Melatonin: A Cutaneous Perspective on its Production, Metabolism, and Functions

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Melatonin, an evolutionarily ancient derivative of serotonin with hormonal properties, is the main neuroendocrine secretory product of the pineal gland. Although melatonin is best known to regulate circadian rhythmicity and lower vertebrate skin pigmentation, the full spectrum of functional activities of this free radical-scavenging molecule, which also induces/promotes complex antioxidative and DNA repair systems, includes immunomodulatory, thermoregulatory, and antitumor properties. Because this plethora of functional melatonin properties still awaits to be fully appreciated by dermatologists, the current review synthesizes the main features that render melatonin a promising candidate for the management of several dermatoses associated with substantial oxidative damage. We also review why melatonin promises to be useful in skin cancer prevention, skin photo- and radioprotection, and as an inducer of repair mechanisms that facilitate the recovery of human skin from environmental damage. The fact that human skin and hair follicles not only express functional melatonin receptors but also engage in substantial, extrapineal melatonin synthesis further encourages one to systematically explore how the skin's melatonin system can be therapeutically targeted in future clinical dermatology and enrolled for preventive medicine strategies.

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Abbreviations: AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; HF, hair follicle; MT1 and 2, melatonin type 1 and 2 receptors; TPH1 and 2, tryptophan hydroxylase 1 and 2

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The paper is dedicated in gratitude to the memory of Aaron B Lerner, who discovered melatonin and initially defined its activity in pigment cells.

MELATONIN: A JOURNEY THROUGH TIME

The methoxyindole, melatonin (*N*-acetyl-5-methoxytryptamine), is produced by at least three clades of bacteria, all clades of eucarya including dinoflagellates, unicellular and multicellular fungi, at least 120 plant species, and animal species including simple and complex vertebrates and invertebrates (Back et al., 2016; Hardeland, 2016; Hardeland et al., 1995; Pöggeler et al., 1991; Tan et al., 2013, 2014).

The presence of melatonin in Alphaproteobacteria and Cyanobacteria indicates an early appearance of this multifunctional serotonin derivative in the evolution of life (Tan et al., 2012, 2013). The discovery of antioxidative properties of melatonin (Hardeland, 2005, 2017; Hardeland et al., 2011; Reiter, 1998; Tan et al., 2002) is consistent with an ancient role of this molecule in contributing to survival under high oxygen levels or exposure to UVR. Because of melatonin production in mitochondria (Reiter et al., 2017b; Suofu et al., 2017; Tan et al., 2013), scavenging of free oxygen radicals has likely been a primary role of melatonin in evolution (Hardeland et al., 1995) originating around 2.5–3 billion years ago (Reiter et al., 2017; Tan et al., 2015).

In all organisms, melatonin is formed from tryptophan via serotonin, with taxon-specific variations in the sequence of steps and intermediates (Back et al., 2016; Hardeland, 2015, 2016; Tan et al., 2012, 2014, 2015, 2016) (Supplementary Text S1 online).

In vertebrates, melatonin is mainly perceived as the hormone of the pineal gland (Lerner et al., 1958; Reiter, 1991). The mammalian pineal gland represents the major source of melatonin in the blood, and in the cerebrospinal fluid of the third ventricle of the brain, where it contributes to the regulation of the circadian system (Reiter et al., 2014). Melatonin is also synthesized in numerous extrapineal sites such as brain, Harderian gland, retina, lens, cochlea, immune system, lung, gastrointestinal tract, liver, kidney, thyroid, pancreas, thymus, spleen, carotid body, reproductive tract, endothelial cells, and skin (Acuña-Castroviejo et al., 2014; Hardeland et al., 2011; Slominski et al., 2008; Venegas et al., 2012). Melatonin levels are regulated by its rapid metabolism in the liver or peripheral organs including skin (Slominski et al., 2017b).

Since its origin in early unicells, melatonin, while protecting against oxidative stress, has acquired numerous functions that are taxon-, species-, and tissue-specific. In vertebrates, these functions are manifold and complex, with melatonin acting as a pleiotropic regulator of numerous parameters that orchestrate complex cell and tissue responses, both directly and indirectly (via the circadian system) (Hardeland et al., 2011; Tan et al., 2015). A full record of these functions is summarized elsewhere (Hardeland et al., 2011; Pandi-Perumal et al., 2006; Tan et al., 2015).

MELATONIN IN THE SKIN

Production and metabolism

Under physiological and pathological conditions, skin not only expresses key enzymatic elements of the pathway but can also produce serotonin, *N*-acetylserotonin, and melatonin with its metabolites (Kim et al., 2013, 2015a, 2015b; Kobayashi et al., 2005; Nordlind et al., 2008; Schallreuter et al., 2012; Semak et al., 2004; Slominski et al., 1996, 2002a, 2002b, 2002c, 2003a, 2005c, 2008) (Supplementary Figure S1 online). Skin can also synthesize/recycle the (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin, a cofactor for tryptophan hydroxylase (TPH) (Grando et al., 2006; Schallreuter et al., 1997, 1998). Hydroxytryptophan can also be generated in skin nonenzymatically through H_2O_2 and UVA-induced free-radical-mediated oxidation of L-tryptophan (Schallreuter et al., 2008). In addition to widespread *TPH1* gene expression in skin cells (Slominski et al., 2002b, 2003a, 2005c), we also detected *TPH2* in melanocytes, dermal fibroblasts (Slominski et al., 2014b), and retinal pigment epithelium (Zmijewski et al., 2009). Skin can also express alternatively spliced forms of the melatonin-synthesizing pathway enzymes: TPH, arylalkylamine *N*-acetyltransferase, and *N*-acetylserotonin-*O*-methyltransferase (Slominski et al., 2002b). Cutaneous *N*-acetylserotonin is produced by both arylalkylamine *N*-acetyltransferase and arylamine *N*-acetyltransferase (Semak et al., 2004; Slominski et al., 2002a, 2003b).

