MINI REVIEW
Melatonin and the hair follicle

Abstract: Melatonin, the chief secretory product of the pineal gland, has long been known to modulate hair growth, pigmentation and/or molting in many species, presumably as a key neuroendocrine regulator that couples coat phenotype and function to photoperiod-dependent environmental and reproductive changes. However, the detailed effects and mechanisms of this surprisingly pleiotropic indole on the hair follicle (HF) regarding growth control and pigmentation have not yet been completely understood. While unspecific melatonin binding sites have long been identified (e.g., in goat and mouse HFs), specific melatonin membrane MT2 receptor transcripts and both protein and mRNA expression for a specific nuclear melatonin binding site [retinoid-related orphan receptor α (RORα)] have only recently been identified in murine HFs. MT1, known to be expressed in human skin cells, is not transcribed in mouse skin. After initial enzymologic data from hamster skin related to potential intracutaneous melatonin synthesis, it has recently been demonstrated that murine and human skin, namely human scalp HFs in anagen, are important sites of extrapineal melatonin synthesis. Moreover, HF melatonin production is enhanced by catecholamines (as it classically occurs in the pineal gland). Melatonin may also functionally play a role in hair-cycle control, as it down-regulates both apoptosis and estrogen receptor-α expression, and modulates MT2 and RORα expression in murine skin in a hair-cycle-dependent manner. Because of melatonin's additional potency as a free radical scavenger and DNA repair inducer, the metabolically and proliferatively highly active anagen hair bulb may also exploit melatonin synthesis in loco as a self-cytoprotective strategy.

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Introduction

Observations suggesting that the pineal gland and its chief secretory product, melatonin [1, 2], are involved in the regulation of hair growth and pigmentation date back several decades, and have long-intrigued chronobiologists, animal scientists, veterinarians, endocrinologists, dermatologists and researchers in the wool-industry among others [3–9]. Thus, it is well-recognized that melatonin can alter wool and cashmere production, the development and cycle frequency of pelage, seasonal molting and coat color in several species, presumably as a major neuroendocrine regulator that couples coat phenotype and function to photoperiod-dependent environmental and reproductive changes [10, 11].

Yet, the understanding of the role of melatonin in hair follicle (HF) biology is still very limited. Because of the complexity of melatonin interactions and metabolism [11–18] and the substantial, often seemingly contradictory species-, gender-, and dose-dependency of melatonin-related hair effects [7, 10, 11, 19–26], the picture of the exact functions of melatonin in hair biology seems to be still a blurred and confusing one.

However, the recent discovery that mammalian skin not only is a target of melatonin bioactivity, but also an important extrapineal site of its synthesis, regulation and metabolism [13, 27–29], and that additionally even in HFs of normal mouse skin and human scalp melatonin was detected [11, 18, 30], has re-vitalized general interest in melatonin as a modulator of hair growth and/or pigmentation.

On this background, after summarizing some salient features of melatonin biology that are most pertinent in the current context, this review summarizes the available evidence indicating a significant role of melatonin in hair biology. We will interpret this evidence in view of emerging concepts on the role of melatonin in general skin biology. Major open questions and unresolved controversies are defined and particularly promising avenues for future research into the 'melatonin-hair connection' and its potential clinical implications are delineated.

Melatonin biology 'in a nutshell'

Melatonin is a phylogenetically ancient, highly conserved indole with astoundingly pleiotropic biologic effects on

multiple cells, tissues and organisms. Because of its highly lipophilic chemical structure, it easily penetrates cell membranes and organelles where it, as well as its metabolites, protects intra- and extracellular components from oxidative damage [31–36]. Melatonin is generated enzymatically in a cascade of reactions beginning with uptake of the essential amino-acid L-tryptophan and the enzymatic formation of 5-hydroxytryptophan by trypto-phan hydroxylase (TPH) via its essential co-factor (6R) 5,6,7,8-tetrahydrobiopterin (6-BH$_4$) [37–39]. Decarboxyl-ation produces serotonin and further synthesis requiring the alleged rate-limiting enzyme arylalkylamine-*N*-acetyl-transferase (AANAT, EC 2.3.1.87) [2, 40–43] leads to the formation of *N*-acetylserotonin. Further methylation by hydroxy-indol-*O*-methyltransferase (HIOMT) produces melatonin [18, 27] (Fig. 1). While decarboxylases are available in most tissues, the enzymes TPH, AANAT and HIOMT have to be present locally to enable melatonin synthesis [44]. In mammals, melatonin was long thought to be secreted predominantly by the pineal gland, but several important extrapineal sites of melatonin synthesis are now recognized as well, as shown recently for most cell types and tissues of cutaneous origin and even for murine and human HFs [11, 27].

Metabolism of melatonin can occur in an organ- and/or compartment-dependent manner via systemic metabolism of melatonin after oral intake or by release from the pineal gland mediated in the liver by cytochrome p-450 and 6-hydroxylase to produce 6-hydroxymelatonin (6-OH-mel). 6-OH-mel is the main systemic metabolite found in the human body and is further conjugated in the kidney by sulfate to be excreted in the urine as 6-sulphathoxymela-tonin [45–47]. Alternative pathways degrade melatonin to 5-methoxytryptamine (5-MT), 5-methoxyacetaldehyde, 5-methoxy-indol-acetic acid and 5-methoxytryptophol [18]. Oxidation of melatonin by reactive oxygen species (ROS) [48–50], or enzymatically by 2,3-dioxygenase, myeloperoxidase or oxyferrylhemoglobin, leads to the formation of the intermediates 2-hydroxy and 4-hydrox-ymelatonin (2-OH/4-OH-mel) and finally to N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK). The latter is further degraded by catalase to N^1-acetyl-5-methoxykynur-amine (AMK) [13, 17, 51, 52]. Alternatively, AMK has recently been found to be also produced by mitochondrial cytochrome c oxidation [14].

