to the stage of the disease as it progresses, from the mild to moderate stage and finally, to severe late stage. As a result, treatment for AD is multimodal as it involves a combination of clinical and caregiver care along with pharmacological management. Despite the presumptive effect of these therapies in treating dementia, they do not prevent progression to late stages of the disease (Korolev 2014; Scheltens et al. 2016; Wang et al. 2017). The development of multitarget agents is highly needed given the complex pathogenesis of this disease that has shown to integrate contribution from the CNS as well as the peripheral system (Wang et al. 2017).

Post-mortem samples of AD brains have served as a tool to delineate whether CB use is an ideal option to treat AD patients. Concerning receptor expression, research findings show overexpression of CB2R levels, yet a downregulation of CB1R levels (Solas et al. 2013), although for CB1Rs, findings are controversial (Lee et al. 2010) and may be dependent on disease progression or their role in regulating cognitive functions (Manuel et al. 2014; Ramirez 2005). Also, eCBs as well as exogenous CBs

have demonstrated to be neuroprotective by preventing Aβ-induced neurotoxicity in cellular and animal models of AD (Koppel and Davies 2008; Maroof et al. 2013). Interestingly, although memory retention has been achieved in animal models that received brain injections of Aβ peptide, the subsequent increases of the 2-AG and CB2R levels detected appear to be dependent on elevations in the expression of the CB1R which increases at later stages halting the improvement in memory retention (Bisogno and Di Marzo 2008). Another possibility for the early CB2R increases is the result of an immediate inflammatory response activation (Ehrhart et al. 2005; Koppel and Davies 2008). This is consistent with the presence of high levels of CB2R in microglia surrounding senile plaques analyzed from post-mortem AD brains (Aso and Ferrer 2016).

Despite the evidence of eCBs as potential regulators of AD pathology and progression, further studies are required to clarify the mechanisms through which CBR modulation by agonists or antagonists regulate brain cognitive functions, neuroprotection, inflammation, and oxidative stress. Up-to-date, CB-derived therapeutic interventions (Nabilone and Dronabinol), in clinical trials pipeline for AD, are tailored to improve neuropsychiatric symptoms, such as agitation, as well as improve appetite and relieve pain effects (Cummings et al. 2018). However, since 2003 no drug has been approved by the Food and Drug Administration (FDA) for the treatment of AD (Cummings et al. 2018), thereby highlighting the need for novel interventions that enable the proper diagnosis and prevention of AD progression.

4.8 Cannabinoids and Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder (Ferreira and Romero-Ramos 2018) distinguished by a decrease in dopaminergic neurons in the substantia nigra (Ferreira and Romero-Ramos 2018; Obeso et al. 2008) and the appearance of Lewy bodies (i.e. aggregations of α-synuclein in brain areas). The loss of dopaminergic neurons leads to a malfunction of the basal ganglia circuitry (Obeso et al. 2008), and results in the classic symptoms of PD: tremors, bradykinesia, muscular rigidity, and postural instability (Aarsland et al. 2018; Chen-Plotkin et al. 2018; Gopalakrishna and Alexander 2015). For these symptoms to reflect in movement and motor function impairment, loss of dopaminergic neurons needs to exceed over 60% (Rodriguez-Oroz et al. 2009). Nevertheless, individuals with PD can present non-motor symptoms, such as cognitive decline and dementia (Aarsland et al. 2018).

Currents treatments for PD aim to decrease the symptoms and the progression of the condition. A dopamine agonist (Levodopa) is the goal standard drug prescribed to PD patients to help control the tremors (Connolly and Lang 2014). Non-pharmacological treatments are also available to can be provided to PD patients if the sole intake of pharmaceutical agents is not efficient. Examples include cognitive training, physical exercise, and neurostimulation (Aarsland et al. 2018).

Studies done in mice and humans have improved our understanding of the mechanistic factors leading to PD pathology (Bilkei-Gorzo 2012) and the potential effects of CBs in improving the outcomes of this disease (Fernández-Ruiz et al. 2007; García-Arencibia et al. 2009; Lago and Fernandez-Ruiz 2007; Lim et al. 2017; Price et al. 2009; Sagredo et al. 2007). The rationale for this relies on the well-supported findings that show a significant increase of CB1R in PD-associated brain areas, such as the basal ganglia where neurons interact with excitatory, inhibitory and dopaminergic neural circuits responsible for the control of movement (Fernández-Ruiz et al. 2013; Turner et al. 2017) and the cerebellum (Price et al. 2009). Furthermore, CBs are capable of disrupting protein aggregation, serving as preclearance and as an anti-oxidant agent (Dando et al. 2013; Silvestri and Di Marzo 2013). In contrast to the CB1R expression in pre- and postsynaptic neuronal sites and to a lesser level in glial cells (Pazos et al. 2005; Stella 2010), CB2Rs localize in the

dorsal root ganglia, in granules and Purkinje cells of the cerebellum (Beltramo et al. 2006; Hsieh et al. 2011; Rodríguez-Cueto et al. 2014; Skaper et al. 1996). Despite the discrepancy in published reports, CB2Rs have also been documented in perivascular microglial cells of the cerebellum, in pyramidal neurons of the medial prefrontal cortex, in nigrostriatal dopaminergic neurons, in inhibitory GABAergic neurons as well as in neurons localized in the CA1 hippocampal and substantia nigra (Benito et al. 2003; den Boon et al. 2012; Lanciego et al. 2011; Morgan et al. 2009; Núñez et al. 2004; Onaivi 2011; Sagredo et al. 2009; Stampanoni Bassi et al. 2017). When considering the expression profile and potential functional implications, the best therapeutic target for PD appears to be the CB1Rs which if absent in PD animal models and humans, results in motor deficits that improve after modulation of dopaminergic pathways (Lane et al. 2010; Pérez-Rial et al. 2011). However, a neuroprotective role is strongly imparted by CB2Rs. They not only obstruct the production of neurotoxins by glia cells, but they also counteract the high oxidative stress atmosphere that results from dopaminergic neurodegeneration in PD and prevents blood-brain barrier leakage (Chung et al. 2016; Javed et al. 2016).

Research areas that explore the possibility of using CBs are an alternative or adjuvant therapy for PD need to consider remaining challenges. First, even when studies show that nicotine and marihuana smoke improves motor functions (Babayeva et al. 2016; Page et al. 2003), smoke in itself is a risk factor for the development of other diseases like cancer (Al-Obaide et al. 2018). Second, although CBs ameliorates motor dysfunction, in the case of PD, surviving dopaminergic neurons compensate for the disease-induced dopaminergic neuronal loss, hence enabling dopamine availability in the brain (Turner et al. 2017) which in turn reduces the need for a possible cannabinoid demand. Third, non-cannabinoid receptors that can be activated by cannabinoid ligands (Stampanoni Bassi et al. 2017; Turner et al. 2017) and may mask the efficiency of CB1 or CB2 activation in PD models.

Despite these challenges, the studies discussed in this section as well as others argue for therapeutic value in the modulation of the CBRs for controlling PD symptoms.

4.9 Cannabinoids and Epilepsy

Epilepsy (Epi) is a brain disorder that predisposes an individual of any age, to generate epileptic seizures due to excessive neuronal activity (Duncan et al. 2006) and unpredicted interruptions of normal brain function (Fisher et al. 2005). Risk factors involved in the development of epilepsy include head trauma, infections in the CNS, cerebrovascular disease, and tumors (Duncan et al. 2006). Although seizures can occur at any age during development, they are more common in children than are less than 2 years and adults older than 55 years of age (Epilepsy Foundation 2018).

Seizures can be presented according to the location of onset in the brain, dissemination patterns, brain maturity, among other factors (Fisher et al. 2005). The most common symptoms in Epi are loss of consciousness, impaired movements, impaired vision and hearing, a deficit in cognitive functions, and sudden extension or flexion of the body extremities (Stafstrom and Carmant 2015).

Current treatment for this neurological disorder is based on anti-epileptic actions, the goal is to decrease the abnormal exacerbated neuronal activity, hence resulting in a reduction of the patient's symptoms. Anti-epileptic drugs work by blocking sodium or calcium channels, which prevents the depolarization of neurons while enhancing the function of the potassium channels (Stafstrom and Carmant 2015). A primary outcome of the anti-epileptic drugs is that they promote gamma–aminobutyric acid (GABA) neurotransmitter to inhibit neuronal activity, and inhibit glutamate neurotransmitter, which is the major excitatory neurotransmitter responsible for the induction of neuronal activity (Stafstrom and Carmant 2015). Although there are various treatments for seizures, no drug fully prevents the development of Epi (Rosenberg et al. 2015).

Throughout history, cannabis has been used to treat seizures. Mechanistically, CBs regulate neuronal activation and excitability (Soltesz et al. 2015) and this has shown promise in disorders like Epi. In a survey conducted in Australia, the principal reason for the use of cannabis in patients with Epi was to have better control of treatment-resistant Epi and to reduce the side effects that are induced by the majority of anti-epileptic drugs (Suraev et al. 2017). In animal models, administration of THC to treat seizures induced anti-epileptic actions (Devinsky et al. 2014) consistent with the effects observed in humans exposed to CBD (Hill et al. 2012). To support this view, a study conducted by Jones et al. (2010) was able to validate the anti-seizure properties of CBD using both *in vitro* and *in vivo* models (Jones et al. 2010). In the *in vitro* model using extracellular multi-electrode array recordings, the results showed that CBD was able to decrease the epileptiform activity in the mammalian hippocampus, decreasing the amplitude and duration of the local field potential burst, therefore, decreasing the neuronal hyperactivity. In an animal model, CBD also exerted anti-epileptic effects, lowering the incidence of seizures when compared to the untreated animals (Jones et al. 2010). In this report, the CBD-induced anti-epileptic role was surprisingly independent of mechanisms mediated by CB1R, which is highly expressed in the hippocampus (Jones et al. 2010). Instead of binding to CB1R, CBD activates CB1R non-associated pathways (Devinsky et al. 2014).

Following the use of CBD for Epi, a recent clinical study aimed to determine the effect of highly purified CBD (Epidiolex) in epileptic patients, characterized the adverse effects, seizure severity, and seizure frequency in the individuals (Szaflarski et al. 2018). The outcomes of the trial showed that during week twelve there was a significant improvement of adverse effects, seizure severity, and seizure frequency in patients (Szaflarski et al. 2018). Extending other studies that suggested CBD as an alternative treatment for patients that are resistant to standard therapies for Epi, Devinsky and colleagues investigated the effects of CBD in children and young adults that were not optimistically affected by anti-epileptic drugs (Devinsky et al. 2016). The results of this study show that CBD reduces seizure frequency in patients (Devinsky et al. 2016), and complementary clinical work that evaluated the safety of CBD in epileptic children showed safety and efficacy (Chen et al. 2018). Overall, these findings demonstrate that CBD adverse effects can be manageable, and that some of the adverse effects reported by patients and caregivers may not be related to CBD (Chen et al. 2018).

In a separate study, a group of scientists determined the role of CB2R using the maximal dentate activation (MDA) rat model of human partial Epi (Rizzo et al. 2014). The authors evaluated the anti-epileptic action of the agonist WIN 55,212–2 and the antagonist AM630, which is specific for CB2R. Results demonstrated that distinct from animals that displayed a decrease in epileptic activity after treated with WIN 55,212–2 alone, or in combination with AM630, those exposed to AM630 alone did not have an anti-epileptic effect (Rizzo et al. 2014). These outcomes could imply that when CB2R is antagonized by AM630, more WIN 55,212–2 is linking to the CB1R (Rizzo et al. 2014), enhancing the response of CBRs as anti-epileptic agents, consistent with previous studies that suggested CB1R involvement in the reduction of seizures (Deshpande et al. 2007). According to these findings, the authors suggested that CB2R has no direct effect in the reduction of epileptic activity in the MDA model (Rizzo et al. 2014). Even when further research needs to define if CB1R, CB2R, or both are good regulators of epileptic events, convincing evidence highlight the potential of CBs as a treatment for Epi.

4.10 Cannabinoids and Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized mainly by repetitive patterns of behaviors, impaired reciprocal social interactions, impaired communication, stereotypical motor behaviors, restricted interests, and cognitive deficits (Gu 2017; Habib et al. 2017; Khalil 2012; Quattrocki and Friston 2014).

The signs and symptoms of patients with ASD vary, but there are two important areas affected in these patients: social communication and sensory-motor behaviors (Lord et al. 2018). Other indicators for ASD are attention deficit, affective behavior deficit, poor response to his/her name, poor visual attention, and mood variation (Zwaigenbaum et al. 2009).

The gold standard treatment for ASD is directed toward occupational and physical therapy in combination with antipsychotic drugs. The drugs used in ASD patients target mostly agitation, irritability, and attention deficit hyperactivity disorder (ADHD) (Lord et al. 2018). However, none of these therapies for ASD are completely effective (Gu 2017).

The use of CBs in patients with ASD is quite controversial when compared to its use in other brain disorders, since the majority of individuals are diagnosed during childhood or at times, adolescent periods (Fig. 4.1). In 2010 a case-study of an autistic child aimed to evaluate the use of THC as a supplementary therapy. Results demonstrated a reduction in hyperactivity, lethargy, irritability, and improvement in the child's speech (Kurz and Blaas 2010). Anecdotal reports suggested that the use of CBD is a viable treatment for anxiety, convulsions, and the motor and behavioral dysfunctions observed in ASD patients (Gu 2017). Consistent with this, a study using an animal model showed that small doses of CBD improved social interactions that are characteristics of this disorder (Kaplan et al. 2017).

Currently, different theories suggest that a dysregulation or dysfunction of the oxytocin system could be involved in the development of ASD (Quattrocki and Friston 2014). Oxytocin is a neuropeptide hormone indispensable for social behavior, and so far it seems to improve social behavior in humans (Yatawara et al. 2016) and animal models (Peñagarikano et al. 2015). Previous evidence supports the idea that the activation of CB1R via anandamide is necessary for an improvement in social interaction (Wei et al. 2015). Moreover, the signaling mechanism mediated by anandamide and CB1R is associated and driven by oxytocin (Wei et al. 2015). The exposure to oxytocin improves anandamide mobilization to the nucleus accumbens, which is a vital region of the CNS involved in the reward circuit (Wei et al. 2015), and critical for normal social skills. The dysfunction of this signaling mechanism and the reward circuit could explain impaired social behaviors in patients with ASD.

Consistent with previous studies of the CBR's role in the immune system, their anti-inflammatory actions in ASD are also a target considering that neuroinflammation has been identified in the post-mortem brains of ASD patients (Vargas et al. 2005). In examining peripheral inflammatory responses, a study in children with ASD sought to determine the association between the eCB system and the immune response of peripheral blood mononuclear cells. Researchers found that the mRNA and protein levels of CB2R were upregulated in peripheral blood mononuclear cells of ASD patients (Siniscalco et al. 2013). Although these results may be indicative of a role of CB2Rs in ASD pathogenesis (Siniscalco et al. 2013), another interpretation of the findings is that CB2Rs could be imparting a protective role in ASD, inhibiting pro-inflammatory cytokines (Pacher 2012). Overall, better understanding and validation of this data could help in the development of a diagnostic tool using CB1R and/or CB2R expression as potential biomarkers for ASD.

4.11 Conclusion

The studies discussed in this chapter support the involvement of CBR activation regulating CNS disorders that develop throughout the life span. Their altered expression during the development and progression of neuropathologies seems to be in many cases the result of the brain system's defense mechanisms against threatening events. As part of this defense mechanism, CBR stimulation is apt to protect the cells of the brain, as well as to encourage an appropriate homeostatic environment that enables proper neuronal function partly facilitated by the dynamic supporting role of glia cells. Due to the high level of complexity of the eCB system, major challenges that need to

be addressed in future research include a full characterization of the system that helps us understand: (1) how are the components of the eCB system expressed in neurons and glial cells in distinct brain regions throughout development?; (2) how can CBRs activate non-CBRs?; (3) is it possible that the activation of non-CBRs mask the stimulation of CBRs after a pathological manifestation of the CNS?; (4) is there a correlation between the expression levels of CBRs or their ligands with specific stages of a disease?; (5) what determines if one CBR overexpresses to compensate for the loss of the other CBR? These are some questions that need attention. Should they be answered, they could prompt the beginning of a new era for the development of personalized CBR-based medicines that target CNS disorders from a neuroprotective and/or inflammatory approach.

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Sleep-Wake Neurobiology

5

Giancarlo Vanini and Pablo Torterolo

Abstract

Sleep and wakefulness are complex, tightly regulated behaviors that occur in virtually all animals. With recent exciting developments in neuroscience methodologies such as optogenetics, chemogenetics, and cell-specific calcium imaging technology, researchers can advance our understanding of how discrete neuronal groups precisely modulate states of sleep and wakefulness. In this chapter, we provide an overview of key neurotransmitter systems, neurons, and circuits that regulate states of sleep and wakefulness. We also describe long-standing models for the regulation of sleep/wake and non-rapid eye movement/rapid eye movement cycling. We contrast previous knowledge derived from classic approaches such as brain stimulation, lesions, cFos expression, and single-unit recordings, with emerging data using the newest technologies. Our understanding of neural circuits underlying the regulation of sleep and wakefulness is rapidly evolving, and this knowledge is critical for our field to elucidate the enigmatic function(s) of sleep.

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5.1 Introduction

Sleep and wakefulness are cyclic physiological processes that occur in virtually all animal species (Siegel 2008). Wakefulness is a behavioral state during which an organism exhibits purposeful, coherent motor activity in response to stimuli that are either internally generated or from the environment. Sleep is, on the other hand, classically defined by reduced body movement, closed eyes, resting body posture, and increased threshold to external stimuli; non-mammalian sleep is mainly defined by inactivity or a prolonged rest period. While the functions of sleep remain an enigma, there is no doubt that sleep is essential for optimal brain development (Frank 2011), cognitive function (Abel et al. 2013; Wild et al. 2018), brain clearance of potentially toxic products generated during wakefulness (Xie et al. 2013), immune responses (Fondell et al. 2011; Brager et al. 2013), metabolism (Broussard et al. 2012; Hart et al. 2013), cardiovascular (Altman et al. 2012; Kim et al. 2013; Tobaldini et al. 2013) and mental health (Lee et al. 2012; Okun et al. 2013). The regulation of sleep and wakefulness is tightly

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orchestrated by several, redundant neural circuits and molecules that are distributed along the brain and brain stem. Understanding the mechanisms controlling sleep and wakefulness is of paramount scientific and clinical importance. This knowledge is critical for understanding the neuropathological basis of sleep disorders, as well as for a scientifically-based development of better therapeutic interventions to treat these disorders. In this chapter, we outline the fundamental mechanisms that regulate mammalian states of sleep and wakefulness, with emphasis on recent research using new neuroscience tools such as optogenetics and chemogenetics that have allowed a more precise interrogation of brain circuits and their role in sleep and wake control.

5.2 Characteristics of Sleep and Wakefulness States

States of sleep and wakefulness can be objectively identified by polysomnography; a simultaneous recording of the electroencephalogram (EEG), electromyogram (EMG), electrooculogram, electrocardiogram, and respiration. During wakefulness, the EEG is comprised of low-amplitude, fast frequencies, associated with high muscle tone evidenced by varying degrees of EMG activity (Fig. 5.1). During non-rapid eye movement (NREM) sleep, the EEG shows high-amplitude, low frequencies with a prominent peak in the delta range (0.5 to 4.0 Hz), and a marked reduction in EMG activity (Fig. 5.1). In humans, NREM sleep is classified into 3 sub-stages; N1 is a transitional state from wakefulness, N2 is characterized by k-complexes typically followed by sleep spindles (a brief spindle-shaped EEG oscillation of 12.0 to 15.0 Hz), and N3 is defined by delta waveforms (Carskadon and Dement 2001). The amplitude of delta oscillations (or slow wake activity) during NREM sleep is highest at sleep onset, gradually declines overnight, and is used as a biomarker of sleep intensity. The initial amplitude depends on the duration and quality of previous wakefulness. However, it is important to note that slow wave activity (SWA) during NREM sleep can be influenced by several factors (i.e., age, drugs, circadian clock, stress, and others) independent of wakefulness duration (Dijk and Czeisler 1995; Davis et al. 2011). During rapid eye movement (REM) sleep, the EEG shows low-amplitude, fast frequencies similar to that one during wakefulness, accompanied by rapid eye movements and sustained muscle atonia (Carskadon and Dement 2001) (Fig. 5.1). Based on the wakefulness-like EEG, REM sleep is also called "paradoxical" sleep. In rodents, EEG theta oscillations (4.0 to 9.0 Hz) become evident during the transition between NREM and REM sleep and remain dominant during the entire REM sleep episode (Fig. 5.1). Besides, oneiric activity (i.e., dreams) can be initiated during NREM sleep but occurs primarily during REM sleep (Siclari et al. 2017). The suppression of motor activity during REM sleep (i.e., motor atonia) is a protective mechanism to prevent the enactment of our dreams.

Human sleep alternates between NREM and REM sleep for about 90 minutes, and each cycle repeats four to five times during the night (Carskadon and Dement 2001).

5.3 Mechanisms of Sleep and Wakefulness

5.3.1 Circadian and Homeostatic Regulation of Sleep and Wakefulness

Sleep timing and intensity (NREM delta amplitude) are regulated by two interacting processes, a homeostatic component (Process S) and a component regulated by the circadian pacemaker (Process C) (Borbely 1982). Mathematical models that include these two processes successfully simulate the timing and intensity of sleep in individuals under different experimental conditions. In diurnal animals, Process S determines the increase in sleep pressure during wakefulness and its gradual decline during subsequent sleep, whereas Process C determines the oscillation between periods of high and low sleep propensity. In mammals, the suprachiasmatic nucleus (SCN) of the

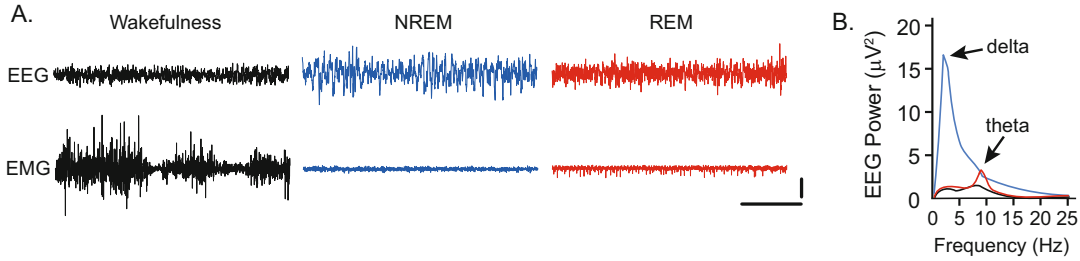

Fig. 5.1 (**a**) Recordings of mouse electroencephalogram (EEG) and electromyogram (EMG) used to identify states of wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. Bars = 300 μV and 5 s. (**b**) EEG power spectra during states of wakefulness (black), NREM sleep (blue) and REM sleep (red) calculated from the EEG traces in A. Arrows = peaks corresponding to EEG delta (0.5–4 Hz) during NREM sleep and theta (5–8 Hz) during REM sleep

hypothalamus is the "master clock" that dictates the precise circadian timing of sleep/wake oscillations (Process C) (Mistlberger 2005). Both gene expression and activity of the neurons in the SCN (mostly GABAergic) oscillate with 24 hours, even in the absence of environmental cues (Welsh et al. 2010). The SCN receives information about the environmental light intensity directly from the retina. In diurnal animals such as humans, SCN neurons promote wakefulness, probably by facilitating and inhibiting, respectively, the activity of the arousal-promoting and sleep-promoting regions. The subparventricular zone and the dorsomedial nuclei of the hypothalamus are relevant targets by which the SCN regulates the timing of sleep and wake behaviors (Chou et al. 2003; Vujovic et al. 2015). The homeostatic process S is modulated by substances, such as the sleep-promoting neuromodulator adenosine, that are released during wakefulness into specific regions of the central nervous system including (among many other regions) the basal forebrain and cerebral cortex (Basheer et al. 2004; Huang et al. 2011).

5.3.2 Local Sleep

Growing evidence suggests that sleep may not just be a global process that simultaneously involves the entire brain. Rather, subsets of neurons that are restricted to a cortical area -but not in adjacent ones- show pauses in discharge activity during periods of extended wakefulness, as they do during NREM sleep (Vyazovskiy et al. 2011). The incidence of these off-periods increases as a function of time awake and they are accompanied by slow wave activity in localized cortical areas, while the animal's global EEG activity and behaviors are those characteristically observed during wakefulness. This phenomenon is called "local sleep" and is associated with progressive impairment in performance during cognitive tasks (Vyazovskiy et al. 2011). Also, elegant studies in rats showed that stimulation of the vibrissae increases slow wave activity in specific cortical columns in a "use-dependent" manner (Rector et al. 2005). More recently, a sleep-like state was also demonstrated in vitro, in a network formed by cultured cortical neurons and glia (Hinard et al. 2012; Jewett et al. 2015). Evidence of local sleep has also been demonstrated in neurosurgical patients with recordings of the scalp and intracerebral EEG, and extracellular neuronal activity across states of sleep and wakefulness (Nir et al. 2011; Nobili et al. 2011). These data challenge our concept of sleep as a global process and brings in a new perspective for understanding the underlying causes of some sleep disorders such as sleepwalking, as well as fatal consequences of drowsy driving.

5.3.3 Brain Regions and Circuits Regulating Wakefulness

In the early 1900s, an epidemic of encephalitis lethargica spread around Europe causing -among other symptoms- severe hypersomnia in those individuals who survived the acute phase of the disease. The discovery of localized injuries in the midbrain and posterior hypothalamus of these patients, lead the Austrian neurologist Constantine von Economo to propose that these two regions contained wakefulness-promoting circuits (von Economo 1930a). Later studies by Moruzzi and Magoun demonstrated electrical stimulation of the brain stem reticular formation in anesthetized cats caused EEG activation similar to that one characteristic of wakefulness (Moruzzi and Magoun 1949). Furthermore, under light anesthesia, the activation of the EEG was accompanied by behavioral arousal. This influential work led to the concept of the ascending reticular activating system, a diffuse network localized to the core of the brain stem that generates wakefulness (Moruzzi and Magoun 1949). Current knowledge indicates that wakefulness-promoting networks are widely distributed and composed of several neurochemically diverse neuronal subgroups. These networks are located within the midbrain and rostral pons, posterolateral hypothalamus, and basal forebrain.

Wakefulness-promoting neurons send ascending projections to the thalamus and cortex, organized in a dorsal and ventral pathway. The dorsal pathway projects directly to the thalamus and facilitates sensory processing, locomotor responses, and cognition. The ventral pathway, which is formed by a larger contingent of fibers reaches the lateral hypothalamus, basal forebrain, and cortex, and is essential for maintaining wakefulness (Fuller et al. 2011). The wakefulness-promoting neuronal subgroups and projections are shown in Fig. 5.2. It is important to point out that these neuronal subgroups can operate together to generate and maintain wakefulness. However, wakefulness is a highly heterogeneous state and each arousal promoting subgroup can also operate independently as a function of the context. For example, noradrenergic neurons in the locus coeruleus increase wakefulness in response to salient, novel stimuli when selective attention is required, but also modulate motor activity and mood. Similarly, dopaminergic and hypocretinergic neurons promote wakefulness in response to highly motivating and rewarding stimuli. It is thus reasonable to assume that the behavioral state of wakefulness generated by these arousal-promoting subgroups is a prerequisite for an appropriate response to an alert signal (i.e., pain or presence of a predator) and to satisfy physiological demands. In the following subsections, we provide an overview of the main neuronal subgroups that contribute to generate wakefulness.

5.3.3.1 Monoaminergic Neurons

Monoaminergic neurons use norepinephrine, dopamine, serotonin or histamine as neurotransmitters, and project diffusely to the thalamus, lateral hypothalamus, basal forebrain, and cerebral cortex. Consistent with an arousal-promoting role, monoaminergic neurons are -in general- maximally active during wakefulness.

Norepinephrine-containing neurons are almost exclusively located in the locus coeruleus (LC), a bilateral nucleus within the dorsolateral mesopontine region of the brain stem (Fig. 5.2). LC neurons project diffusely to the neocortex, forebrain, brain stem, and spinal cord to increase wakefulness and muscle tone. These neurons discharge tonically and at the highest rates during wakefulness (Aston-Jones and Bloom 1981) when cortical norepinephrine release is maximal (Berridge and Abercrombie 1999), reduce their discharge rates during NREM sleep, and cease firing during REM sleep. LC neurons increase wakefulness in response to novel and salient stimuli, as well as to stressors (Aston-Jones and Cohen 2005). This notion is further supported by evidence that rats with LC lesions had substantially less wakefulness than controls when placed in a novel environment (Gompf et al. 2010). Optogenetic stimulation of LC-NE neurons wakes mice from sleep with a short latency, whereas optogenetic inhibition of NE neurons

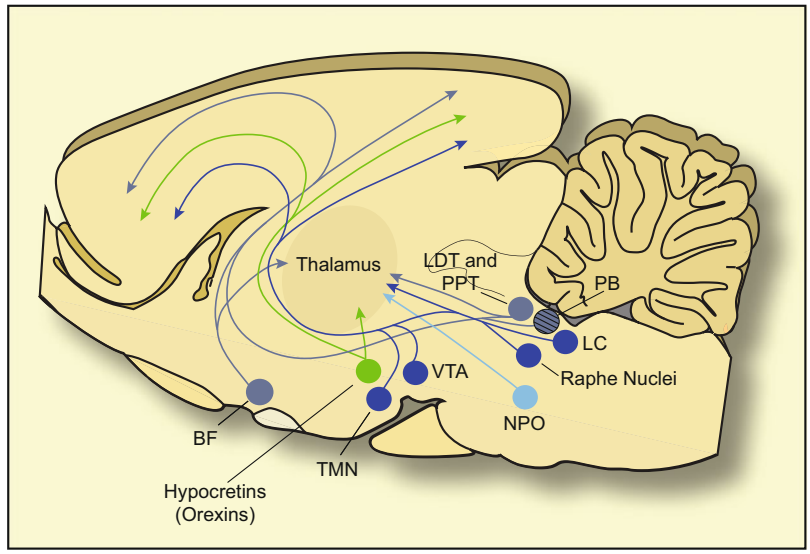

Fig. 5.2 Neuronal distribution and relevant projections of main arousal promoting systems. Wakefulness and EEG activation are generated by monoaminergic cell groups that produce norepinephrine in the locus coeruleus (LC), serotonin in the median and dorsal raphe nuclei, dopamine in the ventral tegmental area (VTA), and histamine in the tuberomammillary nucleus (TMN), cholinergic cells groups in the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT), and basal forebrain (BF), glutamatergic neurons in the nucleus pontis oralis (NPO), as well as glutamatergic neurons in the parabrachial nucleus (PB)

promotes the transition into NREM sleep and reduces the time spent in wakefulness (Carter et al. 2009).

Dopamine-containing neurons are located in mesencephalic substantia nigra pars compacta and ventral tegmental area (VTA) (Fig. 5.2). The VTA dopaminergic neurons project to the prefrontal cortex, and both VTA and substantia nigra neurons project to the striatum (Oades and Halliday 1987), and other arousal promoting nuclei such as the dorsal raphe, locus coeruleus, and the laterodorsal and pedunculopontine tegmental nucleus (Monti and Monti 2007). Unlike the other monoaminergic subgroups, dopaminergic neurons do not change their firing rate across the sleep-wake cycle (Miller et al. 1983). Dopaminergic neurons are mainly active during wakefulness with rewarding behavior (Mirenowicz and Schultz 1996) and discharge at a high frequency in "burst or trains" in response to a motivational stimulus that causes the release of dopamine into the extracellular space (Schultz et al. 1993; Wightman and Robinson 2002). This same firing pattern is observed during REM sleep in VTA dopaminergic neurons, and dopamine release into the nucleus accumbens (ventral striatum) also increases during REM sleep (Lena et al. 2005; Dahan et al. 2007). The ventral subdivision of the periaqueductal gray contains a third group of dopaminergic neurons that may play a role in the generation of wakefulness (Lu et al. 2006). These neurons project to several areas related to sleep and wake control, express c-Fos exclusively during wakefulness, and their selective lesion increases sleep (Lu et al. 2006). Evidence that brain administration of dopamine receptor agonists increases wakefulness and suppresses sleep (Monti et al. 1990; Isaac and Berridge 2003) supports the idea that dopamine promotes wakefulness. Additionally, dopamine transporter knockout mice exhibit increased wakefulness and decreased sleep, presumably caused by an increase in extracellular dopamine levels (Wisor et al. 2001). Genetic polymorphism of the dopamine transporter and dopamine D2 receptor genes are also associated with sleep duration (Rhodes

et al. 2018), and the neurobehavioral and neurophysiological consequences of sleep deprivation in humans (Holst et al. 2017). Recent optogenetic studies lend additional support to the conclusion that dopaminergic neurons promote wakefulness. Optostimulation of VTA-dopaminergic neurons causes a rapid transition from NREM sleep to wakefulness (Eban-Rothschild et al. 2016), as well as the transition from an anesthetized state to wakefulness (Taylor et al. 2016). Similarly, chemogenetic activation of dopaminergic neurons in the VTA, but not in the substantia nigra, increases wakefulness, and this effect is mediated by D2/D3 receptors (Oishi et al. 2017). Conversely, optoinhibition of VTA neurons reduces wakefulness, especially during highly motivated states (Eban-Rothschild et al. 2016).

Serotonin is produced by neurons within the raphe nuclei of the brain stem (Jacobs and Azmitia 1992; Monti 2010, 2011b). The most relevant for the regulation of arousal states are the dorsal and medial raphe; these nuclei are located in the mesopontine region and project directly to the thalamus and cortex (Fig. 5.2). Serotonergic neurons share the typical state-specific pattern of activity with most monoaminergic cells. They are active during wakefulness and stop firing during REM sleep (McGinty and Harper 1976), and the release of serotonin is consistent with this pattern of activity (Portas et al. 2000). Subgroups of serotonergic neurons are specifically active during stereotyped movements, such as locomotor activity or grooming (Jacobs and Fornal 2008). The electrical stimulation of the dorsal raphe produces a marked activation (desynchronization) of the EEG (Dringenberg and Vanderwolf 1997). Optogenetic activation of serotonergic neurons in the dorsal raphe nucleus increases wakefulness, decreases sleep time by fragmenting (i.e., reduces episode duration) NREM sleep (Ito et al. 2013).

Histaminergic neurons are exclusively located in the tuberomammillary nucleus of the posterior hypothalamus (Monti 2011a) (Fig. 5.2). These neurons are active during wakefulness and do not fire during REM sleep (wake-on/REM-off) (Takahashi et al. 2006), and innervate the cortex and thalamus. Histamine receptor antagonists that act at brain H1 receptors induce sleepiness, and "knock-out" mice lacking histidine decarboxylase (an enzyme involved in the synthesis of histamine) or the H1 receptor are unable to stay awake when placed in a new environment (Parmentier et al. 2002; Parmentier et al. 2016). Drugs that increase synaptic levels of histamine increase wakefulness (Kalivas 1982). Administration of histamine into the basal forebrain increases wakefulness and decreases sleep (Ramesh et al. 2004). In anesthetized rats, the pharmacologic stimulation of H1 receptors in the basal forebrain causes EEG activation and reduces the time to transition from the anesthetized state to wakefulness (Luo and Leung 2009). In vitro optogenetic stimulation of histaminergic neurons inhibits GABAergic neurons (sleep-active) in the ventrolateral preoptic nucleus of the hypothalamus, supporting the sleep-wake "flip-flop switch" hypothesis and a role for histamine in stabilizing the switch to generate wakefulness (Williams et al. 2014).

5.3.3.2 Cholinergic Neurons in the Brain Stem and Basal Forebrain

Wakefulness-related cholinergic neurons are localized within the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT), as well as in the basal forebrain (Fig. 5.2) (Satoh and Fibiger 1986; Semba 2000; Jones 2005). Neurons in the LDT and PPT project into the thalamus, hypothalamus, and basal forebrain, whereas neurons in the basal forebrain project to the cerebral cortex (Hallanger and Wainer 1988). The cholinergic neurons discharge during wakefulness and REM sleep, and suppress their discharge during NREM sleep; cortical acetylcholine release is also consistent with this firing pattern (Marrosu et al. 1995; Lee et al. 2005; Boucetta et al. 2014; Cisse et al. 2018). In fur seals, cortical acetylcholine release is higher during wakefulness and REM sleep, lower during NREM sleep, and becomes lateralized during unihemispheric NREM sleep with a greater release in the hemisphere with a wake-like activated EEG (Lapierre et al. 2007). Administration of the cholinesterase inhibitor physostigmine (i.e., increasing acetylcholine levels by

preventing its degradation) reverses general anesthesia in humans (Plourde et al. 2003). Chemogenetic activation of cholinergic neurons in the PPT suppresses EEG slow activity during NREM sleep (Kroeger et al. 2017). Results from optogenetic stimulation of LDT and PPT neurons are congruent with these findings. Activation of cholinergic neurons during sleep caused a shift from slow wake activity to theta and enhanced EEG gamma activity, but without causing behavioral arousal (Cisse et al. 2018). These data suggest that cholinergic neurons in the LDT and PPT promote cortical activation during wakefulness and REM sleep.

