Endocannabinoids and Skin Barrier Function: Molecular Pathways and Therapeutic Opportunities

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Endocannabinoids and Skin Barrier Function: Molecular Pathways and Therapeutic Opportunities

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Abstract

As an interface between the organism and its environment, skin is continuously subjected to a myriad of insults that could impair its structural and functional integrity, ultimately leading to clinical manifestations. Skin reacts to external stresses through a vast array of cellular and molecular components, which form a highly sophisticated and well-organized signaling network. Our knowledge of the molecular pathways underlying this complex interplay and accounting for skin homeostasis, both under normal and pathological conditions, is still in its infancy. Among these pathways, the endocannabinoid system has emerged as a key actor in skin biology, by controlling epidermal barrier formation and maintenance, modulating growth and terminal differentiation of cutaneous cells, and regulating skin inflammatory responses via pleiotropic mechanisms. This chapter summarizes our current knowledge of the manifold effects of endocannabinoids in skin, in order to put in a better perspective their potential as next-generation therapeutics against disorders of this organ, particularly those involving dysregulation of immune system and epidermal barrier, such as allergic and atopic dermatitis, localized scleroderma and psoriasis.

Keywords

Endocannabinoids
Endocannabinoid system
Skin biology
Homeostasis
Psoriasis
Atopic dermatitis
Allergic contact dermatitis
Localized scleroderma
Skin barrier
Immunomodulation

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15.1. Introduction

Skin, or cutis, is the outermost and largest organ in the body and serves as primary barrier against physical, chemical and biological insults. The physical barrier of the skin is almost exclusively provided by the stratum corneum, the outer part of the epidermis, consisting of corneocytes, i.e., keratinocytes terminally differentiated in horny lamellae, made of specialized proteins and lipids. In addition to provide a passive

physical barrier, the skin also contains elements of innate and adaptive immune systems, such as macrophages, dendritic cells and lymphocytes, which allow an active fight against infections (Bos 2005).

To support its specialized functions, the skin possesses a highly dynamic structure with remarkable self-renewing properties, which allow cutaneous barrier to continuously regenerate and to repair upon damage. Skin homeostasis is tightly regulated by multiple molecular and cellular mechanisms, such as the release of pro-inflammatory or anti-inflammatory cytokines, proliferation and differentiation of keratinocytes and melanocytes, as well as the recruitment and activation of immune cells. Each of these activities is controlled and integrated by a number of distinct cellular signal transduction systems, which allow skin cells to appropriately cope with a variety of stresses.

In the last few years, the endocannabinoid (eCB) system has emerged as a prominent lipid signaling system widely expressed in the body and involved in multiple adaptive responses to stressful internal and/or environmental insults (Maccarrone et al. 2015). By virtue of its capability of modulating cell functions, generally in a pro-homeostatic manner, the eCB signaling is currently regarded as a promising target for multimodal drug approaches for the treatment of an equally wide variety of pathological conditions, including neurodegenerative, cardiovascular and metabolic disorders (Di Marzo 2008).

In this chapter, we highlight the relevance of eCB signaling in skin physiology, and its therapeutic potential in maintaining/restoring the cutaneous barrier function in a vast range of skin diseases. We begin with a brief overview of the eCB system, giving a summary of its key components as well as of the main signaling pathways that it can elicit. Then, we focus on the possible role of eCBs in skin homeostasis, describing how these bioactive lipids may regulate the biological processes involved in the structural and functional integrity of the skin, such as keratinization, melanogenesis, sebogenesis, dermal fibrogenesis and immune response. Finally, we also describe current evidence supporting that targeted manipulation of eCB signaling by eCB-oriented medicines may represent a valuable strategy for a broad variety of dermatoses.

15.2. The Endocannabinoid System

Fifty years ago (1964) the psychoactive ingredient of cannabis (*Cannabis sativa*), Δ^9-tetrahydrocannabinol (THC), was isolated, and approximately 30 years later the endogenous counterparts of THC, collectively termed eCBs, were discovered: *N*-arachidonoylethanolamine (AEA), also known as anandamide, in 1992, and 2-arachidonoylglycerol (2-AG) in 1995 (Mechoulam and Parker 2013). Since then, many research efforts have shed light on the impact of eCBs on human health and disease, identifying an ensemble of proteins that bind, synthesize and degrade them, and that altogether form the eCB system (Di Patrizio and Piomelli 2012; Galve-Roperh et al. 2013; Maccarrone et al. 2014). The eCBs control basic biological processes, including cell-choice between survival and death, immune response, neuronal development, neurotransmission, energy homeostasis and reproduction, just to mention a few. Unsurprisingly, in the last two decades they have been recognized as key mediators of several aspects of human pathophysiology, and thus have emerged among the most versatile signaling molecules discovered at the end of the past millennium. On this basis, a better understanding of the key-factors that drive at the right time one eCB to the right target among many available candidates in the same cell, holds potential to decipher basic molecular details of energy homeostasis and drug dependence, as well as to develop more effective therapeutics against a variety of human diseases, stress conditions and pain (Di Marzo 2008).

The regulation of eCB levels by biosynthetic and hydrolyzing enzymes, and their mode of action, are being uncovered and appear characterized by an increasing degree of redundancy of pathways and promiscuity of molecular targets. The main elements of this "eCB system" are summarized in Table 15.1.