In mammals, melatonin, modifies numerous physiological processes, of which seasonal biological rhythms [53, 54], daily sleep induction, and modulation of immunological
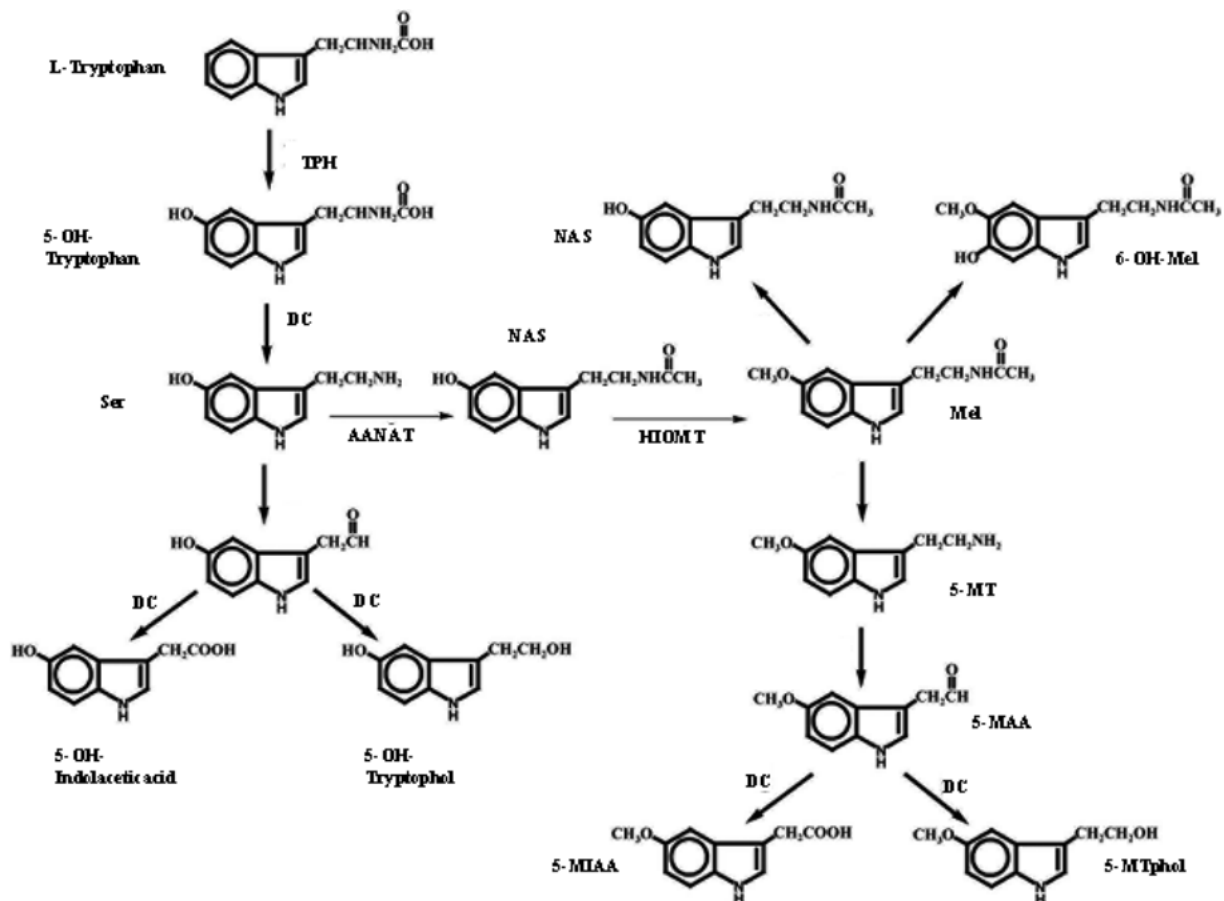

Fig. 1. Pathway of melatonin synthesis and metabolism. TPH, tryptophane hydroxylase; DC, decarboxylase; AANAT, arylalkyl-*N*-acetyl-transferase; HIOMT, hydroxy-indol-*O*-methyltransferase; Ser, serotonin; NAS, *N*-acetylserotonin; Mel, melatonin; 5-MT, 5-methoxy-tryptamine; 5-MAA, 5-methoxyacetaldehyde; 5-MIAA, 5-methoxy-indol acetic acid; 5-MTphol, 5-methoxy-tryptophol; 6-OH-Mel, 6-hydroxymelatonin.

defense reactions [55] represent but a few prominent examples. Furthermore, melatonin exerts anti-carcinogenic activities both in vitro and in vivo, that can be enhanced by expression of MT1, MT2 or retinoid-related orphan receptor α (RORα) receptors depending on the cell line [56–61].

The exceptionally wide range of documented biological activities of melatonin in different systems, cells, and species is further complicated by many (biologically active) derivatives that are generated in vivo from melatonin [16]. In addition to its mainly receptor-mediated functions, melatonin also exerts direct receptor-independent chemical effects, which render it a potent radical scavenger [12, 62–64] as well as a chemocytotoxicity-preventive substance [62, 65].

Melatonin binding protein have been first described in murine skin in the epidermis and the epithelial bulb of the HF [66]. At present, melatonin receptors can be specifically identified as membrane-bound, cytosolic and nuclear receptors [67–69] (Table 1). MT1 and MT2 receptors (formerly Mel1a and Mel1b) are membrane-bound, G protein-coupled receptors that were initially thought to be expressed primarily in the central nervous system (first identified as MT1 in the retina and MT2 in the brain of chicken and hamster. As then, melatonin membrane receptors have been associated with many different sites and functions, e.g., MT1 transcripts have now also been found in murine heart, kidney, liver, and lung tissue, while MT2 mRNA was also detected in mouse lung [56, 68, 70, 71].