Several lines of evidence from experiments using optogenetic and chemogenetic modulation of cholinergic neurons in the basal forebrain demonstrated that activation of these neurons reduces EEG slow wave activity during NREM sleep, whereas their inhibition increases the slow oscillation without causing a substantial change in in the amount of wakefulness (Han et al. 2014; Irmak and de Lecea 2014; Anaclet et al. 2015; Shi et al. 2015; Xu et al. 2015; Chen et al. 2016). By using a dual optostimulation and microdialysis probe, Zant et al. demonstrated that selective stimulation of cholinergic neurons in the basal forebrain increases local acetylcholine levels, increasing wakefulness (Zant et al. 2016). Cholinergic neurons in the medial septum and the vertical limb of the diagonal band of Broca contribute to the generation of theta activity during active W and REM sleep. In addition to their arousal-promoting role, cholinergic neurons in the basal forebrain contribute to regulate sensory processing, attention, memory, and plasticity.

5.3.3.3 GABA in the Pons, Basal Forebrain and Lateral Hypothalamus

GABAergic neurons are widely distributed throughout the central nervous system. GABA is classically considered a sleep-promoting substance; the sedative effect of ethanol or sleep medication such as benzodiazepines supports this idea. However, the role of GABA in the regulation of sleep and wakefulness vary on a brain-to-brain region basis (Brown and McKenna 2015). Pharmacologic studies showed that the administration of $GABA_A$ receptor agonists into the nucleus pontis oralis (or its rodent homologue, the oral pontine reticular nucleus) increases wakefulness and suppresses sleep (Xi et al. 1999; Vanini and Baghdoyan 2013). Conversely, the microinjection of a $GABA_A$ receptor antagonist into the same pontine region decreases wakefulness and increases REM sleep (Xi et al. 1999). Endogenous GABA levels in the pons vary in a state-specific manner, being highest during wakefulness and lowest during REM sleep (Vanini et al. 2011). Also, general anesthesia causes the loss of consciousness by reducing extracellular GABA levels in the oral pontine reticular nucleus, and pharmacologically increasing or decreasing endogenous GABA levels, increases or decreases the time needed to induce general anesthesia, respectively (Vanini et al. 2008; Vanini et al. 2014).

GABAergic neurons in the VTA also increase their firing rate during arousal (and REM sleep) (Lee et al. 2001). It has been hypothesized that these neurons generate reward-related arousal (Brown and McKenna 2015). A subset of GABAergic neurons in the basal forebrain also contributes to generating wakefulness. Opto- and chemogenetic stimulation of GABAergic neurons that are parvalbumin-positive increases wakefulness and enhances EEG gamma activity (Anaclet et al. 2015; Kim et al. 2015; Xu et al. 2015; Anaclet et al. 2018). Wake promoting GABAergic neurons are also found in the lateral hypothalamus. Ventral GABAergic neurons may induce wakefulness by inhibiting sleep-promoting neurons in the ventrolateral preoptic area, while dorsal GABAergic neurons inhibit the reticular thalamic nucleus that is critical for the generation of sleep spindles (Herrera et al. 2016; Venner et al. 2016).

5.3.3.4 Hypocretins (Orexins)

The hypocretins 1 and 2 (also called orexins A and B) are neuropeptides used as non-classical neurotransmitters or neuromodulators by neurons located in the posterior and lateral region of the hypothalamus (de Lecea et al. 1998; Sakurai et al. 1998) (Fig. 5.2). Hypocretinergic neurons project

diffusely to the cortex, thalamus, and other areas related to sleep and wake regulation. Intracerebral or intraventricular administration of hypocretins promotes wakefulness (Piper et al. 2000). Single unit recordings revealed that hypocretinergic neurons discharge during active wakefulness or the transition from sleep to waking in association with changes in muscle tone, and virtually stop firing during sleep (Lee et al. 2005; Takahashi et al. 2008). Furthermore, hypocretinergic neurons are active (assessed by cFos expression) during wakefulness with motor activity related to the motivation to explore a new environment, but not during quiet wakefulness or forced locomotion (Torterolo et al. 2003). Genetic ablation of hypocretinergic neurons significantly delays the recovery from general anesthesia (Kelz et al. 2008). Opto- and chemogenetic activation of hypocretinergic neurons promotes wakefulness (Adamantidis et al. 2007; Carter et al. 2009; Sasaki et al. 2011) and optogenetic inhibition induces NREM sleep during the light phase only (Tsunematsu et al. 2011). The hypocretinergic system has great clinical importance because the degeneration of hypocretinergic neurons is implicated in the pathogenesis of the sleep disorder narcolepsy (Mignot 2011a, b).

5.3.4 Brain Regions and Circuits Regulating Sleep

The devastating epidemic of encephalitis lethargica in the early twentieth century led Von Economo to the discovery that the preoptic area of the rostral hypothalamus is a key node for sleep generation (Von Economo 1930b). Subsequent research has demonstrated that sleep-promoting neurons are also found in the basal forebrain, posterior hypothalamus, and brain stem.

5.3.4.1 The Preoptic Region

The median preoptic nucleus (MnPO) and ventrolateral preoptic nucleus (VLPO) contain neurons that are critical in the generation and maintenance of NREM sleep (Szymusiak and McGinty 1986; Torterolo et al. 2009; Benedetto et al. 2012) (Fig. 5.3). Lesions of the MnPO and VLPO produce long-lasting insomnia (Lu et al. 2000) and acute resistance to anesthetic induction (Moore et al. 2012). Studies using extracellular unit recordings show that the preoptic region and adjacent basal forebrain contain neurons that are active during both NREM and REM sleep (Szymusiak and McGinty 1986; Sakai 2011). Recent research using cFos-dependent activity-tagging techniques demonstrated that sleep-promoting neurons are found in all the preoptic areas, regions dorsal to the preoptic area, and several septal nuclei (Zhang et al. 2015). Most sleep-active preoptic neurons are GABAergic, and neurons in the VLPO also co-localize with the neuropeptide galanin (Sherin et al. 1998). Preoptic neurons send direct (monosynaptic) projections to most major arousal-promoting nuclei including the hypocretinergic lateral hypothalamic area, the tuberomammillary nucleus, the dorsal raphe nucleus, and the locus coeruleus (Sherin et al. 1998). Consistent with this anatomical evidence, electrical stimulation of the preoptic area and adjacent basal forebrain inhibits the arousal-promoting neurons and induces NREM sleep (McGinty and Szymusiak 2005a, b). Conversely, neurons from arousal-promoting systems inhibit hypnogenic regions (Gallopin et al. 2000; Williams et al. 2014). This reciprocal inhibition between activating and hypnogenic regions is critical for the transition between sleep and wakefulness states, and represents the foundation of the flip-flop state switch model (Saper et al. 2010). Optogenetic activation or inhibition of the GABAergic VLPO neurons that project toward the TMN histaminergic neurons, enhances or decreases sleep, respectively (Chung et al. 2017). Additionally, low-frequency optogenetic stimulation as well as chemogenetic stimulation of VLPO galanin-positive neurons promotes sleep and heat loss (Kroeger et al. 2018). Chemogenetic activation of a subset of nitrergic-glutamatergic neurons in the MnPO and medial preoptic -identified by cFos-dependent activity-tagging- also promotes sleep coupled with body cooling (Harding et al. 2018). Together, these studies demonstrate an underlying neural mechanism linking sleep and temperature regulation.

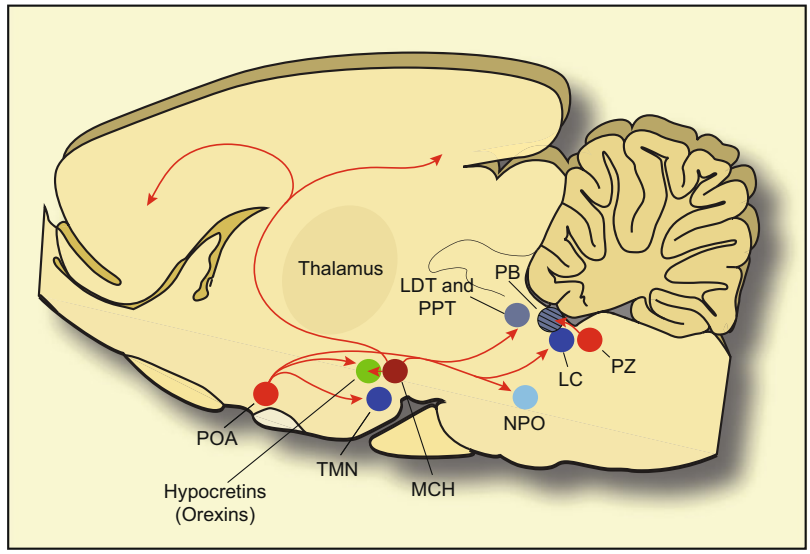

Fig. 5.3 Neuronal distribution and relevant projections of NREM sleep-promoting systems. Major arousal-promoting cell groups are inhibited by widely distributed neural circuits that generate and maintain NREM sleep such as GABAergic neurons in the preoptic area (POA) and adjacent basal forebrain, as well as in the parafacial zone (PZ) of the brain stem. In addition, neurons containing melanin-concentrating hormone (MCH) also contribute to generate NREM sleep by inhibiting neighboring hypocretinergic neurons in the hypothalamus and, via descending projections, monoaminergic neurons in the raphe nuclei and locus coeruleus (LC). Other abbreviations: TMN, tuberomammillary nucleus; LDT and PPT, laterodorsal and pedunculopontine tegmental nuclei; PB, parabrachial nucleus; NPO, nucleus pontis oralis

5.3.4.2 Melanin-Concentrating Hormone

Melanin-concentrating hormone (MCH) is a neuropeptide produced and used as a neuromodulator by neurons in the posterolateral hypothalamus and incerto-hypothalamic area (Torterolo et al. 2006; Torterolo et al. 2011; Monti et al. 2013; Bittencourt and Diniz 2018). These neurons project diffusely throughout the central nervous system including most major arousal-promoting nuclei (Bittencourt and Diniz 2018; Costa et al. 2018) (Fig. 5.3). These neurons fire scarcely during wakefulness, increase their firing rate during NREM sleep, and reach a maximum during REM sleep (Hassani et al. 2009). Both optogenetic and chemogenetic studies strongly suggest that this system plays a critical role in the generation of sleep (Vetrivelan et al. 2016; Blanco-Centurion et al. 2018). Microinjection of MCH into the cerebral ventricles, preoptic area, basal forebrain, dorsal raphe nucleus, locus coeruleus, and NPO facilitates the generation of NREM sleep and/or REM sleep (Verret et al. 2003; Lagos et al. 2009; Torterolo et al. 2009; Lagos et al. 2012; Benedetto et al. 2013; Monti et al. 2015). These data suggest that MCH-containing neurons inhibit the activity of arousal-promoting systems and/or activate hypnogenic circuits to promote sleep. Consistent with this hypothesis, MCH inhibits serotonergic neurons in the dorsal raphe and decreases serotonergic release (Devera et al. 2015; Urbanavicius et al. 2016); this finding may explain, at least in part, the mechanism by which MCH induces REM sleep (Lagos et al. 2009). Importantly, the suppression of the serotonergic activity by MCH could explain also the pro-depressive effect of this neuropeptide (Lagos et al. 2011; Lopez Hill et al. 2013; Urbanavicius et al. 2014; Torterolo et al. 2015). A recent chemogenetic study suggests that when MCHergic neurons are physiologically recruited, NREM sleep depth is increased and the extinction of NREM sleep episodes is accelerated,

strengthening the probability for natural NREM to REM sleep transition (Varin et al. 2018).

5.3.4.3 Mesopontine Reticular Formation

The mesopontine reticular formation (Fig. 5.4) contains the neural networks that are necessary and sufficient for the generation and maintenance of REM sleep (Siegel 2011). Two models, that are not mutually exclusive, provide a potential circuit-based explanation on how REM sleep is generated: the cholinergic-aminergic and the GABAergic-glutamatergic models.

Cholinergic-aminergic model. The reciprocal interaction model is the first structural and mathematical model that describes the interaction between REM-promoting (cholinergic) and REM-antagonizing (aminergic) neurons which generates the ultradian occurrence of REM sleep (McCarley and Hobson 1975). An updated version of this model is discussed in (McCarley 2007). As reviewed above, noradrenergic neurons in the locus coeruleus and serotonergic neurons in the dorsal raphe nucleus are wake-active and stop their discharge during REM sleep ("REM-off" neurons). These neurons are considered "permissive" for the generation of REM sleep; i.e. for REM sleep to occur, these neurons must be inhibited. Several lines of evidence show that during wakefulness, these aminergic neurons inhibit "REM-on" cholinergic neurons in the laterodorsal and pedunculopontine tegmental nucleus, as well as neurons in the nucleus pontis oralis (Luebke et al. 1992; Williams and Reiner 1993; Crochet and Sakai 1999). Cholinergic neurons in the laterodorsal and pedunculopontine tegmental nucleus (Fig. 5.4) are maximally active during wakefulness and REM sleep (Boucetta et al. 2014). Selective optogenetic stimulation of cholinergic neurons in the laterodorsal or pedunculopontine tegmental nucleus promote the transition from NREM to REM sleep (Van Dort et al. 2015). Cholinergic "REM-on" neurons project to the nucleus pontis oralis, the REM sleep "executive area", and pharmacologic stimulation of this area with cholinergic agonists promotes REM sleep (Kubin 2001; Chase and Morales 2005). Cholinergic terminals from neurons in the laterodorsal and pedunculopontine tegmental nucleus activate glutamatergic neurons of the NPO, which are active during REM sleep and orchestrate the generation of this state (Kubin 2001; Clement et al. 2011; Weng et al. 2014). For example, glutamatergic neurons in the dorsolateral pons produce muscle atonia and EEG activation during REM sleep, respectively, by descending projections to glycinergic neurons in the medullary reticular formation and ascending projections to the thalamus. A more current version of this model includes mesopontine GABAergic neurons that inhibit monoaminergic neurons during REM sleep, and/or inhibit "REM-on" neurons in the nucleus pontis oralis during wakefulness (Xi et al. 1999; Torterolo et al. 2000, 2001; Torterolo et al. 2002; Torterolo et al. 2002; McCarley 2007; Vanini et al. 2007; Sapin et al. 2009; Torterolo and Vanini 2010).

GABAergic-glutamatergic model. This model is based on direct projections from neurons in the ventrolateral subdivision of the periaqueductal gray (vlPAG) to the REM sleep effector region, the nucleus pontis oralis (or rodent sublaterodorsal nucleus) (Luppi et al. 2006). According to this model, REM-on glutamatergic neurons in the nucleus pontis oralis are inhibited during wakefulness by wake-active (REM-off) GABAergic neurons in the vlPAG (Lu et al. 2006; Luppi et al. 2006; Sapin et al. 2009) (Fig. 5.4). The release of this tonic inhibitory drive would thus permit the generation of REM sleep. Pharmacological evidence that bilateral inhibition by microinjection of a $GABA_A$ receptor agonist into the vlPAG and adjacent reticular formation promotes REM sleep supports this model (Vanini et al. 2007). Besides, calcium imaging and unit recordings across sleep-wake states show that most GABAergic neurons in the vlPAG stop discharging at the onset of REM sleep and resume firing upon its termination, and optogenetic activation suppresses REM sleep initiation through projections to the dorsolateral pons (Weber et al. 2018). Of note, this model is primarily supported by experiments in rodents and considers the cholinergic influence on REM sleep not to be executive, but only modulatory (Grace and Horner 2015).

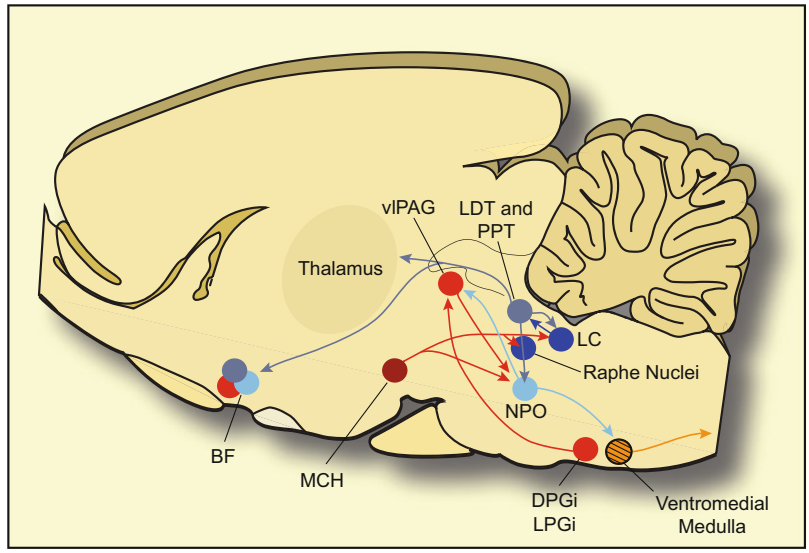

Fig. 5.4 Neuronal distribution and relevant projections of REM sleep-promoting systems according to the models described in the text. REM sleep is generated by the removal of a tonic inhibitory drive from wake-active GABAergic neurons in the ventrolateral periaqueductal gray (vlPAG) onto REM-promoting glutamatergic neurons in the nucleus pontis oralis (NPO). GABAergic neurons in the dorsal and lateral paragigantocellular nuclei (DPGi and LPGi) inhibit wake-active vlPAG neurons to generate REM sleep. REM-promoting neurons in the NPO then generate EEG activation via ascending projections to the thalamus, and muscle atonia by descending projections to glycinergic neurons in the ventromedial medulla that inhibit spinal motor neurons during REM sleep. Neurons containing melanin-concentrating hormone (MCH) also facilitate REM sleep by inhibition of monoaminergic nuclei in the brain stem. Additionally, reciprocal inhibition of mesopontine cholinergic neurons and monoaminergic neurons in the pons determine the timing and duration of REM sleep. Wake-active monoaminergic neurons inhibit cholinergic neurons in the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT). During REM sleep, monoaminergic neurons stop firing (LDT and PPT neurons contribute to their inhibition), and REM-active LDT and PPT neurons promote EEG activation as well as activation of REM-promoting neurons in the NPO. Other abbreviations: BF, basal forebrain; LC, locus coeruleus

5.3.4.4 Medullary Reticular Formation

Early transection and stimulation studies suggested that the lower brain stem contained neural circuits that contribute to the generation of NREM sleep (Batini et al. 1958). In a recent study, Anaclet et al. identified a group of medullary sleep-promoting neurons located ventral and lateral of the genu of the facial nerve, the parafacial zone (Anaclet et al. 2012) (Fig. 5.3). GABAergic/glycinergic neurons within this area are active during NREM sleep, and selective activation or inhibition of these neurons promotes or suppresses NREM sleep, respectively (Anaclet et al. 2012; Anaclet et al. 2014). Furthermore, parafacial zone neurons directly inhibit the wake-promoting medial parabrachial area. However, recordings of neuronal discharge activity across spontaneous sleep-wake states in mice and rats have yielded conflictive results. While parafacial neuronal discharge in both rats and mice are heterogeneous, only rats showed NREM-active neurons (Sakai 2017; Alam et al. 2018). Additional studies are needed to reach a definite conclusion about these neurons.

The medullary reticular formation plays a critical role in the generation of motor atonia during REM sleep. A long-standing model proposes that motor atonia is generated by direct projections of glutamatergic neurons within the nucleus pontis oralis (the sublaterodorsal nucleus in rodents) onto glycinergic neurons in the ventromedial medulla (Fig. 5.4). These medullary neurons produce a tonic glycinergic post-synaptic inhibition of alpha motoneurons during REM sleep (Chase

2013). GABAergic neurotransmission may also play a role in the generation of REM sleep atonia (Sapin et al. 2009; Brooks and Peever 2012). Additionally, GABAergic neurons in the dorsal and lateral paragigantocellular nuclei inhibit "REM-off" neurons in the mesopontine region and contribute to the generation of REM sleep (Goutagny et al. 2008; Weber et al. 2015).

5.4 Conclusions

We now have a better understanding of the molecules, neuronal subtypes, and networks that regulate sleep and wakefulness at a global, regional, and local scale. Together, growing evidence that sleep (or a sleep-like state) is present in most species in the animal kingdom, and the well-known deleterious health consequences associated with sleep loss, support the idea that sleep is a vital physiological process. With the rapid development of neuroscience technologies, we can now hope that our field will continue to advance our knowledge on the mechanisms that regulate sleep and wakefulness, and ultimately elucidate the enigmatic function(s) of sleep.

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Cannabinoids and Sleep/Wake Control

6

Mónica Méndez-Díaz, Alejandra E. Ruiz-Contreras, Jacqueline Cortés-Morelos, and Oscar Prospéro-García

Abstract

The sleep-wake cycle is a complex process that includes wake (W), non-rapid-eye-movement (NREM) and rapid-eye-movement (REM) sleep. Each phase is regulated by specialized brain structures that, by means of different neurotransmitters, maintain the constant expression of the sleep-wake cycle. Molecules like orexin, serotonin, noradrenaline, histamine, for waking; GABA, adenosine, prostaglandins, for NREM sleep and acetylcholine and glutamate for REM sleep, among other molecules are responsible for the expression and maintenance of each phase. When the endocannabinoid system was being described for the first time, almost three decades ago, oleamide's sleep promoting properties were highlighted. Nowadays, enough evidence has been cumulated to support the endocannabinoid system role in the sleep-wake cycle regulation. The endocannabinoids oleamide anandamide, and 2-arachidonylglycerol promote NREM and/or REM sleep via the CB1R, thereby making this system a target to treat sleep disorders, such as insomnia.

Keywords

Non-rapid-eye-movement (NREM) sleep · Rapid-eye-movement (REM) sleep · Anadamide · 2-arachidonylglycerol · Oleamide · Cannabinoid receptor 1 · Insomnia

6.1 Introduction

The sleep-wake cycle is a complex process that includes at least three states. Waking (W): this is a state in which different levels of alertness occur, depending on the behavior the subject is performing. This stage is modulated by neurons located mainly in the lateral and posterior hypothalamus (Fig. 6.1a), that synthesize neurotransmitters, such as orexins/hypocretins (ORX), and histamine, and brainstem neurons synthesizing noradrenaline (Locus coeruleus), serotonin (dorsal raphe nucleus), and acetylcholine (peduncle pontine tegmental nucleus) (Siegel 2009; Luppi and Fort 2011). Non-rapid-eye-movement (NREM) sleep is not a homogeneous state. In humans it is divided into three stages: N1, N2, and N3 (American Academy of Sleep Medicine, 2015), while in rats it is frequently

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divided into slow-wave sleep 1 (SWS1) and slow-wave sleep 2 (SWS2). NREM sleep is promoted by the anterior hypothalamus (medial and ventrolateral preoptic area) (Fig. 6.1b). GABA, a major inhibitory neurotransmitter, adenosine, a nucleoside, and PGD2, a prostaglandin, participate in its regulation (Siegel 2009; Luppi and Fort 2011; Kumar et al. 2013). In rapid-eye-movement (REM) sleep, the subject exhibits eye movements and postural atonia. In this stage, humans have more vivid dreams. REM sleep is promoted mainly by nuclei located in the brainstem, i.e., peduncle pontine tegmental (PPT), laterodorsal tegmental (LDT), and pontis oralis nuclei (PON), among others (Fig. 6.1c and d) and neurotransmitters, such as acetylcholine and glutamate, and neuropeptides, such as melanin-concentrating hormone (MCH) (Siegel 2009; Luppi and Fort 2011).

In the last 30 years, experimental evidence has been supporting the notion that endocannabinoids may be also modulating the sleep-wake cycle. In the following section, we discuss evidence supporting such a notion.

6.2 The Endocannabinoid System

Once delta-9-THC (THC) was isolated from the Cannabis plant, it took a little bit more than two decades of scientific research to reveal that this molecule has specific binding sites in the brain (Devane et al. 1988). A year later, the cannabinoid 1 receptor (CB1R) was cloned (Matsuda et al. 1990). Soon after, a second receptor, CB2R, was also cloned (Munro et al. 1993). A crucial piece of evidence to confirm the existence of an endocannabinoid system was discovered by Mechoulam's group when they isolated a molecule from the porcine brain, an ethanolamine of arachidonic acid (AEA), which not only binds to these receptors (Devane et al. 1992) but induces similar THC effects. This molecule was named anandamide (AEA), based on the Sanskrit word, Ananda, which means 'bliss'. The second endocannabinoid was described by Lerner's group; they reported the existence of a lipid isolated from the cerebrospinal fluid of sleep-deprived cats identified as cis-9,10-octadecenoamide (oleamide, ODA) (Lerner et al. 1994; Cravatt et al. 1995). The physiological and pharmacological properties of ODA are similar to those observed for AEA such as analgesia, motor depression, catalepsy, and hypothermia; hence, it met the criteria to be considered an endocannabinoid (Mechoulam et al. 1997). Later on, another endocannabinoid, 2-arachidonylglycerol (2-AG), was isolated from canine intestines (Mechoulam et al. 1995) (Fig. 6.2). More endocannabinoids have thus far been identified: arachidonoyl-ethanolamine (virodhamine), N-arachidonoyldopamine (NADA), N-arachidonoylglyceryl ether (NAGE) and 2-arachidonyl glyceryl ether (2-AGE).

To date, we can consider that this system is amply described with the identification of the enzymes involved in the synthesis and degradation of them. For the synthesis of AEA, we have N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD), glycerophosphodiesterase-1 (GDE1) and protein tyrosine phosphatase N22 (PTPN22). While fatty acid amide hydrolase (FAAH) is the one in charge of breaking it up. 2-AG is synthesized by diacylglycerol lipase (DGL) alpha and its degradation is the responsibility of monoacylglycerol lipase (MAGL) and FAAH. ODA is synthesized by Cytochrome C and also hydrolyzed by FAAH (see Prospéro-García et al. 2016).

The basal level of 2-AG is about 1000 times higher than AEA in the brain. AEA turns out to be a high-affinity, partial agonist of CB1R, and almost inactive at CB2R; whereas 2-AG acts as a full agonist at both CBRs with moderate-to-low affinity. The release of AEA and 2-AG, occurs from the postsynaptic sites to the synaptic cleft, in response to elevation of intracellular calcium and they, then, act as retrograde neurotransmitters on presynaptically located cannabinoid receptors to maintain homeostasis and prevent excessive neuronal activity (Howlett 2002; Terry et al. 2009). The first conclusive evidence supporting retrograde endocannabinoid signaling came from the observation of depolarization-induced suppression of inhibition (DSI)/excitation (DSE). Later, it was discovered that the endocannabinoid

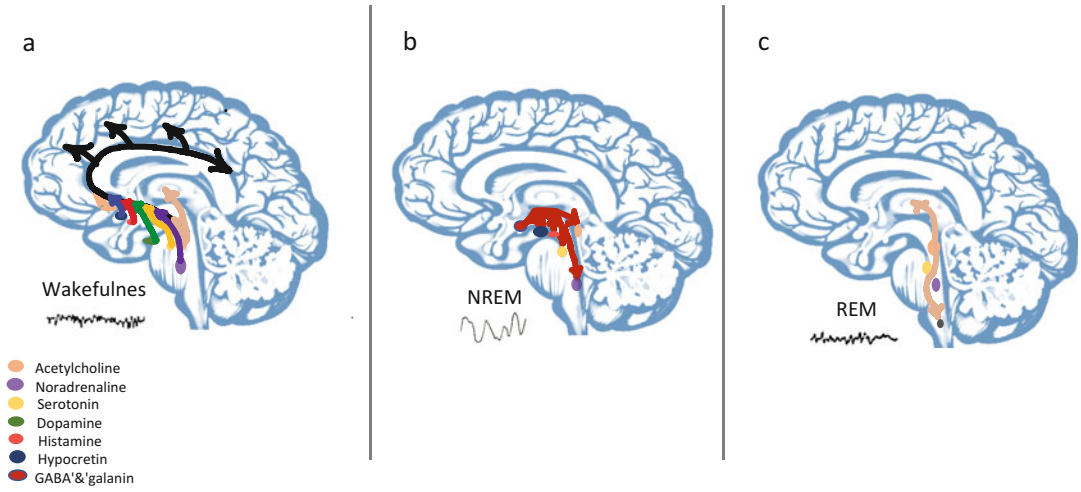


Fig. 6.1 Main brain structures regulating the sleep-wake stages. (**a**) Wake: typical EEG trace, structures and neurotransmitters involved in its modulation. (**b**) Non-rapid-eye-movement (NREM) sleep: typical EEG trace, structures and neurotransmitters involved in its modulation, and (**c**) Rapid-eye-movement (REM) sleep: typical EEG trace, structures and neurotransmitters involved in its modulation

system is involved, not only in short-term depression but also in long-term depression (LTD) at both excitatory and inhibitory synapses.

6.3 Cannabinoid Receptor 1 (CB1R)

The CB1R is the most abundant G-protein–coupled receptor expressed in the brain (Fig. 6.3), it is a seven-transmembrane domain receptor coupled to Gi/o proteins. CB1R is encoded by gene CNR1 and consists of 472 amino acids in humans (473 amino acids in rat and mouse, with 97–99% amino acid sequence identity among these species). It is located pre-synaptically defining retrograde neurotransmission.

CB1R activation inhibits neurotransmitter release at synapses through two main mechanisms; for short-term plasticity, the mechanism involves direct G protein-dependent

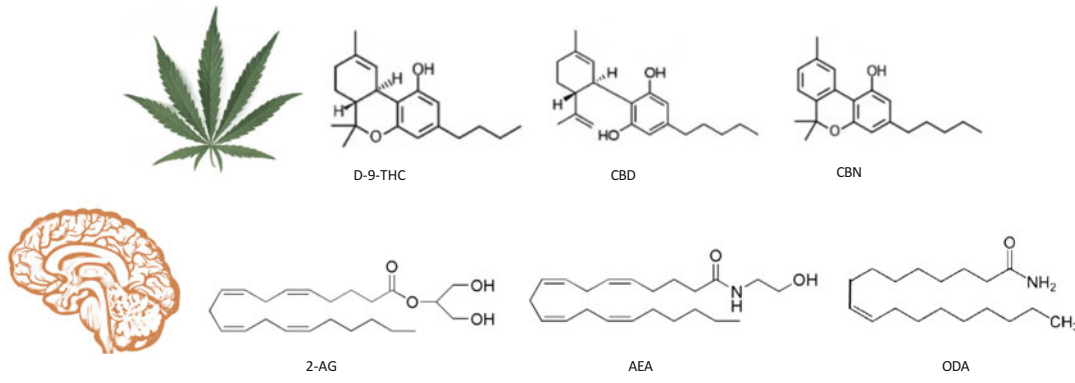


Fig. 6.2 Plant and brain derived cannabinoids. D-9-THC, delta-9-tetrahidrocannabinol. CBD, cannabidiol. CBN, cannabinol. 2-AG, 2-Arachidonoylglycerol. AEA, Anandamide, also known as N-arachidonoylethanolamine. ODA, cis-9-10-octadecenoamide, also known as Oleamide

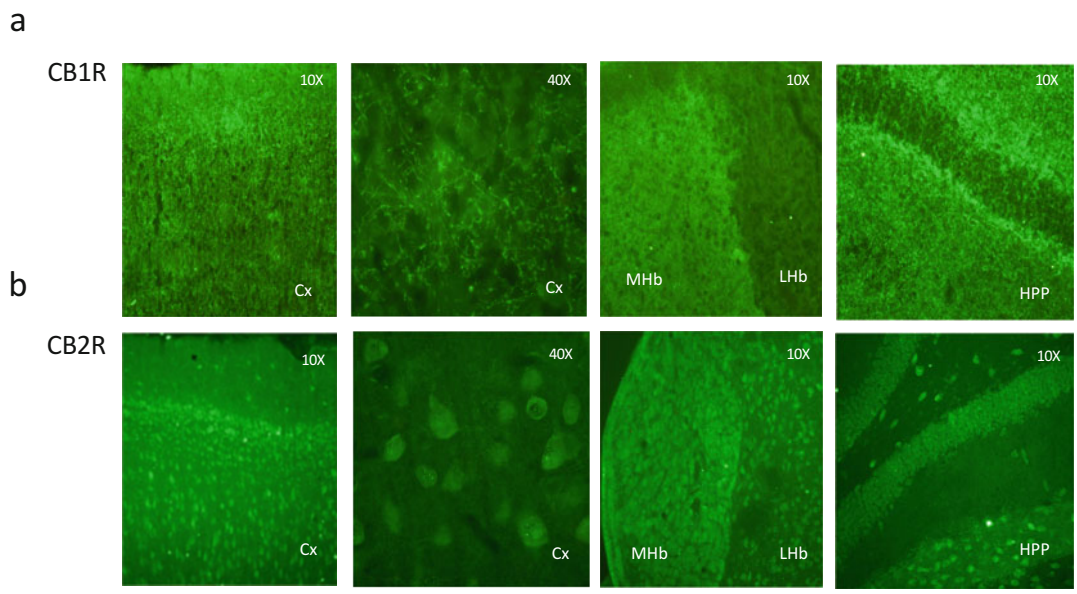


Fig. 6.3 Brain cannabinoid receptors (**a**) CB1R expression in cortex (Cx), medial habenula (MHb), lateral habenula (LHb) and hippocampus. (**b**) CB2R expression in cortex (Cx), medial habenula (MHb), lateral habenula (LHb) and hippocampus. (Immunofluorescence)

inhibition of presynaptic Ca2+ influx through voltage-gated Ca2+ channels (VGCCs) while facilitating K+ conductance. For long-term plasticity, the predominant mechanism requires inhibition of adenylyl cyclase and downregulation of the cAMP/PKA pathway via the αi/o (for details Castillo 2012) resulting in the inhibition of the release of neurotransmitters (Fig. 6.4). CB1R has been found to inhibit GABA and glutamate release from presynaptic terminals, which confers to the CB1R the ability to modulate neurotransmission. This has been proposed as a plausible underlying mechanism of CB1R-mediated neuroprotection against excitotoxicity, a prominent pathological process of many neurological disorders, including epilepsy and neurodegenerative diseases. CB1R has a dense expression in (ranked in order): the substantia nigra, globus pallidus, hippocampus, cerebral cortex, putamen, caudate, cerebellum, and amygdala.

CB1R may form heteromers, with two or more other GPCRs co-expressed. CB1R also can form heteromers with dopamine D2 receptors, or dopamine D2 and adenosine A2A (Navarro et al. 2003). Likewise, CB1Rs form heteromers with CB2Rs in the brain, and the agonist coactivation of CB1Rs and CB2Rs results in negative crosstalk in AKT1 phosphorylation (Callén et al. 2012).