Table 15.1

The main elements of the eCB system

Member	Description	Function
AEA	*N*-Arachidonoylethanolamine	Bioactive lipid
2-AG	2-Arachidonoylglycerol	Bioactive lipid

CB_1	Type-1 Cannabinoid receptor	G protein coupled receptor, associated with Gi/o, Gs and Gq/11
CB_2	Type-2 Cannabinoid receptor	G protein coupled receptor, associated with Gi/o, Gs and Gq/11
TRPV1	Type-1 Transient receptor potential vanilloid channel	Non-selective cation channel with a preference for Ca^{2+}
PPARs	Peroxisome proliferator-activated receptors	Ligand-activated transcription factors
NAPE-PLD	NArPE-selective phospholipase D	Ca^{2+}-dependent hydrolase responsible for AEA synthesis
DAGL	Diacylglycerol lipase	Ca^{2+}-dependent hydrolase responsible for 2-AG synthesis
FAAH	Fatty acid amide hydrolase	Hydrolase responsible for AEA degradation
MAGL	Monoacylglycerol lipase	Hydrolase responsible for 2-AG degradation

15.2.1. Metabolism

The eCBs are produced from membrane lipid precursors by multiple biosynthetic pathways “on demand”, namely when and where needed upon (patho)physiological stimuli (Mechoulam and Parker 2013).

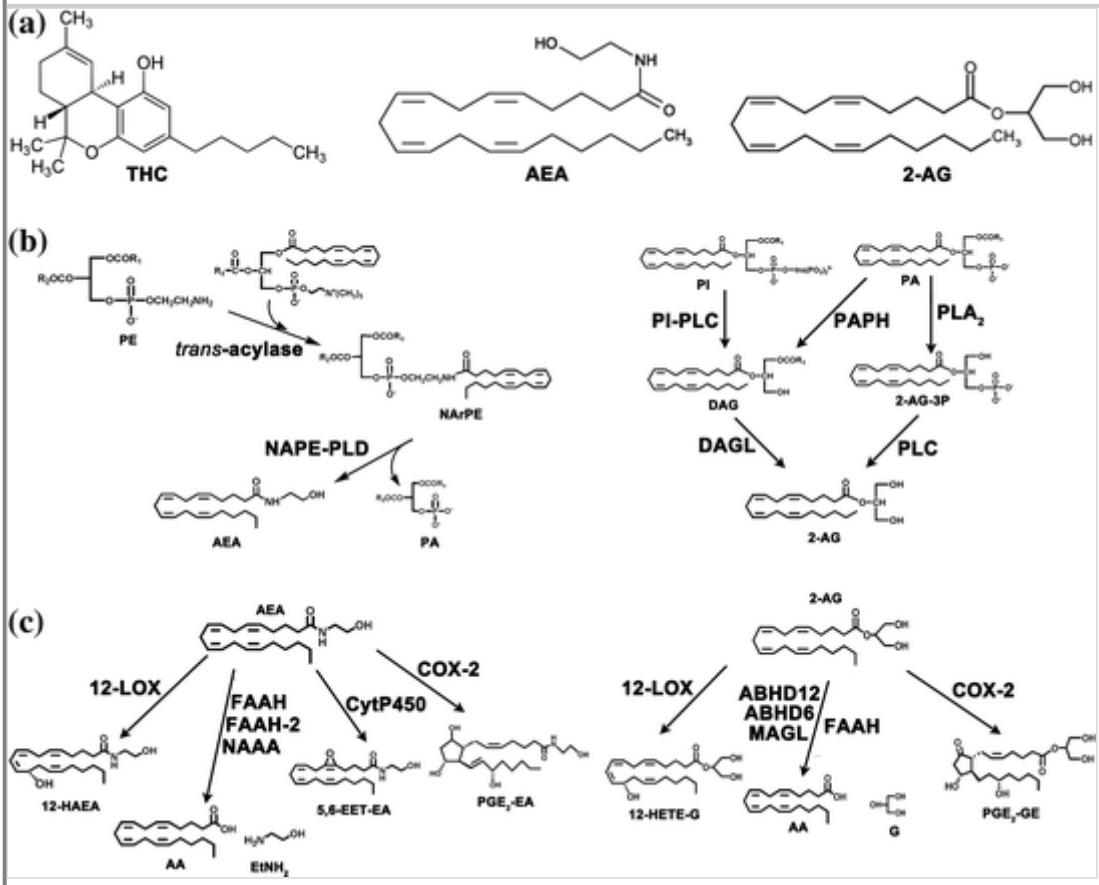
AEA is produced from the hydrolysis of the corresponding *N*-acyl-phosphatidyl-ethanolamine (NArPE). This can occur in one step, when catalyzed by the NAPE-selective phospholipase D (NAPE-PLD) enzyme, or in two or three steps through alternative routes. 2-AG is produced in one step from the hydrolysis of diacylglycerols (DAGs) by either of two *sn*-1-diacylglycerol lipases (DAGLs), namely DAGLα and DAGLβ (Fezza et al. 2014).

The signaling action of eCBs is terminated by cellular uptake and intracellular degradation by specific hydrolases. The mechanism by which cells take up these bioactive lipids has not been fully understood yet, but it is likely to involve membrane transporters, intracellular carriers and endocytosis (Maccarrone et al. 2010; Nicolussi and Gertsch 2015). The most relevant AEA-catabolizing enzyme is the serine hydrolase fatty acid amide hydrolase (FAAH), an endo-membrane-bound enzyme that hydrolyzes AEA to release arachidonic acid and ethanolamine (Cravatt and Lichtman 2002). 2-AG is mainly hydrolyzed by a monoacylglycerol lipase (MAGL) by other emerging lipases, like αβ-hydrolases 6 (ABH6) and 12 (ABH12), and FAAH (Ueda et al. 2011). Additionally, both AEA and 2-AG are also oxidized by the cyclooxygenase 2 (COX2), lipoxygenases 12 (12-LOX) and 15 (15-LOX), and cytochrome P450 to a series of hydroperoxy, hydroxy and epoxy derivatives with distinct biological properties compared to the parent compounds (Kozak et al. 2002; Snider et al. 2010; Kuc et al. 2012; Urquhart et al. 2015). The metabolism of AEA and 2-AG is schematically depicted in Fig. 15.1.

