2.1 An Overview

It has been documented that skin resident cells can produce and further metabolize catecholamines, serotonin, and histamine (Fitzpatrick et al. 1993; Gillbro et al. 2004; Schallreuter et al. 1995; Slominski et al. 2005c). These biogenic amines not only regulate phenotype of skin cells cultured in vitro but also can affect skin functions and may have systemic effects (Schallreuter et al. 1997; Slominski and Wortsman 2000; Slominski et al. 2005c). The functional activity of biogenic amines in the skin is mediated through the interactions with specific cell surface receptors (Gillbro et al. 2004; Nordlind et al. 2008; Slominski et al. 2003d); however, non-receptor effects are also considered.

2.2 Catecholamines

2.2.1 Production and Metabolism

Nonessential aromatic amino acid L-tyrosine, depending on the cell type and enzymatic context, serves as a direct precursor to catecholamines, tyramine/octopamine (Yen 2001), and melanin pigment (Slominski et al. 2004c). To serve these diverse functions, L-tyrosine is either delivered through the gastrointestinal tract (GI) or produced through phenylalanine hydroxylase (PH)-mediated hydroxylation of L-phenylalanine (Blau et al. 2010; Schallreuter et al. 2008b). L-tyrosine is hydroxylated to L-dihydroxyphenylalanine (L-DOPA) by either tyrosine hydroxylase (TH) or tyrosinase (Tyr), or decarboxylated to tyramine by L-amino acid decarboxylase (AAD) (Fig. 2.1). L-DOPA is further decarboxylated to dopamine by AAD with subsequent hydroxylation and methylation reactions to generate norepinephrine or epinephrine, all of them being oxidated by monoamine oxidase (MAO) or methylated by catechol-methyl transferase (COMT) (Fig. 2.1). L-DOPA...
Fig. 2.1 Catecholamine synthesis in the skin. The common pathway in the skin requires its consecutive hydroxylations of L-phenylalanine [mediated by phenylalanine hydroxylase (PH)] to L-tyrosine with following hydroxylation by tyrosine hydroxylase (TH) or tyrosinase to produce L-dihydroxyphenylalanine (L-DOPA). L-DOPA is either oxidized to DOPA quinone with following multistep transformation to melanin or serves as a substrate for synthesis of catecholamines. The skin expresses complete enzymatic machinery required for dopamine synthesis (L-amino acid
and catecholamines can also be oxidized by either tyrosinase or metal cations to form melanin and neuromelanin pigments (Fitzsimons et al. 2002; Lassalle et al. 2003; Park et al. 2009; Slominski et al. 2004c) (Fig. 2.1).

Human epidermal keratinocytes and melanocytes have the capability to synthesize the catecholamines from L-tyrosine with sequential production of L-DOPA, dopamine, norepinephrine, and epinephrine through the action of classical enzymes listed above with the subsequent inactivation of catecholamines by MAO or COMT (Fig. 2.2) (Fuziwara et al. 2005; Gillbro et al. 2004; Schallreuter et al. 1992, 1995). Interestingly, acetylation of dopamine to N-acetylDOPA has also been described in the hamster skin (Gaudet et al. 1993). Activity of TH and PH depends on local availability of their essential cofactor/electron donor, i.e., 6R-L-erythro-5, 6, 7, 8-tetrahydrobiopterin (6BH4) as demonstrated for the first time by Schallreuter’s group (Schallreuter et al. 1994, 1997). Importantly, Schallreuter and coworkers demonstrated de novo synthesis/recycling/regulation of 6BH4 in both human epidermal keratinocytes and melanocytes as well as in hair follicles (Schallreuter et al. 1997, 1998). Furthermore, AAD activity requires pyridoxal phosphate (PP) as the cofactor, the cutaneous availability of which is regulated locally (Coburn et al. 2003). Lymphocytes and other immune cells can also represent an additional source of catecholamines: L-DOPA production with its further transformation to epinephrine and norepinephrine has been shown in human lymphocytes (Musso et al. 1997) as well as in Langerhans cells (Falck et al. 2004). An additional cutaneous source of catecholamines is their dermal release from adrenergic nerve fibers (Fitzpatrick et al. 1993). A challenging task in current skin biology is to determine which skin cells and adnexal structures have similar capability of de novo synthesis of catecholamines and what is the final product in different compartments.