6.4 Cannabinoid Receptor 2 (CB2R)

The CB2R is encoded by gene CNR2, which consists of 360 amino acids in humans. It shares only 44% sequence homology with CB1R at the protein level. CB2R also has greater species differences between humans and rodents in comparison to CB1R, as the amino acid sequence homology is slightly above 80% between humans and rodents. Similarly to CB1R, CB2R is a seven-transmembrane domain receptor coupled to Gi/o proteins, the activation of which inhibits adenyl cyclase activity and initiates mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-Akt pathways. CB2R expression has been shown in the cerebral cortex, hippocampus, striatum, amygdala, thalamic nuclei, periaqueductal grey, cerebellum, on glial, and neurons (Fig. 6.3). Recent studies support a role

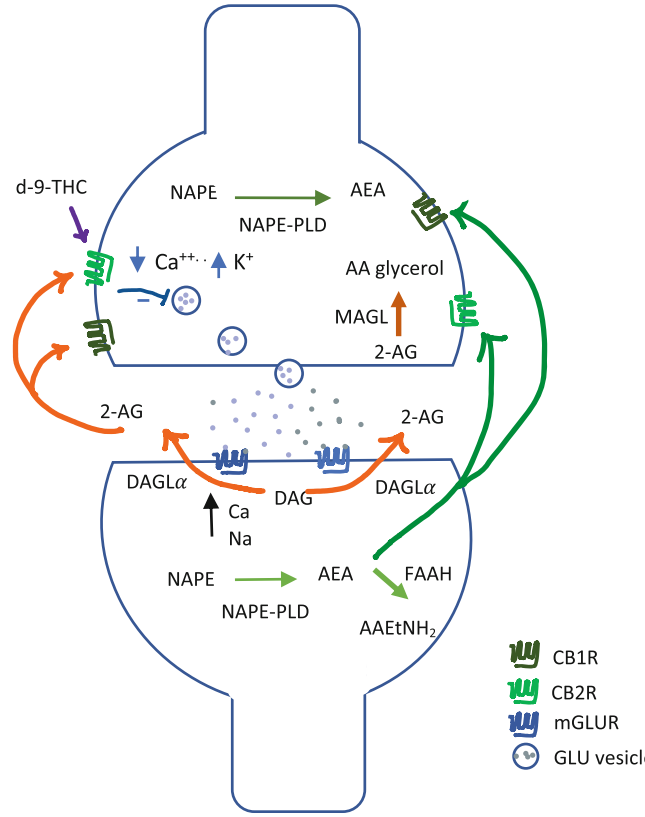


Fig. 6.4 Cannabinergic retrograde transmission in synapses. Illustration of endocannabinoids synthesis, degradation and mechanism of presynaptic inhibition

for these receptors in the CNS (Xi et al. 2011; Romano-López et al. 2012; Amancio-Belmont et al. 2017). CB2R is of considerable interest because all the psychoactive effects of THC in humans can be abolished by selective antagonism of the CB1R, implying that THC activation of CB2R does not produce psychoactive effects.

6.5 Other Receptors

In addition to CB1R and CB2R, AEA, 2-AG, NAGE, THC, and CBD may also be ligands at G protein-coupled receptor 55 (GPR55) as well as GPR18, GPR119, and other transient receptor potential ion channels TRPV (TRPV1, TRPV2, TRPA1, TRPM8) that have actions similar to capsaicin. ODA also interacts with serotoninergic 5HT2c receptors, and with the peroxisome proliferator activating receptor α (PPARα) and gap-junctions (Takao et al. 2015; Juszczaka and Swiergiela 2009).

The endocannabinoid system is widely distributed in the brain and the body and is involved in the regulation of diverse functions like food ingestion (Amancio-Belmont et al. 2017), learning and memory (Rueda-Orozco et al. 2017), immune system (Hernández-Cervantes et al. 2017), motivation, reward, addictions, and sleep.

6.6 Cannabinoids and Sleep

An extensive amount of anecdotal reports and some studies lacking methodological strictness convinced the population that marihuana affects sleep. The use of the electroencephalogram was essential to describe the effect of THC on the sleep pattern in humans. Barratt et al. (1974), reported that smoking two marijuana cigarettes

(equivalent to approximately 0.2 mg/kg THC) for 10 nights increases delta sleep (stages 3–4) during the first 4 drug nights, followed by a subsequent decrease. While Feinberg et al. (1975), found that THC significantly reduced eye movement activity during sleep with rapid eye movements (REM) and, to a lesser extent, the duration of REM itself.

After the discovery of CB1R, a useful pharmacological tool to elucidate the physiological role of CB1R was developed by Rinaldi and cols. (1994) and was referred to as SR141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehy-hidrochloride]; the first selective and orally active CB1R antagonist. It was shown that SR141716A antagonizes the inhibitory effects of CB1R agonists, on mouse vas deferens contractions and adenylyl cyclase activity in rat brain membranes. Systemic administration of SR141716A antagonizes the classical pharmacological and behavioral effects of cannabinoid receptor agonists (Rinaldi-Carmona et al. 1994). Two years later Santucci et al. (1996) described that SR141716A increases the time rats spent awake at the expense of both non-rapid eye movement sleep (NREM) and REM sleep. They suggested that an endogenous cannabimimetic system may regulate the organization of the sleep–wake cycle. Recently, similar results have been obtained with AM251 (Goonawardena et al. 2015). We do not know if SR141716A and AM251, by affecting NREM sleep expression, are preventing REM sleep occurrence. It is very unlikely that a normal subject could fall into REM sleep without a previous period of NREM sleep. Hence, if NREM sleep occurrence is reduced, the probability of having REM sleep is reduced as well.

In this context, AEA involvement in sleep modulation was investigated. Our group showed that AEA increases both NREM and REM sleep in rats, when injected into the cerebral ventricles (Murillo-Rodriguez et al. 1998) (Fig. 6.5). Moreover, we have also shown that SR141716A inhibits AEA-induced sleep in normal rats, supporting the notion that AEA's effect is mediated by CB1R (Murillo-Rodríguez et al. 2001). Further studies have shown that AEA increases the amount of adenosine in the basal forebrain (Murillo-Rodriguez et al. 2003), suggesting that adenosine may also be mediating AEA's effect, although this finding has not been replicated.

Adenosine is a byproduct of the metabolism of adenosine monophosphate, the concentration of which in the anterior hypothalamus increases in direct proportion to the amount of waking and decreases in direct proportion to the amount of NREM sleep (Porkka-Heiskanen et al. 2000). It has been suggested that the drive to sleep is partially dependent on the accumulation of sleep-inducing factors, and that adenosine seems to behave as such and the time in the circadian rhythm.

To test if endocannabinoids were one of these sleep-inducing factors, we selectively deprived rats of REMS for 24 h and studied the changes induced by SR141716A. We found that antagonizing the CB1R prevents REM sleep rebound when rats were allowed to sleep. Moreover, we found that 2 h of sleep rebound following a 24 h-period of selective REM sleep deprivation, increased CB1R expression in the pons (Navarro et al. 2003).

Likewise, studies in rats demonstrated that ODA enhances sleep, 2.8, and 5.6 μg injected intraventricularly shortens sleep latency (Basile et al. 1999). In mice, systemic administration (10 mg/kg) reduced sleep latency and increased NREM (Mendelson and Basile 1999). Later on, Huitrón-Reséndiz et al. (2001), discarded that the NREM sleep-inducing effect of ODA occurs as a consequence of affecting blood pressure, heart rate, or core temperature. At that time, the evidence was suggesting that acute administration of AEA or ODA enhances sleep through CB1R. Therefore, we decided to evaluate if the subchronic administration of AEA or ODA would still have sleep-inducing effects. Herrera-Solís et al. (2010) evaluated the effect of subchronic administration of both and found that either AEA or ODA increase REM. Additionally, this effect was prevented by AM251. It is noteworthy that, during withdrawal, rats did not express an abstinence-like syndrome, meaning changes in the sleep-waking cycle (Herrera-Solís et al. 2010).

On the other hand, knowing that PAR1 activation in rat hippocampal neurons in culture triggers synaptic retrograde 2AG signaling, thereby activating CB1R, we decided to test if S1820, a PAR1 agonist, would induce sleep when administered in the lateral hypothalamus. We found that PAR1 stimulation increases REM sleep and such effect was prevented by AM251. Besides, we observed an increase in food ingestion (Pérez-Morales et al. 2012), therefore, when tetrahydrolipstatin (THL), a DAGL inhibitor (this is the 2-AG synthesizing enzyme) was administered, the increase in both REMS and food intake was blocked. Apparently, in the lateral hypothalamus, 2-AG induces food ingestion and facilitates sleep. Further studies in our laboratory have indicated that 2AG increases the activity of Melanin Concentrating Hormone-synthesizing neurons in the hypothalamus (Pérez-Morales et al. 2013). This finding helps to understand how 2AG induces food consumption and REM sleep.

6.7 Cannabinoid System Diurnal Variations

The sleep-waking cycle is a delicate balance of two complementary processes, the homeostatic process (S) which grows as a function of waking, the longer the waking period the stronger process S becomes; hence, process S rises during waking and declines during sleep, and the circadian process (C) that depends on the hour of the day (Borbély 1982). Consequently, sleep occurs with circadian (*circa* = about, and *diem* = day) periodicity. Extensive literature supports the notion that the circadian master clock is the suprachiasmatic nucleus (SCN). In humans, the presence of light synchronizes SCN activity, signaling the appropriate moment for being awake, while darkness, reducing the SCN activity, facilitate sleep. It has been hypothesized that the endocannabinoid system, regulated somehow by the SCN, serves as the link between the SCN and those behavioral and physiological processes that undergo circadian variations, such as the sleep-waking cycle.

For example, the number of endocannabinoids in the brain, their breakdown, and synthesizing enzymes and their receptors, all show diurnal variations, indicating that the endocannabinoid system is under circadian regulation. Likewise, selective REM sleep deprivation causes a reduction of CB1R expression, while a 2 h recovery period causes an elevation. These findings suggest the involvement of the endocannabinoid system in the homeostatic maintenance and recovery of sleep (Martínez-Vargas et al. 2003; Vaughn et al. 2010). Circadian fluctuations of AEA and 2AG content in the cerebrospinal fluid (CSF) have been documented. Also, both endocannabinoids have diurnal variations in the hippocampus, pons, hypothalamus, prefrontal cortex, striatum, and nucleus accumbens, albeit these variations are dependent on the brain structure. For example, AEA exhibits a high release during darkness in these structures, while an opposite pattern is seen in the hypothalamus, where AEA concentrations are higher during the light phase (Murillo-Rodriguez et al. 2006). These studies are suggesting that AEA-mediated signaling varies throughout the day revealing its dependence on the circadian system.

Interestingly, contents of 2AG behave in the opposite fashion of the AEA contents, in brain samples where both were measured, i.e. 2AG was higher during the light phase in the nucleus accumbens, prefrontal cortex, striatum and hippocampus than AEA. In turn, in the striatum, both MGL and DGL activity is higher during the light phase than during the dark phase. In particular, DGL increased activity is consistent with a higher turn-over of 2AG during the light phase in the striatum. In contrast, no changes in MGL or DGL were seen in the hippocampus (Valenti et al. 2004), suggesting the existence of divergent 2AG regulatory mechanisms for the striatum and hippocampus. In humans, 2AG diurnal variations have also been described, the nadir occurring at night, around 2 am, while the acrophase occurring during the day, around 1 pm (Hanlon et al. 2015), which is very similar to the fluctuation described in rats (Valenti et al. 2004). These findings suggest that 2AG may not be involved in promoting sleep but, rather, wakefulness.

Sanford et al. (2008), showed that the SCN of hamsters expresses CB1R, and systemic administration of CP55940 (a CB1R agonist) interfered with the generation of the light-induced phase advance in these animals, while AM251 prevented this effect. Regarding CB1R and CB2R expression, evaluated in the pons of rats throughout the light-dark cycle, results revealed that CB1R expression is higher at 1 pm and lower at 1 am. CB2R does not seem to undergo diurnal fluctuations (Martínez-Vargas et al. 2003). All these findings support the notion that there is a circadian modulation of the components of the endocannabinoid system, i.e. synthesis and breakdown. Hence, all the contribution that the endocannabinoid system is making to regulate sleep expression is also a result of such circadian influence.

6.8 Cannabinoids and Insomnia

Insomnia is one of the most frequent sleep disorders in the general population and represents a frequent reason for visiting the physician's office. The patient complains about his difficulty falling sleep or to maintain its continuity throughout the night. This disorder has a great impact on the subjects' waking state, workability, and quality of life.

Considering that the endocannabinoid system is one of the mechanisms modulating the sleep-wake cycle, as we have tried to explain in this review, its pharmacological modulation could have a very valuable utility for the treatment of patients suffering insomnia or diurnal hypersomnia. In this context, pre-clinical studies have indicated that adult rats subjected to maternal care deprivation (MCD) from postnatal day (PND)2 to PND14 exhibit depression-like (the forced swimming test) and anxiety-like (open arms test) behaviors when they become adults (Lambás-Señas et al. 2009). Moreover, Hofer (1976) had previously shown that rats subjected to MCD for 24 h exhibited a reduction in total sleep time.

Using this knowledge, we analyzed the proficiency of endocannabinoids to restore sleep. Rats submitted to MCD for a daily 3 h-period from PND2-PND16 and were implanted for standard sleep recordings on PND90, to evaluate their spontaneous sleep-wake cycle and ODA effects. As we expected MCD produced a reduction in total NREM and REM sleep (Reyes Prieto et al. 2012). ODA restored NREM and REM sleep in the rats while AM251 increased wakefulness and prevented the effect of ODA. Additional results indicated that there was decreased CB1R expression in the prefrontal cortex and the hippocampus of MCD rats (Fig. 6.4b) (Reyes Prieto et al. 2012). Using the same protocol, 2AG administered into the lateral hypothalamus of MCD rats restores sleep (Pérez-Morales et al. 2014). As we have abovementioned, we previously observed that 2AG into the lateral hypothalamus increased REMS through CB1R activation and by increasing MCH neuron activity as inferred by c-FOS detection in these neurons. In short, we propose that ODA and 2AG restore sleep in insomniac rats, through CB1R (Fig. 6.6).

In humans, there is a high degree of sleep and mood disorder (depression and anxiety) comorbidity (Atalay 2011; Augner 2011). The role of the endocannabinoid system in the modulation of anxiety and depression has also been suggested (Ashton and Moore 2011). Considering that basal ganglia are important in the regulation of the sleep/wake cycle (Lazarus et al. 2012) and emotions (Brown et al. 2012), we explored the effect of AEA and AM251 administered into the entopeduncular nucleus on the sleep-wake cycle, depression and anxiety in rats. AEA increases NREMs expression, while AM251 increases W and decreases both NREM and REM. Besides, AM251 decreases the time rats spend in the open arms of the elevated plus-maze and increases immobility time in the FST. It seems that activation of the CB1R in the EP is important to induce sleep, while its blockade promotes W, as well as anxiety and depression, resembling insomnia in humans (Méndez-Díaz et al. 2013). These results suggest that EP CB1R is modulating sleep and mood (Fig. 6.7).

6.9 Therapeutic Potential

It has been proposed that marihuana would have an effect on sleep and thus could be an effective treatment for patients with insomnia and other sleep disorders. As mentioned above, research exploring the impact of cannabis on sleep started in the 1970s. Results, although controversial, described a decrease in sleep onset latency (Cousens and DiMascio 1973) but caused waking after sleep onset (Pivik et al. 1972). Some other studies did not replicate these findings (Feinberg et al. 1975), while others described an increase in slow wave sleep (Barratt et al. 1974) and a decrease in the REM (Feinberg et al. 1976). Besides, it was reported that chronic use of cannabis was associated with a process of habituation to sleep induction, hence, in the long run, failing to keep inducing it (Barratt et al. 1974). Thus far, the evidence suggested that long-term marijuana use could harm sleep. Not only that, but once individuals got habituated to this effect, they would perhaps use more marijuana in an attempt to obtain the sleep-inducing effect; therefore, escalating its use, and thereby increasing the risk of becoming addicted to it. In this context, marijuana does not seem to be the treatment of choice for insomnia. Clinical research has shown that the type of cannabinoid, i.e. THC, CBD, their concentration ratio in the plant, dosage, route, and even time of administration is critical for the potential effects. In individuals with insomnia, pioneer studies indicated that CBD (160 mg/day) increases total sleep time and decreases the frequency of arousals during the night (Carlini and Cunha 1981). Interestingly, low-dose CBD increases wakefulness (Nicholson et al. 2004; Zuardi 2008). According to other studies (Gorelick et al. 2013), THC decreases sleep latency albeit tolerance to this effect is developed.

Although there is a great percentage of insomniac patients who suffer from an idiopathic form, there are also a great number of patients whose insomnia is secondary to another medical illness. Chronic pain, for example, is one debilitating condition that perturbs sleep. Fibromyalgia, neuropathic pain caused by diabetes complications, or cancer-related pain are among many other pain-causing diseases. Therefore, the use of cannabinoids to treat pain becomes crucial to mitigate not only the suffering caused by pain but to also improve the quality of sleep. Nabilone, a synthetic cannabinoid has been used to treat fibromyalgia and chronic cancer and non-cancer-related pain, but results are not conclusive. Some patients may respond to nabilone while others do not.

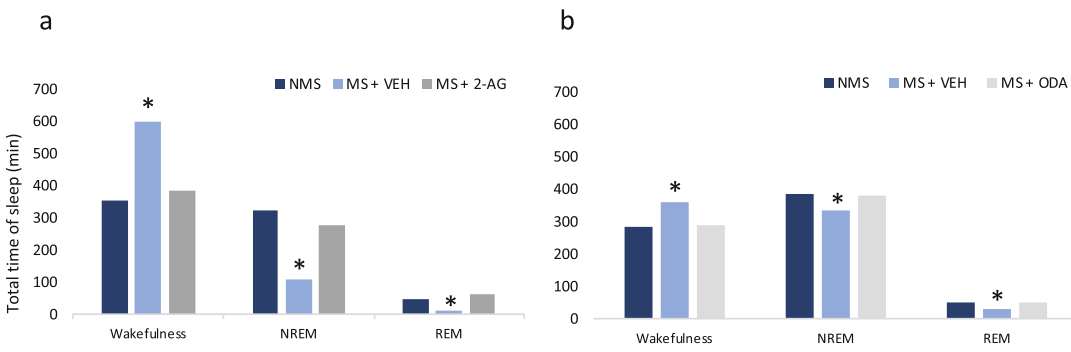
6.10 Discussion and Conclusions

Based on the evidence herein discussed, we conclude that the endocannabinoid system is one of the systems regulating the sleep-wake cycle. Cannabinoids not only participate in sleep modulation but in the regulation of other physiological processes, such as pain sensitivity. Therefore, cannabinoids seem to have some potential to treat patients suffering from insomnia, secondary to chronic pain. Marijuana synthesizes around 400 compounds, including 60–70 cannabinoids. Thus far, the effect induced by each compound

% \ TX	CTL	AEA	SR	AEA + SR
Wakefulnes	46	30 *	47	47 **
NREM	49	59 *	49	47
REM	6	11 *	4	6 **

% of total time of 4 h of sleep recording compare to control group. * $p < 0.05$ vs. CTL, ** $p < 0.05$ *vs.* AEA. Data taken from Murillo-Rodriguez et al., 2001

Fig. 6.5 Effect of anandamide (AEA) and SR (Rimonabant) on sleep-wake cycle



% of total time compare to control group. Data taken from Perez-Morales et al., 2012 and Reyes Prieto et al., 2012.
a) 2-AG (0.001μg) intra lateral hypothalamus. * < p 0.05 *vs.* VEH
b) ODA (1 mg/Kg). * p < *vs.* VEH
NMS no maternal separation, MS maternal separation, VEH vehicle.

Fig. 6.6 2-Arachidonoylglycerol (2-AG) and Oleamide (ODA) sleep induction in *insomniac* rats

	AEA	AM251
Wakefulnes	↓	↑
NREM	↑	↓
REM	↑	↓
Anxiety (time in open arms)	↓	↑
Depresion (immobility time in FST)	↓	↓

All diferences were significatives compare to control group. AEA (5μg) or AM251 (1μg) administered intra entopeduncular nucleus. Modified from Méndez-Díaz et al., 2013.

Fig. 6.7 Entopeduncular endocannabinergic modulation of sleep-wake cycle, anxiety and depression in rats

has not been established. Therefore, we have ahead of us, a great deal of questions to answer before we can say with confidence, that cannabinoids are safe and useful to treat sleep disorders. However, in the best scenario, we are aiming to use compounds isolated from the plant instead of using the plant itself.

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Effects of Cannabinoid Agonists and Antagonists on Sleep in Laboratory Animals

7

Maureen L. Petrunich-Rutherford and Michael W. Calik

Abstract

The cannabinoids are a family of chemical compounds that can be either synthesized or naturally derived. These compounds have been shown to modulate a wide variety of biological processes. In this chapter, the studies detailing the effects of cannabinoids on sleep in laboratory animals are reviewed. Both exogenous and endogenous cannabinoids generally appear to decrease wakefulness and alter rapid eye movement (REM) and non-REM sleep in animal models. In addition, cannabinoids potentiate the effects of sedative-hypnotic drugs. However, the individual contributions of each cannabinoid on sleep processes is more nuanced and may depend on the site of action in the central nervous system. Many studies investigating the mechanism of cannabinoid effects on sleep suggest that the effects of cannabinoids on sleep are mediated via cannabinoid receptors; however, some evidence suggests that some sleep effects may be elicited via non-cannabinoid receptor-dependent mechanisms. More research is necessary to fully elucidate the role of each compound in modulating sleep processes.

Keywords

Laboratory animals · In vivo · Cannabinoids · Cannabinoid receptors · Sleep

7.1 Introduction

The term "cannabinoids" refers to endogenously-produced, plant-derived, or synthetically-produced oxygen-containing C21 aromatic hydrocarbon compounds. The stereotypical and most widely known cannabinoid is Δ9-tetrahydrocannabinol (Δ9-THC), which is the major psychoactive constituent in the plant *Cannbis sativa* (also referred as cannabis, marijuana, etc.). Due to the high lipid solubility and low water solubility of cannabinoids, it was long believed that the pharmacological actions of cannabinoids were due to the disruption of phospholipids in the cell membrane (Pertwee 2005) Though there were hints that cannabinoids might bind to a receptor (Edery et al. 1971), it was not until the early 1990s that that two cannabinoid receptors were cloned, CB_1 (Matsuda et al. 1990) and CB_2 (Munro et al. 1993). Though other putative cannabinoid receptors have been described (Laprairie et al. 2017), the pharmacological

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actions of cannabinoids occur primarily at the CB_1 and CB_2 receptors, which are inhibitory G protein-coupled receptors (Howlett 2002).

Cannabinoids can be divided into two groups. The endogenous cannabinoids, referred to as endocannabinoids, comprise a class of lipophilic compounds based on the general structure of modified arachidonic acid (AA) derivatives (e.g. anandamide, 2-arachidonoylglycerol, etc.) that are naturally produced by cells, while exogenous cannabinoids represent the plant-derived or synthetically-produced compounds that can be either ingested, injected, or inhaled (Childers 2006; Martin et al. 2018). Both endogenous and exogenous cannabinoids bind to either CB_1, CB_2 or both CB_1/CB_2 receptors located on many tissues with varying degrees of affinity (Pertwee 2005). Moreover, both endogenous and exogenous cannabinoids can allosterically modulate other receptors, channels, and enzymes (Pertwee 2005; Hourani and Alexander 2018).

The effects of cannabinoids in health and disease are widely known (Lu and Anderson 2017). Here, we review the effects of cannabinoids on sleep in laboratory animals (Table 7.1).

7.2 Before the Discovery of Cannabinoid Receptors

7.2.1 Exogenous Cannabinoids

The very first studies of sleep and cannabinoids in laboratory animals involved the "classical" exogenous cannabinoids (e.g. Δ9-THC, Δ8-THC, cannabidiol, cannabinol, etc). This group consists of cannabinoids that are either cannabis-derived compounds (phytocannabinoids) or their synthetic analogues (Pertwee 2005). At a minimum, it was known that these classic cannabinoids caused a sedative, depressive, or cataleptic state in various laboratory animals, including nonhuman primates, dogs, cats, rats, mice, rabbits, and gerbils, though specific and detailed changes in sleep were not studied (Sassenrath and Chapman 1975; Grunfeld and Edery 1969; Edery et al. 1971; Scheckel et al. 1968; Carlini et al. 1970; Lipparini et al. 1969; Mechoulam and Gaoni 1967). After these preliminary experiments, researchers further examined the effects of cannabinoids on sleep.

In cats administered an oral form of a marijuana distillate daily for 180 days, a decrease in slow-wave sleep (SWS) and an increase in "drowsy-light" sleep occurred at the 20th day of drug administration. Moreover, these changes in sleep persisted 40 days after cessation of the drug. There were also changes in time spent awake and in rapid eye movement (REM) sleep on isolated days, but no significant and consistent changes were observed throughout the drug trial (Barratt and Adams 1973). Similar findings were found in squirrel monkeys, where orally administered Δ9-THC decreased SWS and increased drowsy sleep and awake time (Adams and Barratt 1975). Rabbits administered intravenous (IV) Δ9-THC had a decreased number of REM bouts on the first day that returned to normal on the third day post-IV injection (Fujimori and Himwich 1973). In a similar study, cats administered Δ8-THC IV or intraperitoneal (IP) had fewer but longer REM sleep bouts (Wallach and Gershon 1973). These early studies provided evidence that cannabinoids modulate sleeping patterns in laboratory animals.

Most of the later studies with cannabinoids have been completed in rat and mouse models. Many of these studies investigated the potentiation of barbiturate or sedative-hypnotic drug-induced sleeping time. The use of "sleeping time" produced by a subsequent IP injection of a barbiturate/sedative-hypnotic was a measure of central nervous system (CNS) activity produced by either: the stimulant or depressive effects on the CNS of the co-administered drug, the co-administered drug increasing or decreasing penetration of the barbiturate into the CNS, or the modulation of metabolism of the barbiturate derived from the co-administered drug (Stevenson and Turnbull 1974). Since the cannabinoid receptors were not yet known, it was thought that cannabinoids modulated sleeping time though modifying metabolism or penetration of the barbiturates. Various THC isomers and their derivatives/metabolites, either inhaled or injected IP, increased barbiturate-induced sleeping time (Stevenson and Turnbull 1974; Berger

Table 7.1 Summary of cannabinoids and their effect on sleep

	Wakefulness	Total sleep	NREM/SW sleep	REM sleep	Drug-induced sleeping time
Exogenous agonists					
Δ9-THC	NC	Increased/ NC	Decreased/NC	Decreased	Increased
Δ8-THC	–	Increased/ NC	Decreased/NC	Decreased	Increased
Marijuana distillate	–	–	Decreased	Decreased	Increased
CBN	–	Increased	–	Decreased	Increased/NC
CBD	Increased	–	Decreased	Decreased	Increased
Cannabichromene	–	–	NC	NC	Increased
Cannabigerol	–	Increased	–	NC	–
CP47,497	–	–	Decreased/ increased	NC	–
WIN55,212	Decreased	Increased	Increased	Decreased	–
ACEA	–	Increased	–	–	–
HU-210	–	Increased	–	–	–
HU-310	–	Increased	–	–	–
PhAR-DBH-Me	NC	–	NC	Decreased	–
Endocannabinoids					
2-AG					
Infused	NC	–	NC	Increased	–
Synthesis inhibited	Increased	–	Decreased	NC	–
Anandamide					
Infused	Decreased	–	Increased	Increased	Increased
Infused Precursor: AA	Increased	–	Decreased	NC	–
Reuptake inhibitor: VDM-11	Decreased	–	Increased	Increased	–
Reuptake inhibitor: OMDM-2	Decreased	–	Increased	Increased	–
Oleamide	Decreased	Increased	Increased	Increased/ decreased	Increased
FAAH inhibitors					
URB597	Increased	–	Decreased	–	–
AM3506	–	–	Increased	Decreased	–
AA-5-HT	Decreased	–	Increased	Increased	–
FAAH knockout	Decreased	–	Increased	–	–
CB receptor knockout	Increased	–	Decreased	Decreased	–
Antagonists/inverse agonists					
Compound 64	Increased	–	Decreased	Decreased	–
SR141716	Increased	–	Decreased	Decreased	–
AM251	Increased/ NC	NC/ Decreased	Increased/NC	Decreased	–
AM281	–	–	NC	–	–
ABD459	NC	NC	NC	Decreased	–
AM630	NC	NC	NC	NC	–

The effects of compounds listed above on wakefulness, total sleep, NREM/slow-wave (SW) sleep, REM sleep, and on drug-induced sleeping time. These compounds either increased, decreased, or had no change (NC)
Δ8-THC Δ8-tetrahydrocannabinol, *Δ9-THC* Δ9-tetrahydrocannabinol, *2-AG* 2-arachidonoylglycerol, *AA* arachidonic acid, *AA-5-HT* N-arachidonoyl-serotonin, *ACEA* arachidonyl-2-chloroethylamide, *CB* cannabinoid, *CBD* Cannabidiol, *CBN* cannabinol, *FAAH* fatty acid amide hydrolase

and Krantz 1972; Bhattacharyya et al. 1980; Bose et al. 1963; Lazaratou et al. 1980; Hatoum et al. 1981; Kaneto and Nagaoka 1981; Katsunori et al. 1993; Martin et al. 1975; Oishi et al. 1988; Sofia and Knobloch 1973, 1974; Sofia 1977; Rating et al. 1972; Segelman et al. 1974; Siemens et al. 1974; Stone et al. 1976; Paton and Pertwee 1972; Watanabe et al. 1980, 1982, 1987, 1990; Kubena and Barry 1970; Giusti et al. 1980; Chiarotti et al. 1980; Yoshimura et al. 1978; Siemens and Kalant 1974; Sofia and Barry 1983; Fujimoto 1972; Narimatsu et al. 1983, 1984, 1985). Similarly, Δ9-THC also increased alcohol-induced sleeping time (Friedman and Gershon 1974). Dogs trained to inhale marijuana smoke also showed an increase in barbiturate-induced sleeping time (Sullivan and Willard 1978). Cannabidiol (CBD) and its derivatives and/or metabolites have been shown in multiple studies to increase barbiturate sleeping time, in part, by modifying liver metabolism of the barbiturate (Stone et al. 1976; Bornheim et al. 1981; Borys et al. 1979; Carlini et al. 1975; Karler et al. 1979; Leite et al. 1982; Yamamoto et al. 1988, 1991). Another cannabinoid, cannabichromene, also increased barbiturate sleeping time (Hatoum et al. 1981). For cannabinol (CBN), there was either no increase or slight increase in barbiturate sleeping time (Fernandes et al. 1974a; Chesher et al. 1974). In combination studies, CBD with Δ9-THC increased barbiturate sleeping time compared to Δ9-THC alone, while CBN with Δ9-THC decreased the sleeping time (Fernandes et al. 1974a, b; Chesher et al. 1974; Krantz et al. 1971; Takahashi and Karniol 1975; Karniol and Carlini 1973). Though the original hypothesis of cannabinoids increasing barbiturate-/sedative-hypnotic-induced sleeping time by decreasing metabolism of the barbiturates/sedative-hypnotics was incorrect, it is now known that the increased sleeping time is due to the increased depressant effect of barbiturates and cannabinoids via their respective receptors (Szabo and Schlicker 2005; Jembrek and Vlainic 2015).

More detailed studies on the effect of cannabinoids on sleep have been completed. Δ9-THC, CBN and cannabigerol injected IP increased total sleep and REM sleep onset in rats; however, Δ9-THC and CBN only decreased time spent in REM (Colasanti et al. 1984a, b). In another study that used single IP injection of Δ9-THC, Δ8-THC, or marjuana distillate, all three compounds reduced SWS and REM sleep, and no REM sleep rebound was observed 5 days post injection. That same study also investigated chronic use (i.e. 20 days) of Δ9-THC, Δ8-THC, or marijuana distillate, and found that REM sleep returned to normal on the fourth day, thus the rodents developing tolerance to the cannabinoids (Moreton and Davis 1973). Δ9-THC injected IP was also associated with EEG changes during SWS and REM sleep (Buonamici et al. 1982). Another cannabinoid, cannabichromene, had no effect (Colasanti et al. 1984a). CBD, after single doses, decreased sleep-wave sleep latency at 20 mg/kg, while at 40 mg/kg, increased SWS time. Following chronic injections of CBD, tolerance developed (Monti 1977). These earlier studies, once again, showed that cannabinoids modulate sleep.

7.3 After the Discovery of Cannabinoid Receptors

7.3.1 Exogenous Cannabinoid Agonists

After the cloning of the cannabinoid receptors in the early 1990s, investigations of cannabinoid agonists and antagonists centered around the role of these receptors in the CNS in the various stages of sleep. In addition, the specificity of these receptors in central sleep-wake centers have also been examined.

In congruence with earlier studies, systemic administration of CBD modulates sleep, as high doses of CBD injected IP increases the percentage of sleep time but increases the latency to REM (Chagas et al. 2013). CBD and some halogenated derivatives of this molecule potentiated the effects of barbiturates on sleep time in mice when injected IV (Usami et al. 1999). However, CBD generally appears to increase wakefulness when injected centrally. In rats, intracerebroventricular (ICV) injection of CBD increased

wakefulness and decreased REM compared to sham or vehicle-injected groups (Murillo-Rodríguez et al. 2006). CBD also increased wakefulness and decreased SWS and REM when injected into the lateral hypothalamus (Murillo-Rodríguez et al. 2008a, 2011) or dorsal raphe (Murillo-Rodríguez et al. 2008a) of rats. These findings are supported by sleep quality studies that demonstrate that injection of CBD into the lateral hypothalamus or dorsal raphe nuclei increased alpha power, yet decreased delta and theta power (Murillo-Rodríguez et al. 2008a). In addition, CBD dose-dependently prevented sleep rebound in sleep-deprived rats (Murillo-Rodríguez et al. 2011).

CBD may increase wakefulness by increasing the activation of wake centers in the hypothalamus or dorsal raphe, as CBD administered ICV increased c-Fos expression in these regions (Murillo-Rodríguez et al. 2006, 2008a). CBD may also work, at least in part, to enhance monoamine transmission, as CBD administration increased extracellular levels of norepinephrine, epinephrine, dopamine, and serotonin in the nucleus accumbens (Murillo-Rodríguez et al. 2006, 2011). CBD injected into the lateral hypothalamus also increased adenosine levels in the nucleus accumbens a few hours post-injection (Mijangos-Moreno et al. 2014). Thus, site of CBD administration must be taken into consideration when interpreting the findings of these studies.

CBD may also work indirectly to reverse stress-induced alterations in sleep by modulating anxiety rather than influencing sleep. Rats repeatedly exposed to an open field increases anxiety-like behavior and decreases REM sleep; injection of CBD into the central amygdala decreases open field anxiety-like behavior and decreased stress-induced REM suppression (Hsiao et al. 2012).

No recent studies have focused on the specific, direct effects of CBN on sleep architecture, although CBN and some halogenated derivatives of CBN potentiated the effects of barbiturates on sleep time (Yoshida et al. 1995).

Δ9-THC appears to modulate REM sleep, as IP injection decreased REM (Calik and Carley 2017; Carley et al. 2002). However, this effect does not appear to be mediated by either CB_1 or CB_2 receptors, as the effects of Δ9-THC on REM sleep was not blocked by either AM251 or AM630, CB_1 and CB_2 receptor antagonists, respectively (Calik and Carley 2017). Δ9-THC may also play a more general role in sedation, as Δ9-THC and some halogenated derivatives of Δ9-THC potentiated the effects of barbiturates on sleep time (Usami et al. 1998).

CP47,497, a potent cannabinoid CB_1 receptor agonist, had a circadian-dependent effect on sleep in mice. Activation of CB_1 receptors with CP47,497 induced more non-rapid eye movement (NREM) sleep and increased NREM bout duration during the dark phase but reduced NREM sleep and decreased NREM bout duration during the light phase. These effects were abolished by CB_1 antagonism with AM281 (Pava et al. 2016). WIN55,212, another potent cannabinoid CB_1 receptor agonist, increased total sleep time, increased NREM sleep, and reduced wakefulness and REM sleep in mice. WIN55,212 also decreased latency to NREM sleep and increased NREM sleep bout duration, while having the opposite effects on REM sleep latency and duration. WIN 55,212 caused a global suppression of normalized spectral power (Goonawardena et al. 2015). Other cannabinoid agonists, arachidonyl-2-chloroethylamide (ACEA), HU-210 (R(−)-7-hydroxy-delta-6-tetrahydrocannabinol-dimethylheptyl), and HU-310 (2-O-arachidonoylglycerylether) increased sleep duration in mice, which was partially mediated by CB_1 receptors (Schuster et al. 2002).

Some newer cannabinoid receptor-targeting drugs also appear to modulate sleep. IP injection of PhAR-DBH-Me ((R,Z)-18-((1S,4S)-5-methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)-18-oxooctadec-9-en-7-yl phenylacetate PhAR-DBH-Me), a putative CB_1 receptor agonist, increased REM sleep in rats. This effect was blocked by AM251, indicating that CB_1 may be mediating

the effects of PhAR-DBH-Me on REM sleep (López et al. 2010).

7.3.2 Endogenous Cannabinoid Agonists

The endogenous cannabinoids, or endocannabinoids, were discovered shortly after the cloning of the cannabinoid receptors (Mechoulam et al. 2014). Since then, numerous studies have investigated the involvement of endocannabinoids on sleep.