Fig. 15.1

Endocannabinoid metabolism. Chemical structures of THC and two prominent eCBs (**a**). Alternative biosynthetic and degradative pathways of AEA (**b**) and 2-AG (**c**). Abbreviations: *2-AG-3P* 2-arachidonoylglycerol-3-phosphate; *12-HAEA* 12-hydroxyanandamide; *12-LOX* 12-lipoxygenase; *5,5-EET-EA* 5,5-epoxyeicosatrienoyl ethanolamide; *12-HETE-G* 12-hydroxyarachidonoyl-glycerol; *AA* arachidonic acid; *ABHD6/12* α/β-hydrolase domain 6/12; *Cyt P450* cytochrome P450; *COX-2* cyclooxygenase-2; *DAG* diacylglycerol; *DAGL* diacylglycerol lipase; $EtNH_2$ ethanolamine; *FAAH* fatty acid amide hydrolase; *FAAH-2* fatty acid amide hydrolase-2; *G* glycerol; *MAGL* monoacylglycerol lipase; *NAAA* *N*-acylethanolamine-hydrolyzing acid amidase; *NArPE* *N*-arachidonoyl-phosphatidylethanolamine; *NAPE-PLD* *N*-acyl-phosphatidyl-ethanolamines-hydrolyzing phospholipase D; *PA* phosphatidic acid; *PAPH*

phosphatidic acid phosphohydrolase; *PI* phosphatidylinositol; *PE* phosphatidylethanolamine; *PGE_2-G* prostaglandin glycerol E_2; *PLA_2* phospholipase A_2; *PLC* phospholipase C



15.2.2. Molecular Targets

AEA and 2-AG bind with different affinity and potency type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors (Pertwee et al. 2010), two well-characterized 7-transmembrane G protein-coupled receptors (GPCRs). In addition, eCBs are described to interact with several non-cannabinoid receptors. Some of them have an intracellular binding site, like the transient receptor potential vanilloid type-1 (TRPV1) ion channel (Di Marzo and De Petrocellis 2010). Others are localized in the nucleus, like the peroxisome proliferator-activated receptors (PPAR) α and γ (Pistis and Melis 2010).

15.2.2.1. Cannabinoid Receptors

CB_1 and CB_2 receptors are members of the rhodopsin-like family of heptahelical transmembrane-spanning GPCRs. They are encoded for by different genes, and exhibit 44 % amino acid identity throughout the whole protein, and 68 % within the transmembrane domains. These receptors show a quite broad, and sometimes overlapping, distribution (Pertwee et al. 2010).

CB_1 receptors are predominantly found in the central nervous system, where they are highly expressed in brain areas associated with cognition, memory, emotionality, motor function, and pain, including cortex, limbic system, hippocampus, cerebellum and several nuclei of the basal ganglia. CB_1 receptors are also expressed in non-neuronal cells, including immune cells (Chiurchiù et al. 2015).

CB_2 receptors are mainly expressed in the immune system, including the marginal zone of the spleen and macrophages, generally mediating anti-inflammatory and immunosuppressive effects. There is also evidence that CB_2 receptors are also expressed in central and peripheral neurons, both under physiological conditions and upon stress (Van Sickle et al. 2005; Viscomi et al. 2009; Onaivi et al. 2012).

Cannabinoid receptors can couple to multiple heterotrimeric G-proteins to elicit specific signaling pathways,

often in a cell-type-specific, agonist-specific or dose-dependent manner (Howlett 2005). Typically, their stimulation has been shown to lead to: inhibition or, less often, stimulation of adenylyl cyclase (AC), via Gi/o or Gs proteins, with subsequent inhibition, or stimulation, of protein kinase A (PKA), respectively; (ii) activation of different members of the mitogen-activated protein kinase (MAPK) family, such as extracellular signal-regulated kinase (ERK) (Bouaboula et al. 1995), c-Jun N-terminal kinase (JNK) and p38 MAPK (Liu et al. 2000), via Gi/o proteins; (iii) activation of phospholipase C (PLC) via either Gq/11 or βγ subunits of Gi/o proteins (Howlett 2005). Finally, CB_1 receptors can cause Gi/o-mediated inhibition of voltage-gated Ca^{2+} channels (L, N, and P/Q-type) and activation of A-type and inwardly rectifying K^+ channels (Howlett 2005). CB_2 receptors share with CB_1 receptors similar signal transduction mechanisms in terms of their actions on adenylyl cyclase and MAPKs, but they seem to be unable to regulate ion channels as do CB_1 receptors. However, it has been demonstrated that CB_2 receptors are coupled, via inositol trisphosphate receptor, to Ca^{2+}-activated Cl^- channels in pyramidal neurons of the rodent medial prefrontal cortex, suggesting that also these receptors may regulate ion homeostasis and cell excitability (den Boon et al. 2012).

15.2.2.2. Transient Receptor Potential Vanilloid Type-1 Channel

The best-established non-cannabinoid receptor for eCBs is the TRPV1 channel, previously discovered as the receptor for capsaicin, the pungent agent in hot-chilli peppers (Caterina et al. 1997). TRPV1 is a non-selective cation channel with a preference for Ca^{2+}, that is involved in the transmission and modulation of pain (nociception), as well as in the integration of diverse painful and inflammatory stimuli. Therefore, it senses, directly or indirectly, a wide range of cellular and environmental signals, including noxious temperature, mild acidification, and local mediators of inflammation, such as histamine, bradykinin, nerve-growth factor, eicosanoids, ADP and ATP (Szallasi et al. 2007). TRPV1 is primarily expressed in primary sensory neurons, but it is also widely expressed in the body, including the central nervous system (Szallasi et al. 2007).

15.2.2.3. Peroxisome Proliferator-Activated Receptors

Endogenous, synthetic, and natural cannabinoids are also reported to activate PPARs, ligand-activated transcription factors that are primarily involved in the regulation of metabolism and energy homeostasis, cell differentiation, and immune function (Kersten et al. 2000). Three different PPAR isoforms are known (α, γ and δ), that are ubiquitously expressed, even though with a different tissue distribution. PPARα is mainly expressed in liver and brown adipose tissue, and to a lesser extent in kidney, heart and skeletal muscle; PPARγ is expressed in adipose tissue and colon, immune system and retina; PPARδ is widely expressed in the body, with the highest expression in heart, gut and kidney (Kersten et al. 2000).