Melatonin in skin cells is rapidly metabolized through indolic, kynuric, and P450-dependent pathways or via nonenzymatic processes induced by UVR or free radicals (Fischer et al., 2006a; Kim et al., 2013; Slominski et al., 1996, 2017b) (Supplementary Figure S1).

Although immunocytochemistry identified *N*-acetylserotonin and melatonin antigens in epidermal and follicular keratinocytes and melanocytes, adnexal structures, fibroblasts, endothelial cells, and mast cells (Kobayashi et al., 2005; Slominski et al., 2005c, 2008), only recently mass spectrometry quantified melatonin and its metabolites in human epidermis (Kim et al., 2015a, 2015b). Epidermal melatonin production depends on race, gender, and age with the highest melatonin levels in African Americans. Among metabolites, 6-hydroxymelatonin showed the highest levels followed by 5-methoxytryptamine, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), and N^1-acetyl-5-methoxykynuramine (Kim et al., 2015a, 2015b). Levels of AFMK and N^1-acetyl-5-methoxykynuramine were the highest in African Americans, and no racial difference was seen for 6-hydroxymelatonin and 5-methoxytryptamine. However, skin pathology-related changes in the epidermal content of melatonin and its metabolites remain to be systematically explored to dissect the relationship between their endogenous production and metabolic consumption and defined skin disorders.

Mechanism of action

The potential intracellular targets for melatonin action are shown in Figure 1. The receptor-dependent regulatory functions of melatonin are mediated through interactions with G protein-coupled melatonin type 1 and 2 receptors (MT1 and MT2) (Cecon et al., in press; Slominski et al., 2012). The latter predominantly work by inhibiting the production of second messengers (cAMP, cGMP), thereby modifying signaling pathways downstream of protein kinases A and C, and cAMP response element-binding protein (Dubocovich et al., 2010; Slominski et al., 2012) and by activating MAP kinases (discussed in Hardeland, 2009). MT2 shows a 60% homology in structure to MT1 (Reppert et al., 1996). MT1 and MT2 homodimerize as well as heterodimerize (Jockers et al., 2008), which can affect the pharmacological properties of the receptors (Jockers et al., 2008; Legros et al., 2014). In addition, their activity is modulated by C-terminal phosphorylation, and, in the case of MT1, by stabilization via the scaffolding protein MUPP1 and inhibition by heterodimerization with GPR50 (Hardeland, 2009).

MT1 and MT2 receptors have been detected in mammalian skin (Fischer et al., 2008a; Kobayashi et al., 2005; Singh and Jadhav, 2014; Slominski et al., 1994, 2005c, 2008). Human skin predominantly transcribes the *MT1* gene, with *MT2* showing restricted or conditional expression (Slominski et al., 2003c, 2005a). Gene expression and production of alternatively spliced or aberrant forms of MT receptors is modulated by UVB and skin pathology (Slominski et al., 2003c, 2005a). In contrast, murine skin showed exclusive expression of the *MT2* gene (Kobayashi et al., 2005; Slominski et al., 2004a). By immunocytochemistry, MT1 was detected in the differentiating layers of the epidermis, outer and inner root sheaths of the hair follicle (HF), eccrine glands, and blood vessels, whereas MT2 was detected in inner root sheath, eccrine glands, and blood vessels of human skin (Fischer et al., 2008a; Slominski et al., 2005a, 2005c).

It remains to be clarified whether there is also a nuclear receptor for melatonin, because the originally proposed nuclear melatonin receptor candidate, RORα, which is expressed in skin and HFs (Brozyna et al., 2016; Kobayashi et al., 2005; Slominski et al., 2005a, 2014a), has turned out to be a receptor for sterols and secosteroids, but not for melatonin (Slominski et al., 2014a, 2016, 2017a).

Melatonin and its metabolites act as free radical scavengers and protectors against oxidative stress (Fernández et al., 2015; Fischer et al., 2006c; Galano et al., 2013; Hardeland, 2017; Hardeland et al., 2011). Melatonin and its precursor *N*-acetylserotonin bind to several regulatory proteins including quinone reductase 2 (Jockers et al., 2008; Nosjean et al., 2000). Quinone reductase 2 protects cells against oxidative stress (Boutin, 2016; Hardeland, 2009), against dimethylbenz(a)anthracene-induced skin cancer (Shen et al., 2010), and is required for tumor necrosis factor-α-induced apoptosis in keratinocytes (Ahn et al., 2007). The capability of melatonin binding to this enzyme (see Kleszczynski et al., 2016) requires additional studies.

Calmodulin is another melatonin-binding protein, which may gain physiologically relevant affinity after Ca^{2+} binding and interaction with calmodulin-controlled enzymes, such as calmodulin kinase II and calcineurin, which regulate intracellular calcium homeostasis (Fernández et al., 2015; Fukunaga et al., 2002; Hardeland et al., 2009; León et al., 2000; Lu et al., 2015; Romero et al., 1998). These observations may partially explain the roles of melatonin in the endoplasmic reticulum stress response, regulation of apoptosis and autophagy, and mitochondrial homeostasis (Fernández