Injection of 2-arachidonoylglycerol (2-AG) into the lateral hypothalamus increased REM, but had no effect on waking or SWS. The effects of 2-AG appear to be mediated by CB_1 receptors, as increased REM sleep induced by 2-AG is blocked by AM251 (Pérez-Morales et al. 2013). Diacylglycerol lipase (DAGL) is an enzyme involved with the synthesis of 2-AG. Inhibition of DAGL by a compound injected into the lateral hypothalamus decreased SWS duration, increased wakefulness, and increased latency to REM (Pérez-Morales et al. 2014a). These findings support the role of 2-AG in serving as a sleep-enhancing molecule. 2-AG has also been shown to be present in the laterodorsal tegmentum, a brainstem area involved with arousal and sleep (Soni et al. 2017). Direct injection of 2-AG into the lateral hypothalamus increased c-fos expression in melanin-concentrating neurons of the hypothalamus, an area that increases firing during REM (Pérez-Morales et al. 2013).

Infusions of 2-AG have also been shown to reverse the effects of early life separation on sleep states. Early life maternal separation increased wakefulness, and decreased NREM and REM sleep in adulthood in male rats. 2-AG injection into the lateral hypothalamus of rats subjected to maternal separation decreased wakefulness, and increased NREM and REM. These effects are likely mediated by CB_1 receptors, as the effects of 2-AG in maternally separated rats are blocked by administration of AM251 (Pérez-Morales et al. 2014b). It is unknown, in this early life stress paradigm, whether 2-AG enhances sleep directly or indirectly by altering anxiety-inducing processes, as has been proposed for CBD (Hsiao et al. 2012).

Anandamide (N-arachidonoylethanolamide) is an endocannabinoid that enhances the effects of sleep. Anandamide is present in the laterodorsal tegmentum, a brainstem area involved with arousal and sleep (Soni et al. 2017). Anandamide administered to the cerebral ventricles in male rats decrease waking, increases SWS, and increases REM (Murillo-Rodríguez et al. 1998, 2001). Systemic injection of anandamide also decreases waking and increases SWS (Murillo-Rodriguez et al. 2003) and prolonged pentobarbital-induced sleep time (Watanabe et al. 1999). Some of the effects of anandamide on sleep stages appear to be mediated by CB_1 receptors, as the effects on waking, SWS, and REM are blocked by administration of the CB_1 receptor antagonist SR141716 (Murillo-Rodríguez et al. 2001, 2003).

Anandamide may be working to modulating sleep by specifically targeting various regions involved in sleep. Intra-hippocampal injection of anandamide in rats increases REM but does not alter wake time or SWS. This effect was blocked by AM251. The sleep-inducing effect may be somewhat specific to the hippocampus, as the effects of anandamide on sleep was not observed when anandamide was injected into the cortex (Rueda-Orozco et al. 2010). Other regions may also be mediating the effects of anandamide. Anandamide injected into the entopeduncular nucleus increases NREM and REM (Méndez-Díaz et al. 2013). The effects of anandamide may partially mediated by the pedunculopontine tegmental nucleus, but not the hypothalamic medial preoptic area (Murillo-Rodríguez et al. 2001).

Altering the processes of endogenous anandamide regulation supports the role of anandamide in modulating sleep, although the results are less straightforward. AA, a precursor for anandamide, administered ICV, increases waking and decreases SWS with no change in REM sleep (Murillo-Rodríguez et al. 1998). VDM-11, an inhibitor of facilitated membrane transport of anandamide, decreases wake time, and increases SWS and REM time. However, the effects of VDM-11 on the length of these parameters were

not completely blocked by SR141716, indicating that processes involved with enhancing sleep quantity may not be entirely mediated by CB_1 receptors. On the other hand, CB_1 receptors may be more involved with regulating sleep quality. VDM-11, presumably by increasing anandamide content, increased delta and theta power. When VDM-11 was combined with SR141716, delta and theta power was partially decreased relative to VDM-11 alone (Murillo-Rodríguez et al. 2008b). Additionally, VDM-11 enhanced c-Fos expression in the anterior hypothalamic area, paraventricular thalamic nucleus, and pedunculopontine tegmental nucleus, all brain areas involved in sleep regulation, and reduced the extracellular levels of dopamine collected from nucleus accumbens (Murillo-Rodríguez et al. 2008b, 2012). OMDM-2, another anandamide reuptake inhibitor, also decreased wakefulness and increased NREM and REM sleep, and was also associated with reduced extracellular dopamine levels (Murillo-Rodríguez et al. 2012).

There are several proposed mechanisms for the effects of anandamide on modulating sleep. The effects of anandamide may involve the activity of phospholipase C (Murillo-Rodríguez et al. 2001). Anandamide administration was associated with an accumulation of adenosine in the lateral preoptic area, which may inhibit cholinergic wake-active neurons (Murillo-Rodriguez et al. 2003).

Oleamide (cis-9,10-octadecenamide, also known as cerebrodiene) was first isolated and identified in the cerebrospinal fluid (CSF) of sleep-deprived cats (Lerner et al. 1994). Sleep deprivation did not increase oleamide in plasma, suggesting that deprivation-induced increases in oleamide are specific to the CNS (Basile et al. 1999). Fatty acid amide hydrolase (FAAH), the enzyme associated with the catabolism of oleamide and anandamide, was identified in rat choroid plexus, which may regulate oleamide content in the CSF (Egertová et al. 2000).

Oleamide appears to have a hypnotic effect when administered exogenously and can also dose-dependently potentiate barbiturate-induced sleeping time and decrease sleep latency induced by a subthreshold dose of barbiturate (Yang et al. 1999). When oleamide was administered either centrally or systemically, sleep in rodents increased (Cravatt et al. 1995). Administration of oleamide decreased sleep latency, an effect that was blocked by the antagonism of CB_1 receptors with SR141716 (Mendelson and Basile 1999). Oleamide administered IP decreased wake time and sleep latency, increased NREM and total sleep, and decreased REM in rodents (Laposky et al. 2001; Yang et al. 2003; Huitrón-Reséndiz et al. 2001). However, the effects of oleamide appear to be dependent on the dose, as low doses decreased wake and increased REM, and high doses increased NREM and REM (Carley et al. 2002). Thus, more work is needed to clarify this potential biphasic effect of oleamide on sleep regulation.

Maternal separation (MS) in the early life period increases waking, decreases NREM and REM sleep during adulthood in male rats. Oleamide restored the parameters of MS rats to the same levels observed in non-separated siblings (NMS), but did not alter sleep parameters in NMS rats. The effects of oleamide were not blocked by AM251 in MS rats (Reyes Prieto et al. 2012), indicating a CB_1-receptor independent mechanism.

Further support for endogenous cannabinoid regulation of sleep comes from studies manipulating the enzymatic regulation of endocannabinoids. The FAAH inhibitor, URB597, when injected ICV in male rats, dose-dependently increased wake time, decreased SWS, but had no effect on REM. URB597 also increased c-Fos in the hypothalamus and dorsal raphe. URB597 increased dopamine content and decreased L-DOPA in the nucleus accumbens (Murillo-Rodríguez et al. 2007, 2016). URB597 blocked sleep rebound in sleep-deprived rats (Murillo-Rodríguez et al. 2016). However, systemic administration of URB597 had no effect on sleep (Pava et al. 2016). When a longer lasting FAAH inhibitor, AM3506, was used, NREM sleep increased and REM sleep decreased (Pava et al. 2016). N-arachidonoyl-serotonin (AA-5-HT), another inhibitor of FAAH, dose-dependently decreased waking, increased SWS, and increased REM during the dark phase. These

effects were associated with decreased alpha EEG power spectra, and increased delta and theta power spectra. Administration of AA-5-HT increased adenosine, but decreased dopamine, norepinephrine, epinephrine, and serotonin in the nucleus accumbens. AA-5-HT blocked the effects of CBD and modafinil, a putative dopamine transporter inhibitor, on sleep parameters, EEG power spectra, and extracellular levels of other sleep-modulating neurotransmitters during the lights-on period. CBD and modafinil prevented sleep rebound induced by sleep deprivation, but AA-5-HT blocked these effects (Murillo-Rodríguez et al. 2017). In studies using mice knocked out for FAAH enzyme, FAAH(−/−) mice had decreased brief awakenings, decreased wake time, and increased duration of SWS bouts during the light period compared to wild-type littermates. FAAH(−/−) mice also had decreased EEG power density during wake and REM, while EEG power density was increased during SWS. There was no genotype-specific effects observed in recovery from sleep deprivation (Huitron-Resendiz et al. 2004).

7.4 Antagonists/Inverse Agonists of Cannabinoid Receptors

Numerous antagonists have been investigated to clarify the role of cannabinoid receptors in sleep-associated processes. Compound 64, a potent and selective CB_1 receptor inverse agonist, decreased REM and NREM sleep in rats while increasing wakefulness (Jacobson et al. 2011). SR141716A, a CB_1 receptor antagonist/inverse agonist, increased wakefulness at the expense of SWS and REM sleep, delayed the occurrence of REM sleep, and decreased EEG spectral power during SWS, in part, by increasing adenosine (Murillo-Rodriguez et al. 2003; Jacobson et al. 2011; Santucci et al. 1996). However, other studies using SR141716A showed no effect on sleep parameters (Mendelson and Basile 1999; Navarro et al. 2003). In another study, SR141716 blocked sleep rebound after sleep deprivation by increasing dopamine, norepinephrine, epinephrine, serotonin, and adenosine levels in the brain (Murillo-Rodríguez et al. 2016). AM281, another CB_1 antagonist/inverse agonist, caused fragmented NREM sleep, depending on the time of day AM281 was administered. AM281 also produced broadband changes in EEG power spectral features, and did not reduce NREM sleep rebound caused by sleep deprivation (Pava et al. 2016). A more frequently studied CB_1 receptor antagonist/inverse agonist is AM251, with conflicting reports on its effects on sleep. In regards to total sleep time, AM251 has been shown in two reports to have no change on total sleep time (Calik and Carley 2017; Schuster et al. 2002), while one report showed a decrease in sleep time (Goonawardena et al. 2015). Consistently, AM251 has been shown to decrease REM sleep, while increasing wakefulness (Calik and Carley 2017; Goonawardena et al. 2015; Pérez-Morales et al. 2013; Méndez-Díaz et al. 2013; Reyes Prieto et al. 2012; Herrera-Solís et al. 2010). However, one studied showed no change in wakefulness with AM251 (Calik and Carley 2017). Another study also showed a decrease in REM sleep, but it did not reach statistical significance (López et al. 2010). AM251 has inconsistently been shown modulate NREM sleep, with some studies showing AM251 increasing NREM sleep (Méndez-Díaz et al. 2013; Reyes Prieto et al. 2012; López et al. 2010), while others showing no effect on NREM sleep (Calik and Carley 2017; Goonawardena et al. 2015; Pérez-Morales et al. 2013; Herrera-Solís et al. 2010). AM251 also has been shown to decrease latency to NREM sleep, while increasing latency to REM sleep, and modifying EEG spectral power (Goonawardena et al. 2015). These conflicting results with AM251 could be due to differences between rodent models or dosage of AM251, since AM251 is known to allosterically modulate non-cannabinoid receptors (Baur et al. 2012). ABD459, a neutral antagonist of the CB_1 receptor, only decreased REM sleep, and had no effect on total sleep time or NREM sleep (Goonawardena et al. 2015). AM630, a CB_2 receptor antagonist, had no effect on sleep parameters (Calik and Carley 2017).

7.5 Allosteric Modulation

Cannabinoids are known to allosterically modulate non-cannabinoid receptors (Pertwee 2005). In mice with a targeted mutation at the GABA-A receptor, oleamide administration failed to increase NREM sleep at the expense of wakefulness as seen in the wild-type mice. It is interesting to note that the mutant and wild type mice had no difference in baseline physiological sleep parameters (Laposky et al. 2001). Indeed, work in vitro on GABA-A receptors shows that oleamide is a non-selective modulator of inhibitory ionotropic receptors (Coyne et al. 2002; Lees and Dougalis 2004).

Similarly, oleamide-induced increases in NREM sleep was prevented by serotonin reuptake inhibitors and by activation of serotonin 1A (5-HT_{1A}) receptors. Blockade of the 5-HT_{1A} receptor by WAY100635, a selective antagonist, rescued the oleamide-induced sleep changes (Yang et al. 2003), indicating that serotonergic modulation is involved with cannabinoid effects on sleep. Oleamide may enhance the function of 5-HT_2 receptors (Cheer et al. 1999) and/or GABA receptors (Coyne et al. 2002). In GABA-Aβ3 knockout mice, the effects of oleamide on sleep parameters are not observed at low doses. Only high doses of oleamide administered to these knockout animals were associated with decreased REM and increased sleep latency (Mendelson and Basile 1999).

7.6 Effect of Cannabinoid Receptors on Sleep

Endocannabinoid signaling is important for sleep architecture. A strategy to investigate endocannbinoids' effect of sleep was to knockout the CB_1 receptor. Genetic deletion of CB_1 receptor in mice exhibited increased wakefulness as a result of reduced NREM and REM sleep with no change in NREM delta power (Silvani et al. 2014). These results can be attributed to endocannabinoids modulating up-/down-state transitions in pyramidal neurons (Pava et al. 2014). These studies using targeted genetic manipulations demonstrate the importance of endocannabinoids in modulating sleep.

7.7 Conclusion

Decades of research has shown that cannabinoids, both exogenous and endogenous, modulate sleep in laboratory animals (Table 7.1). Cannabinoids have been shown to potentiate sleeping time induced by other drugs. More directly, introducing exogenous cannabinoid agonists into laboratory animals generally decreased wakefulness, increased NREM sleep, and decreased REM sleep, though in a minority of studies there were conflicting results. Similar results were obtained if endogenous cannabinoids were increased, with the exception that both NREM and REM sleep were increased. These effects of exogenous and endogenous were partially mediated by cannabinoid receptors, though some evidence points to cannabinoids allosterically modulating other receptor systems to affect sleep. Moreover, cannabinoid antagonists, or if cannabinoid receptors were removed, generally increased wakefulness. Though some work has teased out mechanisms with which cannabinoids modulate the sleep systems in the CNS, more work needs to be conducted to clarify the sleep-inducing effects of cannabinoids.

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8 Modulation of Noradrenergic and Serotonergic Systems by Cannabinoids: Electrophysiological, Neurochemical and Behavioral Evidence

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Abstract

The main noradrenergic and serotonergic nuclei in the central nervous system (CNS) are the locus coeruleus (LC) and the dorsal raphe nucleus (DRN). These brain areas, located in the brainstem, play a pivotal role in the control of various functions and behaviors that are altered by cannabinoids (i.e., pain, arousal, mood, anxiety, or sleep-wake cycle). Anatomical, neurochemical, and functional data suggest that cannabinoids regulate both central noradrenergic and serotonergic neurotransmission. Thus, strong evidence has shown that the firing activity of LC and DRN monoamine neurons or the synthesis/release of noradrenaline (NA) and serotonin (5-HT) in the projection areas are all affected by cannabinoid administration. Herein, we propose that interaction between the endocannabinoid system and the noradrenergic-serotonergic systems could account for some of the anxiolytic, antidepressant, and antinociceptive effects of cannabinoids or the disruption of attention/sleep induced by these drugs.

Keywords

Locus coeruleus · Dorsal raphe nucleus · Noradrenaline · Serotonin · Cannabinoid · CB_1 receptor

8.1 Introduction

The main noradrenergic and serotonergic nuclei in the central nervous system (CNS) are the locus coeruleus (LC) and the dorsal raphe nucleus (DRN), both located in the brainstem. These nuclei project to different brain areas and regulate several physiological functions that could be altered by cannabinoid administration. Much evidence from histochemical, anatomical, and functional studies has indicated that cannabinoids modulate serotonin (5-HT) and noradrenaline (NA) neurotransmission. This chapter integrates findings from different studies on the involvement of the endogenous cannabinoid system in the regulation of the neuronal activity and the synthesis/release of NA or 5-HT, together with its relevance in cannabinoid-induced behavioral effects. Thus, the interaction between the endocannabinoid system and noradrenergic/serotonergic systems could account for anxiolytic, antidepressant, and antinociceptive effects or explain the disruption of attention or sleep induced by cannabinoid administration.

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8.2 Modulation of Locus Coeruleus/Noradrenergic System by Cannabinoids

8.2.1 The Locus Coeruleus: Anatomy, Physiology, and Role in the Sleep/Wake Cycle

The LC contains the majority of brain noradrenergic neurons and tyrosine hydroxylase (TH)-containing cells in mammalian tissues. It is located on both sides of the dorsorostral pons, immediately ventrolateral to the fourth ventricle (Bucci et al. 2017). It receives two major afferents: an excitatory amino acid input from the lateral paragigantocellular nucleus (PGi) and a gamma-aminobutyric acid (GABA) inhibitory input from the nucleus prepositus hypoglossi (PrH) (Williams et al. 1991; Samuels and Szabadi 2008). The LC is also a major target for orexinergic innervation from the lateral hypothalamus (HT) (Marcus et al. 2001). Axons arising from the LC or the subcoerulear area extensively innervate different brain regions including the cerebellum, thalamus and HT, prefrontal cortex (PFC; the main efferent of the LC), hippocampus (HC), brainstem and dorsal and ventral horns of the spinal cord (Berridge and Waterhouse 2003).

LC neurons display a spontaneous firing activity that is tonically maintained by intrinsic membrane currents (Williams et al. 1984) and can be affected by synaptically released neurotransmitters such as glutamate, GABA, or NA. The excitatory response is mediated by NMDA type (Olpe et al. 1989) and non-NMDA type receptors (Williams et al. 1991), although the stronger influence of non-NMDA receptors (AMPA receptors) on LC cells has been reported under physiological conditions (Cherubini et al. 1988; Ennis et al. 1992). LC cells are also stimulated by endogenous neuropeptides such as orexin-A (Hagan et al. 1999). The inhibitory response is triggered, among others, by GABA, opioid peptides or NA, which mainly act through $GABA_A$ receptors (Osmanovic and Shefner 1990; Williams et al. 1991), MOR receptors (Medrano et al. 2017) and α_2-adrenoceptors (Pineda et al. 1997; Arima et al. 1998), respectively.

In the CNS, the LC is involved in the regulation of different emotional states and physiological behaviors, including depression, pain, arousal, sleep-wake cycle, anxiety, or stress (Samuels and Szabadi 2008). Concerning the role of the LC in emotional states, a supersensitivity of α_2 adrenoceptors associated with a decrease in NA concentration in LC projection areas was observed in depressive disorders. Furthermore, antidepressants down-regulated the inhibitory control of LC neurons (Cottingham and Wang 2012). Concerning pain, electrical stimulation of specific subpopulations of LC neurons induces an antinociceptive response in the rat (West et al. 1993; Hickey et al. 2014) and reduces the response of the dorsal horn neurons to noxious stimuli (Llorca-Torralba et al. 2016). Regarding arousal, it is well known that LC fires in phasic and tonic modes depending on the task performance. The phasic mode representing burst activity occurs during glutamate release, which acts onto AMPA receptors (Ennis et al. 1992). This mode is associated with good task performance and focused attention. In contrast, the tonic mode is related to poor performance and scanning attentiveness (Aston-Jones et al. 1999). A high ratio of phasic to tonic activity would favor performance on tasks that require focused attention (Aston-Jones and Cohen 2005). The LC nucleus is one of the major wakefulness-promoting brain areas (Takahashi et al. 2010). Stimulation of noradrenergic neurons of the LC induces cortical excitability and consequently sleep-to-wake switch, while inhibition of LC neurons reduces wakefulness (Carter et al. 2010). The firing activity of LC noradrenergic neurons changes during the sleep/wake cycle, with high firing rates during wakefulness, lower firing activities during slow-wave sleep, and completely cessation during rapid eye movement (REM) sleep (Kayama and Koyama 2003). The progressive reduction of noradrenergic neuron activity is required for the generation of REM sleep. Consistent with the progressive reduction in the firing rate of LC across the sleep-wake cycle, levels of NA change in the projection areas of the LC (e.g. PFC or

nucleus accumbens). Thus, NA levels remain the highest during waking and the lowest during REM sleep (Léna et al. 2005).

It is known that the decrease of the firing activity of LC cells throughout the sleep/wake cycle occurs via activation of the $GABA_A$ receptor (Kaur et al. 1997; Gervasoni et al. 1998; Mallick et al. 2012). Stimulation of PrH, the main GABAergic input to the LC, inhibits the neuronal activity of noradrenergic neurons and increases REM sleep, while application of the $GABA_A$ receptor antagonist picrotoxin into the LC decreases REM sleep (Kaur et al. 2004; Mallick et al. 2012). In line with this, suppression of inhibitory GABAergic input to the LC, which increases the firing activity of noradrenergic cells, could lead to wakefulness. Furthermore, activation of the orexinergic system enhances the neuronal activity of LC neurons by inhibition of GABA neurotransmission (Tortorella et al. 2013; Kargar et al. 2018) and promotes wakefulness reducing REM sleep (Bourgin et al. 2000).

8.2.2 Distribution of the Endocannabinoid System in the Locus Coeruleus/ Noradrenergic System

Autoradiography techniques in vitro have demonstrated a low to moderate CB_1 receptor binding in the rat LC (Herkenham et al. 1991). Scattered mRNA labeling and CB_1-receptor-like immunoreactivity are present in the proximity of the nucleus (Matsuda et al. 1993; Tsou et al. 1998). As in most brain areas, CB_1 receptors are located at inhibitory or excitatory axon terminals in the LC, probably corresponding to GABAergic or glutamatergic cells (Scavone et al. 2010). CB_1 positive axon profiles mainly constitute inhibitory type synapses (~71%). Immunofluorescence and immunoelectron microscopy studies remark the presence of CB_1 receptors at the presynaptic level but also at somatodendritic (postsynaptic) sites on noradrenergic neurons. Moreover, Scavone et al. (2010) identified CB_1 receptors, apart from at the cell membrane of noradrenergic cells, within the cytoplasm, although the functional role of the cytoplasmic receptors has not been characterized yet. To date, no other components of the cannabinoid system have been identified in the LC such as the main endocannabinoids anandamide (AEA) and 2-arachidonoylglicerol (2-AG) or the enzymes involved in their synthesis or degradation. However, allosteric peptide endocannabinoids that negatively regulate the CB_1 receptor were detected by immunohistochemistry in the LC and in other noradrenergic brain areas (Hofer et al. 2015). Finally, CB_1 receptors have also been shown at noradrenergic terminals arising from the LC, for instance, in the frontal cortex (FC), the main projection area of the LC (Oropeza et al. 2007; Page et al. 2007, 2008; Carvalho et al. 2010).

8.2.3 In Vivo Evidence for Interactions Between the Locus Coeruleus/ Noradrenergic and Endocannabinoid Systems

8.2.3.1 Electrophysiological Findings

There is strong evidence for a modulation of the neuronal activity of LC cells by cannabinoids via CB_1 receptors in rodents. Acute intravenous administration of increasing doses of the CB_1/CB_2 receptor agonists WIN 55212-2, CP55940, Δ^9-THC or a single dose of URB597, an enhancer of endocannabinoid tone, increases the spontaneous firing activity of LC cells (Gobbi et al. 2005; Mendiguren and Pineda 2006; Muntoni et al. 2006) (Table 8.1). Interestingly, noradrenergic cells are not affected by the administration of WIN55212-2 through the pipette (Mendiguren and Pineda 2006), suggesting that the systemic stimulatory effect of cannabinoids does not arise from activation of local CB_1 receptors in the LC. No change (Mendiguren and Pineda 2006) or an increase (Muntoni et al. 2006) in the firing activity of LC cells has been shown after administration of WIN 55212-2, CP 55040 or Δ^9-THC in the lateral ventricle (Table 8.1). It is widely known that the reduction of $GABA_A$ receptor activation in the LC increases the tonic activity

Table 8.1 Electrophysiological evidences for the effects of cannabinoids on the noradrenergic cells of the locus coeruleus and serotonergic neurons of the dorsal raphe nucleus in rodent brain

Treatment	Brain area	Effect	Reference
In vivo			
WIN 55212-2			
0.03–0.5 mg/kg, i.v.	Locus coeruleus	↑ Firing rate (35%)	Mendiguren and Pineda (2006)
0.06–1.0 mg/kg, i.v.	Locus coeruleus	↑ Firing rate (42%)	Muntoni et al. (2006)
10–25 μg, i.c.v.	Locus coeruleus	↑ Firing rate (84%)	Muntoni et al. (2006)
10–20 μg, i.c.v.	Locus coeruleus	= Firing rate	Mendiguren and Pineda (2006)
8.3–31.3 pmol, local.	Locus coeruleus	= Firing rate	Mendiguren and Pineda (2006)
0.05–0.2 mg/kg, i.v.	Dorsal raphe nucleus	↑ Firing rate (96%)	Bambico et al. (2007)
0.4 mg/kg, i.v.	Dorsal raphe nucleus	↓ Firing rate (43%)[a]	Bambico et al. (2007)
0.2 mg/kg, 3 times/day, i.p.	Dorsal raphe nucleus	↑ Firing rate (~124%)	Bambico et al. (2007)
0.2 mg/kg, 20 days, i.p.	Dorsal raphe nucleus	↓ Firing rate (~41%)	Bambico et al. (2010b)
1 mg/kg, 20 days, i.p.	Dorsal raphe nucleus	↓ Firing rate (~41%)	Bambico et al. (2010b)
2 mg/kg, 3 times/day, i.p.	Dorsal raphe nucleus	↓ Firing rate (~63%)	Bambico et al. (2007)
CP 55940			
0.03–0.5 mg/kg, i.v.	Locus coeruleus	↑ Firing rate (57%)	Mendiguren and Pineda (2006)
20–40 μg, i.c.v.	Locus coeruleus	= Firing rate	Mendiguren and Pineda (2006)
Δ^9-THC			
0.06–1 mg/kg, i.v.	Locus coeruleus	↑ Firing rate (21%)	Muntoni et al. (2006)
25 μg, i.c.v.	Locus coeruleus	↑ Firing rate (29%)	Muntoni et al. (2006)
1, 2, 4 mg/kg, i.p.	Dorsal raphe nucleus	= Firing rate	Bambico et al. (2012)
1 mg/kg, i.p., 5 days.	Dorsal raphe nucleus	↑ Firing rate (~60%)	Bambico et al. (2012)
URB597			
0.1 mg/kg, i.v.	Locus coeruleus	↑ Firing rate (55%)	Gobbi et al. (2005)
0.1 mg/kg, i.p., 4 days.	Locus coeruleus	↑ Firing rate (~118%)	Gobbi et al. (2005)
0.03–0.3 mg/kg, i.v.	Dorsal raphe nucleus	↑ Firing rate (81%)	Gobbi et al. (2005)
0.1 mg/kg, i.p., 4 days.	Dorsal raphe nucleus	↑ Firing rate (~67%)	Gobbi et al. (2005)
Cannabidiol			
0.1–1 mg/kg, i.v.	Dorsal raphe nucleus	↓ Firing rate (100 %)[a]	De Gregorio et al. (2018)
Rimonabant			
0.62–1 mg/kg, i.v.	Locus coeruleus	↓ Firing rate (10%)	Muntoni et al. (2006)
2 mg/kg, i.p.	Locus coeruleus	= Firing rate	Mendiguren and Pineda (2006)
In vitro			
ACEA			
1 μM	Dorsal raphe nucleus	↑ Firing rate (11%)	Mendiguren and Pineda (2009)
Methanandamide			
10 μM	Dorsal raphe nucleus	= Firing rate	Mendiguren and Pineda (2009)
AEA			
10 μM	Locus coeruleus	↑ NMDA effect (41%)	Mendiguren and Pineda (2004)
10 μM	Dorsal raphe nucleus	↓ Firing rate (43%)[a]	Mendiguren and Pineda (2009)
10–30 μM	Dorsal raphe nucleus	= Membrane potential	Haj-Dahmane and Shen (2009)
AM404			
30 μM	Locus coeruleus	↑ NMDA effect (17%)	Mendiguren and Pineda (2004)
WIN 55212-2			
10 μM	Locus coeruleus	= Firing rate	Mendiguren and Pineda (2006)
10 μM	Locus coeruleus	↑ NMDA effect (13%)	Mendiguren and Pineda (2004)
10 μM	Locus coeruleus	↓ KCl effect (42%)	Mendiguren and Pineda (2007)
10 μM	Dorsal raphe nucleus	= Membrane potential	Haj-Dahmane and Shen (2009)
CP 55940			
10–30 μM	Locus coeruleus	= Firing rate	Mendiguren and Pineda (2006)
30 μM	Locus coeruleus	↑ NMDA effect (35%)	Mendiguren and Pineda (2004)

(continued)

Table 8.1 (continued)

Treatment	Brain area	Effect	Reference
Rimonabant			
1 μM	Dorsal raphe nucleus	↓ Firing rate (53%)	Mendiguren and Pineda (2009)
AM 151			
1 μM	Dorsal raphe nucleus	↓ Firing rate (62%)	Mendiguren and Pineda (2009)

ACEA, CB_1 receptor agonist; AEA: the endogenous cannabinoid anandamide; AM404, inhibitor of AEA transport and degradation; CP 55940, CB_1/CB_2 receptor agonist; Δ^9-THC, CB_1/CB_2 receptor agonist; URB597, inhibitor of fatty acid amide hydrolase (FAAH); WIN 55212-2, CB_1/CB_2 receptor agonist; AM 251 and rimonabant, CB_1 receptor antagonists. *i.c.v.* intracerebroventricular, *i.v.* intravenous, *i.p.* intraperitoneal
[a]Non CB_1 receptor mediated effects

of noradrenergic cells. Therefore, one of the mechanisms that have been proposed for the stimulatory effect of cannabinoids observed in vivo is the inhibition of GABA release by activation of CB_1 receptors located in the PrH, the main GABAergic afferent to the LC (Muntoni et al. 2006) (Fig. 8.1). On the other hand, a decrease in blood pressure elicited by activation of peripheral CB_1 receptors could also contribute to the stimulation of noradrenergic neurons via the vagus nerve (Niederhoffer et al. 2003). Acute systemic application of the CB_1 receptor antagonist rimonabant leads to no change (Mendiguren and Pineda 2006) or to a slight but significant reduction of the firing activity of noradrenergic cells (Muntoni et al. 2006) (Table 8.1), which reveals that the LC is under tonic regulation of endocannabinoids or that CB_1 receptors are constitutively active in this nucleus. The deletion of the CB_1 receptor disrupts LC noradrenergic neuron regulation leading to an increase in the firing rate in male rodents. However, this effect was related to the alteration of corticotrophin-releasing factor (CRF) (Wyrofsky et al. 2018). Finally, an increase of LC cell activity via the CB_1 receptor has been shown in rats after 4 days of treatment with the FAAH inhibitor URB597 (Gobbi et al. 2005). However, more studies are needed to further explore the chronic effects of cannabinoids on the neuronal activity of noradrenergic cells. Overall, acute systemic administration of cannabinoids increases the neuronal activity of LC cells and this effect does not occur through the activation of local CB_1 receptors.

8.2.3.2 Neurochemical Findings

As in electrophysiological studies, systemic administration of the classical CB_1/CB_2 receptor agonists WIN 55122-2 and CP 55940 increases, via the CB_1 receptor, the expression of c-Fos protein in the LC, indicating neuronal activation (Patel and Hillard 2003; Oropeza et al. 2005) (Table 8.2). Accordingly, systemic administration of CB_1/CB_2 receptor agonists increases TH, the enzyme that limits the synthesis of NA, and the synthesis of NA in the LC and its projection areas such as HC, cerebral cortex (CC), HT and cerebellum (Moranta et al. 2004; Page et al. 2007) (Table 8.2). However, activation of the CB_1 receptor by local administration of URB597 or application of the CB_1 receptor allosteric modulator cannabidiol (CBD) fails to significantly change c-Fos expression in the LC (Murillo-Rodríguez et al. 2006, 2007). The release of NA in noradrenergic terminals (i.e. FC) is enhanced by acute administration of cannabinoid agonists in vivo (Oropeza et al. 2005) (Table 8.2) but not by chronic treatment with WIN 55212-2 or by inhibition of the endocannabinoid catabolism with the FAAH inhibitor URB597 (Gobbi et al. 2005; Page et al. 2007).

On the other hand, systemic administration of CB_1 receptor antagonists does not change c-Fos expression or NA synthesis in the projection areas of the LC such as the FC or HC (Moranta et al. 2004; Oropeza et al. 2005), indicating the absence of tonic activation of CB_1 receptors. When the CB_1 receptor was deleted, TH expression in the LC and NA levels in the mPFC were increased

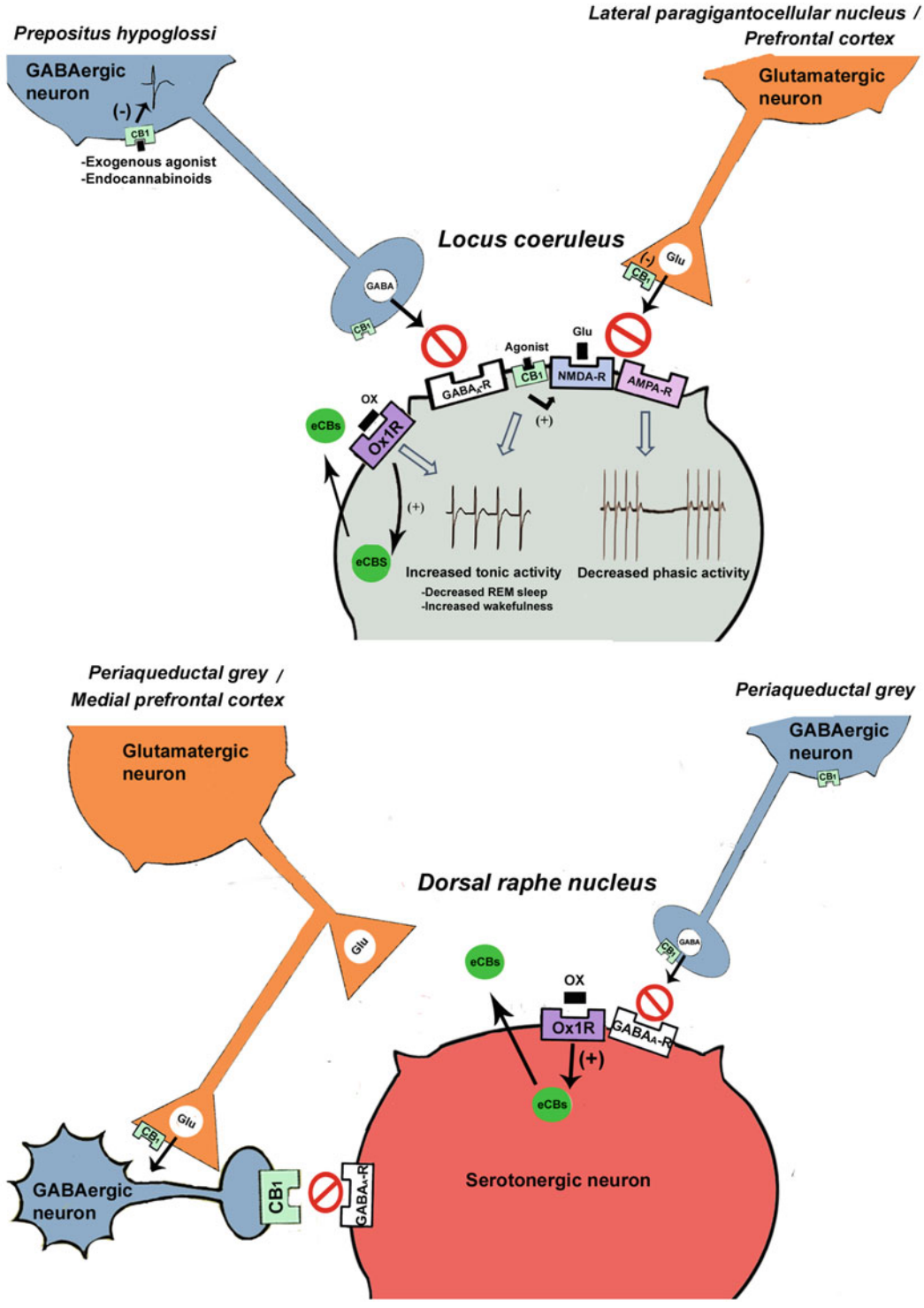

Fig. 8.1 General regulation of noradrenergic neurons in the LC and serotonergic cells in the DRN by CB_1 receptors located on GABAergic and glutamatergic systems. In the LC, CB_1 receptor-mediated inhibition of GABAergic input from the PrH to NA cells and postsynaptic CB_1 receptor-mediated enhancement of glutamatergic NMDA-type responses from the lateral PGi or PFC contribute to an increase of NA cells firing activity. Moreover, activation of orexinergic system may increase the firing activity of LC cells by direct depolarization of NA cells or

(Wyrofsky et al. 2018). Finally, different authors have reported discrepancies on the effect of CB_1 receptor antagonists on NA release in LC projection areas, which could arise from the differences in noradrenergic innervation observed among the rat strains used in the studies. Thus, no change in NA release was reported in the FC (Oropeza et al. 2005), while an increase of release was shown in the HC (Tzavara et al. 2001).