A third specific melatonin binding site, initially named MT3, was later identified as the cytosolic enzyme, NRH: quinone oxidoreductase 2 (NQO2, EC 1.6.99.2), a flavoprotein that catalyzes the reduction of quinones and therefore is related to the redox status of the cell [67, 72, 73]. To date, the biological role of NQO2 is poorly understood, but there is some evidence for association with anti-carcinogenic effects, as NQO2 knockout mice are significantly more sensitive to skin tumor induction by carcinogens compared with normal mice [74]. In functional cell growth assays of malignant cells (e.g., melanoma), NQO2 correlated with tumor suppressive effects of melatonin [60] and NQO2 is also involved in the protection of cells by melatonin from oxidative damage [75]. Thus, it might be hypothesized that NQO2 may play a role in the prevention of (oxidative?) stress-induced HF catagen regression, and this is supported by the wide expression of the NQO2 gene in human skin [30] (Table 1). However, next to nothing is yet known about the NQO2 hair-connection.

The nuclear receptors for melatonin belong to the RORα that is a member of the RZR/ROR subfamily. This subfamily consists of at least four splicing variants: RORα1, RORα2, RORα3 and RZRα (RORα4) [69, 76, 77]. We recently suggested to change the nomenclature of the last isoform (RZRα) to RORα4 for consistent terminology, as RZRα and RORα4 differ only by a single nucleotide substitution [30]. RORα appears to be widely expressed, with the highest levels found in leukocytes and skin [78]. While classical chronobiology considers melatonin exclusively a hormone occurring in the plasma at daytime levels of 20–50 pg/mL in mammals including

humans, recent data have revealed a variety of compartments including bile, bone marrow, cerebrospinal fluid, and gastric mucosa [79–82] that not only represent important sites of extrapineal melatonin synthesis in situ, but even more surprisingly reveal melatonin concentrations at orders of magnitudes higher than those in the plasma. These data therefore support the view that melatonin might occur at tissue-specific concentrations in different compartments where it exerts biologically-relevant effects at both physiological and pharmacological concentrations [83, 84].

While the relevance of melatonin has been systematically investigated in different organ systems, including ovary [85], eye [86], gut [82, 87, 88], bone marrow [79] as well as in lymphocytes [89], and skin (reviewed in [12, 18, 30, 90]), detailed, systematic knowledge of melatonin in hair biology remains rather limited.

Melatonin receptor expression in the hair follicle

Some of the reported hair growth- and/or pigmentation-modulatory effects of melatonin might result from receptor independent, direct effects of melatonin, while others are likely to result from signaling via functional melatonin receptors expressed by HFs.

Genes encoding the MT1 receptor have been identified in HF keratinocytes and dermal papilla fibroblasts, but not in HF melanocytes [28] (Table 1). Moreover, an aberrant form of MT2 has been identified in dermal papilla fibroblasts, but was not expressed by HF keratinocytes or melanocytes. Hair-cycle-dependent MT2 and RORα mRNA transcription [as assessed by reverse transcriptase polymerase chain reaction (RT-PCR)] has been reported in C57BL/6 mouse skin, although not in single murine HFs, where MT2 expression was up-regulated in late-anagen and catagen, and down-regulated in telogen (Table 1). Alternatively, RORα was down-regulated in late anagen and up-regulated in late catagen and decreased in telogen [11]. In contrast to human cell lines, MT1 expression was not found in mouse skin and no high affinity melatonin binding site was found in cashmere goat skin [11, 91] (Table 1).

Prominent RORα-like immunoreactivity (IR) was detected in the mesenchymal dermal papilla and the epithelial inner and outer root sheaths of C57BL/6 mouse pelage HFs in situ [11]. While MT1-like IR in human skin has but yet been detected in HFs, this receptor has been detected in keratinocytes of the differentiating layers of the epidermis and in eccrine sweat glands. MT2 receptor IR has only been shown in eccrine sweat glands (Fig. 2). However while melatonin receptors are quite likely to exhibit functional effects on human HF cycling and growth regulation, their precise expression pattern and proof of their functional activity is still lacking.

Interaction of melatonin with androgen receptor- and estrogen receptor-mediated signaling

Melatonin not only interacts with its cognate receptors but surprisingly can interact also with androgen- and estrogen receptor-mediated signaling pathways. This may be highly relevant, given the central importance of androgens and estrogens in hair growth control [92–94]. Melatonin is

3

Table 1. Expression of genes encoding melatonin receptors in single cells of skin and hair follicle origin, mouse and human skin

Cell/tissue type		Species	Detection	Melatonin binding site	MT1	MT2	NQO2 (MT3)	RORα	RORα1	RORα2	RORα3	RORα4 (RZR1)	Ref.
Keratinocytes	Epidermal keratinocytes	Human	RT-PCR		+	−	+	+	−	−	−	+	[28, 30]
	Immortalized keratinocytes (HaCaT)	Human	RT-PCR		−	Aberrant	+	+	−	−	−	+	[28, 30]
	HF keratinocytes	Human	RT-PCR		+	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[28]
		Human	RT-PCR		+	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[28]
Melanocytes	Epidermal melanocytes	Human	RT-PCR		−	Aberrant	+	+	−	+	−	−	[28, 30]
	Immortalized melanocytes (PIG-1)	Human	RT-PCR		−	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[112]
	Immortalized normal melanocytes	Mouse (C57BL/6)	RT-PCR		−	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[28, 30]
Fibroblasts	HF melanocytes	Human	RT-PCR		−	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[28, 30]
	Adult dermal fibroblasts	Human	RT-PCR		+	−	+	+	+	−	−	+	[28, 30]
	HF fibroblasts	Human	RT-PCR		+	Aberrant	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[28, 30]
Skin	Epidermis	Mouse (C57BL/6)	In situ autoradiography	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[66]
Skin	HF (epithelial bulb)	Mouse (C57BL/6)	In situ autoradiography	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[66]
Skin	−	Goat (Cashmere/Angora)	In situ autoradiography	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[91]
Skin	−	Mouse (C57BL/6)	RT-PCR	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[111]
Skin	−	Mouse (C57BL/6)	RT-PCR		b.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[112]
Skin	−	Mouse (C57BL/6)	In situ immunoreactivity		−	+ h.c.d.	n.d.	+ h.c.d.	n.d.	n.d.	n.d.	n.d.	[11]
HF	−	Mouse (C57BL/6)	In situ immunoreactivity		n.d.	−	n.d.	+ h.c.d.	n.d.	n.d.	n.d.	n.d.	[11]
Normal skin	−	Human	RT-PCR		+	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[28]
Scalp skin	Epidermis	Human	In situ immunoreactivity		+	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[30]
HF		Human	In situ immunoreactivity		+ (upper ORS, IRS)	+ (IRS)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[30]