Once activated by ligand binding, PPARs heterodimerize with the receptor of 9-*cis*-retinoic acid, and bind to specific peroxisome proliferator response elements to regulate the transcription of target genes. Endogenous ligands for PPARs are eicosanoids, fatty acids and fatty acid derivatives, including eCBs (Pistis and Melis 2010).

It has been described that AEA and 2-AG, at micromolar concentrations, directly bind to and activate both PPARα and PPARγ. In particular, AEA induces expression of several PPARγ-sensitive genes in 3T3-L1 fibroblasts (Bouaboula et al. 2005), and causes inhibition of interleukin (IL)-2 expression by activating PPARγ in primary splenocytes (Rockwell and Kaminski 2004). Finally, AEA controls adipocyte differentiation by activating PPARγ (Gasperi et al. 2007) and induces vasorelaxant responses on the ophthalmic artery through activation of PPARα (Romano and Lograno 2012).

15.3. The Cutaneous Endocannabinoid System

15.3.1. Expression

As in other parts of the body, the eCB system is ubiquitously expressed by all cellular components of the skin (Oddi and Maccarrone 2014). The eCBs are produced and released by different skin cell populations, including keratinocytes (Maccarrone et al. 2003; Toth et al. 2011; Magina et al. 2011), sebocytes (Dobrosi

et al. 2008), melanocytes (Pucci et al. 2012), sweat gland epithelial cells (Czifra et al. 2012), and macrophages (Jiang et al. 2009), under basal and stimulated conditions. The levels of AEA and 2-AG have been measured in rodent paw skin (Felder et al. 1996; Khasabova et al. 2012), reaching pmol/g and nmol/g concentrations, respectively, similar to those found in the brain.

The presence in the skin of diacylglycerol lipase, DAGL, and monoacylglycerol lipase, MAGL, the main enzymes involved in 2-AG biosynthesis and catabolism, respectively, has been assessed in cultured keratinocytes (Berdyshev et al. 2000; Maccarrone et al. 2003; Oddi et al. 2005), melanocytes (Hamtiaux et al. 2012; Pucci et al. 2012), sebocytes (Dobrosi et al. 2008), and fibroblasts (McPartland 2008). Also AEA-metabolizing enzymes, namely FAAH and NAPE-PLD, have been documented in rodent skin (Karsak et al. 2007; Petrosino et al. 2010; Khasabova et al. 2012).

Immunohistological investigations of the precise localization of CB_1 and CB_2 receptors in sections of human and rodent skin revealed that both receptors are expressed in virtually all skin cell populations. In particular, both receptors are present in keratinocytes, cutaneous nerve fibers, dermal cells, and specialized cells with adnexal structures (Casanova et al. 2003; Ibrahim et al. 2005; Stander et al. 2005). Notably, in one of these immunohistochemical studies on human skin, CB_1 and CB_2 have been found to be distributed in a complementary fashion in epidermis, hair follicle, and sebaceous gland, with CB_1 and CB_2 being predominantly expressed in differentiated and undifferentiated cells, respectively. Therefore, it can be suggested that these two receptors play nonredundant roles during differentiation of keratinocytes and sebocytes. In the skin, CB_2 is expressed beyond the basal layer, but fairly uniformly distributed throughout the epidermis (Ibrahim et al. 2005). In the dermis, CB_1 and CB_2 are both expressed in the myoepithelial cells of the secretory portion of eccrine sweat glands, but not in secretory cells (Stander et al. 2005). Positive immunoreactivity for CB_1 and CB_2 has been also documented in mast cells and in most (but not all) CD68-positive macrophages (Stander et al. 2005; Sugawara et al. 2012). In mouse skin, CB_2 is present in myofibroblasts and vascular smooth muscle cells (Zheng et al. 2012). Concerning their localization in human primary sensory nerves, CB_1 and CB_2 are expressed in large (myelinated) and thin (unmyelinated) calcitonin gene-related peptide positive nerve fibers (Stander et al. 2005). Finally, both receptors are functionally expressed in cultured melanocytes (Magina et al. 2011; Pucci et al. 2012), fibroblasts (McPartland 2008), sebocytes (Dobrosi et al. 2008) and sweat gland epithelial cells (Czifra et al. 2012).

A wide expression in both neuronal and non-neuronal cells of the skin has also documented for TRPV1 and all the isotypes of PPARs, in several cases with an overlapping expression CB_1 and CB_2 receptors. TRPV1 is located in a neurochemically heterogeneous population of small-diameter primary afferent fibers and with small-diameter nerve fibers in the skin of rodents and humans (Guo et al. 1999; Stander et al. 2004; Bodo et al. 2004). Moreover, TRPV1 ion channels have been described in numerous non-neuronal cell types, including keratinocytes, mast cells and dendritic cells (Stander et al. 2004; Bodo et al. 2004). PPARδ is present throughout all epidermal layers, whereas PPARα and PPARγ are mainly located in suprabasal compartments (Di-Poi et al. 2004).

15.3.2. Endocannabinoids and Skin Barrier Function

15.3.2.1. Activity in Epidermal Barrier Formation

Growing evidence supports a functional role of eCB signaling in regulating key biological processes of keratinocytes, such as proliferation, differentiation and apoptosis, which are essential for formation and maintenance of epidermal barrier structure and function.