On the whole, neurochemical variations observed in LC neurons and its projection areas after administration of cannabinoids are in line with the increase in the firing activity observed in vivo and could be of relevance for some of the cannabinoid-induced effects (see Sect. 8.2.3.3). For instance, the cannabinoid-induced increase of the firing activity of LC noradrenergic cells and the subsequent NA release could affect the waking-REM sleep phases (Léna et al. 2005).

8.2.3.3 Behavioral Findings

Cannabinoids produce antidepressant and antinociception through the noradrenergic system or by activation of the LC nucleus (Morrish et al. 2009; Dogrul et al. 2012). Mood-related effects of cannabinoids appear to be signaled by the NA system since the administration of the CB_1 receptor agonist HU-210 produces an antidepressant effect in the forced swimming test (FST) via adrenoceptors (Morrish et al. 2009). Furthermore, the application of URB597 induces antidepressant-like effects in the FST and tail suspension test, which was suggested to be due to an increase in LC cell activity (Gobbi et al. 2005). Concerning antinociception, reduction in brain NA content by neurotoxin injection results in a significant decrease of the antinociceptive effect of cannabinoids (Korossy-Mruk et al. 2013), while local administration of a CB_1 receptor antagonist increases nociception (Kargar et al. 2015).

Finally, cannabinoids alter sleep phases such as REM sleep, sleep onset, or slow wave sleep (Babson et al. 2017; Calik and Carley 2017). No studies have directly linked the effects of cannabinoids on sleep with the LC. However, cannabinoid-induced suppression of the PrH inhibits LC neurons and increases REM sleep, while antagonism of the GABAergic system decreases REM sleep (Mallick et al. 2012). Muntoni et al. (2006) demonstrated, by electrophysiological techniques, that cannabinoids inhibit GABAergic system to the LC. Therefore, one hypothesis that could be proposed is that some cannabinoids may decrease REM sleep by CB_1 receptor-mediated inhibition of PrH neurons and the subsequent increase in the firing activity of LC cells. Moreover, the role of cannabinoid receptors in orexin effects in the LC may support a further local mechanism. Thus, it has been proposed that orexin-A inhibits, via CB_1 receptors, GABA release, thereby increasing the firing activity of LC cells (Kargar et al. 2018). The enhancement of the neuronal activity of noradrenergic cells by CB_1 receptors could account for the promotion of wakefulness and reduction of REM sleep observed after intracerebroventricular administration of orexin-A in the LC (Bourgin et al. 2000).

Fig. 8.1 (continued) by an indirect inhibitory effect of endocannabinoids on GABA release through presynaptic CB_1 receptors. The converged stimulation of LC cells would result in a decrease of REM sleep and an increase of wakefulness. On the other hand, activation of CB_1 receptors on glutamatergic terminals onto NA cells may decrease phasic activity of LC cells due to a reduction of glutamate acting on the AMPA receptor. In the DRN, serotonergic cells may be regulated by CB_1 receptors located on axons from GABAergic interneurons, GABAergic projections from the PAG or on glutamatergic terminals targeting GABAergic interneurons. Activation of the CB_1 receptor would probably reduce GABAergic input to the DRN serotonergic cells, leading to an activation of these cells. On the other hand, serotonergic cells may be also regulated by the orexinergic system via retrograde signalling mediated by endocannabinoid release

Table 8.2 Neurochemical evidences for acute and chronic effects of cannabinoids on the locus coeruleus and its projection areas

Treatment	Species/brain area	Effect	References
CB_1/CB_2 receptor agonists			
In vivo			
WIN 55212-2			
3 and 15 mg/kg, i.p.	Rat/FC	↑ NA release	Oropeza et al. (2005)
	Rat/LC	↑ cFos expression	Oropeza et al. (2005)
10 mg/kg, i.p.	Mouse/LC	↑ cFos expression	Patel and Hillard (2003)
2 and 4 mg/kg, i.p.	Rat/LC, HC, HT, CC, cerebellum	↑ NA synthesis	Moranta et al. (2004)
8 mg/kg, i.p.	Rat/HC, CC, cerebellum	↑ NA synthesis	Moranta et al. (2009)
4 mg/kg, i.p.	Rat/CC, HC, HT, cerebellum	↓ NA content	Moranta et al. (2004)
2–8 mg/kg, i.p., 5 days.	Rat/HC, cerebellum	↑ NA synthesis	Moranta et al. (2009)
2–8 mg/kg, i.p., 5 days.	Rat/CC	~ NA synthesis	Moranta et al. (2009)
3 mg/kg, i.p., 8 days.	Rat/FC	= NA release	Page et al. (2007)
	Rat/LC	↑ TH expression	Page et al. (2007)
CP 55940			
1, 10 mg/kg, i.p.	Mouse/LC	↑ cFos expression	Patel and Hillard (2003)
Δ^9-THC			
5 mg/kg, i.p.	Rat/PFC	↑ NA turnover	Jentsch et al. (1997)
10 mg/kg, i.p.	Rat/CC, HC, HT, cerebellum	↓ NA content	Moranta et al. (2004)
5, 10, 20 mg/kg, i.p.	Rat/LC, HC, CC,HT	↑ NA synthesis	Moranta et al. (2004)
In vitro			
WIN 55212-2			
0.1 nM–1 μM	Mouse/CC	= NA release	Trendelenburg et al. (2000)
1 μM	Guinea pig/cerebellum, CC, HT	↓ NA release	Schlicker et al. (1997)
	Human/HC	↓ NA release	Schlicker et al. (1997)
	Rat, Mouse/HC	= NA release	Schlicker et al. (1997)
3 μM	Rat/PFC	↓ NA release	Richter et al. (2012)
0.1–10 μM	Guinea pig/HC	↓ NA release	Kathmann et al. (1999)
100 μM	Rat/FC	↑ NA release	Page et al. (2008)
CP 55940			
1 μM	Rat/HC	= NA release	Gifford et al. (1997)
0.1–10 μM	Guinea pig/HC	↓ NA release	Kathmann et al. (1999)
CB_1 receptor antagonists			
In vivo			
Rimonabant			
0.3, 1, 3 mg/kg, i.p.	Rat/HT	↑ NA release	Tzavara et al. (2001)
0.2 mg/kg, i.p.	Rat/FC	= NA release	Oropeza et al. (2005)
	Rat/FC	= cFos expression	Oropeza et al. (2005)
3 mg/kg, i.p.	Rat/HC	↑ NA release	Tzavara et al. (2001)
10 mg/kg, i.p.	Rat/CC, HC, corpus striatum	= NA synthesis	Moranta et al. (2004)
AM 281			
10 mg/kg, i.p.	Rat/CC, HC, corpus striatum	= NA synthesis	Moranta et al. (2004)
In vitro			
Rimonabant			
0.1 and 0.3 nM	Rat/HC	= NA release	Gifford et al. (1997)
300 μM	Rat/FC	= NA release	Page et al. (2008)

(continued)

Table 8.2 (continued)

Treatment	Species/brain area	Effect	References
CB_1 receptor allosteric modulator			
In vivo			
Cannabidiol			
10 μg/5 μl i.c.v.	Rat/LC	= cFos expression	Murillo-Rodríguez et al. (2006)
FAAH inhibitor			
In vivo			
URB597			
10 μg/5 μl, i.c.v.	Rat/LC	= cFos expression	Murillo-Rodríguez et al. (2007)
0.1 mg/kg, i.p.	Rat/PFC	= NA release	Gobbi et al. (2005)
0.1 mg/kg, i.p., 4 days.	Rat/PFC	= NA release	Gobbi et al. (2005)
5, 10, 20 mg/kg i.p.	Rat/NAcc	↑ NA content (after sleep deprivation)	Murillo-Rodríguez et al. (2016)

CC cerebral cortex, *DRN* dorsal raphe nucleus, *FC* frontal cortex, *HC* hippocampus, *HT* hypothalamus, *NAcc* nucleus accumbens, *PFC* prefrontal cortex, *i.c.v.* intracerebroventricular, *i.p.* intraperitoneal

8.2.4 In Vitro Evidence for Interactions Between the Locus Coeruleus/ Noradrenergic and Endocannabinoid Systems

8.2.4.1 Electrophysiological Data

In brain slice preparations containing the LC, administration of WIN 55212-2 and CP55940 fails to directly modify the firing activity of noradrenergic cells (Mendiguren and Pineda 2006). However, as next shown, cannabinoids may have indirect local effects on the LC, by modulating postsynaptic and presynaptic glutamatergic responses through CB_1 receptors. Concerning the postsynaptic regulation, perfusion with AEA, an inhibitor of its transport (AM404), WIN 55212-2 or CP55940, enhances NMDA-induced excitation of noradrenergic cells (Mendiguren and Pineda 2004) (Table 8.1; Fig. 8.1). The enhancement of NMDA effect produced by cannabinoids could contribute to the overall excitation of LC neurons observed in vivo and may result from a direct interaction between CB_1 and NMDA receptors or be due to indirect mechanisms (i.e. regulation of intracellular calcium or the GABAergic system). On the other hand, cannabinoids regulate presynaptic excitatory responses in the LC, as observed in a pharmacological model of presynaptically KCl-evoked excitation of LC neurons. The glutamatergic component of KCl-evoked stimulation of LC neurons was decreased by perfusion with WIN 55212-2 (Mendiguren and Pineda 2007) (Fig. 8.1), which indicates that activation of the presynaptic CB_1 receptor on glutamatergic terminals in the LC would lead to an inhibition of glutamate release and a decrease on the phasic activity of noradrenergic cells (Fig. 8.1).

8.2.4.2 Neurochemical Data

To date, no studies have been performed to investigate the effect of cannabinoids on NA release in the LC in vitro. However, there is remarkable evidence for the regulation of NA release by cannabinoids in the projection areas of the LC. Administration of WIN 55212-2 or CP 55940 failed to change NA release in the rodent HC (Gifford et al. 1997; Schlicker et al. 1997), while it decreased NA release in the HC of other species (Schlicker et al. 1997; Kathmann et al. 1999) (Table 8.2). In the cortex, WIN 55212-2- increased (Page et al. 2008), decreased (Schlicker et al. 1997; Kathmann et al. 1999; Richter et al. 2012) or failed to change NA release (Trendelenburg et al. 2000). Additionally, the

local application of rimonabant did not change NA release in the HC (Gifford et al. 1997) or the FC (Page et al. 2008). On the whole, data obtained in vitro describe discrepant results in comparison to those in vivo. Differences between in vivo and in vitro could arise from the interaction between several neurotransmission systems or a combination of direct and indirect mechanisms occurring in vivo.

8.2.5 Concluding Remarks

Cannabinoid receptor agonists stimulate the tonic firing activity of LC noradrenergic neurons in vivo but not in vitro. The general stimulation of the firing rate of LC cells would lead to an increase in the activity of TH and NA efflux in the brain, which could contribute to cannabinoid-induced effects (i.e. antidepressant, antinociceptive effects and alteration of wakefulness and REM sleep). Cannabinoids increase the firing activity of LC cells through direct and indirect mechanisms. The *indirect mechanism* may involve the activation of CB_1 receptors located at either the peripheral nervous system or other brain nuclei different from the LC. The central indirect mechanism would occur through the activation of CB_1 receptors located in the PrH and the subsequent inhibition of GABA release in the LC. The latter effect could contribute, in part, to the disruption in REM sleep observed with some cannabinoids, since the reduction of REM occurs after blockade of the GABAergic afferent projection to the LC. The *direct mechanism* to explain the stimulatory effect of cannabinoids in the LC would imply the enhancement of NMDA-induced responses by CB_1 receptors located on the somatodendritic site of noradrenergic cells. All these effects would lead to an increase of NA release in the main projection area of the LC, the PFC.

In vitro data remark a modulation of the glutamatergic system by cannabinoids in the LC through a local circuit. Activation of CB_1 receptors would reduce glutamate release onto AMPA receptors located on LC noradrenergic cells and subsequently decrease the phasic activity of LC cells. A high ratio of phasic to tonic activity would favor performance on tasks that require focused attention (Aston-Jones and Cohen 2005). Cannabinoids would lead to an unfavorable (low) ratio of phasic to tonic activity in the LC leading to disruption of attention since they reduce glutamate release but increase the tonic firing activity of LC cells in vivo. Therefore, this regulation of LC noradrenergic cells by cannabinoids could be relevant to explain some side effects (i.e. disruption of attention) reported after acute cannabis administration.

8.3 Modulation of Dorsal Raphe Nucleus/Serotonergic Systems by Cannabinoids

8.3.1 The Dorsal Raphe Nucleus: Anatomy, Physiology, and Role in Sleep/Wake Cycle

In the vertebrates, most serotonergic neurons are restrained to the raphe nuclei of the brainstem. The DRN is located in the ventromedial part of the midbrain periaqueductal gray (PAG) and it is composed of different subregions with different cell density, morphology, and projections (Piñeyro and Blier 1999). The DRN contains serotonergic neurons and non-serotonergic cells, such as GABAergic (Nanopoulos et al. 1982) or glutamatergic (Clements et al. 1991) neurons. Serotonergic neurons are modulated by Gi/Go protein-coupled 5-HT_{1A} receptors (Aghajanian and Lakoski 1984), which activation leads to hyperpolarization of serotonergic cells (Courtney and Ford 2016). Non-serotonergic cells synapse with serotonergic neurons to directly regulate their firing activity within the nucleus (local circuitry). These cells are mainly GABAergic (Jolas and Aghajanian 1997; Piñeyro and Blier 1999; Adell et al. 2002). The DRN also receives glutamatergic inputs from the PFC. PFC terminals predominantly synapse onto GABA immunolabeled cells rather than onto 5-HT immunoreactive neurons (Jankowski and Sesack 2004). Finally, the DRN is innervated by orexinergic terminals from the lateral HT (Wang

et al. 2005). Activation of postsynaptic orexin 1 receptor (OX1R) depolarizes serotonergic cells (Soffin et al. 2004).

The serotonergic system is involved in the regulation of a wide range of functions associated with different brain regions. This is due to its diffuse projections to the brain. In the CNS, it plays a crucial role in the control of pain, anxiety, mood, or sleep-wake cycle (Berger et al. 2009; Luo et al. 2016). Thus, stimulation of DRN induces antinociceptive (Zhao et al. 2015), anxiolytic (McDevitt and Neumaier 2011), and antidepressant effects (Piñeyro and Blier 1999; Zhao et al. 2015) in behavioral tests.

Concerning the involvement of DRN in sleep-wake regulation, it is well known that the firing activity of serotonergic neurons changes during the sleep/wake cycle (Monti 2010a). Single-unit electrophysiological recordings of DRN cells in vivo demonstrated that the firing activity of serotonergic neurons decreases from waking to sleep and is absent during REM sleep (Guzmán-Marín et al. 2000; Monti 2010b). The role of serotonergic neurons as wake-inducing cells during sleep/wake cycle is controlled by the GABAergic (Monti 2010b), the orexinergic (Wang et al. 2005) or the serotonergic systems (Cespuglio 2018). Thus, the inhibitory GABA terminals induce a decrease in the firing activity of DRN serotonergic neurons during sleep/wake cycle (Gervasoni et al. 1998; Fiske et al. 2006). Moreover, the hyperpolarizing effect produced by the $GABA_A$ receptor agonist muscimol is greater in the sleep phase than during daytime in rodents (Kim et al. 2018). Both muscimol or local injection of 5-HT_{1A} receptor agonists in the DRN increase REM sleep (Monti and Monti 2000; Monti 2010b).

8.3.2 Distribution of the Cannabinoid System in the Dorsal Raphe Nucleus/Serotonergic System

Immunocytochemical analysis has shown the presence of CB_1 receptors, the FAAH enzyme, and endocannabinoids in the DRN (Tsou et al. 1998; Moldrich and Wenger 2000; Egertová et al. 2003; Häring et al. 2007). Double in situ hybridization experiments that detect coexpression of the marker of serotonergic cells tryptophan hydroxylase and CB_1 mRNA, revealed that more than 20% of serotonergic cells express mRNA for CB_1 receptors in rodents. Besides, CB_1 mRNA was found at non-tryptophan hydroxylase 2 positive cells in the DRN (Häring et al. 2007), which are likely to be GABAergic interneurons (Piñeyro and Blier 1999). CB_1 receptors are also found in glutamatergic afferents to the DRN arising from the PFC (Marsicano and Lutz 1999), which mainly synapse with GABAergic neurons (Jankowski and Sesack 2004).

8.3.3 In Vivo Evidence for Interactions Between the Dorsal Raphe Nucleus/Serotonergic and Endocannabinoid Systems

8.3.3.1 Electrophysiological Findings

Several electrophysiological data remarks that CB_1 receptors regulate the neuronal activity of DRN serotonergic neurons. Acute intravenous or intraperitoneal administrations of WIN 55212-2 enhance, through CB_1 receptor activation, the spontaneous firing rate of DRN serotonergic neurons in rats (Bambico et al. 2007). In accordance, the elevation of endocannabinoid tone by inhibition of the FAAH or its genetic deletion increases the neuronal activity of DRN serotonergic cells (Gobbi et al. 2005; Bambico et al. 2010a) (Table 8.1). Additionally, systemic administration of the partial agonist for the CB_1/CB_2 receptor, Δ^9-THC, fails to change the firing activity of DRN serotonergic cells (Bambico et al. 2012) probably due to its lower efficacy (Table 8.1). However, acute systemic administration of CBD decreases the neuronal activity of DRN serotonergic cells but this effect has been proposed to occur via activation of the 5-HT_{1A} receptor or the vanilloid receptor 1 (De Gregorio et al. 2018). The fact that CBD affects the firing activity of DRN serotonergic cells by a non-CB_1 receptor-mediated mechanism makes it

interesting for its future use in therapeutics due to its lack of psychoactive effects.

Local infusion of WIN 55212-2 in the DRN causes variable effects in the firing activity of serotonergic cells. Thus, an increase, a decrease, or no response has been shown in a small number of cells that were recorded in vivo (Bambico et al. 2007). Interestingly, local microinfusion of WIN 55212-2 in the mPFC enhances, via CB_1 receptor activation, the firing activity of most of the DRN serotonergic neurons, which indicates that CB_1 receptors located at mPFC play an important role in the stimulatory effect of WIN 55212-2 on the DRN. Accordingly, lesions of the mPFC block the increase in the firing rate elicited by systemic administration of WIN 55212-2- (Bambico et al. 2007).

Finally, chronic treatments (4–5 days) with Δ^9-THC or URB597 or repeated treatments (three times per day) with low doses of WIN 55212-2 increase the firing activity of DRN serotonergic neurons in rodents by a CB_1 receptor-mediated mechanism (Gobbi et al. 2005; Bambico et al. 2007, 2012). However, subchronic treatments (23 h, 5 h, and 0.75 h before recordings) with high doses of WIN55212-2 or long-term treatments (20 days) reduce the firing activity of DRN serotonergic cells (Bambico et al. 2007, 2010b), although it is unknown whether these effects are mediated by activation of the cannabinoid system since CB_1 receptor antagonists have not been tested.

Overall, acute systemic activation of the CB_1 receptor increases the firing activity of DRN serotonergic cells. Both the glutamatergic (mPFC) and the GABAergic neurotransmission (see Sect. 8.3.4) could contribute to the increase in the firing activity produced by cannabinoids.

8.3.3.2 Neurochemical Results

In consonance with electrophysiological data in vivo**,** intracerebroventricular administration of URB597 enhances cFos expression in the DRN (Murillo-Rodríguez et al. 2007). Accordingly, activation of CB_1/CB_2 receptors increases 5-HT release in the DRN, and it enhances or does not change 5-HT levels in different brain areas (Table 8.3). Interestingly, in some projection areas of the DRN such as the nucleus accumbens, the increase of 5-HT levels produced by URB597 also occurs after sleep deprivation (Murillo-Rodríguez et al. 2016). In consonance, the deletion of FAAH also increases 5-HT tone in different brain areas in rodents (Cassano et al. 2011).

Intraperitoneal administration of the CB_1/CB_2 receptor agonists WIN 55212-2 or Δ^9-THC decreases, via CB_1 receptor activation, 5-HT synthesis in the projection areas of the DRN such as the LC, HC, CC, cerebellum and corpus striatum (Moranta et al. 2004) (Table 8.3). In line with this action, activation of the cannabinoid system decreases (HC, FC, NAcc, HT) or fails to change (PFC, HC) 5-HT release in different projection areas of the DRN (Table 8.3). Exceptionally, an increase of 5-HT release was reported in the NAcc after the administration of CP55940 (Tao and Ma 2012). The blockade of the CB_1 receptor does not change the production of 5-HT (Moranta et al. 2004) but produces an increase (PFC, NAcc, and LC) or no change (HT, CC, NAcc) of 5-HT release (Table 8.3). Similarly, genetic deletion of CB_1 receptors or central application of the CB_1 receptor antagonist AM-251 increases 5-HT release in the PFC (Aso et al. 2009) and the HT (Merroun et al. 2009). Taking into account that the cannabinoid effect on serotonergic cells is stimulatory, these neurochemical actions could be the result of local inhibitory mechanisms related to the CB_1 receptor in the projection areas. On the other hand, the application of the non-psychoactive cannabinoid CBD enhances cFos expression in the DRN (Murillo-Rodríguez et al. 2006). However, in contrast to classical cannabinoids, CBD does not increase the firing activity of DRN serotonergic cells (see Sect. 8.3.3.1).

Concerning the chronic neurochemical effects of cannabinoids on the serotonergic system, repeated injection of WIN 55212-2 fails to change 5-HT synthesis in several projection areas of the DRN (CC, HC, and cerebellum), which indicates that chronic stimulation of CB_1 receptors induces neuroadaptation (Moranta et al. 2009) (Table 8.3).

Therefore, heterogeneous neurochemical data have been observed concerning cannabinoid

Table 8.3 Neurochemical evidences for acute and chronic effects of cannabinoids on the dorsal raphe nucleus and its projection areas

Drug and treatment	Species/brain nucleus	Effect	References
CB_1/CB_2 receptor agonists			
In vivo			
WIN 55212-2			
2 and 4 mg/kg, i.p.	Rat/LC, HC, CC, cerebellum, corpus striatum	↓ 5-HT synthesis	Moranta et al. (2004)
8 mg/kg, i.p.	Rat/CC, HC, cerebellum, corpus striatum	↓ 5-HT synthesis	Moranta et al. (2009)
1 mg/kg, i.p.	Rat/PFC	~5-HT release	Kleijn et al. (2011)
2.5 and 5 mg/kg, i.p.	Rat/DRN	~5-HT release	Tao and Ma (2012)
	Rat/NAcc	↑ 5-HT release	Tao and Ma (2012)
2–8 mg/kg, i.p., 5 days.	Rat/CC, HC, cerebellum	~ 5-HT synthesis	Moranta et al. (2009)
1 μg/5 μl, i.c.v.	Rat/HT	↓ 5-HT release	Merroun et al. (2009)
4 mg/kg, i.p.	Rat/LC, CC, HC, HT, cerebellum	= 5-HT content	Moranta et al. (2004)
CP 55940			
0.1 mg/kg i.p.	Rat/HT	↑ 5-HT content	Arévalo et al. (2001)
0.5 mg/kg, i.p.	Rat/DRN	~ 5-HT release	Tao and Ma (2012)
0.5 mg/kg, i.p.	Rat/NAcc	↑ 5-HT release	Tao and Ma (2012)
Δ^9-THC			
5 mg/kg, i.p.	Rat/PFC	~ 5-HT turnover	Jentsch et al. (1997)
	Rat/HC, substantia nigra	↑ 5-HT content	Molina-Holgado et al. (1993)
	Rat/DRN, LC, HT	~ 5-HT content	Molina-Holgado et al. (1993)
6 mg/kg, i.p.	Rat/HC	↓ 5-HT release	Egashira et al. (2002)
	Rat/HC	↑ 5-HT content	Egashira et al. (2002)
	Rat/DRN	~ 5-HT content	Egashira et al. (2002)
	Rat/FC	↓ 5-HT release	Ferreira et al. (2012)
10 mg/kg, i.p.	Rat/LC, CC, HC, HT, cerebellum	~ 5-HT content	Moranta et al. (2004)
	Rat/NAcc	↓ 5-HT release	Sano et al. (2008)
5, 10, 20 mg/kg, i.p.	Rat/LC, HC, CC, cerebellum, corpus striatum	↓ 5-HT-synthesis	Moranta et al. (2004)
10 mg/kg, i.p., 8 days.	Rat/PFC	↑ 5-HT content	Sagredo et al. (2006)
	Rat/DRN, HC, HT	~ 5-HT content	Sagredo et al. (2006)
In vitro			
WIN 55212-2			
1 μM	Mouse/CC	↓ 5-HT release	Nakazi et al. (2000)
100, 300, 1000 μM	Rat/DRN	↑ 5-HT release	Tao and Ma (2012)
	Rat/NAcc	↓ 5-HT release	Tao and Ma (2012)
CP 55940			
1 μM	Mouse/CC	↓ 5-HT release	Nakazi et al. (2000)
CB_1 receptor antagonists			
In vivo			
Rimonabant			
0.3, 1, 3 mg/kg, i.p.	Rat/HT	~ 5-HT release	Tzavara et al. (2001)
1 mg/kg s.c.	Rat/PFC	~ 5-HT release	Kleijn et al. (2011)
3 and 10 mg/kg, i.p.	Rat/PFC	↑ 5-HT release	Tzavara et al. (2003)
10 mg/kg, i.p.	Rat/NAcc	↑ 5-HT release	Tzavara et al. (2003)

(continued)

Table 8.3 (continued)

Drug and treatment	Species/brain nucleus	Effect	References
	Rat/LC, PFC	↑ 5-HT release	Ortega et al. (2013)
	Rat/CC, HC, corpus striatum	~ 5-HT synthesis	Moranta et al. (2004)
	Rat/LC, CC, HC, HT, cerebellum	~ 5-HT content	Moranta et al. (2004)
SR 147778			
5 mg/kg, i.p.	Rat/NAcc	~ 5-HT release	Tao and Ma (2012)
AM 251			
5 mg/kg, i.p.	Rat/NAcc	~ 5-HT release	Tao and Ma (2012)
1 μg/5 μl, i.c.v.	Rat/HT	↑ 5-HT release	Merroun et al. (2009)
AM 281			
10 mg/kg, i.p.	Rat/CC, HC, corpus striatum	~ 5-HT synthesis	Moranta et al. (2004)
	Rat/LC, CC, HC, HT cerebellum	~ 5-HT content	Moranta et al. (2004)
In vitro			
Rimonabant			
0.032 and 0.32 μM	Mouse/CC	~ 5-HT release	Nakazi et al. (2000)
CB_1 receptor allosteric modulator			
In vivo			
Cannabidiol			
50 mg/kg, i.p.	Rat/PFC	~ 5-HT release	Linge et al. (2016)
50 mg/kg, 3 days +10 mg/kg i.p., 11 days.	Rat/PFC	↑ 5-HT release	Linge et al. (2016)
10 μg/5 μl, i.c.v.	Rat/DRN, HT	↑ cFos expression	Murillo-Rodríguez et al. (2007)
FAAH inhibitor			
Anandamide membrane transporter blocker			
In vivo			
URB597			
0.1 mg/kg, i.p., 4 days.	Rat/HC	↑ 5-HT release	Gobbi et al. (2005)
	Rat/PFC	~ 5-HT release	Gobbi et al. (2005)
0.1 mg/kg, i.p., once.	Rat/HC, PFC	~ 5-HT release	Gobbi et al. (2005)
10 μg/5 μl, i.c.v.	Rat/DRN, HT	↑ cFos expression	Murillo-Rodríguez et al. (2007)
5, 10, 20 mg/kg i.p.	Rat/NAcc	↑ 5-HT content (after sleep deprivation)	Murillo-Rodríguez et al. (2016)
VDM-11			
5, 10, 20 mg/kg i.p.	Rat/NAcc	↓ 5-HT content (after sleep deprivation)	Murillo-Rodríguez et al. (2016)

CC cerebral cortex, *DRN* dorsal raphe nucleus, *FC* frontal cortex, *HC* hippocampus, *HT* hypothalamus, *5-HTP* 5-hydroxytryptophan, *NAcc* nucleus accumbens, *PFC* prefrontal cortex, *i.c.v.* intracerebroventricular, *i.p.* intraperitoneal, *s.c.* subcutaneous

effects on the DRN and its projection areas. These differences may be due to variations in the density of CB_1 receptors, which could change between different brain regions and, in each area, between cell populations (serotonergic, GABAergic or glutamatergic neurons). However, irrespective of the variable actions of cannabinoids, they may underlie cannabinoid-induced effects including behavioral states of wake and sleep. Indeed, changes in brain levels of monoamines (5-HT and NA) could generate or suppress stages of wake and sleep. The transition between wakefulness and REM sleep is accompanied by changes

in 5-HT and NA, which are highest in wakefulness or are absent during REM sleep.

8.3.3.3 Behavioral Findings

Variation in the firing activity of DRN serotonergic cells produced by cannabinoids and the changes in 5-HT release in its projection areas could contribute to cannabinoid-induced effects. Administration of cannabinoid agonists induces antidepressant and anxiolytic-like responses (McLaughlin and Gobbi 2012). In this regard, some behavioral evidences support the involvement of the serotonergic system or 5-HT_{1A} receptors in the antidepressant and anxiolytic effects produced by cannabinoids. Thus, inhibition of 5-HT synthesis abolishes the antidepressant effects of Δ^9-THC or URB597 in the FST in rodents (Häring et al. 2013; Bambico et al. 2016). This effect may be of relevance, given the depressive side effects caused by the CB_1 receptor antagonist rimonabant, which led to its withdrawal after being commercialized in 2006 for the treatment of obesity. Similarly, inhibition of 5-HT synthesis decreases the anxiolytic effect produced by URB597 (Bambico et al. 2016). Moreover, 5-HT_{1A} receptors mediate the anxiolytic effects induced by CP55940, Δ^9-THC, and anandamide transport inhibitor AM404 (Marco et al. 2004; Braida et al. 2007). The non-psychoactive cannabinoid CBD also elicits anxiolytic (Campos and Guimarães 2008; Resstel et al. 2009; Gomes et al. 2011; Fogaça et al. 2014) and antidepressant effects (Zanelati et al. 2010; Linge et al. 2016; Sartim et al. 2016) by activation of 5-HT_{1A} receptors. Interestingly, the anxiolytic effect caused by CB_1/CB_2 receptor agonists or CBD via 5-HT_{1A} receptor could be beneficial for anxiety-induced sleep disturbances. The non-psychoactive CBD has been shown to alleviate the alteration of REM sleep due to its anxiolytic effect (Hsiao et al. 2012). Interestingly, CBD administration also produces other effects by activation of 5-HT_{1A} receptors such as anticataleptic (Gomes et al. 2013; Sonego et al. 2016), antiepileptic (Devinsky et al. 2014), neuroprotective (Fernández-Ruiz et al. 2013; Pazos et al. 2013), antiemetic (Rock et al. 2012; Bolognini et al. 2013) and antinociceptive (Ward et al. 2014) effects.

Other studies point to direct involvement of the DRN in cannabinoid-induced effects. Thus, local injection of cannabinoids into the nucleus elicits antinociception (Martin et al. 1999; Lichtman et al. 1996), catalepsy (Lichtman et al. 1996), hypothermia (Lichtman et al. 1996) and impairment of locomotor activity (Egashira et al. 2002). Finally, increasing anandamide tone in the DRN disrupts sleep homeostasis by promoting waking and decreasing slow-wave and REM sleep (Murillo-Rodríguez et al. 2011).

8.3.4 In Vitro Evidence for Interactions Between the Dorsal Raphe Nucleus/Serotonergic and Endocannabinoid Systems

In contrast to electrophysiological assays in vivo, administration of CB_1 receptor agonists in brain slices fails to affect or slightly changes the excitability of serotonergic cells (Haj-Dahmane and Shen 2009; Mendiguren and Pineda 2009) (Table 8.1). The lack of agonist effects may be secondary to the existence of a saturating endogenous cannabinoid tone that activates DRN serotonergic cells since CB_1 receptor antagonists inhibit the firing activity of serotonergic cells in the DRN (Mendiguren and Pineda 2009) (Table 8.1). As the synaptic function and structure are preserved in the entire animal (in vivo), the endogenous cannabinoid tone is weaker and thereby the stimulatory effect of exogenous cannabinoids on the firing activity of DRN serotonergic cells becomes visible. According to our results, the high endogenous cannabinoid tone presumed in vitro would cause a maximal inhibition of the GABAergic system in the DRN via CB_1 receptors, making the serotonergic neurons be highly activated. CB_1 receptors would probably be located at GABAergic axon terminals (Fig. 8.1) since perfusion with the CB_1/CB_2 receptor agonist WIN 55212-2 did not induce any change in the excitability of non-serotonergic neurons (GABAergic

interneurons). Interestingly, CB_1 receptor-mediated inhibition of GABAergic interneurons in the DRN has been proposed by Tao and Ma (2012) as a mechanism to explain the increase of 5-HT release observed in the projection area of the DRN after systemic administration of WIN 55212-2.

On the other hand, cannabinoids suppress excitatory postsynaptic currents (EPSCs) through CB_1 receptor-mediated inhibition of glutamate release, by which they regulate the strength and plasticity of synapsis in the DRN (Haj-Dahmane and Shen 2009). Besides, endogenous cannabinoids have been involved in the inhibition of glutamate release produced by orexins in the DRN (Haj-Dahmane and Shen 2005). Given the contribution of glutamatergic and orexinergic systems to the control of sleep homeostasis, these electrophysiological data in vitro could support a role for the endocannabinoids in the regulation of the sleep/wake cycle.

About neurochemical data in vitro, local infusion of the CB_1/CB_2 receptor agonists WIN 55212-2 or CP55940 has been shown to decrease 5-HT release in the projection areas of the DRN (i.e., CC and the NAcc) (Nakazi et al. 2000; Tao and Ma 2012) (Table 8.3).

Overall, electrophysiological findings in vitro reveal the presence of an endogenous cannabinoid tone in the DRN that may explain some behavioral effects elicited by cannabinoids.

8.3.5 Concluding Remarks

In the DRN, cannabinoids affect serotonergic neurons by modulation of excitatory and inhibitory neurotransmission. Regulation of inhibitory input to serotonergic cells may be attributed to activation of CB_1 receptors located at GABAergic or glutamatergic projections making synapses with GABAergic neurons. Thus, activation of CB_1 receptors would probably reduce GABAergic input to DRN serotonergic neurons and lead to an excitatory effect on these cells. The CB_1 receptor could be located at local GABAergic projections from the PAG or glutamatergic terminals from the mPFC or the PAG targeting GABAergic interneurons (Fig. 8.1). On the other hand, direct regulation by an excitatory input to serotonergic cells may involve activation of CB_1 receptors present at the mPFC that directly innervate serotonergic cells. The indirect regulation of serotonergic cells seems more likely for several reasons. First, glutamatergic synapses onto GABA neurons are more sensitive to CB_1 receptor-mediated inhibition (Geddes et al. 2016). Second, CB_1 receptor antagonists inhibit the firing rate of DRN serotonergic cells through GABA neurotransmission. In sum, CB_1 receptors could regulate glutamatergic neurons that make synapse with GABAergic interneurons, which finally would regulate the firing activity of DRN serotonergic cells (Fig. 8.1).

The functional evidence also indicates the presence of a strong endogenous cannabinoid tone in vitro that indirectly activates serotonergic neurons by restraining the GABA-dependent inhibition of serotonergic cells in the DRN. The strong cannabinoid tone would be relieved by the administration of CB_1 receptor antagonists, which may trigger the GABAergic inhibition of serotonergic cells in the DRN (Fig. 8.1). In vivo, the basal cannabinergic tone would be weaker and so it could be further enhanced by the administration of cannabinoid agonists, leading to stimulation of the firing activity of serotonergic cells.