An important alternative source of L-DOPA for cutaneous catecholamines is its production via the tyrosine hydroxylase activity of tyrosinase that, depending on the intracellular environment including acidic pH, may not undergo oxidation but will diffuse or be transported to other cells or systemic circulation (Slominski et al. 2004c, 2011a). In fact, diffuse “melanocytic organ” can provide DOPA or its adducts to systemic circulation to serve either as a precursor for further modifications or as a bioregulator (Slominski et al. 1993a, 2011a; Zmijewski and Slominski 2009a). A role for tyrosinase-derived L-DOPA is supported by findings that retinal network adaptation to bright light requires tyrosinase-dependent production of DOPA (Page-McCaw et al. 2004). This phenomenon represents the TH-independent pathway of peripheral dopamine synthesis (Eisenhofer et al. 2003) and

Fig. 2.1 (continued) decarboxylase, AAD) and its subsequent conversion into norepinephrine (dopamine β-hydroxylase) and methylation (phenylethanolamine N-methyltransferase) to form epinephrine. An alternative pathway of catecholamine synthesis involves decarboxylation of L-tyrosine to tyramine, which in turn is hydroxylated by TH (and Cyp2D) or dopamine β-hydroxylase to octopamine or dopamine, respectively. Octopamine could be metabolized to norepinephrine by TH. This alternative pathway which is present in invertebrates remains to be tested in the skin. Catecholamines also undergo oxidation to corresponding quinones with further multistep transformation to neuromelanin, a process similar to melanogenesis starting from L-DOPA.
it can regulate activities of melanocytes and immune cells (Slominski and Paus 1990; Slominski et al. 1998c). These findings are in agreement with our hypothesis that L-tyrosine and L-DOPA can have hormone- and neurotransmitter-like roles (Slominski and Paus 1990, 1994; Slominski et al. 2011a), with melanocytes acting as important regulators of catecholamines’ availability in the skin (Slominski et al. 1993a).

### 2.2.2 Bioregulatory Role of Catecholamines in the Skin

#### 2.2.2.1 Dopamine Receptors

There are five subtypes of dopamine receptors, and they have been categorized into two families, i.e., D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4) (Watson 1994). The D1-like receptor agonists stimulate Gs-dependent intracellular production of cAMP (Missale et al. 1998). The D2-like receptor agonists activate Gi proteins and inhibit intracellular cAMP signaling pathway (Missale et al. 1998; Watson 1994). In addition, via Gβγ subunits, D2-like receptors are capable of inhibiting N- and L-type calcium channels which results in the
activation of G-protein-regulated inwardly rectifying potassium channels (GIRKs) (Beaulieu and Gainetdinov 2011). After D2-like receptors were identified in the keratinocytes (Fuziwara et al. 2005) they were found to play a significant role in the maintenance of epidermal barrier homeostasis. Application of D2-like receptor agonists accelerated barrier recovery, whereas D2-like receptor antagonists delayed it. Actual receptor subtypes localize to different parts of the epidermis: D4 is localized in the uppermost part of the epidermis and D2 is localized in the basal layer of the epidermis where it plays a role in the regulation of cell proliferation (Fuziwara et al. 2005). It remains to be tested whether dopamine is also regulating epidermal and follicular pigmentary systems as well as adnexal functions including hair follicle.

Dopamine receptors on lymphocytes exert differential effects. Dopaminergic signaling through D2-like receptors of T lymphocytes showed an immunostimulatory effect (Besser et al. 2005), whereas signaling through D1-like receptors had immunoinhibitory effect (Saha et al. 2001). Dopamine also inhibits proliferation of human lymphocytes and causes apoptosis of peripheral blood mononuclear cells (Bergquist et al. 1997). IL-6 (and other cytokines) stimulates a development of a subtype of T lymphocytes capable of producing IL-17 (and other cytokines), i.e., Th17 lymphocytes. Th17 lymphocytes constitute relatively recently described branch of immune responses (Harrington et al. 2006). Dopamine released by dendritic cells induces IL-6–Th17 axis and upregulates synovial inflammation (Nakano et al. 2011). The IL-6–Th17 axis plays a role in the pathogenesis of inflammatory diseases including rheumatoid arthritis. It can therefore be deduced that dopamine may also have various differential modulatory roles in the skin immune system.