For example, AEA inhibits the formation of cornified envelope in differentiating keratinocyte, through a CB_1-dependent reduction of transglutaminase and protein kinase C activities (Maccarrone et al. 2003), suggesting an important role of eCB signaling in epidermal differentiation. This anti-differentiative activity of AEA has been found to be associated with the silencing of genes involved in keratinocyte differentiation (i.e., keratin 1, keratin 10, involucrin, and transglutaminase 5) by p38-MAPK-mediated DNA methylation of their promoters (Paradisi et al. 2008). On the other hand, in hair follicles it has been demonstrated that CB_1 activation by AEA inhibits hair shaft elongation and the proliferation of hair matrix keratinocytes, and also induces intraepithelial apoptosis and premature hair follicle regression (Telek et al. 2007). Interestingly, these cells failed to respond to 2-AG stimulation, highlighting the nonredundancy of these two prototypic

eCBs (Telek et al. 2007). Consistent with an inhibitory role of AEA in keratinocyte growth, CB_1 activation by AEA has been found to markedly suppress proliferation and induce cell death in both human cultured keratinocytes and skin organ-culture models (Toth et al. 2011), possibly by elevating intracellular Ca^{2+} concentration through activation of TRPV1.

Histological phenotyping of CB_1 and CB_2 deficient mice revealed that these two receptors operate in an opposite manner to regulate epidermal barrier homeostasis and epidermal differentiation (Roelandt et al. 2012). In particular, the loss of CB_1 receptor enhances proliferation and reduces differentiation of keratinocytes, causing the formation of a thicker epidermis with altered lipid bilayer structures. Conversely, the targeted disruption of CB_2 leads to the formation of a thinner epidermis with reduced proliferative rates of keratinocytes, paralleled by a strong expression of the main epidermal differentiation markers: involucrin, loricrin, filaggrin and caspase 14 activation (Roelandt et al. 2012). Moreover, functional data from these mouse models demonstrated that the recovery of the permeability barrier function of the epidermis following acute removal of corneocytes from the stratum corneum is impaired in $CB_1^{-/-}$, whilst it was enhanced in $CB_2^{-/-}$ mice. These findings strongly suggest that CB_1 signaling is a positive regulator, whereas CB_2 signaling is a negative regulator, of epidermal permeability barrier and stratum corneum structure (Roelandt et al. 2012).

Since it is well-established that an increase of intracellular 3′,5′-cyclic adenosine monophosphate (cAMP) in epidermal keratinocytes delays barrier recovery, that instead is accelerated by cAMP antagonists (Denda et al. 2004), it is tempting to speculate that the opposite effects exerted by cannabinoid receptors on epidermal homeostasis could be due to their different regulation of adenylyl cyclase activity. Furthermore, it should also be kept in mind that Ca^{2+} is a central regulator of keratinocyte differentiation (Elsholz et al. 2014) and that the eCBs can strongly influence cytosolic Ca^{2+} levels via cannabinoid receptors and TRPV1, which are co-expressed in keratinocytes. However, additional studies are needed to better clarify the physiological relevance of eCB signaling in keratinocyte differentiation, as well as in epidermal barrier homeostasis.

15.3.2.2. Activity in Non-keratinocyte Skin Cells

Beyond the pivotal role played by keratinocytes, structure and function of the skin barrier are both strongly influenced by the activity of many other specialized cells, such as sebocytes, melanocytes and sweat gland cells, that regulate the content of sebum, melanin pigmentation and hydration of the skin, respectively. Moreover, dermis, the collagen-rich connective tissue of the skin, is produced, organized and maintained by the activity of fibroblasts, the main resident cells of the dermis.

On these cells, most likely because of the variety of their targets and of their underlying signaling pathways, eCBs and their synthetic counterparts evoke complex, and even biphasic, actions. In particular, it has been documented that human sebocytes express CB_2 but not CB_1 receptors, and that AEA stimulates lipid production at low concentrations, but induces apoptosis at higher levels, in a CB_2-mediated manner (Dobrosi et al. 2008). Similarly, in melanocytes AEA produces melanogenic, mitogenic, and dendritogenic effects at low doses (via CB_1) and proapoptotic effects at higher doses (via TRPV1) (Pucci et al. 2012). Additionally, activation of CB_1 inhibits basal and ultraviolet B-induced melanogenesis in a human melanoma cell line (Magina et al. 2011). In human eccrine sweat gland epithelial cells both AEA and 2-AG are able to (i) suppress proliferation, (ii) induce apoptosis, (iii) alter expression of various structural proteins (i.e., involucrin, filaggrin, loricrin, and keratins), and (iv) upregulate lipid synthesis; remarkably, all these effects have been found to be exerted in a CB_1-, CB_2-, and TRPV1-independent manner (Czifra et al. 2012). In dermal fibroblasts, it has been reported that synthetic cannabinoid agonists, acting via non-CB receptors, limit extracellular matrix (ECM) production by disrupting the transforming growth factor β (TGFβ) cascade, and downregulating proliferation and activation (Servettaz et al. 2010; Balistreri et al. 2011). On the same cells, it has been demonstrated that AjA, a synthetic THC analogue, inhibits collagen synthesis through activation of PPARγ in skin fibroblasts (Garcia-Gonzalez et al. 2012).

15.3.2.3. Activity in Skin Immunity

In addition to being a physical barrier, the skin is an immunological barrier, consisting in a combination of specialized cell types with distinct roles in innate and adaptive immunity, including macrophages, mast cells, dendritic cells, Langerhans cells, keratinocytes, fibroblasts, as well as B and T lymphocytes.

It is well-established that CB_2, and to a lesser extent CB_1, receptors are expressed in several cell lineages of the immune system (Chiurchiù et al. 2015). It is also widely recognized that their stimulation by exogenous and/or endogenous cannabinoids generally suppresses acute and chronic inflammatory conditions. Although the precise mechanisms of (e)CB-induced immunomodulation have not been fully elucidated yet, it is likely that they depend on the ability of the eCB system to manipulate those signaling cascades (such as cAMP, Ca^{2+} and MAPK cascades), that are essential for maturation and function of immune cells (Chiurchiù et al. 2015).