In summary, the effect of cannabinoids on the activity of DRN serotonergic neurons will depend on the balance between excitatory and inhibitory inputs. When the administration of CB_1/CB_2 receptor agonists is systemic, the stimulatory effect of serotonergic neurons is predominant in the DRN. The variable effects of cannabinoids on 5-HT release observed after systemic cannabinoid administration in vivo will probably result from the activation of CB_1 receptors located on different neuronal populations. In any case, the electrophysiological and neurochemical effects of cannabinoids on serotonergic neurons of the DRN would account for cannabinoid-induced behavioral effects, since changes in neuronal activity of these cells have been linked to some mood or attention disorders (see Sect. 8.2.3.3).

8.4 General Conclusions

CB_1/CB_2 receptor activation or rising the endogenous cannabinoid tone leads to an enhancement of the firing rate of LC and DRN neurons in vivo by CB_1 receptor-mediated mechanisms. The increase in the firing rate produces NA and 5-HT release in the projection areas of these monoaminergic nuclei such as PFC or HC. The interaction between the endocannabinoid system and noradrenergic/serotonergic systems mainly occurs via activation of CB_1 receptors located at excitatory or inhibitory synapses.

In summary, cannabinoids regulate synaptic transmission in the two of the main brain areas related to sleep/wake cycle: LC and the DRN. This regulation could be of interest to the development of new therapeutic approaches to sleep disorders, anxiety, depression, or pain. Additionally, the modulation of DRN and LC nuclei by cannabinoids could be relevant to understand some side effects produced by cannabinoids such as disruption of attention.

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Natural Cannabinoids as Templates for Sleep Disturbances Treatments

9

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Abstract

The sleep-wake cycle is a complex composition of specific physiological and behavioral characteristics. In addition, neuroanatomical, neurochemical and molecular systems exerts influences in the modulation of the sleep-wake cycle. Moreover, homeostatic and circadian mechanisms interact to control the waking or sleeping states. As many other behaviors, sleep also develops pathological features that include several signs and symptoms corresponding to medical conditions known as sleep disorders.

In addition to the neurobiological mechanisms modulating sleep, external elements also influence the sleep-wake cycle, including the use of *Cannabis sativa* (*C. sativa*). In this regard, and over the last decades, the interest of studying the pharmacology of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent of *C. sativa,* has been addressed. Moreover, in recent years, the focus of scientific interest has moved on to studying the second plant constituent with non-psychotropic pharmacological properties: Cannabidiol (CBD).

The pharmacological and pharmaceutical interest of CBD has been focus of attention due to the accumulating body of evidence regarding the positive outcomes of using CBD for the treatment of several health issues, such as psychiatric and neurodegenerative

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disorders, epilepsy, etc. Since the most prominent sleep disruptions include excessive daytime sleepiness (EDS), current treatments include the use of drugs such as stimulants of antidepressants. Notwithstanding, side effects are commonly reported among the patients under prescription of these compounds. Thus, the search of novelty therapeutical approaches aimed to treat ESD may consider the use of cannabinoid-derived compounds, such as CBD. In this chapter, we will show experimental evidence regarding the potential role of CBD as a wake-inducing compound aimed to manage EDS.

Keywords

Cannabis sativa · Disturbance · Drug · Medical · Wakefulness

9.1 Introduction

Wide varieties of compounds have been used for managing excessive daytime sleepiness (EDS). For instance, stimulants (methylphenidate [Ritalin] or modafinil [Provigil]), antidepressants (fluoxetine [Prozac]) or sodium oxybate [Xyrem]) have shown positive outcomes managing sleepiness (Avellar et al. 2016; Chen et al. 2016; de Biase et al. 2017; Mason et al. 2013; Moresco et al. 2018; Murillo-Rodríguez et al. 2018a; Proserpio et al. 2018; Saini and Rye 2017; Shen et al. 2018; Trotti et al. 2016). However, most of the patients report undesirable side effects after using these drugs (Goulas et al. 2018; Schmidt et al. 2018; Zhang et al. 2018). Thus, aimed to develop a novel therapeutic approach targeting the EDS, several pharmaceutical efforts have explored the use of different compounds. In this regard, and in despite of its controversy, the use of Cannabis or cannabis-derived products for medical purposes has been described in the last years. Interestingly, at this date, over 40 states in the USA have passed legislation approving the use of Cannabis for medical issues (Han et al. 2018; Hunt and Pacula 2017; Keyes et al. 2016; Paschall et al. 2017; Sarvet et al. 2018). In similar scenario, several countries have legislations regarding the use of Cannabis-derived compounds for health problems (Abuhasira et al. 2018; Mechoulam 2015; Fischer et al. 2015; Vujcic et al. 2017). Legal permissions were based in the evidence about the positive effects of Cannabis-derived compounds, such as CBD for managing health issues, including epilepsy (Devinsky et al. 2014, 2016; Friedman and Devinsky 2015; Rosenberg et al. 2015; Szaflarski and Bebin 2014).

Research of using CBD is expanding for the use this compound to manage a wider health spectrum including multiple sclerosis, anxiety, pain, inflammation, bipolar disorders, dystonia, Parkinson's disease and schizophrenia as well as epilepsy (Boggs et al. 2018; Chagas et al. 2014a, b; Couch et al. 2017; Cuñetti et al. 2018; Fogaça et al. 2018; Guinguis et al. 2017; Khoury et al. 2017; Koppel 2015). Evidence for its efficacy in treating these disturbances is growing. Progress towards the use of CBD in additional health issues should incorporate an understanding of the mechanisms of action of this compound. Due to this enhancement of evidence with positive outcomes of medical uses of CBD, we will discuss in the following sections the current knowledge about the role of CBD on the control of the sleep-wake cycle and the potential use of this compound for managing sleep disorders, specifically targeting the control of EDS.

9.2 The Neurobiology of Sleep

Due that in previous chapters of this book is described the mechanisms of sleep-wake cycle modulation, we will avoid fully descriptions of the neurobiological mechanism of action for sleep modulation. Briefly, sleep is a phenomenon described in most of the species studied so far. It is accepted that sleep is a behavioral state characterized with reduced motility and diminished responsiveness to external sensory stimulus (Carskadon and Dement 2005; Murillo-Rodríguez et al. 2009). Under normal conditions, the sleep-wake cycle is integrated of two basic forms of sleep: Slow wave sleep (SWS) and rapid

eye movement (REM) sleep (Eban-Rothschild et al. 2018; Murillo-Rodríguez et al. 2009). By specific polysomnographic (electroencephalographic [EEG] and electromyographic [EMG]) traces the sleep-wake cycle has been characterized (Fig. 9.1). The neurobiological mechanism that modulate the transitions between these sleep stages require the engagement of complex brain circuitry, including neuroatanomical, neurochemical and genetically elements (Assimakopoulos et al. 2018; González et al. 2018; Kim et al. 2017; Monti 2013; Saper and Fuller 2017; Zaki et al. 2018). In the following sections, we will focus on the putative use of natural cannabinoid for putative therapeutical approaches aimed to treat sleep disturbances, making emphasis in the role of CBD in sleep control.

9.3 Molecular Components of *Cannabis sativa*

The diversity of compounds present in *C. sativa* include molecules, such as Δ^9-THC, CBD, cannabigerol, cannabichromene, tetrahydrocannabivarian, cannabinol, (−)-trans-Δ^8-tetrahydrocannabinol, cannabidiolic acid, cannabigerolic acid, and Δ^9-tetrahydrocannabinolic acid-A (Gul et al. 2018; Hanuš et al. 2016; Mechoulam et al. 1970; Mechoulam 1970; Sharma et al. 2012; Turner et al. 2017). Since the two most abundant and most studied components of the plants are Δ^9-THC and CBD (Fig. 9.2), the experimental evidence available at this date is limited about the physiological role of the rest of compounds.

Regarding the mechanism of action of cannabinoids, it is known that Δ^9-THC is responsible for the psychoactive effects due to the activation of the specialized receptor named CB_1 cannabinoid receptor located in the several organs, including the brain (Hu and Mackie 2015; Kendall and Yudowski 2017). Contrarily, CBD does not induce psychotropic effects or binds to the CB_1 cannabinoid receptor. Current studies are providing evidence that CBD's effects may be mediated via transient receptor potential cation channel subfamily V member 1, voltage gated potassium and sodium channels, as well as cannabinoid receptor G Protein-coupled Receptor 55 (Gaston and Friedman 2017; Pisanti et al. 2017; Turner et al. 2017). Limited evidence does not allow to fully describing the mechanism of action of CBD modulating physiological processes.

Lastly, as mentioned, Δ^9-THC has been the most studied compound in multiple experimental paradigms, including sleep. Classical experiments have reported that this cannabinoid promotes sleep (Feinberg et al. 1975, 1976). Next, the interest to study the effects of CBD on sleep raised in the recent years.

9.4 The Sleep-Wake Cycle Under the Influence of Cannabidiol

Since other chapters of this book describe the mechanisms of action of cannabis in detail, then we will focus on the current evidence regarding the effects of cannabidiol on sleep modulation. While the mechanisms of action of CBD is described fully, multiple studies have shown that CBD exerts effects in several neurobiological processes including pain perception (Hammell et al. 2016; Philpott et al. 2017), learning and memory (Lee et al. 2017; Loureiro et al. 2016), and sleep (Babson et al. 2017; Murillo-Rodríguez et al. 2006, 2008, 2014). Regarding this last point, our group has reported that administrations of CBD in rats during the lights-on period enhanced wakefulness, but decreased sleep (Murillo-Rodríguez et al. 2006, 2008, 2014).

In concrete, i.c.v. administrations of CBD (10 μg/5 μL) in rats during the lights-on period increased wakefulness, but decreased REM sleep. Similar effects on sleep were observed if CBD is injected into the lateral hypothalamus. Moreover, molecular evidence has shown that CBD promotes *c*-Fos expression in neurons placed in wake-related area, such hypothalamic nuclei and dorsal raphe nuclei. It is worthy to mention that the mentioned effects of CBD on sleep have been found after either systemic of central injections, as well as after intracerebral perfusions (Murillo-Rodríguez et al. 2006, 2008, 2011,

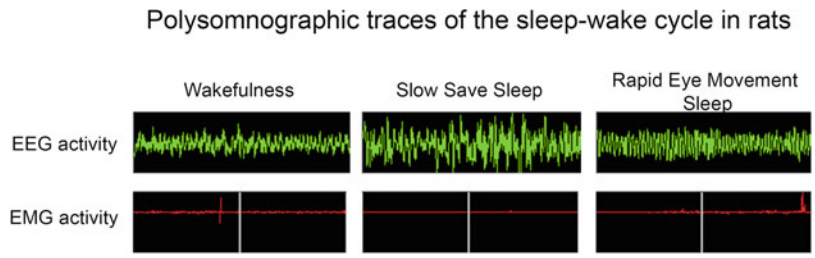

Fig. 9.1 Polysomnograph traces of the sleep-wake cycle in rats: Electroencephalogram (EEG) and electromyogram (EMG). During wakefulness, the EEG shows cortical activation as well as muscle activity in the EMG. The sleep onset shows wave forms such as "sleep spindles", K complex, and high-voltage slow waves present in the EEG. Next, a progression in the EEG activity from slow wave sleep (SWS) to rapid eye movement (REM) sleep is observed in EEG and EMG signals. During SWS and REM sleep, the activity of EMG is decreased

2014, 2017; Mijangos-Moreno et al. 2014; Fig. 9.3).

To provide a putative mechanism of action of CBD's effects on sleep, we have drawn a possible pathway by demonstrating that this cannabinoid may involve the activity of wake-related neurotransmitters in its actions. For example, administrations of CBD also enhances the extracellular levels of dopamine (Murillo-Rodríguez et al. 2006, 2008, 2014), adenosine as well as monoamines (Murillo-Rodríguez et al. 2011, 2017; Mijangos-Moreno et al. 2014). In addition, we have found that CBD-treated rats displayed a significant enhancement on acetylcholine [an additional neurotransmitter linked with waking (Holst and Landolt 2018; Schwartz and Kilduff 2015)] contents collected from the basal forebrain (Murillo-Rodríguez et al. 2018b), a brain area linked to wake control (Blake and Boccia 2018; Bringmann 2018; Yamakawa et al. 2016). Importantly, due to the wide spectrum of actions of CBD, we should consider alternative neurobiological pathways engaged in this phytocannabinoid's effects by engaging the activity of peptides, hormones, lipids, etc.

9.5 The Putative Therapeutic Use of Cannabidiol on Sleep Disturbances

As diverse physiological functions, the sleep-wake cycle also develops pathological features (Khoury and Doghramji 2015; Ramar and Olson 2013). Currently, the characterization of the group of pathologies related to sleep have been integrated into the *International Classification of Sleep Disorders* (ICSD), which describes the following categories:

1. *Dyssomnias*.—Contains intrinsic/extrinsic sleep disorders and circadian rhythm sleep disorders)

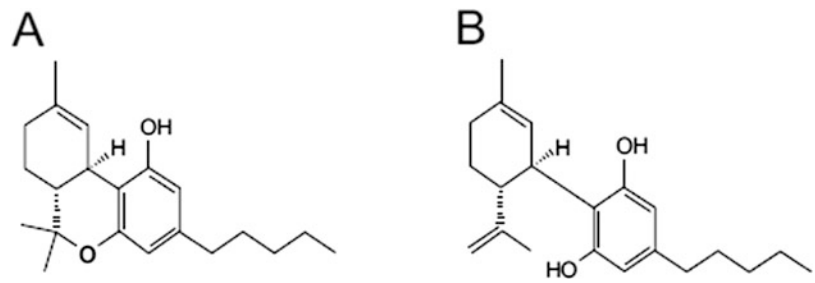

Fig. 9.2 Molecular structures of the two most abundant and most studied components of Cannabis sativa: Δ^9-tetrahydrocannabinol (Δ^9-THC; Panel **a**) and Cannabidiol (CBD; Panel **b**)

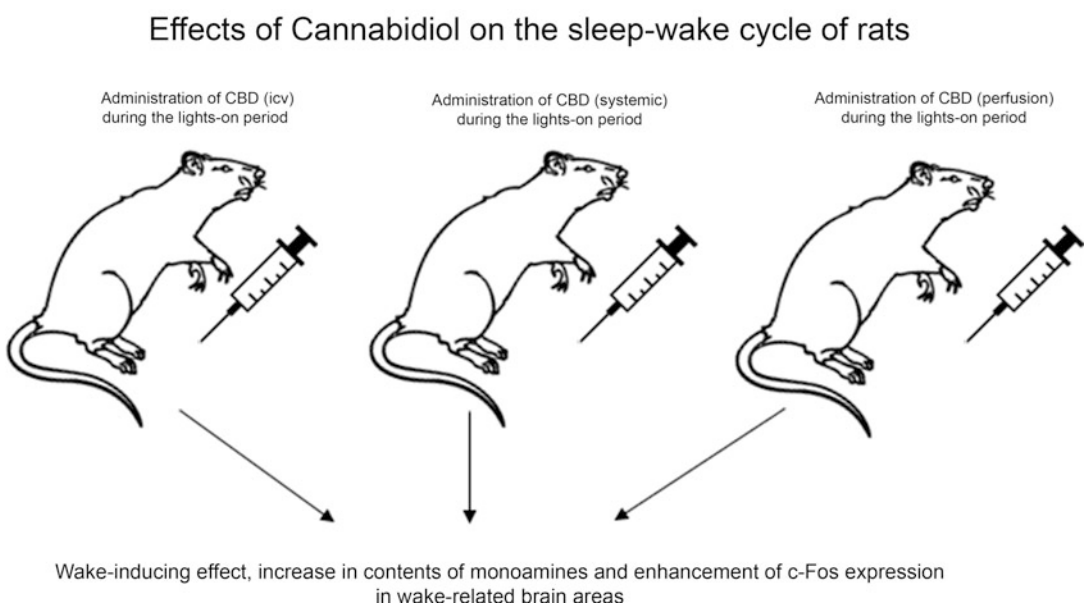

Fig. 9.3 Summarize of the effects of cannabidiol on sleep. Administrations (icv, systemic or perfused into the brain) of CBD in rats during the lights-on period enhance wakefulness, promotes *c*-Fos expression in neurons placed in wake-related area, such hypothalamic nuclei and dorsal raphe nuclei and promote the extracellular levels of monoamines

2. *Parasomnias*.—Comprises arousal disorders, sleep-wake transition disorders, parasomnias usually associated with REM sleep, and other parasomnias)
3. *Sleep disorders linked with mental, neurologic, or other medical disturbances*
4. *Proposed sleep disorders*

It is worthy to recall that EDS is among the primary complains betwixt many patients, and this sleep disorder is understood **as the difficult to** fall asleep during daytime (Khoury and Doghramji 2015; Ramar and Olson 2013). The etiology of EDS might be complex due to the influence of additional sleep disorders such as insomnia, obstructive sleep apnea, narcolepsy, or restless leg syndrome, just to mention a few. In addition, EDS is also the result of other pathological conditions, such as Parkinson's disease (Macêdo et al. 2017; McCarter et al. 2016; Ng et al. 2017; Shen et al. 2018). In sum, the coexistence of medical issues with EDS has long been recognized. As one can expect, the approaches for a proper therapy for EDS is complex. In the present, there are behavioral, psychological, and pharmacological treatments aimed at control EDS (Abad and Guilleminault 2017; Alves et al. 2013; Bekfani and Abraham 2016; Bertisch 2015; de Biase et al. 2017; Ebben 2017; Picchietti et al. 2017; Rosenberg 2015; Saini and Rye 2017).

As mentioned previously, administrations of CBD increase wakefulness and decrease sleep (Murillo-Rodríguez et al. 2006, 2008, 2011, 2014, 2017; Mijangos-Moreno et al. 2014). This scenario suggest us to consider the use of natural cannabinoids, such as CBD, as templates for developing new treatments for sleep disorders. Due to the positive results after administration of CBD in different health conditions (Boggs et al. 2018; Chagas et al. 2014a, b; Couch et al. 2017; Cuñetti et al. 2018; Fogaça et al. 2018; Guinguis et al. 2017; Khoury et al. 2017; Koppel 2015) place plausible proposal to explore the medical properties of CBD in EDS. In recent years, it has been tested the effects of CBD on diseases that are associated with sleep disorders, such as anxiety (Blessing et al. 2015; Jurkus et al.

2016; Patel 2017; Shannon and Opila-Lehman 2016).

Although there is evidence in the literature regarding the putative modulatory role of CBD on sleep, some papers suggest that the phytocannabinoid behaves as a wake-promoting compound (Murillo-Rodríguez et al. 2006, 2008, 2011, 2014, 2017; Mijangos-Moreno et al. 2014), while others report sleep promotion after its administration (Babson et al. 2017; Chagas et al. 2013, 2014a, b). It is important to remark that in most of the pharmacological studies, variables such as absorption, distribution, and metabolism determine the onset and duration of action of compounds because of several factors. Moreover, experimental design differences contribute to the apparent contradictory results among comparable studies, including route of administration, the vehicle used, sleep measurement procedures, subjects (humans or animal models [strain, sex, weight, age, etc]), just to mention a few elements. Finally, it has been suggested that the critical nature of dose and preparation are additionally important in pharmacological experiments (Iffland and Grotenhermen 2017; MacCallum and Russo 2018). In conclusion, CBD's properties on sleep modulation requires further investigation under different experimental designs, such as sleep disorder models. Then, the putative use of natural cannabinoids, such as CBD, as templates for developing new treatments for sleep disorders, should include health disturbances associated with sleep disorders.

9.6 Conclusions and Future Directions

The sleep-wake cycle is controlled by physiological mechanism neuroanatomical, neurochemical, and genetic factors), as well as by homeostatic and circadian elements. Like other behaviors, the sleep-wake cycle displays aberrant features that have been categorized as sleep disturbances. Among the most common sleep pathologies, EDS has been treated by different therapeutical strategies, including wake-promoting compounds. On the other hand, several pieces of evidence have shown that CBD acts as a positive compound since it promotes successfully management in different health conditions, such as psychiatric and neurodegenerative disorders. Due that current evidence has shown that CBD promotes wakefulness, it could be plausible to consider the use of CBD to explore its medical properties managing EDS. The current chapter reviewed the pharmacological evidence on the effects of CBD on sleep-wake cycle modulation and provided insights regarding a putative therapeutical use of this compound for managing EDS. Indeed, it is needed a fully described mechanism of action for a better understanding of the molecular and neuroanatomical mechanism by which CBD regulates sleep.

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The Effect of Cannabinoids on the Brain's Circadian Clock

10

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Abstract

The circadian rhythm is without a doubt the main element influencing the way human lead their lives. Emerging evidence indicate that cannabinoids affect these routines by regulating neuronal firing within the suprachiasmatic nucleus, the master circadian pacemaker in the brain. These actions of cannabinoids on the brain's clock may also underlie time-wraps commonly experienced by marijuana users.

Keywords

Suprachiasmatic · Cannabinoids · Hypothalamus · Synaptic transmission · Time perception

10.1 Introduction

Sleeping and eating, among other activities, are part of a physiological cycle known as the circadian rhythm. Circadian rhythms are biological processes that display the biological period of about 24 h and are mainly controlled by a tiny region located just above the optic chiasm at the base of the anterior hypothalamus, the suprachiasmatic nucleus (SCN) (Saper 2013). The SCN receives prominent inputs from the retina that control photic entrainment, and send abundant direct or indirect efferent connections to other hypothalamic and extra-hypothalamic regions to control circadian rhythms of physiology and behavior (van den Pol and Dudek 1993; Saper 2013). Many neuromodulators impact the function of SCN neurons and thereby impact biological rhythms. Addictive substances may also impact circadian rhythms, by controlling directly or indirectly the activity of SCN neurons. In this article, I discuss how cannabinoids influence suprachiasmatic neurons. By disrupting the activity of the brain's circadian clock, cannabinoids can not only impact sleep and feeding behaviors, but they also may influence time perception, which is commonly distorted in people that smoke marijuana and hashish where cannabinoids are a the major psychotropic/psychoactive component.

10.2 Cannabinoid Receptors in the SCN

Cannabinoids exert their functions trough two major receptor subtypes: cannabinoid receptors type 1 (CB1) and cannabinoid receptors type 2 (CB2). Within the brain, cannabinoids actions are mainly mediated by CB1 receptors whereas CB2 receptors are highly enriched outside the brain, specifically in the immune system and

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hematopoietic cells (Piomelli 2003). Early studies using in situ hybridization uncovered several hypothalamic cell populations that express mRNA encoding CB1 receptors, including neurons in the SCN (Marsicano and Lutz 1999). More recently, using dual-labeling in situ hybridization, Hrabovszky et al. demonstrated that a subpopulation of GABAergic cells in ventral SCN synthesizes CB1 receptors (Hrabovszky et al. 2012).

In other brain regions CB1 receptors do not localize to the cell body of cells that synthesize them, but selectively target preterminal or terminal axonal domains, where they regulate the dynamics of transmitter release (Nyíri et al. 2005; Kreitzer and Regehr 2001; Wilson and Nicoll 2001; Ohno-Shosaku et al. 2001). To determine the exact location of CB1 proteins in the SCN, Wittmann and colleagues developed a novel, highly-specific CB1 receptor antibody (Wittmann et al. 2007). Using this new antibody, CB1 receptors were found to be heavily expressed in nerve terminals synapsing onto the dorsomedial SCN. This suggests that cannabinoids can selectively modulate the activity of dorsomedial SCN neurons and thereby impact circadian rhythms. As SCN neurons are highly interconnected with each other (van den Pol 1980), these findings also suggest that CB1-receptor synthesizing neurons in the ventral SCN may project their axons to the dorsal SCN, and serve as targets for cannabinoid actions. This hypothesis has not been directly tested yet, but would likely require in vivo single-cell labelling followed by light and electron microscopy reconstruction of synaptic connections between different SCN domains (Mallet et al. 2012), and will surely help understanding how SCN orchestrate biological rhythms and how cannabinoids can disrupt them.

10.3 Cellular and Network Actions of Cannabinoids Within the SCN

Despite of its obvious significance, not many studies have addressed the cellular effects of cannabinoids on the activity of SCN neurons. SCN neurons are spontaneously active both in vitro (Green and Gillette 1982; Welsh et al. 1995) or in vivo (Groos and Hendriks 1979). Indeed, most SCN neurons display characteristic firing patterns, which consist of relatively constant firing rates, with rather fixed inter-spikes intervals. To determine the actions of cannabinoids on SCN neuronal firing, we prepared acute coronal mouse brain slices containing the SCN. Under our experimental conditions, SCN neurons fired at around 4 Hz in vitro (Acuna-Goycolea et al. 2010). These firing frequencies were independent of the method used for recording because similar frequencies were obtained either using cell-attached recordings which prevent alteration of the intracellular milieu or whole cell patch clamp recordings. Remarkably, we found that in the presence of cannabinoids SCN neurons fire about 50% more frequently. These effects were reversed by AM251, a selective CB1 receptor antagonist, indicating that they were mediated by CB1 receptor subtypes. Altogether these experiments indicated cannabinoids have a rather unconventional *excitatory* effect on SCN neuronal activity.

What cellular mechanisms underlie these atypical excitatory actions of cannabinoids within the SCN? These actions of cannabinoids are not cell autonomous, because in the presence of TTX, which blocks spike-mediated transmitter release, cannabinoids were not capable of increasing SCN neuron firing rates. Moreover, cannabinoid effects were not dependent on modulation of retinal glutamatergic synaptic inputs. Rather, our results indicate that cannabinoids control spiking of SCN neurons via a mechanism that involves blockade of tonic inhibitory synaptic input onto SCN cells. Three independent lines of evidence indicate that indeed this appears to be the case. First: In the SCN, cannabinoids receptors are located in GABAergic nerve terminals and their activation leads to decreases in GABA release. Second: SCN neurons are tonically inhibited, due to prominent spontaneous release of GABA from other nerve terminals synapsing onto them, which mostly arise from other SCN GABA cells.

Indeed, blockade of ionotropic GABA receptors with bicuculine increases the firing rate of SCN neurons by about 50%, comparable to the effects of cannabinoids agonist on SCN firing. Third: Importantly, cannabinoids agonists fail to change firing rates of SCN neurons in the presence of GABA receptor antagonist. Altogether, these findings indicate that within the SCN cannabinoids act specifically on CB1 receptors located on GABA terminals, which results in a substantial reduction in spontaneous GABA release, releasing SCN neurons from tonic inhibition and thereby increasing their spike frequency (Acuna-Goycolea et al. 2010). Given the high level of local circuit axons within the SCN (van den Pol 1980), these CB1 receptors could be located on SCN neuron axons as suggested by in situ hybridizations, or could originate from other loci that express CB1 receptors including the dorsal raphe serotoninergic neurons in the midbrain.

10.4 Cannabinoid Actions on Circadian Rhythms and Entrainment

How do cannabinoids impact biological rhythms? In animal models, circadian rhythms are often measured by continuous tracking of locomotor activity. Mice, for instance, are very active at night but mostly inactive during daytime. Circadian rhythms are preserved even in the absence of any sensory input, and result from the activity of an internal clock, the SCN, which can be easily reset by light behavior (van den Pol and Dudek 1993; Saper 2013). For example, when mice are kept in total darkness, their locomotor activity cycles trough active and inactive phases that last slightly more than 12 h each. If light is shined onto these mice when they are just entering their active phase, they become active about 2 h later in the day compared to mice that have not received light activation. This phenomenon is called 'phase delay' and can be used as proxy of light-induced entrainment of the brain internal clock.

To test the impact of cannabinoids on circadian rhythms and entrainment, we implanted 42 mice with intraventricular cannulas and house them in complete dark for 2 weeks (Acuna-Goycolea et al. 2010). We used locomotor activity as a readout of clock-phase. As expected, mice maintained in total darkness synchronized their internal clock and their locomotor activity cycle trough phases of activity and inactivity that last just over 12 h each. We first measured the impact of cannabinoid receptor agonist on clock phasing. In the absence of photic stimulation, infusion of drug vehicle or the CB1 receptor agonist Win55 did not change the clock free-running phase. We then measured the impact of cannabinoids on light-induced phase delays. In control mice infused with drug vehicle, light stimulation resulted in a significant phase delay in activity onset, as expected. In mice infused with cannabinoids (the CB1 agonist Win55, 9 nM), light-induced phase delay in activity was reduced by about 60%. This effect was reversed by intraventricular infusion of the CB1 receptor antagonist AM251 (9 nM). Similar effects of cannabinoids on circadian rhythms and entrainment have been observed in hamsters (Sanford et al. 2008). Altogether, these experiments indicate that cannabinoids markedly alter the ability of light to entrain the core clock timing process in the brain's circadian clock, the suprachiasmatic nucleus.

10.5 Conclusion

Emerging evidence indicates that neuromodulators and drugs of abuse can impact biological rhythms, at least in part by affecting the activity of the suprachiasmatic nucleus, the brain's main biological clock. Along this line, recent studies indicate that cannabinoids, the major psychoactive component of marijuana and hashish, impair normal firing of SCN neuronal networks by acting on CB1 receptors from GABA terminals located in the dorsomedial part of the SCN. Erratic firing of SCN cells triggered by cannabinoids might impact the way in which the SCN clock entrain to environmental light cues. This could potentially underlie distorted time perception commonly reported by smokers

of cannabinoids-containing recreational drugs such as marihuana and hashish.

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Effects of Cannabis Consumption on Sleep

11

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Abstract

Despite the fact that medical properties of Cannabis have been recognized for more than 5000 years, the use of Cannabis for medical purposes have recently reemerged and became more accessible. Cannabis is usually employed as a self-medication for the treatment of insomnia disorder. However, the effects of Cannabis on sleep depend on multiple factors such as metabolomic composition of the plant, dosage and route of administration. In the present chapter, we reviewed the main effect Cannabis on sleep. We focused on the effect of "crude or whole plant" Cannabis consumption (i.e., smoked, oral or vaporized) both in humans and experimental animal models.

The data reviewed establish that Cannabis modifies sleep. Furthermore, a recent experimental study in animals suggests that vaporization (which is a recommended route for medical purposes) of Cannabis with high THC and negligible CBD, promotes NREM sleep. However, it is imperative to perform new clinical studies in order to confirm if the administration of Cannabis could be a beneficial therapy for the treatment of sleep disorders.

Keywords

Marijuana · REM · NREM · Wakefulness · EEG

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11.1 Introduction

Cannabis is the most frequently used illicit recreational drug, with an annual prevalence of 3.8% of the adult population that consumed Cannabis

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in the past year (UNODC 2017). Nowadays, the consumption of Cannabis has been legalized in different countries, both for recreational and medical uses (Hoz Schilling 2015). The use of Cannabis for medical purposes is rapidly expanding (Han et al. 2018; Paschall et al. 2017), and one of the main motivations for its use is to manage insomnia disorders; i.e., to induce and maintain a refreshing sleep (American-Academy-of-Sleep-Medicine 2014; Babson et al. 2017; Belendiuk et al. 2015). However, the effects on sleep both of Cannabis or its compounds are still not clear. Hence, the aim of the present chapter is to review the main effects of Cannabis on sleep. We focused on the effect of "crude or whole plant" Cannabis consumption (i.e., oral, smoked or vaporized), both in humans and in experimental animal models.

11.2 Cannabis Compounds

Cannabis plant yields more than 538 chemicals of various classes, being phytocannabinoids, terpenes and phenolic compounds the most important (Andre et al. 2016). Phytocannabinoids are the most studied group and are responsible for the main pharmacological properties of Cannabis. The two major phytocannabinoids are Δ^9-tetrahydrocannabinol (THC), the main psychoactive compound, and cannabidiol (CBD) (Hložek et al. 2017). On the other hand, terpenes constitute the largest phytochemical group (Singh and Sharma 2015). In fact, the scent of the plant results from about 140 different terpenoids (Brenneisen 2007). Also, it has been demonstrated that these compounds mediate an important number of pharmacological actions (Andre et al. 2016; Clarke and Watson 2007).

Cannabis users usually consume crude Cannabis products (dried chopped leaves and female inflorescences) (Kisseberth and Trammel 1990). However, when studying the effects of Cannabis, most research has focused on isolated cannabinoids; i.e., not considering the possible interaction among the different compounds of the plant. As a matter of fact, it has been already determined that some of these chemicals have synergic effects. Carlini et al. (1974), based on animal and human studies, determined that Cannabis extracts produced effects "two to four times greater than that expected from their THC content". This synergic effect was described as "entourage effect" (Mechoulam and Ben-Shabat 1999).

11.3 Sleep-Wake Cycle

The sleep-wake cycle is the most obvious circadian rhythm in mammals and birds (Torterolo and Vanini 2010). During wakefulness (W), the interaction with the environment is optimum. In humans, W is accompanied by awareness (consciousness) of the environment. On the other hand, sleep is a reversible behavioral state of perceptual disengagement and unresponsiveness. In most mammals, normal sleep comprises two states; slow wave sleep or Non-REM sleep (NREM) and Rapid Eyes Movement sleep (REM) (Carskadon and Dement 2011).

Polysomnography is the gold-standard technique to determine these behavioral states, both in humans and animal models. It implies the simultaneous recording of the electroencephalogram (EEG), electrooculogram (EOG) and electromyogram (EMG) (Chokroverty et al. 2005; Torterolo and Vanini 2010).

Human EEG recording during W is defined by the presence of high frequency (above 15 Hz) and low amplitude oscillations. In spite of this, during relaxed W with the eyes closed, regular alpha (8–12 Hz) activity predominates in the occipital region. In normal adults, the transition from W to sleep is into NREM sleep. In humans, three NREM phases are distinguished according to the depth of the state: N1, N2 and N3. From W, normal adults enter in N1. This transitional state present low-voltage, mixed frequency waves (2–7 Hz range) in the EEG. N2 is characterized by the presence of sleep spindles and K-complexes in the EEG. Sleep spindles are "spindle-like" bursts of oscillatory activity of 11–15 Hz with a duration of 0.5–2 s (Evans and Richardson 1995), whereas K-complex consists of a brief negative sharp high-voltage peak

(usually greater than 100 μV), followed by a slower positive complex and final negative peak. N2 accounts for approximately 50% of the total sleep time during the night (Keenan and Hirshkowitz 2011). N3 is characterized by low frequency (0.5–4 Hz, delta band of frequencies) of high amplitude (>75 μV) waves in the EEG. N3 accounts for 12–20% of the total sleep time (Keenan and Hirshkowitz 2011).

REM sleep (or Stage R) occurs periodically, and in normal adults is always preceded by NREM sleep. Although the EEG is similar to that of W (high frequency and low amplitude oscillations) REM sleep is a deep sleep stage; hence, it is also called "paradoxical" sleep. Moreover, REM sleep is distinguished by saccadic rapid eye movements and muscle atonia evidenced in the EMG channel.

Cognitive activities not only occur during W. Dreams, are present mostly during REM sleep (Dement and Kleitman 1957; Pace-Schott 2011; Siclari et al. 2017), and are considered a special kind of cognitive activity or proto-consciousness (Hobson 2009). REM sleep occupies 20–25% of total sleep in human adults, and initiates about 90 min after sleep onset (Keenan and Hirshkowitz 2011).

11.4 Effects of Cannabis on Human Sleep

Concerning the effects of crude Cannabis on sleep, most studies had analyzed the consequence of smoking marihuana cigarettes. Some of these, reported an acute hypnotic effect; participants declare a decrease in sleep latency, an enhance in sleeping time, an increase on sleep quality, and significantly less dream recall (Barrat et al. 1974; Belendiuk et al. 2015; Chait 1990; Tringale and Jensen 2011). Nevertheless, there are contradictory results. Pivik et al. (1972) administrated oral marihuana extracts with known concentrations of THC to subjects before going to bed and found a reduction in REM amount, with no effects on NREM sleep. In chronic consumption, smoked Cannabis seems to reduce sleep quality (Barrat et al. 1974; Karacan et al. 1976; Ogeil et al. 2015). Therefore, the impact of Cannabis on sleep is influenced many factors such as dosage, ratio of cannabinoids, prior Cannabis exposure, timing and route of administration (Babson et al. 2017). Furthermore, it is necessary to take into account that some of the experimental designs with humans had a substantial risk of bias, since they do not control the concomitantly consumption of other substances, presence of pre-existing sleep disturbances, age and gender of the experimental subjects (Gates et al. 2014). A summary of the main studies is displayed in Table 11.1.