+, present; −, absent; n.d.: not done; b.d.: below detectability; h.c.d.: hair-cycle dependent; ORS: outer root sheath; IRS: inner root sheath; HF, hair follicle; RT, PCR, reverse transcriptase polymerase chain reaction.
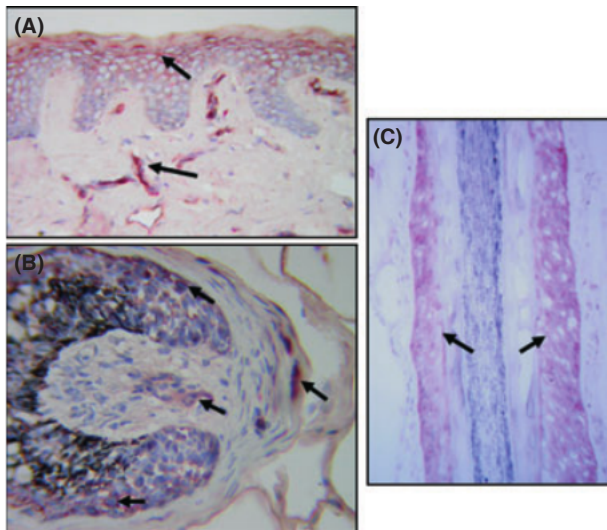
Fig. 2. Localization of immunoreactivity (IR) of melatonin in human scalp skin. (A) Melatonin-IR is mainly expressed in the differentiating keratinocytes of the upper layers of the epidermis (spinous and granular layer) and in the blood vessels. (B) In the hair bulb, melatonin-IR is expressed in the matrix keratinocytes, the blood vessels of the dermal papilla and the connective tissue sheath. (C) In the hair shaft, melatonin-IR is detected in the outer root sheath.

reported to exert anti-androgenic effects on prostate cells in rodents, which are exerted via androgen receptors at the peripheral level [95, 96]. Specifically, melatonin interacts with the nuclear androgen receptor and counteracts its growth stimulatory effects by facilitating translocation of the receptor from the nucleus to the cytoplasm [97]. This translocation is mediated by a melatonin-induced increase in calcium and protein kinase c (PKC) activation [98]. Furthermore, 17-*β*-estradiol-mediated inactivation of melatonin binding to the androgen receptor is ablated by preincubation of prostate cells with a specific inhibitor of PKC [99].

On the other side, human prostate cells express functional melatonin receptors (MT1), and sex steroids reportedly interfere with the melatonin receptor in benign prostatic cells [99, 100], e.g., 17-*β*-estradiol reduces the affinity of the melatonin receptor to [125I]-melatonin, and dihydrotestoterone attenuates the melatonin-mediated inhibitory effects on cell growth [99, 101]. Also, the melatonin-related increase in 3′,5′-cyclic adenosine monophosphate and decrease in 3′,5′-cyclic guanosine monophosphate is attenuated by 17-*β*-estradiol [99].

In other sex-steroid sensitive tissues such as ovarian granulosa cell membranes, MT1 expression and binding may be down-regulated by estradiol and up-regulated by FSH and testosterone [101]. On the other hand, melatonin has direct and indirect effects on the estrogen/estrogen-receptor pathway as shown in human breast cancer cells [102–105]. Growth of human breast cancer cells is inhibited via inactivation of estrogen receptor *α* (but not estrogen receptor *β*) through activation of melatonin membrane receptor MT1 and nuclear receptor RZR*α* [104–106]. This anti-estrogenic effects are mediated by inhibiting the calmodulin-mediated pathway of estrogen receptor activation and gene transcription [104]. Additionally, melatonin interacts on a estrogen presynthesis step by modulating aromatase (the enzyme responsible for local androgen to estrogen transformation) activity and gene expression [107, 108]. Recently, it has been clearly demonstrated in MT1 receptor-transfected breast cancer cells that the MT1 melatonin receptor is a key to reduce aromatase activity and expression, leading to a melatonin-induced inhibition of breast carcinoma cell proliferation [102]. In murine HFs, melatonin has already been shown to inhibit estrogen receptor *α* expression in a hair-cycle-dependent manner, with maximum mRNA reduction in late anagen and telogen, whereas estrogen receptor *α* protein is reduced by melatonin in all hair-cycle phases [11].

From the above observations, several conclusions may be drawn to help explain the effects of melatonin in hair growth regulation: as the high affinity melatonin receptor MT1 expressed in human prostate epithelial cells and breast cancer cells is the same as the MT1 receptor expressed in human skin [28], the anti-androgenic effects of melatonin might be also expressed in the skin. Similar conclusions for the HF might be drawn carefully, as the expression of MT1 has been only shown for single cells of human HF origin (HF keratinocytes, dermal papilla fibroblasts), and in human epidermis [28]. It is hypothesized, although, that melatonin's anti-androgenetic effects could be mediated via the same mechanisms as described for prostate epithelial and breast cancer cells. Such mechanism could very well explain the clinically observed anti-hair loss effects of melatonin in androgenetic alopecia (AGA) [25].

Melatonin and the skin

Over the last decade, increasing evidence has accumulated that melatonin plays a significant role in skin biology – either as an endogenous factor within the melatoninergic functionally active system of the skin or when exogenously administered (reviewed in [12, 13, 18, 27, 28, 30, 90, 109, 110]).

Mammalian skin expresses melatonin binding sites, membrane receptors, cytosolic and nuclear receptors [28, 30, 66, 111, 112]. Whereas mouse skin expresses MT2, but not MT1 receptor [11, 112], human skin shows variable expression of both receptors. Skin-derived cells in vitro mainly express MT1 and an aberrant form of MT2, whereas MT1 is expressed in situ in epidermis, HF, eccrine glands, blood vessel endothelium while and MT2 is only weakly expressed in HF inner-root sheath (IRS), eccrine glands, and blood vessel endothelium (Table 1) [28, 30].