One major mechanism of immunosuppression by (e)CBs is the induction of apoptosis in immune cell populations. For example, activation of cannabinoid receptors (mainly CB_2) by THC and other cannabinoid ligands induces cell death in macrophages, dendritic cells, T- and B-lymphocytes (Klein 2005). Similarly, low doses of AEA cause significant inhibition of proliferation of lymphocytes and dendritic cells, inducing cell death by apoptosis, while 2-AG has been shown to exhibit biological activity in mouse splenocytes, by producing strong immunomodulatory activity on mitogen-induced lymphocyte proliferation (Schwarz et al. 1994; Lee et al. 1995; Do et al. 2004).

Another relevant immunomodulatory action mediated by the eCB system is the regulation of inflammatory cytokine production by immune cells. In particular, THC and eCBs have been shown to cause blockade of pro-inflammatory Th1 cytokines, such as interferon-γ and interleukin (IL)-2, and increase the expression of anti-inflammatory Th2 cytokines, such as IL-4 and IL-10, which are important for humoral immunity, and TGF-α which has immunosuppressive properties (Pacifici et al. 2003). Regarding skin cells, it has been recently shown that CB_1 activation in keratinocytes leads to a reduced secretion of proinflammatory chemokines C–C motif ligand 8 (CCL8) and C–X–C motif ligand 10 (CXCL10) in a mouse model of allergic contact dermatitis. Both substances regulate T cell-dependent inflammation, and attenuate thymic stromal lymphopoietin- (TSLP-) and CCL8-dependent Th2-type allergic inflammatory responses (Gaffal et al. 2013, 2014). Moreover, AEA has been reported to reduce tumor necrosis factor (TNF)-α-induced IL-8 and CCL2 release from keratinocytes, acting in an anti-inflammatory manner (Leonti et al. 2010). Finally, in a very recent study we found that AEA reduces production and release of IL-12 and IL-23 (Th1- and Th17-inducing cytokines, respectively) from inflamed keratinocytes in a CB_1-dependent manner, suggesting that CB_1 could negatively drive the polarization of CD4 naive T cells into Th1 and Th17 (unpublished results).

Regarding the influence of the eCB system on phagocytosis, which is a crucial step in the induction of innate and acquired immunity, it has been shown that 2-AG augments the phagocytosis of HL-60 cells differentiated to a macrophage-like phenotype, via CB_2-mediated signaling, possibly involving Akt and ERK cascades (Gokoh et al. 2007). In contrast, a more recent study reported that CB_1, but not CB_2, enhances phagocytic activity of human macrophages, through Gαi/o-dependent RhoA/ROCK pathways (Mai et al. 2015).

Finally, another cannabinoid-mediated mechanism of immunomodulation involves the ability of cannabinoids to perturb the recruitment of leukocytes at sites of inflammation, by regulating their adhesion to endothelium, extravasation and migration in the tissue (Klein 2005). In particular, there is a large body of data that supports a functional relevance of (e)CBs in inhibiting migratory activities of a diverse array of immune cell types, primarily through CB_2-mediated pathways (Joseph et al. 2004; Kurihara et al. 2006). However, there are also some discrepancies regarding the impact of 2-AG in mediating the trafficking of immune cells. In particular, in peripheral blood eosinophils the activation of CB_2 by 2-AG, but not by AEA, induces cell migration, suggesting that 2-AG acts as a chemotactic agent and may be involved in allergic responses by promoting eosinophil infiltration (Oka et al. 2004). The active involvement of 2-AG into cell migration has been also found in myeloid and normal splenocytes (Jorda et al. 2002), and in other different lymphoid lineages, such as macrophage-like cells HL-60, U937, THP-1, as well as and human peripheral blood monocytes (Kishimoto et al. 2003). This effect of 2-AG appears to occur via CB_2-mediated signaling, that seems to involve Rho kinase and MAPK cascades.

15.4. Therapeutic Opportunities

15.4.1. Atopic Dermatitis

Atopic dermatitis, also known as atopic eczema, is a multifactorial inflammatory skin disorder characterized

by intense itching and recurrent eczematous lesions, that are prone to microbial infections. An altered lipid composition of the stratum corneum is responsible for the xerotic aspect of the skin, and may determine a higher permeability to allergens and pathogens. Atopic dermatitis skin is associated with a type I hypersensitivity reaction characterized by a pro-inflammatory cytokine milieu made by excessive infiltration of immune cells, including eosinophils, mast cells and T-cells, particularly Th2 cells, Th22 cells, and, to a lesser degree, Th1 and Th17 cells (Bos 2005).

Oral or topical administration of cannabinoid-based drugs has been shown to be effective in the treatment of atopic dermatitis-like skin lesions. Indeed, several studies reported beneficial effects of manipulating the eCB system in animal models of this disorder. For example, in an oxazolone-induced dermatitis, topical application of CB_1-specific agonist significantly accelerates the recovery of epidermal permeability barrier function and exerts marked anti-inflammatory activity (Kim et al. 2015). Similarly, mice lacking CB_1 receptors globally, or specifically in keratinocytes, show enhanced Th2-type contact hypersensitivity responses to fluorescein isothiocyanate (FITC), and a delayed epidermal barrier repair when compared with wild-type mice (Gaffal et al. 2014). In particular, mRNA levels for IL-4, TSLP and CCL8, proinflammatory mediators that drive Th2-type skin inflammation in atopic dermatitis, as well as eosinophil activity, are significantly increased in inflamed ear tissue of FITC-challenged $CB_1^{-/-}$ mice, confirming the involvement of CB_1 both in maintaining epidermal barrier homoeostasis and in attenuating Th2-type allergic inflammatory responses (Gaffal et al. 2014).