The influence of isolated cannabinoids on sleep has been deeply investigated. But when analyzing the effect of the consumption of the whole plant, how much are non-cannabinoids compounds influencing the results? Some of the terpenes present in Cannabis have been shown to induce sedative effects (Booth et al. 2017; Do Vale et al. 2002). Additionally, the neurobiological mechanisms by which these compounds exert their action have been studied. For example, terpenes such as αPinene (Yang et al. 2016), myrcene (Do Vale et al. 2002) and phytol (Costa et al. 2014), act on the benzodiazepine binding site of $GABA_A$ receptors, while others like limonene are thought to act on adenosine $A2_A$ receptor (Park et al. 2011); both sites of actions have been related to sleep regulation (Torterolo et al. 2016).

11.5 Cannabis and Human EEG Activity

Psychoactive drugs have been shown to modify the intrinsic electric oscillatory activity of the brain (Blain-Moraes et al. 2014; Dafters et al. 1999; Knott 2000; Schartner et al. 2017). Furthermore, this changes have been correlated with the subjective reports after drug experience (Koukkou and Lehmann 1976; Stuckey et al. 2005). In fact, oral administration of THC extracts (Koukkou and Lehmann 1976), or smoking marihuana cigarettes (Böcker et al. 2010; Struve et al. 1999) modify power and coherence of the alpha, theta and beta bands of the electroencephalogram (EEG).

Table 11.1 Effects of Cannabis on human sleep

Study (first Author and date)	Experimental subjects	Route of administration	Timing	Dose	Sleep measure	Effects on sleep
Barrat et al. (1974)	12 experienced marihuana users	Smoked	Drug administered for 10 nights	2 mg/kg of THC	PSG	Increased SWS (during the first 4 days)
Pivik et al. (1972)	4 adults who had not used drugs for the preceding 2 months	Oral ethanolic crude Cannabis extract	Administered before going to sleep	61–258 μg/kg	PSG	Decreased REM sleep (during the second half of the night for higher doses)
Feinberg et al. (1976)	4 experienced marihuana users	Oral ethanolic crude Cannabis extract	Acute and long term (1 month) administration, 6 doses during the day	70 mg and 210 mg/day of THC	PSG	Increased SWS Decreased REM sleep
Chait (1990)	12 regular smokers	Smoked	One weekend. Five separated smoking periods in afternoon/evening	40 puffs of 2.1% THC marihuana cigarettes	Questionnaires	Greater ease in getting to sleep than usual
Pranikoff et al. (1973)	10 regular smokers	Smoked	2 nights, smoking before bed	Until reach a subjective "high"	PSG	Decreased SWS
Karacan et al. (1976)	32 chronic marihuana users (using for at least 10 years)	Smoked	8 nights. Allow to regular consumption	No data	PSG	Increased REM sleep.
Tringale and Jensen (2011)	Data from 166 subjects from 2 Cannabic clinics	Oral/smoked/vaporized	Retrospective study of marihuana users	No data	Self-questionnaire	Decreased sleep latency Increased sleep quality
Belendiuk et al. (2015)	163 adults purchasing medical cannabis	Smoked	Retrospective study of marihuana users	No data	Self-questionnaire	Decreased insomnia Decreased nightmares
Ogeil et al. (2015)	248 self-identified as alcohol and/or Cannabis users	Smoked	No data	No data	Sleep scales for sleep quality	Decreased sleep quality (chronic Cannabis use)

11.6 Murine Experimental Models for Studying the Effect of Cannabis on Sleep

Since the beginning of the modern sleep research era in the 1950s, the domestic cat has been the animal of choice for experimental neurophysiology; however, for reasons including smaller size, greater ease of acquisition and care, as well as lesser expense, the use of rats has increased sharply in the last decades. In fact, behavioral states in rats can be identified with high confidence with the use of the polysomnographic criteria developed in the cat (Datta and Hobson 2000).

Sleep-wake cycle in rats is polycyclic, with ultradian cycles enclosed in a circadian periodicity. As the rat is a nocturnal animal, during dark phase W is the predominant state (Clancy et al. 1978). During W, the EEG is characterized by low amplitude-high frequency oscillations as well as theta (5–9 Hz) rhythm at the posterior cortices (Núñez-Molina and Amzica 2004). NREM sleep can be divided into two stages; light sleep (LS) and slow wave sleep (SWS). LS involves the transition between W and SWS; it can be evidenced at the EEG by the occurrence of slow and high voltage oscillations associated with lower voltage high frequency activity. As the sleep became deeper, rats enter into SWS. In this stage, oscillations are higher in amplitude with a frequency of 0.5–4 Hz (delta waves). Sleep spindles (10–15 Hz) are intermingled with the delta activity. Additionally, EMG activity is reduced. Finally, during REM sleep, the EEG is similar to the one in W, with high frequency and low amplitude activity as well as theta oscillations generated by the hippocampus. As in humans, during REM there is muscle atony. In Fig. 11.1 the recording of the EEG of different cortices and olfactory bulb (OB) as well as the EMG, are shown for W, NREM (SWS) and REM sleep.

11.6.1 Quantitative Analysis of the EEG Activity During Sleep and Wakefulness in Rodents

Electroencephalography is a graphic representation of the difference in voltage between two different cerebral locations plotted over time (Olejniczak 2006). The EEG signal can be analyzed quantitatively, allowing the assessment of the effect of physiological, pharmacological or pathological conditions on it (Maloney et al. 1997). Spectral analysis is one of the standard methods used for quantification of the EEG. The power spectral density (power spectrum) reflects the 'frequency content' of the signal or the distribution of signal power over frequency (Dressler et al. 2004). The basis of this analysis is the Fourier's theorem which states that any waveform can be decomposed into a sum of sine waves at different frequencies, with different amplitudes and different phase relationships. When summed, these waves reconstitute the original waveform (Cooley and Tukey 1965; Freeman and Quiroga 2013; Walczak 2009). Typically, frequency components of the rat EEG are classified in the following frequency bands; delta (δ, 0.5–4 Hz), theta (θ, 5–9 Hz), sigma (σ, 10–15 Hz), beta (β, 15–30 Hz) and gamma (ϒ, 30–100 Hz) (Maloney et al. 1997; Robbe et al. 2006). Recently, different authors have defined the frequency range between 110 and 160 Hz as "High Frequency Oscillation, HFO" (Cavelli et al. 2017b; Tort et al. 2013). Additionally, for practical reasons, to avoid the alternating current of 50 Hz that can affect the recording quality, gamma frequency can be divided into low gamma (LG, 30–48 Hz) and high gamma (HG, 52–95 Hz). Figure 11.2 shows representative raw recordings of primary somatosensory (S1) and primary visual (V1) cortices for wakefulness (W), NREM sleep and REM sleep (Fig. 11.2a), and the power spectrum of the same cortices (Fig. 11.2b). Note that there are deep changes among different behavioral states.

The functional connectivity among areas in the brain can be determined by the spectral coherence. This is a correlation coefficient that estimates the consistency of relative amplitude and phase between any pair of signals in each frequency band (Srinivasan et al. 2007). Hence, coherence is a measure of synchronization between two signals from distant brain areas based mainly on phase consistency. In other words, two signals may have different phases, but high coherence occurs when this phase difference tends to remain constant (Bullock et al. 2003; Castro Zaballa 2012; Srinivasan et al. 2007). The coherence between S1 and V1 of the same hemisphere is illustrated in Fig. 11.2c.

11.6.2 Effect of Vaporized Cannabis on Sleep and Electrocortical Activity in Rats

In laboratory animals, most research had studied the effect of isolated cannabinoids, synthetic

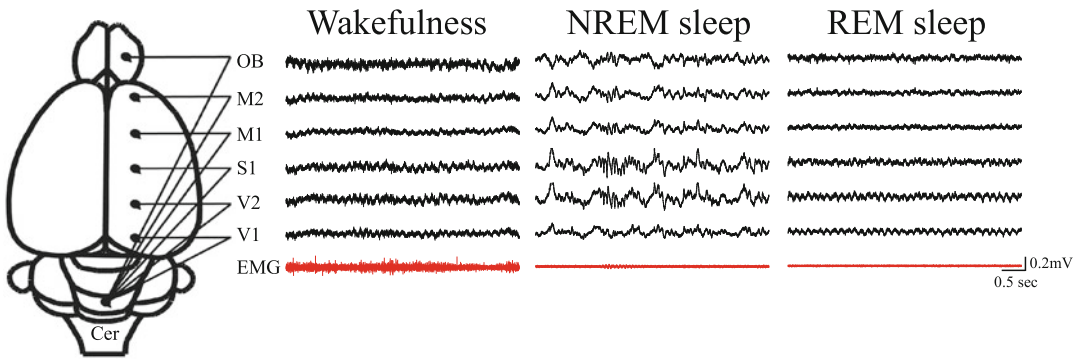

Fig. 11.1 Polysomnographic recordings a representative rat during wakefulness (W), NREM and REM sleep. On the left, a schematic representation of the electrodes position in the rat brain is shown. The reference electrode was localized over cerebellum. W and REM sleep are characterized by high frequency and low amplitude oscillations with clear theta activity (5–9 Hz) primarily in the posterior cortices. While during W the EMG activity is high, REM sleep is characterized by muscle atony as suggested by the lack of activity in the EMG. During NREM sleep, high amplitude slow oscillations (1–4 Hz) and sleep spindles (10–15 Hz) characterize the EEG. OB, Olfactory bulb; M2, secondary motor cortex; M1, primary motor cortex; S1, primary somatosensory cortex; V2, secondary visual cortex; V1, primary visual cortex; Cer, cerebellum; EMG, electromyogram

cannabinoids and agonist or antagonist of the endocannabinoid system. These surveys used injectable routes of administration (Chagas et al. 2013; Murillo-Rodriguez et al. 2003; Murillo-Rodríguez et al. 1998, 2006, 2013). On the other hand, smoking is the most predominant route of Cannabis administration in drug users (Russell et al. 2018). This route has a rapid and good absorption, while oral is slow, unpredictable, and erratic (Lanz et al. 2016; Shiplo et al. 2016). However, smoking of Cannabis is potentially harmful for the consumer, and probably for passive smokers as with tobacco (Borchers et al. 2013; Morioka et al. 2018). Hence, this route is not acceptable for therapeutic purposes (Lanz et al. 2016). An emerging alternative inhalation-based route of Cannabis administration is vaporization. Vaporization provides delivery characteristics that are similar to smoking, without the toxicants that are present due to combustion (Shiplo et al. 2016). Hence, to explore in depth of the effects of vaporized Cannabis on sleep is imperative.

Recently, we characterized in rats the effects of acute administration of vaporized Cannabis on sleep and EEG activity (Mondino et al. 2018). The strain of Cannabis used in this experiment had 11.5% of THC and negligible amounts of CBD. Polysomnographic recordings in chronically prepared rats were performed during 6 h in the light and dark phases. At the beginning of the recordings each rat was placed in a plastic box where 0 mg (C_{sham}), 40 mg (C_{40}), 80 mg (C_{80}) or 200 mg (C_{200}) of Cannabis flowers were vaporized at 180 °C for 10 min. Vaporization was carried out by means of a vaporizer connected to the box. THC plasma concentrations with these doses were low (up to 6.7 ng/mL with C_{200}).

The recordings were performed through a rotating connector, to allow the rats to move freely within the recording box. Total time spent in W, LS, SWS, Non-REM (NREM=LS+SWS) and REM sleep, as well as the duration and the number of episodes over the 6 h recording period, were determined. Sleep latencies (from the beginning of the recording) were also included in the analysis. The time spent in each state during the first recording hour was also analyzed.

When the total recording time of the whole population of animals was analyzed, the administration of C_{40}, C_{80} or C_{200} during the dark phase did not affect W or sleep parameters. During the light phase, no effects were observed with C_{40} and C_{80}, while a decrease in the number of W episodes was observed with C_{200}. When the first

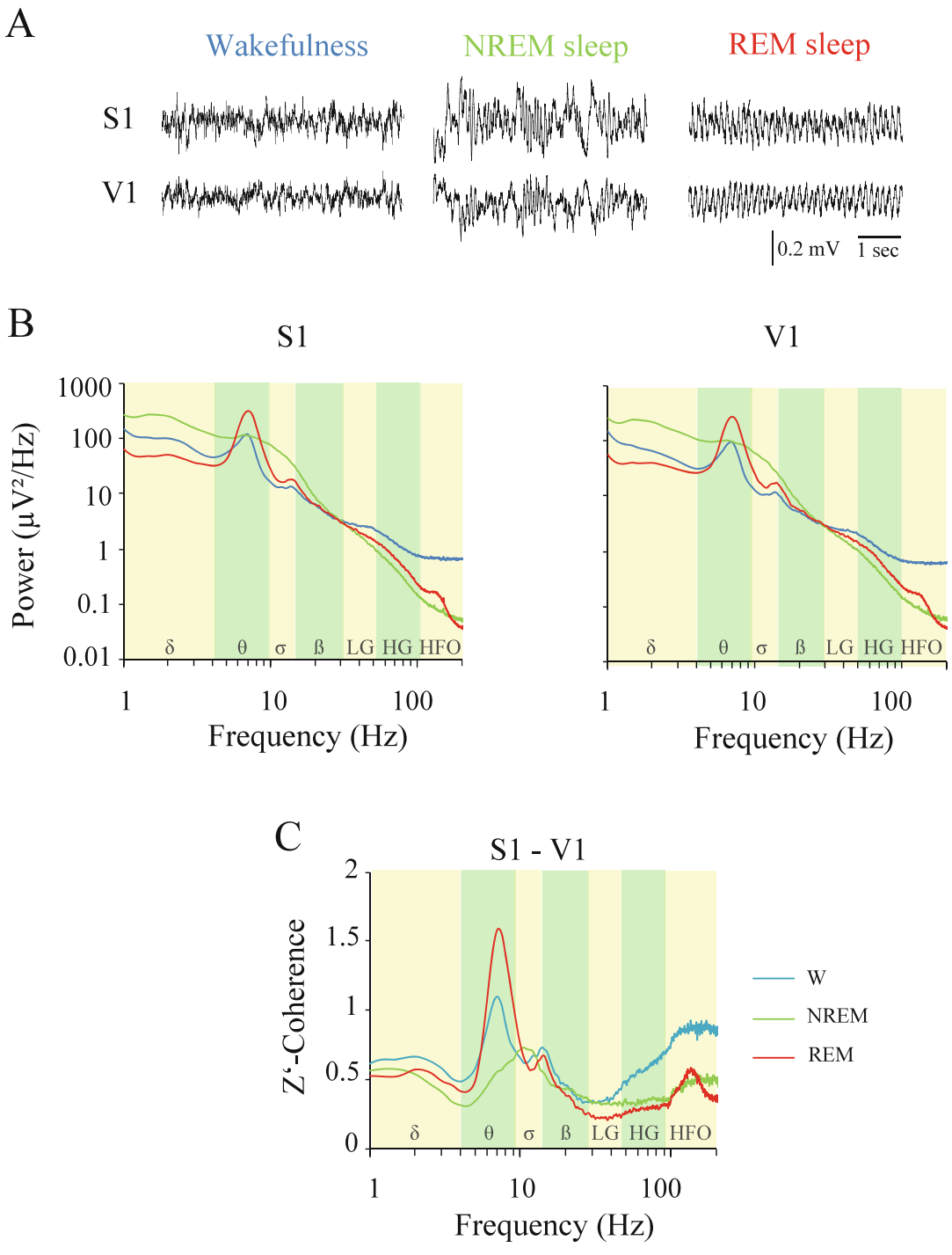

Fig. 11.2 (**a**) Representative raw recording of primary somatosensory (S1) and primary visual (V1) cortices during wakefulness (W), NREM sleep and REM sleep. (**b**) Power spectrum profiles of S1 and V1 cortices. (**c**) Intra-hemispheric Z'coherence profile between S1 and V1. Different states are shown in different colors: blue, W; green, NREM sleep; red, REM sleep. δ, Delta, 1–4 Hz; θ, theta, 5–9 Hz; σ, sigma, 10–15 Hz; β, beta, 16–30 Hz; LG, low gamma 31–48 Hz; HG, high gamma 52–95 Hz; HFO, high frequency oscillations, 105–200 Hz

recording hour was analyzed C_{40} and C_{80} did not affect W or sleep time. However, following C_{200} there was a 70% increment on NREM sleep, but only during the light phase (Fig. 11.3).

11.6.3 Effect of Vaporized Cannabis on Electrocortical Activity in Rats

Since the effect on sleep was observed only for C_{200}, the quantitative analysis of the EEG was restricted to this dose. The electrocortical activity was evaluated through the power spectrum (in each EEG channel) and coherence (between pairs of EEG channels) by means of procedures similar to those done in our previous studies (Cavelli et al. 2017a, b).

The effects of C_{200} on the EEG power and coherence differed drastically depending if it was administered during the day or during the night (resting and active phase, respectively).

11.6.3.1 Light Phase

During W in the light phase, C_{200} produced a reduction in power from theta to LG bands in the OB (Fig. 11.4). In this regard, CB1 receptors had been found in the OB (Moldrich and Wenger 2000). Also, CB1 agonists and antagonists modulate the activity of the periglomerular and external tufted cells in the OB (Wang et al. 2012), and corticofugal feedback axons have CB1 receptors that could regulate the excitation or inhibition of the OB neurons (Pouille and Schoppa 2018). Hence, the C_{200} effect on the OB EEG power may be an evidence of modulation by the endocannabinoid system of sensory processing within the OB.

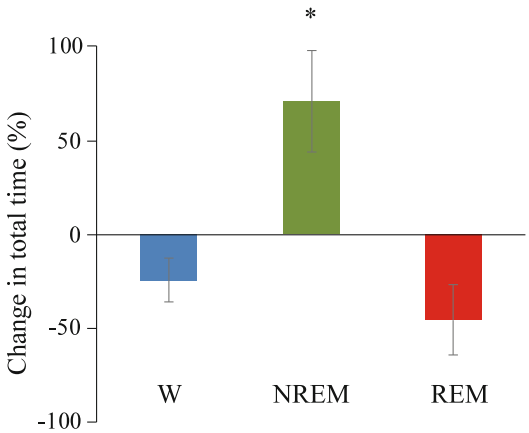

Fig. 11.3 Effects of the administration of 200 mg of Cannabis on sleep and wakefulness (W) during the first recording hour. The graphic chart shows the mean ± SEM of the change in the time (%) spent in W, NREM and REM sleep. The asterisk indicates significant differences ($p < 0.05$)

LG power was also decreased in left primary motor cortex (M1) and there was a tendency to decrease in right M1 during W (Fig. 11.4). This result is in accordance with other authors who demonstrated that THC administration reduces the power of EEG signal in several frequency bands in both hippocampus and neocortex (Willinsky et al. 1975); modifications either in the lower frequencies of the EEG (Böcker et al. 2010; Bounamici et al. 1982; Struve et al. 1999; Willinsky et al. 1975), as well as in LG oscillations have been observed (Cortes-Briones et al. 2015). Furthermore, Robbe et al. (2006) demonstrated that THC and a CB1 receptor agonist (CP559409) damp the power of theta, gamma and HFO in the hippocampus (Robbe et al. 2006).

During REM sleep in the light phase (Fig. 11.5), HFO was reduced in motor cortex and left S1 (also, a tendency to reduce was found in the right S1). HG was also reduced in left M1, while the right M1 tended to decrease.

Both gamma and HFO oscillations are related to cognitive functions (Bosman et al. 2014; Tort et al. 2013). Acute and chronic administration of Cannabis is known to induce cognitive alterations such as memory impairment (Crean et al. 2011; Shrivastava et al. 2011). Therefore, the reduction of HG and HFO power may be related to this effect. The effect Cannabis vaporization on EEG oscillations during REM sleep also suggests that Cannabis may affect dreams. In this regards, it has been hypothesized that the endocannabinoid system may modulate dream activity (Murillo-Rodriguez et al. 2017). In fact, post-traumatic stress disorder (PTSD) patients receiving Nabilone (a synthetic CB1 and CB2 receptor agonist) experienced either cessation or a

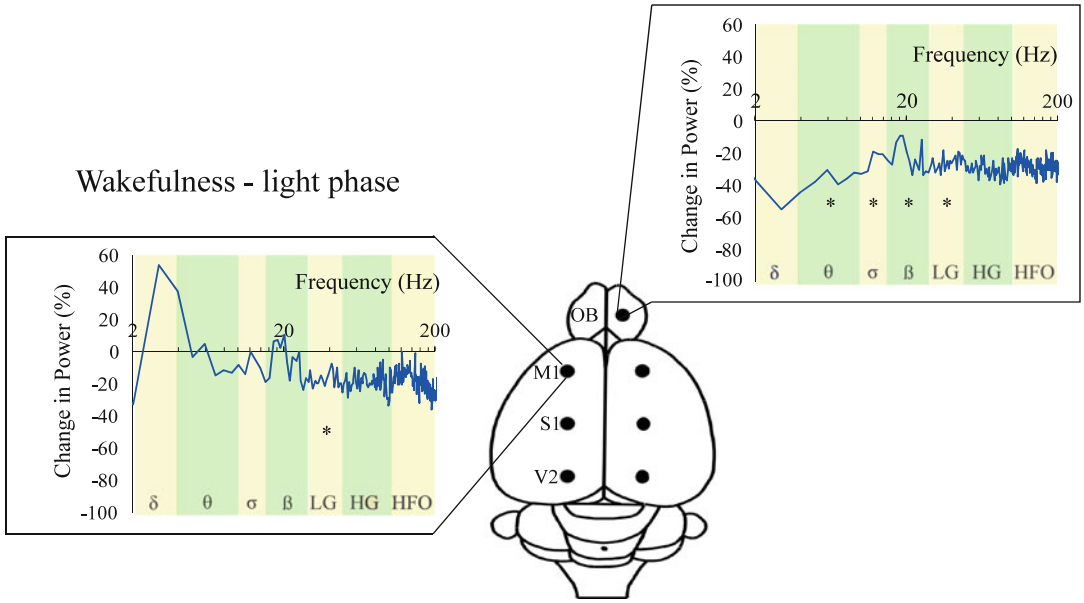

Fig. 11.4 Effects of the administration of 200 mg of Cannabis power spectrum during wakefulness (W) in the light phase. The figure shows a schematic representation of the localization of electrodes in the rat brain, and charts that exhibit the percentage of change in the power spectrum between Cannabis administration vs. control. The asterisks indicate significant differences ($p < 0.05$). OB, olfactory bulb; M1, primary motor cortex; S1, primary motor cortex; V2, secondary visual cortex; δ, Delta, 1–4 Hz; θ, theta, 5–9 Hz; σ, sigma, 10–15 Hz; β, beta, 16–30 Hz; LG, low gamma 31–48 Hz; HG, high gamma 52–95 Hz; HFO, high frequency oscillations, 105–200 Hz

significant reduction in nightmare intensity (Cameron et al. 2014; Fraser 2009).

No significant changes were detected with C_{200} administration in power spectrum during NREM sleep during the light phase.

We also evaluated whether Cannabis affects differentially the right and left hemisphere during the light phase, by means of comparing the power spectrum between right and left cortices following C_{200} administration. As it is shown in Fig. 11.6, no differences were found.

In regards to spectral coherence during the light phase, there was not effect of Cannabis neither during W nor during sleep.

11.6.3.2 Dark Phase

The EEG power was not affected during W or sleep (neither NREM nor REM sleep) in the dark phase. On the contrary, there was a decrease in the intra-hemispheric coherence of the sigma band between S1 and V2 cortices during NREM sleep. This result is illustrated in Fig. 11.7. We mentioned above that sigma band is associated with sleep spindles. Sleep spindles play a role in declarative memory (Fogel and Smith 2011); the phase coupling of hippocampal ripples, sleep spindles and slow oscillations is essential to memory consolidation during sleep (Latchoumane et al. 2017; Siapas and Wilson 1998). Also, spindles are the main factor able to synchronize the occurrence of hippocampal ripples involved in memory replay (Latchoumane et al. 2017). Hence, the reduction in the sigma coherence may be also related to the memory impairment produced by Cannabis (Ashton 2001).

It is interest to note, that patients with schizophrenia showed dramatic reductions of both spindles coherence and sleep-dependent memory consolidation (Wamsley et al. 2012). In this regards, exposure to Cannabis may increase memory deterioration in schizophrenia patients and in individuals with risk to develop psychosis.

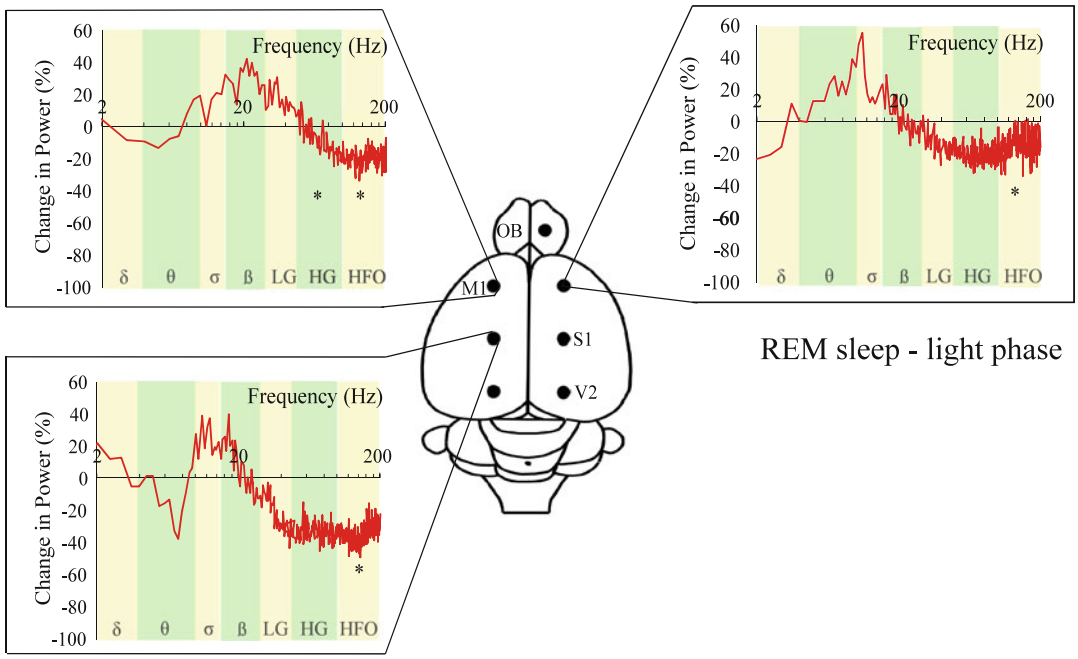

Fig. 11.5 Effects of the administration of 200 mg of Cannabis power spectrum during REM sleep in the light phase. The figure shows a schematic representation of the localization of electrodes in the rat brain, and charts that exhibit the percentage of change in the power spectrum between Cannabis administration vs. control. The asterisks indicate significant differences ($p < 0.05$). OB, olfactory bulb; M1, primary motor cortex; S1, primary motor cortex; V2, secondary visual cortex; δ, Delta, 1–4 Hz; θ, theta, 5–9 Hz; σ, sigma, 10–15 Hz; β, beta, 16–30 Hz; LG, low gamma 31–48 Hz; HG, high gamma 52–95 Hz; HFO, high frequency oscillations, 105–200 Hz

11.6.3.3 Day vs. Night Effects of Cannabis

C_{200} modified NREM sleep time and EEG power only during the light (resting) phase. On the contrary, C_{200} modified the EEG coherence only during the dark (active) period. How could those light/dark differences be explained? It has been demonstrated that the components of the endocannabinoid system show tissue-specific diurnal changes (Martínez-Vargas et al. 2003; Rueda-Orozco et al. 2008; Valenti et al. 2004). During the resting phase of the rats (lights-on period), the CB1 receptor density is higher, while the anandamide concentrations are diminished (Martínez-Vargas et al. 2003; Valenti et al. 2004). As THC is a partial CB1 agonist (Paronis et al. 2012), we hypothesized that it would exert a bigger effect during the lights-on period when the level of CB1 receptors is high, and there is less anandamide to compete with the receptors. Our results suggest that the administration of Cannabis during the "resting" phase of the day may be optimal to treat sleep difficulties. Clinical studies should be carried out to confirm this hypothesis.

11.6.4 Conclusions and Future Directions

Studying the effect of Cannabis on sleep can be challenging due to the high variations of cannabinoids and terpens profiles of Cannabis strains (Casano et al. 2011; Hazekamp et al. 2016). Nonetheless, research of characterizing different strains by metabolomic approaches is increasing (Fischedick et al. 2010; Hazekamp et al. 2016; Hillig and Mahlberg 2004).

The data reviewed demonstrates that in humans and animal models Cannabis modifies sleep and could be a good approach for treatment

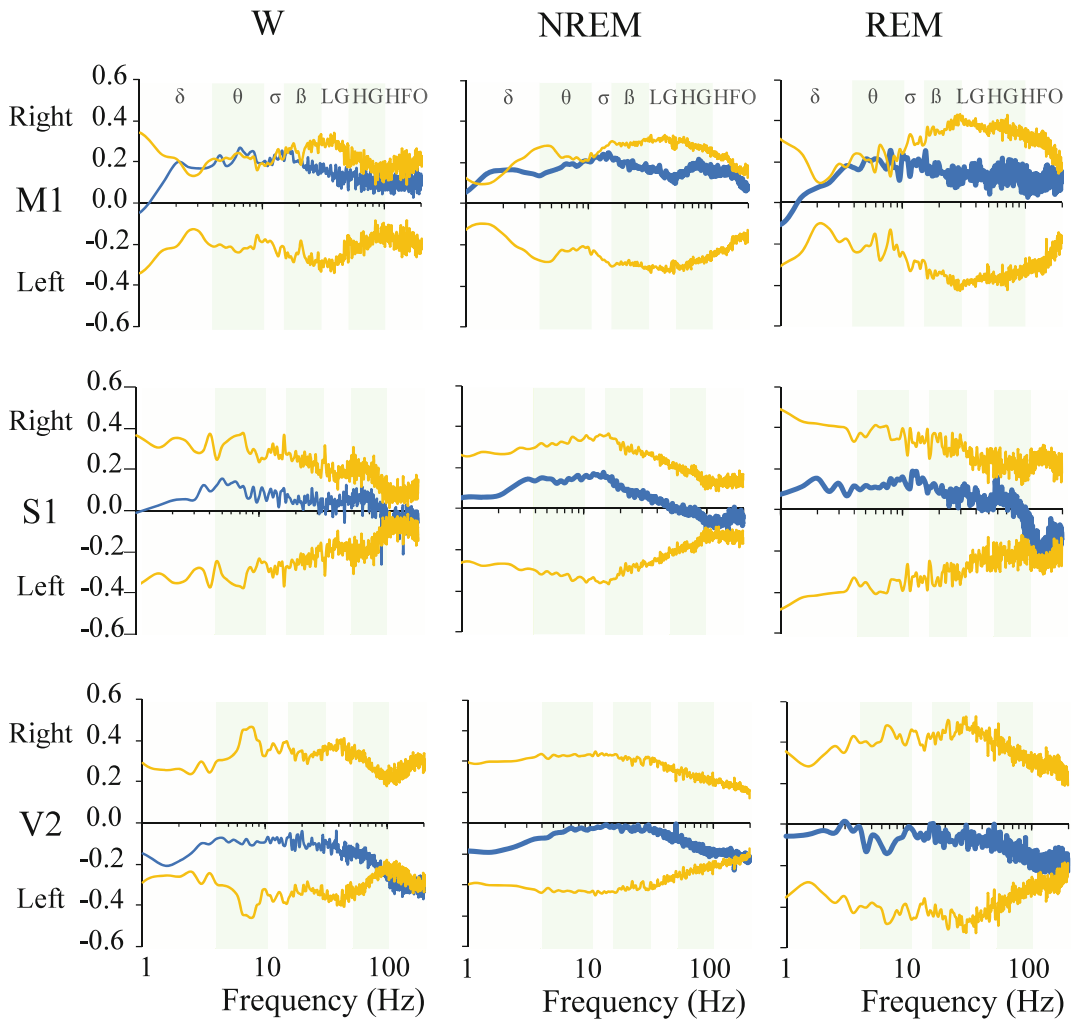

Fig. 11.6 Differential effect of Cannabis on the power spectrum between right and left cortices during wakefulness (W), NREM and REM sleep, in the light phase. Graphic charts show the mean (blue) and ± SD (yellow) of the following equation: (Power of right cortex − Power of left cortex)/(Power of right cortex + Power of left cortex). If power in right cortex is higher than left cortex, values are positive. If left cortex has higher power than right cortex, values are negative. No significant differences were found between right and left cortices. M1, primary motor cortex; S1, primary motor cortex; V2, secondary visual cortex; δ, Delta, 1–4 Hz; θ, theta, 5–9 Hz; σ, sigma, 10–15 Hz; β, beta, 16–30 Hz; LG, low gamma 31–48 Hz; HG, high gamma 52–95 Hz; HFO, high frequency oscillations, 105–200 Hz

of sleep disturbances. However, it is imperative to map the neurobiological effects of different Cannabis strains in order to achieve a more rational use of crude Cannabis for medical purposes.

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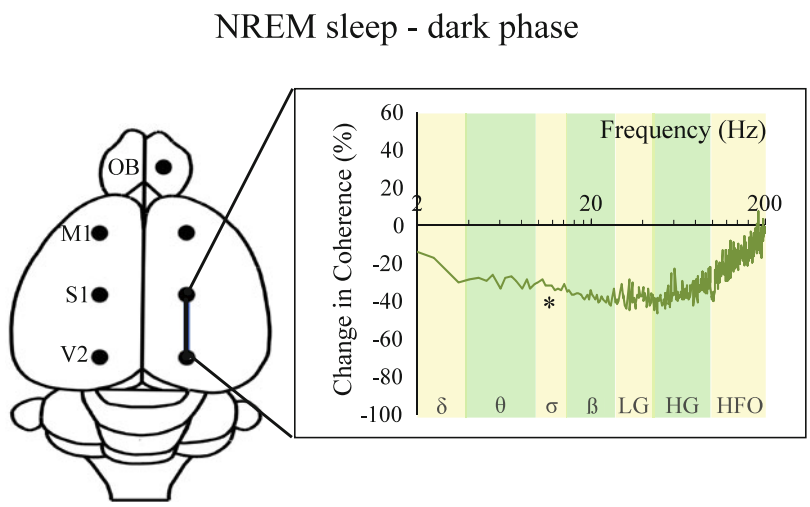

Fig. 11.7 Effects of the administration of 200 mg of Cannabis on the intra-hemispheric Z'coherence between right somatosensory cortex and right visual cortex during NREM sleep, in the dark phase. Graphic chart shows the percentage of change between Cannabis administration and control. The asterisk indicates significant differences ($p < 0.05$). OB, olfactory bulb; M1, primary motor cortex; S1, primary motor cortex; V2, secondary visual cortex; δ, Delta, 1–4 Hz; θ, theta, 5–9 Hz; σ, sigma, 10–15 Hz; β, beta, 16–30 Hz; LG, low gamma 31–48 Hz; HG, high gamma 52–95 Hz; HFO, high frequency oscillations, 105–200 Hz

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Addiction and Sleep Disorders

12

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Abstract

Shared neurophysiology of addiction and sleep disorders results in a bidirectional interplay. Diagnosing and treating primary sleep disorders, particularly in adolescents, can prevent the development of addiction in susceptible individuals. Addressing sleep issues in early recovery, and throughout maintenance, can prevent relapse. Cannabis use for insomnia shows mixed results; assisting with onset sleep latency in early use, this subsides with chronic use and holds addiction risk. Insomnia is a primary complaint of cannabis withdrawal syndrome and a primary cause of relapse in cannabis use disorder. An ideal sleep aid would prevent relapse and have low abuse potential. Pharmaceutical and behavioral options include suvorexant, mirtazapine, trazodone, and aerobic exercise, but clinical trials are lacking to demonstrate efficacy.