The ROR*α* and its isoforms are heterogeneously expressed in different cell lines of cutaneous origin as assessed by RT-PCR (Table 1). While ROR*α*1 and ROR*α*4 are expressed in adult dermal fibroblasts, the isoform ROR*α*2 was detected only in an immortalized melanocyte line (PIG-1). ROR*α*3 has not been detected in any cell line investigated so far, though ROR*α*4 was detected in malignant melanoma cells [30, 60].

The skin – the largest organ of the mammalian body – has been identified as yet another, important site of peripheral, extra-pineal melatonin synthesis. This work

5

was stimulated by the discovery that hamster skin contains activity for AANAT, the key enzyme of melatonin synthesis [113]. This finding prompted a series of further studies that reported expression of a full melatoninergic system in human and rodent skin in situ as well as several of their constituent cell populations in vitro [27, 29, 37, 114, 115] (Fig. 1). Specifically, transcripts of the key relevant enzymes for melatonin synthesis, and the actual protein synthesis and/or activity of these enzymes have been identified both in the intact tissue and in primary cutaneous cell populations prepared from hamster, mouse [11, 115] and human skin [11, 27, 114].

An important exception to the classical pathway of intrapineal melatonin synthesis can be found in the skin of C57BL/6 mouse. These mice have a mutation in the AANAT gene, which results in the production of an inactive enzyme. Here serotonin is acetylated to NAS, the obligate precursor to melatonin by alternative enzyme(s) [115]. Therefore, the C57BL/6 mouse should not any longer be considered a 'natural melatonin knockdown' species, as it is still often claimed [116], because NAS of cutaneous origin may be methylated to melatonin at local or distant tissue sites expressing HIOMT activity [18, 115].

While the skin is richly endowed with the required precursors for melatonin synthesis (e.g., via massive stores of serotonin within murine skin mast cell granules [117]), the essential enzymes for melatonin synthesis have all been identified in mammalian skin, and in a great variety of isolated, cultured human skin cells [27–29, 114]. Redundant to the above mentioned evidence that human or mouse skin actually engages in extrapineal melatonin synthesis, melatonin detection in situ has been missing until recently. However, melatonin-IR has been found in epidermis and blood vessels of human scalp skin as well as in the outer-root sheath (ORS) and the hair-follicle bulb (Fig. 2).

Constitutive melatonin production [18] and UV-induced melatonin metabolism, with additional formation of anti-oxidant degradation products, has been identified in human keratinocytes [13], thereby defining a melatoninergic anti-oxidative system in the skin to protect against sun damage [13]. While melatonin ameliorates UV-induced oxidative stress, it also inhibits melanogenesis and melanocyte growth [61, 118–120].

Melatonin exerts growth regulatory (stimulatory/inhibitory) effects in benign cells (human keratinocytes and fibroblasts) depending on the experimental conditions (serum-free/serum-supplemented, UV-exposed) [28, 34, 121], but shows clearly growth suppressive, anti-tumorigenic effects in malignant melanoma cells [30, 58, 60, 122, 123]. Melatonin has entered clinical use for metastatic malignant melanoma [124–126], but this anti-tumor effect is not limited to pigment cells, but has been found also in breast cancer [56, 127], colon carcinoma [57, 128], and squamous cell carcinoma [30]. In UV-induced damage, melatonin can reduce ROS more effectively than even vitamin C and trolox [33, 129, 130], and promotes cell survival and colony growth by influencing several checkpoints of apoptosis [34, 36]. Indeed, pretreatment of UV-exposed skin with melatonin, either alone or in combination with vitamin C and E, can significantly reduce UV-induced erythema [131–133].

The 'melatonin-hair connection'

Hair follicles and their associated sebaceous glands ('pilosebaceous unit') are the skin's most prominent appendages and, together with the mammary gland, represent one of the defining features of mammals. This mini-organ, which is constructed as the result of complex neuroectodermal-mesodermal interactions [94, 134–136], not only is a target organ for numerous (neuro-)hormones, neuropeptides, neurotrophins, and neurotransmitters, but also produces many of these [137–141]. For example, the HF is both a target and source of prolactin [142, 143], estrogen [144], cortisol [145], CRH [146], thyroid hormones [147], and erythropoietin [148], and exhibits a functional hypothalamic–pituitary–adrenal axis [145] that has been also described for the skin [149]. Thus, the pilosebaceous unit is best viewed as a major neuroendocrine organ. On this emerging background, it is particularly intriguing to evaluate the existing evidence that yet another neuroendocrine key mediator – melatonin – also enjoys intimate connections with the biology of the HF.

Extrapineal melatonin synthesis by human and mouse hair follicles

It is increasingly appreciated that multiple extrapineal sites of melatonin synthesis exist in mammals [79, 80, 86, 88, 89], and mammalian skin has been shown to express the full enzymatic apparatus (and all the substrates and co-factors) necessary for melatonin synthesis [27, 29]. Therefore, it was rather confirmative, when final evidence for actual melatonin synthesis in mammalian skin in situ was generated by showing that mouse and human HFs actively generate this indoleamine under organ-culture conditions [11].

To begin with, prominent melatonin-like IR in human scalp HFs in situ has been independently reported by two laboratories, using different primary antibodies and immunohistological detection techniques [11, 18]. In normal human scalp skin sections, melatonin-like IR is seen in the HF ORS, at lower levels in the keratinocytes of the hair bulb matrix, blood vessels of the connective tissue sheath and in the basal lamina separating the hair bulb matrix from the follicular papilla. Distinct melatonin-like IR was also detected in the ORS of organ-cultured human scalp HFs, and also in the lower IRS and follicular papilla fibroblasts [11]. Interestingly, the latter study also revealed melatonin IR in keratinocytes of the ORS and the lower part of the IRS in murine back skin, as well as in the sebaceous gland and showed discrete, hair-cycle-dependent changes in expression [11]. IR for serotonin-*N*-acetyltransferase in human scalp epidermis and HF epithelium has also been reported [18].