The eCB system has emerged as a key regulator of mast cells, which are key effector cell type in IgE-mediated immediate hypersensitivity and allergic responses in atopic dermatitis (Kawakami et al. 2009). Indeed, natural and synthetic cannabinoids and eCBs exert suppressive activity on mast cell functions, leading to protective effects both in acute and chronic inflammatory pathologies sustained by excessive accumulation and degranulation of these immunocompetent cells (Chiurchiù et al. 2015). More specifically, both the nonpsychotropic CB_2 agonists HU-308 and HU-320 strongly reduce edema in two different murine models of allergic inflammation (Hanus et al. 1999; Sumariwalla et al. 2004). Furthermore, AEA has been shown to inhibit mast cell degranulation, via CB_1-dependent mechanisms, both in vitro and in vivo (Maccarrone et al. 2002; Sugawara et al. 2012).

As compounds that interfere with the pathophysiology of pruritus, cannabinoids could be successfully applied in the management of pruritus commonly occurring in atopic dermatitis. Systemic activation of CB_1 receptor, via either directly-acting receptor agonists (i.e., THC) or inhibitors of AEA degradation, (i.e., by pharmacological blockade or genetic deletion of FAAH) reduces scratching in a murine model of pruritus (Schlosburg et al. 2009). Another study reported that CB_1 activation by HU210 effectively suppresses histamine-induced pruritus in humans (Dvorak et al. 2003). Moreover, oral administration of JTE-907, a selective CB_2 antagonist, significantly inhibits spontaneous scratching behavior in a mouse model of atopic dermatitis (Maekawa et al. 2006), suggesting a yet-to-be-clarified CB_2 involvement in allergic itch. Finally, PAC-14028, a TRPV1 antagonist, suppresses scratching behaviors in two different models of atopic dermatitis (Yun et al. 2011). Interestingly, in a T-cell mediated model of atopic dermatitis, histamine-independent itch induced by IL-31 is significantly reduced in $TRPV1^{-/-}$ mice compared to controls (Cevikbas et al. 2014). These findings suggest that eCB signaling is crucially involved in the neuronal/nonneuronal cellular network of pruritogenic stimuli in the skin, and that its manipulation can represent another therapeutic approach for the management of itching associated to atopic dermatitis, as well as to other inflammatory conditions.

15.4.2. Allergic Contact Dermatitis

Allergic contact dermatitis is a T-cell-mediated inflammatory reaction occurring at the site of challenge with a contact allergen in sensitized individuals. It is characterized by redness, papules, and vesicles, followed by scaling and dry skin. Immunologically, contact dermatitis is a form of delayed type IV hypersensitivity consisting of two phases: sensitisation and elicitation. In the sensitisation phase, occurring at the first contact of the skin with the hapten, the resident antigen-presenting cells, i.e., Langerhans cells and dermal dendritic cells, pick up and process the antigen. Then, hapten-bearing dendritic cells migrate to the draining lymph nodes, where the allergen is exposed to naive T lymphocytes that differentiate into antigen-specific memory T cells. During the elicitation phase, after a new contact with the same antigen, memory T cells are activated and exert direct and indirect cytotoxic effects towards cells of the skin, causing edema, erythema and

induration at the site of contact in sensitized humans or animals (Bos 2005).

Numerous preclinical studies have explored the role of the eCB system in contact dermatitis. Significant alterations in the components of the eCB system have been found in different mice models of this dermatosis. In particular, in an experimental allergy contact dermatitis induced by 2,4-dinitrofluorobenzene (DNFB), tissue levels of AEA and 2-AG were found to be increased, along with a downregulation of CB_1 receptors and an upregulation of NAPE-PLD, TRPV1 and CB_2 receptors (Karsak et al. 2007; Petrosino et al. 2010). An increase of 2-AG, but not of AEA, was also observed in another model of contact dermatitis induced by oxazolone (Oka et al. 2006).

Agonists and antagonists of cannabinoid receptors can produce both anti-allergic- and pro-allergic effects. Studies with animals genetically devoid of CB_1 and CB_2 receptors have generated contradictory findings about the effective contribution of these receptors, particularly of CB_2, to allergic dermatitis. On the one hand, single and double CB_1/CB_2 receptor knockout mice have been found to display exacerbated inflammation following treatment with DNFB, suggesting that both receptors exert a protective role in allergic reaction (Karsak et al. 2007). In particular, it has been reported that $CB_2^{-/-}$ mice experience pronounced chronic inflammation that is alleviated or exacerbated by CB_2 agonists or antagonists, respectively (Karsak et al. 2007). On the other hand, $CB_2^{-/-}$ mice have been found to exhibit a significant suppression of DNFB-induced edema and acanthosis (i.e., diffuse epidermal hyperplasia), and consistently a CB_2-selective antagonist has been found to alleviate chronic inflammation induced by DNFB in wild-type mice (Ueda et al. 2005; Mimura et al. 2012). Moreover, a CB_2-selective agonist induces ear swelling in wild-type mice (Ueda et al. 2007), suggesting that the CB_2 receptor plays a stimulatory role in the sensitization and exacerbation of allergic inflammation. Furthermore, in other contact dermatitis models, where ovalbumin or oxazolone were repeatedly applied, there was a significant decrease in ear swelling and acanthosis in $CB_2^{-/-}$ mice compared with wild-type animals, strongly supporting the notion that CB_2 and its endogenous ligand 2-AG enhance dermal reactions to allergens (Mimura et al. 2012). A possible explanation for these conflicting results could reside in the different doses of inflammatory stimuli used to induce a dermal reaction and, most likely, with the pleiotropic actions that CB_2 exerts on immune cells, including regulation in cell proliferation, activation and migration.

Less controversial seems to be the role of CB_1 receptors in allergic contact dermatitis. Indeed, mice lacking CB_1 in keratinocytes show enhanced and prolonged allergic responses following DNFB challenge (Gaffal et al. 2013). Interestingly, CB_1-deficient keratinocytes produced increased amounts of CXCL10 and CCL8 in response to IFN-γ (Gaffal et al. 2013), suggesting that peripheral CB_1 expressed on these cells helps to limit the secretion of proinflammatory chemokines that regulate T cell-dependent inflammation in the elicitation phase of allergic contact dermatitis.