Keywords

Addiction · Sleep disorders · Insomnia · Relapse · Cannabis · Orexin

We are just beginning to navigate the interface of addiction and sleep disorders. When the neurobiology is considered, it is no surprise an interconnection exists. Both addiction and sleep disorders have their origin in life-preserving, phylogenetically ancient regions of the brain. They share neurotransmitters and neurocircuitry (Table 12.1). This manifests in a physiologic, emotional, and behavioral interplay, which are bidirectional (Ara et al. 2016; Conroy and Arnedt 2014; Pasch et al. 2012). Both conditions have the potential to initiate and exacerbate one another; research has shown sleep issues contributing to addiction (Roane and Taylor 2008; Kenney et al. 2014; Popovici and French 2013; Taylor and Bramoweth 2010; Wong et al. 2010; Hasler et al. 2012) as well as addiction resulting in sleep issues (Johnson and Breslau 2001; Zhabenko et al. 2013; Cohn et al. 2003; Schierenbeck et al. 2008; Chakravorty et al. 2016). Perhaps most importantly, if untreated this interplay can result in relapse (Budney et al. 2008; Babson et al. 2013a; Brower 2003; Brower and Perron 2010) that can be fatal in an individual with addiction.

12.1 Shared Neuroanatomy

Neuroanatomy of sleep is shared with addiction. The comparison shows shared brainstem and midbrain structures, basal ganglia, thalamus, and ascending tracts to the prefrontal cortex. When the development of the human brain is looked at evolutionarily, the most basic life-preserving structures are the oldest: the brainstem, which

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controls breathing and bodily functions; also nuclei for sleep and reward circuits, including basal ganglia (MacLean 1990). Without a primal reward system, an animal would not seek food or reproduction and the species would not survive. This is the same reward system that is hijacked by addiction and altered on a molecular level when chronically overstimulated. The sleep-wake cycle is also embedded in these deep structures of the brain, as being alert is key to survival. Thus both sleep and addiction are closely aligned in structure and function, both systems originating in animals before humans walked the earth.

12.2 Shared Neurotransmitters

Beyond brain structures, there are shared neurotransmitters in addiction and sleep; (Table 12.1) in particular--withdrawal and wakefulness; stimulants and wakefulness; reward and wakefulness; sedatives/alcohol and sleep promotion. Specific stress neurotransmitters of withdrawal originate in the extended amygdala: corticotropin-releasing factor (CRF), norepinephrine (NE), dynorphin, orexin; also NE from the locus coeruleus. This creates a state of dysphoria, irritability, anxiousness, and over-arousal of sympathetic tone (Koob 2010). Not surprisingly, these include neurotransmitters of wakefulness: NE and orexin, which promote alertness, and the sympathetic response that coincides with insomnia. Sleep pathways include inhibition via GABA, the main receptor for alcohol and sedative abuse. Anti-stress neurotransmitters include GABA and endocannabinoids (Kwako and Koob 2017). Worth noting is the role of the endocannabinoid system in the regulation of the circadian sleep-wake cycle, including the maintenance and promotion of sleep (Sanford 2008; Vaughn et al. 2010; Prospéro-García et al. 2016).

12.3 Focus on Orexin

Orexin is described as the "switch" hormone from sleep to wakefulness (Saper et al. 2001). It also functions to modulate reward pathways (Scammell and Saper 2007). The orexin neurons innervate and excite many brain regions that drive arousal and attention, including the locus coeruleus and the dorsal raphe. Orexins enhance signaling of the common reward pathway: dopamine from the mesolimbic projections between the ventral tegmental area and the nucleus accumbens.

Clinically, orexin-antagonists have been successful in treating insomnia (Michelson et al. 2014). Given their functional overlap with reward, there has been recent interest in addiction applications (Baimel et al. 2015). In preclinical animal models, pharmacological antagonism of the OX1 receptor reduced relapse-like behaviors for opiates, psychostimulants, alcohol, and cannabinoids. Antagonism of the OX2 receptor has also been shown to reduce self-administration of both opiates and alcohol (Khoo and Brown 2014).

12.4 Cycles Intertwined

Both addiction and sleep are described as cycles. While sleep clinicians work to regulate the sleep cycle, addictionologist attempt to break the addiction cycle. Koob and Volkow describe the cycle of addiction with three stages, each correlating with major neurocircuits (Koob and Volkow 2010, 2016).

Binge/intoxication (basal ganglia): controls reward and pleasurable effects of use, responsible for the formation of habitual substances taken. Withdrawal (extended amygdala): stress and feelings of unease, anxiety, and irritability that accompany withdrawal, including insomnia. Preoccupation/anticipation (prefrontal cortex): executive function, ability to organize thoughts and make decisions, including exerting control over substance use.

Addiction develops when neuroadaptations occur in the ascending mesocorticolimbic dopamine (DA) system; supraphysiologic levels of DA at the nucleus accumbens with chronic use leads to downregulation of DA2 receptors and a dysfunctional reward system. Conditioned cues

Table 12.1 Shared neurotransmitters (Kwako and Koob 2017; Siegel 2004; Brown et al. 2012; Murillo-Rodríguez et al. 2012)

Wake	*Addiction withdrawal*
DA	CRF
ACh	ACh
NE	NE
Glutamine	Glutamine
Orexin	Orexin
5HT	Glutamate
Hist	Dynorphin
Sleep	*Addiction*
GABA	GABA
Endocannabinoids	Endocannabinoids
Galanine	Endogenous opioids
Adenosine	DA

can still elicit DA release and produce cravings, which can perpetuate the cycle. Most important for this discussion is the Withdrawal stage, where there is increased CRF and other anti-reward neurotransmitters by the amygdala. This drives symptoms such as anxiety, irritability, dysphoria, and insomnia, during acute and protracted withdrawal. This so-called dark side of addiction can also perpetuate the cycle as an individual return to binging for relief (Koob 2009, 2010).

The neuroadaptations that occur, along with the symptoms and lifestyle irregularities that manifest during the cycle of addiction will often disrupt the normal sleep cycle. Additionally when there is a primary sleep disorder, (insomnia, parasomnias, sleep apnea) the shared neurophysiology can set the stage to 1) Initiate use of controlled substances: alcohol, benzodiazepines, cannabis, to address insomnia; use of stimulants for alertness (i.e. sleep apnea, circadian disorders). 2) Exacerbate existing addiction, or push substance abuse to addiction. 3) Push an individual in recovery to relapse: insomnia with irritability; sleep apnea through less energy; restless leg syndrome via poor sleep, etc.

Cycles have been intertwined historically, as a treatment for insomnia for many years was alcohol, opium, and barbiturates. After 1960, this was supplemented by benzodiazepines. All of which are substances of abuse and addiction, with a dependence that can perpetuate further insomnia. This trend continues with large scale use of benzodiazepines (5.6% of Americans in 2013) for anxiety and insomnia (Bachhuber et al. 2016); and belief by the public that smoking cannabis assists with sleep (Bonn-Miller et al. 2014).

12.5 Specific Substances of Abuse

Stimulants (cocaine, methamphetamine, caffeine) create wakefulness to the extreme, disrupting circadian rhythms and resulting in a physiologic and emotional crash. Sedatives (benzodiazepines, barbiturates, alcohol) acutely cause somnolence, and are often used for insomnia; however, with chronic use, GABA receptors are down-regulated and deformed, and glutamate is up-regulated in response to chronic depression. This results in an upsurge in glutamate, hyperactive arousal, and withdrawal symptoms when chronic use stops. Opioids, natural and synthetic, have varying degrees of respiratory depression due to mu receptor activation of the medulla oblongata (Shook et al. 1990) and are associated with increased central sleep apnea. Parasomnias can be present in acute and post-acute withdrawal; "kicking the habit" is derived from kicks of the legs during withdrawal. Insomnia notoriously presents for weeks or months after chronic opioid cessation (Kleber 2007).

12.6 Focus on Cannabis

Cannabis use in the US has increased recently, from 4 to 9.5% during 2001–2013; and the prevalence of cannabis use disorder (CUD) has increased from 1.5 to 2.9% (Hasin et al. 2015). This coincides with a decrease in the perceived risk of harmful effects from cannabis (Hasin et al. 2015; Carliner et al. 2017). Although significant harmful effects have been described (Volkow et al. 2016; Zehra et al. 2018) including acute impairment of learning, attention, and working memory; an amotivational syndrome; and a preventable risk for psychosis (Schizophrenia Commission 2012). Any cannabis use increases the risk of schizophrenia twofold (van Os et al. 2002), and frequent use of high potency cannabis increases the risk of schizophrenia sixfold (Andréasson et al. 1987). One concern with psychosis is increasing psychoactive potency. Average tetrahydrocannabinol (THC) content of confiscated cannabis has increased from 3.8% in the early 1990s to 12.2% in 2014 (Mehmedic et al. 2010). This increase in potency is linked with an increased risk of chronic psychosis (Di Forti et al. 2014).

The previously described cycle of addiction applies to cannabis. Acute THC elicits DA release in the shell of the nucleus accumbens in humans at supraphysiologic levels (Stokes et al. 2010; Bossong et al. 2015; Bloomfield et al. 2016), establishing the cycle of addiction in susceptible individuals. Recent data suggest that 30% of those who use cannabis may have some degree of CUD (Hasin et al. 2015). Those who start using cannabis before the age of 18 are 4–7 times more likely to develop a CUD than adults (Winters and Lee 2008). Cannabis withdrawal, with insomnia as a chief complaint, is well documented and described further on.

12.7 Cannabis and Sleep Disorders

Individuals often report the use of cannabis for insomnia (Bonn-Miller et al. 2014; Tringale and Jensen 2011; Belendiuk et al. 2015). The data on cannabis and insomnia is complex. Early studies with whole-plant cannabis showed mixed results (Babson et al. 2017; Gates et al. 2014), partly due to issues in methodologies (small studies, lack of statistical control), but also due to the heterogeneity of whole-plant cannabis. Recent work continues to show mixed findings--reports of both decreased sleep latency, and poor sleep quality (Tringale and Jensen 2011; Ogeil et al. 2015), suggesting cannabinoid concentration, dose, and route of administration may have differential effects on sleep quality and insomnia symptoms.

A 2017 literature review of cannabis and sleep (Babson et al. 2017), summarized: "Taken together, research suggests that short-term use of cannabis may have a therapeutic impact on sleep, specifically related to sleep onset latency and slow-wave sleep. However, long-term chronic use is associated with habituation to the sleep-enhancing benefits and is associated with increased risk for cannabis dependence. Sleep disruption (self-reported and objective) is a primary withdrawal symptom from cannabis and may play a role in cannabis lapse/relapse during cessation attempts."

There has been an interest in using specific cannabinoids for insomnia. An early study in 1981, reported benefits of 160 mg cannabidiol (CBD) for insomnia in 15 patients who reported more sleep without correlating polysomnography (PSM) (Carlini and Cunha 1981). A preclinical study showed an increase in the total percentage of sleep in rats after administration of mid-range and high dose CBD injections as compared to placebo (Chagas et al. 2013). A recent double-blind, placebo-controlled study compared administration of placebo, 15 mg THC, 5 mg THC/CBD, and 15 mg THC/CBD at bedtime in chronic marijuana users. The study used PSM but included only eight patients. There were no effects of 15 mg THC on nocturnal sleep. With the 5 mg THC/CBD, there was a decrease in stage 3 sleep, and with the 15 mg THC/CBD combination, wakefulness was increased (Nicholson et al. 2004). Another recent double-blind study gave 300 mg of CBD to 27 people with PSM and showed no differences in sleep architecture from placebo (Linares et al. 2018). A 2014 review of

39 studies on the effect of cannabinoid administration on sleep concluded: "Findings were mixed and showed various effects of cannabinoid administration on several aspects of sleep. Methodological issues in the majority of studies to date, however, preclude any definitive conclusion." (Gates et al. 2014).

12.8 Cannabis Withdrawal and Sleep

There is a vast body of data that describes the phenomenon of cannabis withdrawal. A 2017 review of cannabis withdrawal syndrome (CWS) sited 130 reference articles on this subject (Bonnet and Preuss 2017). The review reported consistent evidence of CWS occurring in 90% of patients with cannabis dependence. Clinical diagnosis of cannabis withdrawal includes: irritability, anger, anxiety, decreased appetite, restlessness, depressed mood, tremors, sweating, fever, chills, headache, and sleep disturbances (Karila et al. 2014). Sleep disturbances include: trouble falling asleep, decreased total sleep time, and the presence of nightmares and strange dreams (Gates et al. 2014). Typically symptoms occur 1–2 days after cessation of heavy use and can last between 7 and 45 days (Budney et al. 2003). Self-reported sleep difficulty is considered a hallmark of cannabis withdrawal (Vandrey et al. 2011). Survey studies report sleep difficulty during attempts to quit cannabis occurs in 67–73% (Budney et al. 1999, 2008). Sleep difficulty has been consistently rated as one of the most severe symptoms of cannabis withdrawal (Budney et al. 2001, 2003; Vandrey et al. 2008).

Objective sleep disturbances during CWS have been demonstrated with PSM. A cross-sectional study using PSM (Bolla et al. 2010), demonstrated that abrupt cannabis cessation among heavy users was associated with a decrease in total sleep time, sleep efficiency, and the amount of REM sleep. Besides, increases in wake after sleep onset, sleep onset latency, and periodic limb movements were observed. Another study of PSM-measured sleep in a sample of heavy cannabis users alternated between periods of cannabis use and an abstinence phase supplemented by the administration of either a placebo or zolpidem. Results demonstrated that abrupt cessation was associated with an increase in sleep onset latency and the amount of REM, while a decline in sleep efficiency was observed (Budney et al. 2001).

12.9 Cannabis Relapse and Sleep

Multiple studies indicate poor sleep is a critical risk factor for predicting cannabis relapse (Budney et al. 2008; Babson et al. 2013a, b). Poor sleep quality prior to a quit attempt has also been shown to increase the risk of early relapse to cannabis (Babson et al. 2013a). Not surprisingly, poor sleep after quitting increases the risk of relapse, with 65% of cannabis users reporting poor sleep as the primary reason for relapse by one report (Budney et al. 2008).

12.10 Clinical Considerations

Addressing sleep issues in individuals with addiction, in early recovery, and during disease maintenance, can prevent relapse. When selecting therapeutic interventions for insomnia, agents with low addiction potential and benefits beyond somnolence are preferable. The risk of shifting to another substance of abuse is heightened, due to neuroadaptations described above. Benzodiazepines (including selective receptor subtypes) and barbiturates are not recommended due to high abuse potential. If we can select medications or behaviors that promote sleep and assist abstinence, the bidirectional relationship of shared neurophysiology can work in the individual's favor.

Cannabis withdrawal has insomnia as a chief complaint and a major cause of relapse. An ideal sleep agent would alleviate insomnia, prevent relapse, and have no addiction potential. Cannabinoid agonist treatments for CUD have been considered. Dronabinol (a synthetic form of THC), and nabiximols (1:1 THC:CBD) significantly reduced withdrawal symptoms but did not

reduce cannabis use, or time to relapse (Balter et al. 2014). Nabilone (synthetic TCH analog) was reported to reduce cannabis withdrawal sleep disturbance, along with irritability and anorexia, and reduce relapse in an initial study of 11 individuals (Haney et al. 2013). The orexin antagonist suvorexant (Belsomra) is a new option that may aid sleep and prevent relapse (Baimel et al. 2015). Mirtazapine has been beneficial in reducing CWS insomnia (Haney et al. 2014), can function as an antidepressant, and has low abuse potential. Trazodone is often used as a sleep aid in early recovery, has efficacy with insomnia and low abuse potential. Lofexidine, recently approved in the US for opioid withdrawal, was shown to improve sleep and reduce cannabis relapse in a small study (Haney et al. 2008). Gabapentin is more effective than lorazepam for sleep for people in early recovery (Malcolm et al. 2007; Karam-Hage and Brower 2003), and there is some evidence it can prevent relapse of CUD (Mason et al. 2012). There have been recent concerns about abuse potential (Schifano 2014), thus individual risk/benefit should be weighed; involving family members, and doing pill counts can help in the outpatient setting.

Nonpharmacological treatments with proven benefit for sleep issues in recovery include Mindfulness meditation, Cognitive Behavioral Therapy for insomnia, and good sleep hygiene. Exercise should always be considered; free and without adverse effects, regular aerobic exercise can reduce insomnia (Reid et al. 2010; Passos et al. 2010), reduce relapse (Zschucke et al. 2012; Lynch et al. 2013), and even increase D2 receptor density in the nucleus accumbens for individuals with addiction (Robertson et al. 2016).

12.11 Conclusion

Shared neurophysiology of addiction and sleep disorders results in a bidirectional interplay. Diagnosing and treating primary sleep disorders, particularly in adolescents, can prevent the development of addiction in susceptible individuals. Addressing sleep issues in early recovery, and throughout maintenance, can prevent relapse. Screening for sleep issues in people with addiction should be an integral part of care. Insomnia is a primary complaint of CWS and a primary cause of relapse in CUD. An ideal sleep aid would prevent relapse and have low abuse potential. Pharmaceutical and behavioral options are available, but clinical trials are lacking to demonstrate efficacy. Knowledge of the shared neurocircuitry should assist in the development of agents that can address both sleep issues and addiction.

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Cannabis for Restless Legs Syndrome

13

Imad Ghorayeb

Abstract

Restless legs syndrome (RLS) is a chronic sensorimotor disorder characterized by an urge to move the legs. This urge is often accompanied by pain or other uncomfortable and unpleasant sensations, it either occurs or worsens during rest, particularly in the evening and/or at night, and temporarily improves with activity. Affecting nearly 3% of the North American and European populations in its moderate-to-severe form, RLS has a considerable negative impact on the quality of life, and sleep and is associated with significant morbidity. Although new developments have deepened our understanding of the disorder, yet, the corresponding pathophysiologic features that underlie the sensorimotor presentation are still not fully understood. Usually, symptoms respond well to dopamine agonists (DA), anticonvulsants, or opiates, used either alone or in any combination, but still, a subset of patients remains refractory to medical therapy and serious side effects such as augmentation and impulse control disorder may occur in patients with RLS under DA. Convincing treatment alternative are lacking but recently patients' spontaneous reports of a remarkable and total remission of RLS symptoms following cannabis use has been reported. The antinociceptive effect of marijuana has been documented in many painful neurological conditions and the potential benefit of cannabis use in patients with refractory RLS should, therefore, be questioned by robust clinical trials. Here, we review basic knowledge of RLS and the putative mechanisms by which cannabis may exert its analgesic effects.

Keywords

Restless legs syndrome · Cannabis · Treatment

13.1 Introduction

Restless Legs Syndrome (RLS) is one of the most disabling and sometimes painful sensorimotor ailment of the nervous system that has only in recent years become more widely accepted as a clinical disorder with its distinct features (Barriere et al. 2005). Yet in the literature, evidence of this debilitating condition goes back to 1672 when Sir Thomas Willis first described a medical condition that caused restlessness and unpleasant sensations in the limbs, mostly during rest periods or before sleep, but it was the Swedish neurologist Karl Axel Ekbom whom first defined and used in the

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mid-1940s the term Restless Legs Syndrome for a "*hitherto overlooked disease in the legs*" (Axel 1945). Similarly, the diagnostic criteria of RLS were not well established until 50 years later (Walters 1995) and again update in 2014 (Allen et al. 2014).

13.2 Definition

Restless Legs Syndrome (RLS) is a neurological sensorimotor disease with an unknown natural history that profoundly disturbs sleep and quality of life (Barriere et al. 2005). One of the key diagnostic criteria of RLS is the complaint of an irresistible urge to move the legs. This urge to move is often triggered by uncomfortable, unpleasant, and sometimes painful sensations, it always occurs or worsens at rest, particularly late in the day or at sleep time, and is temporarily relieved with movement. Diagnostic criteria were revised in 2014 by the International Restless Legs Syndrome Study Group (Allen et al. 2014) (Table 13.1). Features supporting the diagnosis of RLS, particularly when there is some lack of diagnostic certainty, are periodic limb movements during sleep (PLMS), a sleep-related phenomenon characterized by periodic episodes of repetitive and highly stereotyped limb movements, as about 88% of RLS patients have an objective motor sign of repetitive PLMS on polysomnography (Montplaisir et al. 1997).

13.3 Epidemiology

With an overall lifetime prevalence of 6–12% in the general Caucasian population, independent of the severity, the RLS is one of the most common neurological disorders (Berger and Kurth 2007). When a differential diagnostic approach is considered, prevalence estimates fall to 1.9–4.6% of European and North American general adult populations and about 2–3% of adults suffer from clinically significant symptoms (Ohayon et al. 2012). Restless Legs Syndrome prevalence in women is approximately double that of men across all populations and ages, it also affects 2–4% of school-aged children and adolescents (Ohayon et al. 2012; Picchietti et al. 2013). The disease-specific, health-related and psychosocial quality of life (QoL) of patients with RLS is reduced compared to the general population and is comparable to that of patients with type 2 diabetes mellitus and osteoarthritis (Allen et al. 2005; Happe et al. 2009). The lifetime prevalence of comorbid depression and anxiety disorders is elevated by odds ratios of 2.1–5.3 in RLS compared to the community at large (Winkelmann et al. 2005; Lee et al. 2008). In addition, it has been documented that comorbidity exists between RLS and obsessive-compulsive disorder and attention-deficit/hyperactivity disorder thereby questioning the existence of distinct RLS phenotypes (Ghorayeb et al. 2017). Several additional factors or comorbidities have been identified (gender, pregnancy, late-stage renal disease) that are associated with RLS symptoms but causality has not yet been confirmed. Although pediatric cases of RLS do exist, the disorder usually emerges later in life, and the lack of a single clear identified trigger or cause suggests that RLS is a disorder of multi-factorial origin. Sleep problems, leg dysesthesias, and the psychological sequelae of the disorder are all particularly implicated in contributing to impair the daily functioning of affected patients (Kushida et al. 2007; Winkelman et al. 2009). The negative impact on QoL is profound and RLS is associated with a substantial economic burden (Durgin et al. 2015).

13.4 Pathophysiology

Despite a gradual and substantial increase in scientific studies over the past decade, our understanding the pathophysiology of RLS is, at best, incomplete (Chokroverty 2014) and attempts to integrate the salient clinical, experimental neurobiological and RLS genetic findings into a heuristic pathogenetic model await a broader scientific consensus (Ferre et al. 2018). Brain iron homeostasis imbalance and consequent dopaminergic neurotransmission abnormalities are thought to play a central role in the pathogenesis of this

Table 13.1 Essential diagnostic criteria for RLS

1. An urge to move the legs usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs.
2. The urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting.
3. The urge to move the legs and any accompanying unpleasant sensations are partially or relieved by movement, such as walking or stretching, at least as long as the activity continues
4. The urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day.
5. The occurrence of the above features is not solely accounted for as symptoms primary to another medical or a behavioral condition.

disorder, possibly along with other non-dopaminergic systems, mainly glutamatergic and adenosinergic (Meiser et al. 2013).

Arguments for a dopaminergic prominent role are to be found in the excellent pharmacological response to low-dose dopaminergic medications. Evidence of biochemical alterations in the dopaminergic system of patients with RLS has been documented with abnormally high levels of the dopamine metabolite 3-ortho-methyldopa (3-OMD) in the cerebrospinal fluid (CSF) (Allen et al. 2009), decreased striatal dopamine transporter (DAT) and dopamine-2 receptor (D_2R) binding potential (Earley et al. 2011, 2013), and a significant increase in tyrosine hydroxylase activity in the striatum and substantia nigra in human tissue (Connor et al. 2009). This would be mostly compatible with a dopaminergic abnormality characterized as an overly activated dopaminergic system at the presynaptic level (Earley et al. 2014). A key factor in the development of a hyperdopaminergic state is the altered brain iron homeostasis that is considered so far as the primary pathogenetic mechanism of RLS (Earley et al. 2014). In patients, low CSF-iron and ferritin concentrations and increased CSF-transferrin suggesting brain iron insufficiency was documented (Earley et al. 2000; Mizuno et al. 2005), and direct magnetic resonance imaging (MRI) assessment of brain regional iron relative concentration further supported these findings (Earley et al. 2006; Allen et al. 2001; Li et al. 2016). Pathological autopsy studies also showed a decreased iron staining in neuropils consistent with MRI data and with impaired brain iron acquisition in RLS (Connor et al. 2003, 2004, 2011; Pittock et al. 2004).

Experimental manipulation of iron in non-human primates (NHP) and rodents highlighted the pivotal role of iron in the modulation of the dopaminergic system since it reproduces the main alterations in dopaminergic transmission observed in RLS patients. In serum iron-depleted macaques, an increase in nigrostriatal dopaminergic function together with a specific alteration of the serotonin/dopamine interaction in the central nervous system was documented (Hyacinthe et al. 2015). In rats with nutritional iron deficiency, an altered synthesis and functioning of DAT were thought to account for the dramatic increase in dopamine extracellular levels in the caudate-putamen (Bianco et al. 2008; Nelson et al. 1997; Beard et al. 1994) and a significant reduction in DAT density was also demonstrated (Erikson et al. 2000). Iron deficiency also decreases D_1 and D_2 receptor density in the caudate-putamen of rat brains (Ashkenazi et al. 1982; Erikson et al. 2001; Ben-Shachar et al. 1985). Similarly, severe iron depletion in rodents was shown to induce a central hypoadenosinergic state with downregulation and upregulation of striatal adenosine A_1 (A_1R) and A_{2A} receptors ($A_{2A}R$), respectively (Quiroz et al. 2016). As adenosine exerts a tight antagonistic control of the dopaminergic system, the relation between adenosine neurotransmission and brain iron status is by now considered as an important pathway by which adenosine may alter the function of the dopaminergic system thus providing the link for a putative unified pathophysiological mechanism of RLS (Ferre et al. 1997, 2018) and supporting, from a pure electrophysiological perspective, a complex sensory-motor disorder in which

cortical, subcortical, spinal cord and peripheral nerve generators are all involved in a network disorder, resulting in enhanced excitability and/or decreased inhibition (Lanza et al. 2017). Further support of disturbed central and peripheral excitability in RLS is provided by the effectiveness of non-pharmacological tools, such as repetitive transcranial magnetic stimulation and transcutaneous spinal direct current stimulation, in transiently modulating neural excitability in RLS patients, therefore extending the therapeutic repertoire (Lanza et al. 2017). Also central to the pathophysiology of RLS, the spinal sympathetic axis seems, in and of itself, to play a role not only in the expression of RLS but also in its persistence through recurrent pathways (Clemens et al. 2006). The neural elements within this loop are a site of convergence for not only dopamine diencephalon-spinal A11 pathway, the dopaminergic source to the spinal cord (Barraud et al. 2010), but additional neurotransmitter pathways originating supraspinally that may have clinical relevance to RLS (Garraway and Hochman 2001). Because dopamine's actions on sympathetic preganglionic neurons in the spinal cord are predominantly inhibitory, its absence would favor increased sympathetic basal tone (Clemens et al. 2006). Anatomic data also indicate that leg muscles and muscle spindles receive significant sympathetic innervation and evidence suggests that sympathetic stimulation increases muscle spindle activity independent of changes in blood supply (Barker and Saito 1981). In this regard, increased sympathetic activity may be indirectly involved in inducing muscle restlessness and pain by excessively increasing neuromuscular derived muscle tone.

Mounting evidence also indicates that altered genetic characteristics of select genes during early development may provide the pathophysiological environment for RLS to emerge later in life. A recent study identified and replicated 13 new risk loci for RLS and confirmed the previously identified six risk loci, *MEIS1, BTBD9, PTPRD, MAP 2K5, SKOR1*, and *TOX3*. Although MEIS1 was confirmed as the strongest genetic risk factor for RLS, mouse models based on decreased *MEIS1* expression failed to recapitulate the most cardinal RLS phenotype and associated neurochemical abnormalities (Salminen et al. 2017).

13.5 Treatment

Management of RLS is primarily pharmacological as nonpharmacological options are, at best, limited and, aside from iron replacement therapy, only symptomatic treatments are used.

Since the serendipitous observation by Akpinar in 1982 of the benefit of levodopa for RLS, dopamine agonists (DA) remain the first-line therapy in de novo patients with severe to moderate RLS although other off-label drugs are increasingly firstly prescribed (Akpinar 1982; Limousin et al. 2018).

Nonergot-derived DA, mainly ropinirole, pramipexole, and rotigotine, show efficacy which is, however, in many cases moderate, and the majority of patients do not experience full remission in drug trials (Winkelmann et al. 2018). Furthermore, long term dopaminergic treatment efficacy is hampered by the gradual tolerance and emergence of adverse effects that cause (i) augmentation, which is a worsening of symptoms severity such as the earlier onset of symptoms at rest, spread to different body parts, increased intensity of RLS severity, and shorter effect of medication, and (ii) impulse control disorders (ICD), such as gambling disorder, compulsive sexual behavior, compulsive shopping, binge eating, or punding. A US community-based study estimated that at least 20% of the patients have definitive or highly suggestive clinical symptoms of augmentation with a yearly incidence rate of approximately 8% (Allen et al. 2011) and another study showed that 20.7% of patients with RLShave developed definitive ICD under DA (Heim et al. 2016). These major side effects often require DA discontinuation leaving the patients helpless.

Alternative off-label drugs, originally developed to combat other diseases including neurological pain conditions, such antiepileptic α2-δ voltage-gated calcium channels ligands, benzodiazepines (clonazepam), or opioids, either prescribed alone or in any combination, are

alternative second-line treatment strategies but mounting evidence recommends α2-δ ligands as first-line treatment of RLS to prevent DA side effects (Chenini et al. 2018).

The efficacy of α2-δ ligands, mainly pregabalin, gabapentin enacarbil, and gabapentin has been documented in many RLS clinical trials. This efficacy is by now considered at least equivalent to therapeutic doses of the DA (Winkelmann et al. 2018). Although clinical experience with very long-term use of α2-δ ligands in RLS has generally not been documented, it is admitted that they do not have the dopaminergic problems of augmentation, compulsive behaviors, or excessive daytime sleepiness.

In refractory RLS, opioids have demonstrated good efficacy (Trenkwalder et al. 2015). However, adverse side effects consistent with the safety profile of opioids such as fatigue, constipation, nausea, headache, somnolence, dizziness, dry mouth and pruritus, may complicate the use of these class drugs, particularly in the elderly. Opioid-induced hyperalgesia was also described in patients with refractory RLS (Chokroverty 2015).

Similar to other opioids, methadone was shown to effectively treat severe refractory RLS (Ondo 2005). Methadone, a long-acting μ-specific opioid agonist, is most frequently used to treat narcotic addiction because it has less abuse potential than other narcotics. In a 10-year, longitudinal assessment of DA and methadone in the treatment of RLS, methadone, in contrast to DA, shows neither augmentation nor major problems with continued efficacy after the first year of treatment (Silver et al. 2011).

Although there is insufficient evidence to support the use of benzodiazepines in RLS, just like α2-δ ligands, clonazepam has been largely used for treating numerous neurological pain conditions including neuropathic pain and RLS. Owing to its central action via the gamma-aminobutyric acid (GABA) receptors, its anxiolytic effect may improve sleep initiation and maintenance in patients with comorbid anxiety (Limousin et al. 2018). In RLS, and to avoid the possible development of tolerance and dependency, clonazepam could be used on an ad hoc basis in case of symptoms severity exacerbation or patients with intermittent symptoms (Limousin et al. 2018; Saletu et al. 2001).

Based on the recent evidence of a central hypoadenosinergic state in rodents with brain iron deficiency, a prospective open-label, non-placebo controlled clinical trial showed mitigated efficacy of dipyridamole, a non-selective inhibitor of adenosine transport, as only 6 out of 13 enrolled patients were full responders (Garcia-Borreguero et al. 2018).

Other avenues, including iron supplementation therapies, are explored to mitigate some of the contributing factors with however unpredictable results. While the benefits of oral iron are limited, intravenous ferric carboxymaltose is considered to be likely efficacious for the treatment of RLS in patients with serum ferritin levels $\leq$100 μg/l with or without anemia, but more data are required to confirm the efficacy and long term tolerance of intravenous iron (Allen et al. 2018).

In conclusion, although medications are available to treat RLS, many patients either experience side effects that prevent them from continuing on the medication or do not sufficiently respond to current RLS medications.

13.6 Cannabis in RLS

Although there is substantial evidence that cannabis is an effective treatment for chronic pain in adults, the analgesic effects of cannabinoids have not been sufficiently beneficial to be used clinically because of their potential for abuse and undesirable central effects (Abrams 2018; Whiting et al. 2015). Interestingly, a recent review on the efficacy of medical marijuana in several neurologic conditions showed that the risk of serious adverse psychopathologic effects was nearly 1% (Koppel et al. 2014). This is far from the estimated 20.7% of patients with RLS that develop definitive impulse control disorders under DA (Heim et al. 2016). Cannabis could also be considered as a potential alternative to prescription opioids for treating chronic pain (Vigil et al. 2017). In RLS, the potential benefit

of cannabis use in RLS is presently unknown. Only one short paper reported the efficacy and tolerability of cannabis in a few patients with refractory RLS (Megelin and Ghorayeb 2017) (Since this fisrt report, I have published a second paper on the efficacy of cannabis in RLS. Would it be possible to add this new reference? so that would make two short papers instead of one). In these patients, compared to previous treatment trials for their RLS, occasional cannabis use was described to be the most effective and the best tolerated with improvement in sleep quality. Whether the latter is related to the potential anxiolytic and sedative-hypnotic effects of cannabis or to PLMS suppression warrants further research (Abrams 2018). The limitations of this report mostly relate to the absence of a polysomnographic control of cannabis efficacy on objective sleep parameters and PLMS. Patients' subjective estimation of the cannabis efficacy may also be skewed by the psychoactive and anxiolytic properties of the drug. Well-controlled clinical trials are therefore required to test the short/long-term effectiveness and safety of medical cannabis in RLS.

13.7 Putative Mechanism of Action of Cannabis on RLS

As a natural component of human physiology, the endocannabinoid system, consisting of the cannabinoid type 1 receptor (CB_1R) and cannabinoid type 2 receptor (CB_2R), endogenous cannabinoid ligands (endocannabinoids), and metabolizing enzymes, is present throughout the pain pathways (Starowicz and Finn 2017). Endocannabinoids function primarily as retrograde messengers that inhibit neurotransmitter release via presynaptic CB_1Rs, which are distributed primarily in GABAergic and to a lesser extent glutamatergic and other presynaptic terminals (Benarroch 2014). Endocannabinoids may also act via postsynaptic CB_1Rs and, depending on neuron type, glutamatergic or GABAergic, activation of CB_1Rs may result in inhibition or activation of the neuronal circuit. Cannabinoid type 1 receptors are widely distributed at peripheral, spinal, or supraspinal sites where cannabinoids exert their analgesic effects likely through inhibition of presynaptic neurotransmitter and neuropeptide release and modulation of postsynaptic neuronal excitability. Unlike CB_1Rs, CB_2Rs are primarily expressed in the periphery, primarily in immune cells (Benarroch 2014). Few CB_2Rs are located in the brain, spinal cord, and dorsal root ganglion, but they increase in response to peripheral nerve damage there by to regulate neuro-immune interactions that mediate the inflammatory hyperalgesia (Starowicz and Finn 2017; Benarroch 2014).

Relevant to RLS hypothetical pathophysiology, complex interactions between endocannabinoids and other neurotransmitter systems, mainly monoaminergic, have been reported (Benarroch 2007). Cannabinoids regulate the neuronal activity of noradrenergic and serotoninergic cells and the release of noradrenaline and serotonin by direct and indirect mechanisms. This may underlie several behavioral effects induced by cannabis, including anxiolytic, antidepressant, and antinociceptive effect (Mendiguren et al. 2018). Evidence also indicates that chronic cannabis use is associated with reduced dopamine synthesis capacity which, in line with the hyperdopaminergic state that may underlie RLS symptoms, may account for the reported efficacy of cannabis in RLS (Megelin and Ghorayeb 2017; Bloomfield et al. 2014). Finally, facilitatory and inhibitory functional interactions between striatal $A_{2A}R$ and CB_1R through heteromeric complexes have also been reported (Moreno et al. 2018). Altogether, these findings may open a new conceptual framework to understand the role of coordinated endocannabinoid signaling in the central nervous system, which may be relevant for the understanding of cannabis efficacy in RLS.

13.8 Conclusion

Cannabinoids have been used for their analgesic and euphoric effects for millennia, and are now being considered in the same way that opioids were decades ago, the combination of a drug

class that is experienced as pain-relieving medication and a banned drug with a high potential for abuse and psychoactive effects. However, the large body of preclinical evidence in support of cannabinoids as potential analgesic agents is so far not supported by high-quality evidence and, although they are involved in critical functions, our understanding of endocannabinoids physiology remains at best limited, necessitating further studies in this field (Hauser and Fitzcharles 2018). Until then, caution must be taken before recommending cannabis use in RLS.

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