However, specific follicular melatonin-like IR in skin and its appendages may represent serum-derived melatonin bound to intrafollicular melatonin receptors/binding sites, and thus does not, by itself, prove intrafollicular melatonin synthesis. Although already much less likely, a similar argument may still be evoked for explaining the intriguing radioimmuno assay (RIA) finding that tissue extracts showed 100–500-fold higher melatonin concentrations in murine vibrissae follicles and human scalp HFs than in

corresponding serum [11]. The most convincing evidence available so far that mouse skin fragments, mouse vibrissae follicles, and human scalp HFs do indeed synthesize melatonin was provided in HF organ culture where melatonin levels were significantly increased after stimulation with norepinephrin [11] – the physiological key stimulus in the β-adrenergic control of intrapineal melatonin synthesis [150].

Hair growth-modulatory effects of melatonin in nonhuman mammals

An indication that melatonin may modulate hair growth in several nonhuman mammalian species was proposed several decades ago. In the late sixties, the first influence of the pineal gland on hair cycle in mice was reported [5], followed by several studies reporting an induction or stimulation of the autumn molt in weasel, mink, red deer, and soay rams [7, 151–153] (Table 2). Thus, mammals exhibit a circadian and seasonal rhythm, which is most evident in those species that modulate their hair/fur growth according to seasonal alteration of the photoperiod (molting). This influence on fur was later described in limousine ram as an melatonin-induced increase of HF activity [154], an increase of growth initializing activity of secondary HFs in situ and hair shaft elongation in cultured HFs from cashmere goat [21, 155] (Table 2). Furthermore, melatonin was reported to induce the pro-anagen phase in the New Zealand goat [22] and to increase pelage development and cycle frequency in pigs [10] (Table 2). Indeed, the list of animal species showing effects of melatonin on hair growth is very extensive, and includes cashmere goat and other goat species [21, 22, 155, 156], ferrets [157], merino sheep [158, 159], mink [19], dogs [24, 160, 161], red deer [20], and others [162]. In many of these species the overcoat and undercoat fur are populated by primary and secondary HFs, and these are altered with change of the seasons and their cyclical activity is further disturbed when the pineal gland is experimentally removed [154].

Dietary supplementation with melatonin can increase the mitosis rate of secondary HF in cashmere goats during spring [21]. Moreover, the administration of melatonin (70 mg/day) over 14 days to New Zealand goats resulted in increased melatonin blood levels (914 pg/mL versus 19.9 pg/mL in controls), and this was associated with the transition of HFs from telogen (resting phase) into the growing pro-anagen phase; HFs of the untreated goats remained in the telogen stage [22]. The hair growth-promoting effect of melatonin is further supported by the finding that it can, dose dependently, stimulate both DNA-synthesis and hair shaft elongation in cashmere goat HFs in a 6-day ex vivo organ culture assay [155] (Table 2).

Melatonin at concentrations of 0.1–10 nM significantly stimulated epidermal keratinocyte DNA synthesis when added to organ-cultured mouse skin with the HFs in the resting phase (telogen), although it did not affect keratinocytes of the HF [66]. However, recent murine skin organ culture data suggest that melatonin can reduce spontaneous apoptosis in HF keratinocytes (as assessed by TUNEL) in un-manipulated organ culture of telogen mouse skin, confirming also the lack of any proliferative effect on HF keratinocytes (as assessed by Ki-67). Interestingly, in this study melatonin also significantly down-regulated the expression of estrogen receptor ERα in the HF matrix and IRS keratinocytes in organ-cultured C57BL/6 mouse skin [11].

Possible mechanisms of melatonin growth stimulatory effects might be deduced from assays using keratinocytes, the cell population that mainly builds the HF, in which melatonin at the concentration of 10 μM to 1 nM increased DNA synthesis, while 1 mM inhibited DNA synthesis. Using the ATP bioluminescence viability assay, melatonin increased cell proliferation at concentrations of 0.032–20μM [121]. However, while melatonin increased DNA synthesis in serum-free media (synchronized cell cycle), melatonin had the opposite effect in growth factor-containing media [28].

Melatonin effects on human hair growth

Reports on the direct effects of melatonin on human hair growth in vitro (using microdissected, organ-cultured anagen VI human scalp HFs) have been conflicting. One organ culture study using female and male HFs from scalp skin reported a stimulation of hair shaft elongation with 30 μM melatonin, while concentrations in the mM range were inhibitory [26]. In the former concentration the stimulatory effect was seen only during the early culture period from day 1–5, and this apparent hair 'growth' stimulation may instead reflect an enhanced protection of melatonin-treated organ-culture HFs from the consequences of general tissue damage after microdissection/wounding. This interpretation concurs with a subsequent independent study that reported no effects of melatonin on human scalp hair growth or hair matrix proliferation in vitro over a wide range of melatonin concentrations [11]. However, at present it has to be stated that melatonin at 10^{-12}– 10^{-6} M does not influence hair growth in vitro, whereas melatonin at 3.0×10^{-5} M does [11, 26] (Table 2).

Data on the clinical effects of melatonin on human scalp hair growth are limited. So far, there has been only a single double-blind, randomized, placebo-controlled trial in 40 women aged 20–70 years diagnosed with diffuse alopecia (AD) or AGA [25]. In this study, 1 mL of a 0.1% melatonin-containing alcohol solution was topically applied each evening for 6 months. To evaluate the effect of melatonin treatment on hair growth, trichograms were taken in defined areas on the frontal and occipital region of scalp hair before treatment and after 3 and 6 months of treatment. After 6 months of treatment, the occipital trichograms from women with AGA treated with melatonin showed an increase in the anagen rate from 76.3% to 85% (+8.7%) while the placebo showed only an increase from 78.22% to 82.11% (+3,89) (odds ratio 1.90; $P = 0.012$). In women with AD, however, the increase of anagen rate was from 82.2% to 83.8% (+1.6%) while there was a reduction of the anagen rate from 83.16% to 81.13% (−2.03%) in women treated with placebo (odds ratio of 1.41; $P = 0.046$). Thus, growth modulation induded by melatonin was slightly relevant in AGA, while in AD only marginal, however statistical significant in both cases [25].