15.4.3. Localized Scleroderma

Localized scleroderma is a rare disease of unknown etiology, characterized by inflammation and thickening of the skin from excessive collagen deposition. Available data suggest that the mechanism of pathogenesis of scleroderma is complex. Vessels, immune system and extracellular matrix are affected and may contribute to the development of the disease. Early stages of scleroderma are characterized by an infiltration of affected skin by inflammatory cells, mostly macrophages and activated T cells. Later stages of the disease are characterized by an excessive collagen synthesis and deposition by fibroblasts, resulting in pathologic dermal fibrosis (Bielsa 2013).

Activation of the eCB system can exerts both pro-fibrotic and anti-fibrotic−effects, by acting through CB_1 and CB_2 receptors, respectively. Indeed, $CB_1^{-/-}$ mice, or controls treated with CB_1 antagonists, are protected from bleomycin-induced skin fibrosis, exhibiting a reduced dermal thickening, associated with a decreased number of myofibroblasts, infiltrating T cells and macrophages. In marked contrast, $CB_2^{-/-}$ mice, or controls treated with CB_2 antagonists, are more susceptible to the same model of scleroderma, with clinical symptoms markedly worsened (Akhmetshina et al. 2009). Similarly, $CB_2^{-/-}$ mice develop a more exacerbated hypochlorite-induced fibrosis compared with wild-type animals (Servettaz et al. 2010). Experiments involving bone marrow transplantation revealed that these two receptors indirectly regulate the activation of fibroblasts by orchestrating the infiltration of leukocytes into lesional skin (Servettaz et al. 2010; Marquart et al. 2010). Finally, increased levels of AEA induced by inactivation of FAAH exacerbate experimental

fibrosis primarily via activation of CB_1 fibrosis, suggesting that CB_1 is the predominant receptor for eCBs in skin fibrosis, and that AEA exerts fibrogenic activity (Palumbo-Zerr et al. 2012).

From these preclinical data it is arguable that selective antagonism and agonism of CB_1 and CB_2 receptors, respectively, may have therapeutic potential for the treatment of early inflammatory stages of skin scleroderma.

15.4.4. Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by sharply demarcated erythematous plaques appearing on the surface of the epidermis. These lesions result from intense skin inflammation with infiltration of inflammatory cells into the dermis and epidermis and keratinocyte hyperproliferation. Although the mechanism underlying psoriasis remains elusive, there is growing evidence that T-cell-mediated immune mechanisms, particularly those mediated by Th1 and Th17 cells, play a major role in the pathology of this disease (Bos 2005).

Although direct studies using (e)CBs on preclinical models of psoriasis have not yet been conducted, the above-mentioned biological effects of these compounds on keratinocytes, and their potential in regulating the activation and balance of Th1/Th2 cells, suggest that the eCB system could be a potential target for the development of new pharmacological approaches against this skin disease.

15.5. Concluding Remarks

There is a huge need for the development of new treatments for managing chronic skin disorders, such as psoriasis, atopic dermatitis, allergic contact dermatitis and localized scleroderma. The increased knowledge of the pathogenesis of these dermatoses has highlighted several molecular and cellular mechanisms, on which it would be advisable to intervene with higher selectivity and efficacy through novel pharmacological approaches. There are many good reasons to believe that (e)CBs could be a new valuable generation of dermatological drugs. In the first place, they modulate the activity of a signaling pathway, the so-called eCB system, which is involved in skin homeostasis by orchestrating anti-proliferative, anti-fibrotic and immunomodulatory actions. In the second place, these substances, by acting as direct or indirect agonists and antagonists of cannabinoid receptors, produce beneficial effects on relevant animal models of different skin disorders, also providing proof of concept that the eCB system has a pleiotropic homeostatic function in the skin (Table 15.2). In the third place, (e)CBs have favorable drug-safety profiles, being generally well-tolerated and showing low toxicity and moderate adverse effects. Finally, as lipophilic compounds, (e)CB-based drugs can be easily administered topically, for example in the form of cream, lotion or ointment. On the basis of accumulated evidence summarized in this chapter, there is certainly a hope that in the next future at least some of (e)CB-related compounds could contribute to expand the therapeutic arsenal of clinical dermatology.

Table 15.2

Preclinical evidence for a possible therapeutic role of the eCB system in skin diseases

Disorder	Preclinical model	Role of the eCB system	Receptors involved	Reference
Atopic dermatitis	Fluorescein isothiocyanate-induced atopic dermatitis	Edema is strongly exacerbated in $CB_1^{-/-}$ mice	CB_1	Gaffal et al. (2014)
Allergic contact dermatitis	2,4-Dinitrofluorobenzene-induced contact dermatitis	Edema is slightly exacerbated in $CB_1^{-/-}$, $CB_2^{-/-}$ and double $CB_1^{-/-}$ $CB_2^{-/-}$ mice THC decreases ear swelling and granulocyte infiltration	CB_1, CB_2	Karsak et al. (2007)
		Edema is suppressed in $CB_2^{-/-}$ mice		

	Oxazolone-induced contact dermatitis Ovalbumin-induced contact dermatitis	CB_2 and 2-AG play important stimulative roles in the sensitization, elicitation, and exacerbation of allergic inflammation	CB_2	Mimura et al. (2012), Oka et al. (2006)
Scleroderma	Bleomycin-induced fibrosis Hypochlorite-induced fibrosis	Skin fibrosis is reduced in $CB_1^{-/-}$ mice and slightly exacerbated in $CB_2^{-/-}$ mice CB_1 and AEA play important stimulative roles in skin fibrogenesis	CB_1, CB_2	Akhmetshina et al. (2009), Servettaz et al. (2010), Marquart et al. (2010), Palumbo-Zerr et al. (2012)

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