In this pilot study, melatonin did not influence the rate of anagen hair growth in HF located in the frontal scalp area

8

Table 2. Effects of melatonin on hair growth and pigmentation

Species	Effect	Ref.
Growth		
Mouse	Influence on the hair cycle by the pineal gland	[5]
Weasel	Induction of molt	[152]
Mink	Induction of autumn molt	[7]
Red deer	Premature moulting of summer pelage and reduced serum prolactin concentrations	[20]
Soay rams	Stimulation of moulting	[151]
Limousine ram	Increased HF activity and reduced prolactin plasma levels	[154]
Mink	Induction of winter fur growth (supposedly by inhibition of prolactin)	[19]
Cashmere goat	Increase of growth initializing activity of secondary HFs in spring time	[21]
New Zealand goat	Induction of pro-anagen phase	[22]
Cashmere goat (cultured HFs)	Increase of hair shaft elongation and DNA-synthesis	[155]
Domestic pig	Increase of pelage development and cycle frequency	[10]
Ferret	Earlier change of winter and consecutive spring coat	[157]
Raccoon dogs	More rapid shedding of mature underfur hairs and growth of new underfur hairs; suppression of prolactin levels	[160]
Merino sheep	No influence of pinealectomy on wool growth and hair density	[159]
Siberian Husky dogs	No change in hair growth or anagen rate (topical administration)	[23]
Human (cultured HFs)	Increase of hair shaft elongation (30 μM); Decrease of hair shaft elongation (1–5 mM)	[26]
Human (cultured HFs)	No influence on hair shaft elongation, matrix keratinocyte proliferation/apoptosis and hair cycling (10^{-12}–10^{-6} M)	[11]
Human (trichograms)	Slight increase of anagen hair rate in women with androgenetic and diffuse alopecia	[25]
Pigmentation		
Weasel	Induction of hair color change	[152]
Mammalians	Effects on hair color	[178]
Djungarian hamster	Pattern of melatonin release induced by experimentally induced photoperiods modifies molt into summer pelage	[6]
Siberian hamster (cultured HFs)	Post-tyrosinase inhibition of melanogensis (10^{-10}–10^{-6} M)	[118]
Yellow mice (C3H/He-A*vy)	Slight reduction of coat darkening	[176]
Mountain hares	Season-dependent effects of melatonin on fur color	[9]
Djungarian hamster	Induction of the winter molt and pelage color change	[8]
Djungarian hamster	Change of fur color	[54]
Mouse	Inhibition of melanogenesis	[66]
Human (cultured HFs)	No effect on pigmentation (10^{-12}–10^{-6} M)	[11]

HF, hair follicle.

of women with AGA – the area mostly affected by hair thinning in this disorder, while the less androgen-sensitive occipital scalp skin area appeared to be positively influenced by melatonin. This effect might be interpreted as induction of hair growth by prolongation of the anagen phase, in part via retardation of the transition to catagen and/or by promotion of the transition from telogen to anagen, as has been observed in animals [22]. However, as the effects of melatonin in this study were only tested in six patients with AGA and 14 patients with AD (against equal number of patients treated with placebo), this study would require to be repeated with a larger number of patients for one diagnosis, and for a longer period. Moreover, it may also benefit from being complemented with additional hair growth parameters (e.g., phototrichogram, global hair photography, effluvium count, hair number, and shaft diameter), before sound conclusions can be drawn on the clinical efficacy of topical melatonin as an agent in the management of defined hair loss disorders. Also, while cutaneous penetration of topically applied melatonin has been reported [163, 164], the depth of melatonin penetration and the exact concentrations that are reached in the HF, especially the matrix keratinocytes remain open questions. However, topically applied melatonin may trigger complex secondary signaling cascades (from epidermis) that may then affect the pilosebaceous unit also indirectly.

The impact of melatonin on hair pigmentation

Melatonin effects on pigmentation have been reviewed in detail, focusing on skin [3] and the HF [4]. Hair shaft pigmentation is generated by specialized melanocytes of the HF pigmentary unit, whose melanogenic activity is strictly coupled to HF cycling (i.e., anagen III–VI) [165–170]. Growth, survival, and melanogeneic activity of these specialized melanocytes underlies complex, species- site- and HF type-dependent controls, which are only partially understood, and can not simply be equated with those recognized for epidermal melanocytes [3, 171–173] (Table 2). While melanocortins like alpha-melanocyte-stimulating hormone (a-MSH) and adrenocorticotrophic hormone (ACTH) have been the main focus of endocrinologists interested in hair pigmentation, many additional (neuro-)hormones, neurotrophins, neuropeptides and neurotransmitters are involved in the control of hair pigmentation in various mammalian species (e.g., beta-endorphine, histamine, estrogen, POMC, and NGF, to name but a few prominent examples) [4, 174–177]. Melatonin has been described to increase number of melanocytes in culture [120].

Early observations in farm and laboratory animals have reported that pinealectomy and/or melatonin administration altered hair shaft color in addition to hair growth, cycling or molting [6, 8, 10, 54, 152, 178] (Table 2). These observations have long suggested that melatonin may be one such neuroendocrine regulator of HF pigmentation. However, the literature continues to paint a rather confusing picture, and so, evidence that melatonin is indeed an important regulator of follicular melanogenesis under physiological conditions remains inconclusive.

While the classical 'skin lightening' effects of melatonin, which reflect primarily the induction of melanosome aggregation e.g., in frog melanophores, are well-known from work in amphibian skin [2, 179], much less is known on the effect of melatonin on mammalian melanocytes [3, 120, 165, 180, 181]. Given the numerous biological differences between epidermal and HF melanocytes [3, 172], however, it is quite unclear whether these findings are at all relevant to hair pigmentation. Evidently, this is even more the case for the reported inhibitory effects of melatonin on melanoma cell melanogenesis and/or growth, which may be antagonized in part by α-MSH [119, 182]. Therefore, the best currently available evidence for pigmentary effects comes from organ culture studies using hamster, mouse and human HFs – all of which are hampered by the shortcomings and limitations that are inherent to such complex assays [6, 8, 118, 176, 183].

Melatonin (0.1 nM–1 μM) reportedly inhibits the post-tyrosinase steps of melanogenesis in hamster HFs [118], and we have found that high dose-melatonin (0.01–100 μM) can inhibit follicular tyrosinase activity in organ-cultured mouse skin with all HFs in anagen growth phase [66] (Table 2). Thody and co-workers reported that melatonin administration slightly reduced coat darkening in young mice in vivo, when hair re-growth after shaft plucking was examined [176]. However, when we checked the effect of 0.001–1000 nM melatonin on organ-cultured human scalp HFs in anagen, no consistent and significant effects on the histochemcially detectable melanin content of human anagen VI hair bulbs in situ could be identified (as assessed by quantitative Masson-Fontana histochemistry) [11] (Table 2). While this study certainly does not rule-out effects of melatonin on human HF pigmentation under physiological conditions, it makes it likely that this indole is not a major modulator of human hair pigmentation. This conclusion is further supported by the lack of case reports of pigmentary effects induced by melatonin dietary supplementation, despite the copious, almost 'epidemic' consumption of sometimes massive oral doses of melatonin worldwide.

Conclusions and perspectives

In summary, murine HFs express transcripts and protein for the melatonin membrane receptor (MT2) and mRNA for the putative nuclear melatonin receptors (RORα) [11]. These intra-follicular melatonin receptors may be functionally active, as their stimulation by melatonin can down-regulate both HF keratinocyte apoptosis and estrogen receptor-α expression in situ [11]. Together with the fact that MT2 and RORα expression in murine skin are strikingly hair-cycle dependent, this raises the possibility that melatonin is somehow involved in hair-cycle control. Even more importantly, murine and human HFs are important sites of extrapineal melatonin synthesis and display a genuine melatoninergic system, which can be stimulated by catecholamines [11].

The two most significant remaining questions are: (i) What is the principal requirement for melatonin by HFs under physiological and pathological conditions and (ii) can melatonin administration be therapeutically exploited for the clinical management of hair growth disorders? Despite much suggestive in vivo evidence from the older literature of melatonin being an important

modulator of hair growth, cycling, molting and pigmentation in selected species (Table 2), the available evidence that melatonin substantially and reproducibly alters hair growth, pigmentation and/or cycling in mouse or human HFs under *physiological* conditions remains unsatisfactory.

Because of the potency of melatonin as a free radical scavenger [12, 63, 64], its anti-apoptotic properties in some systems [28, 34, 184, 185] and its proposed capacity to stimulate DNA repair [62, 186], the metabolically active and proliferatively active (but exceptionally damage-sensitive) anagen hair bulb may exploit melatonin synthesis in loco as a cytoprotective and apoptosis-suppressive strategy [11]. This concept deserves systematic exploration. If confirmed, it may become exploitable in the context of chemotherapy-induced alopecia [187–189]. Given that anagen termination by premature entry into apoptosis-driven HF regression (catagen) lies at the heart of essentially all of the clinically most relevant hair loss disorders [94, 190], it therefore certainly is a key challenge for future, clinically relevant research into the 'melatonin-hair connection' to clarify whether and under which circumstances defined doses of melatonin effectively inhibit human HF keratinocyte apoptosis in situ.

Also, the documented down-regulatory effect of melatonin on ER-α expression may render the HF less sensitive to stimulation by estrogens [144]. In addition to the intriguing endocrine link between estrogens and melatonin, another one exists between prolactin and melatonin. Melatonin serum levels have long been recognized to modulate pituitary prolactin secretion [22, 154]. In view of our recent finding that both murine pelage HFs and human scalp HFs express prolactin and prolactin receptors and employ prolactin receptor stimulation to induce catagen [142, 143], it will be interesting to study whether exogenous melatonin and/or melatonin generated by the HF itself has any impact on follicular prolactin synthesis.

This begs the question: does melatonin exert its most important hair growth-modulatory properties in vivo and in physiological concentrations *indirectly*, e.g., via the estrogen/prolactin axes sketched here? Perhaps, this explains, at least in part, why it has been so difficult to actually prove hair growth- and/or pigmentation-modulatory effects of melatonin? Moreover, given the well-recognized regulation of clock gene expression and activity by melatonin (e.g., in birds, fish, mice nonhuman primates [191–194], and the potential importance of clock genes in hair-cycle control [195], species-dependent hair-cycle-regulatory effects of intrafollicularly generated melatonin may also result from targeting the expression/ activity of clock genes, some of which may actually be expressed in the HF.

Apart from its evident relevance for the – as yet unknown – auto-regulation of intrafollicular melatonin synthesis the stimulation of HF melatonin synthesis by catecholamines raises the question whether this melatoninergic system primarily has inducible, hair growth-regulatory functions, or serves to protect the HF against systemic stressors (sensed and activated by high noradrenaline levels [138]. If the latter speculation holds true, stress-induced hair loss might result from an imbalance between increased systemic noradrenalin levels and the HF's inability to protect itself via the production of sufficient melatonin.

Exploration of the melatonin-hair connection likely holds lessons to better understand the role of melatonin in other skin appendages as well – especially the largest one of all: the mammary gland! It deserves mentioning here that melatonin has long been recognized as an inhibitor of mammary gland development and growth [196, 197].

In short, with the recent recognition of melatonin receptor expression and melatonin synthesis in the HFs of mouse and human, and the tremendous recent progress in understanding the molecular mechanisms which underlie melatonin's vexingly pleiotropic functions (amply documented on the pages of this journal throughout the past decade), it has now become fascinating, clinically important, and scientifically productive to systematically follow-up, at long last, the existing ancient leads to an important role for melatonin in hair biology.

Acknowledgments

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