2.2.2.2 Adrenergic Receptors

The adrenergic receptors belong to the classic seven-transmembrane G-protein-coupled receptor (GPCR) family. These receptors respond to catecholamines and can be subdivided into subtypes of \( \alpha \) and \( \beta \) families, based on their differential pharmacological responses and protein sequences (Lands et al. 1967). More specifically, these receptors are defined, in part, by their endogenous ligand affinity to \( \beta \) receptors having a higher affinity to epinephrine when compared to norepinephrine, and to \( \alpha \) receptors having a higher affinity for norepinephrine. Alpha adrenergic receptors can be further subdivided into \( \alpha_1 \) and \( \alpha_2 \), and \( \beta \) receptors can be further subdivided into \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \) subtypes. The \( \alpha_1 \) (\( \alpha_1a \), \( \alpha_1b \), and \( \alpha_1d \)) receptors couple to phospholipase C via \( G_{Q\alpha} \) and stimulate the formation of diacylglycerol and inositol trisphosphate (Cotecchia 2010). The \( \alpha_2 \) (\( \alpha_2a \), \( \alpha_2b \), and \( \alpha_2c \)) receptors couple to \( G_{i\alpha} \) and inhibit the formation of cAMP, whereas \( \beta \) receptors are positively coupled to the formation of cAMP via \( G_{s\alpha} \) (Hein 2006).

Various receptors of both \( \alpha \) and \( \beta \) subfamilies of adrenergic receptors are present on epidermal and dermal cells (Grando et al. 2006; Schallreuter et al. 1995). As expected, \( \alpha \) and \( \beta \) receptors are also expressed in dermal blood vessels. Their
activation by catecholamines causes vasoconstriction and decreases vascular permeability (Ding et al. 1995; Harada et al. 1996).

Keratinocytes express mainly β2 receptors and also α1 receptors (Steinkraus et al. 1992; Drummond et al. 1996; Sivamani et al. 2007). Stimulation of β-adrenergic receptors in epidermal keratinocytes results in increased cAMP production, activation of protein kinase C, and formation of inositol-1,4,5-trisphosphate, calcium influx, and extracellular signal-related kinase (ERK) dephosphorylation through the action of serine/threonine phosphatase PP2A (Chen et al. 2002; Pullar et al. 2001; Schallreuter et al. 1995). Catecholamines stimulate keratinocyte differentiation with increased expression of keratins 1, 10, involucrin, and transglutaminase (Mammone et al. 1998; Schallreuter et al. 1995). Moreover, there is a local gradient of receptor expression with the highest level in basal keratinocytes and decreasing level toward the surface of the epidermis (Schallreuter et al. 1995). This indicates a potential stimulatory functional role of catecholamines in the process of keratinocytes’ differentiation. Catecholamine-β2 adrenergic system has been implicated in the pathogenesis of atopic dermatitis, psoriasis, and vitiligo (Sivamani et al. 2007). Expression of β2 receptors is increased in vitiligo and decreased in psoriasis (Schallreuter et al. 1993; Takahashi et al. 1996). In vitiligo, there is an overproduction of 6-BH4 leading to a dysregulation of catecholamine biosynthesis with increased plasma and epidermal norepinephrine levels. This is associated with high numbers of β2 adrenoceptors in differentiating keratinocytes and with a defective calcium uptake in both keratinocytes and melanocytes (Schallreuter et al. 2008a). In atopic eczema, a point mutation in the beta 2-adrenoceptor gene could alter the structure and function of the receptor, thereby leading to a low density of receptors on both keratinocytes and peripheral blood lymphocytes (Schallreuter et al. 1997). It is also known that catecholamines and β receptors have a role in wound healing although their exact role is far from being clarified (Ghoghawala et al. 2008; Pullar et al. 2008) (see also discussion of fibroblast below). The adrenergic beta-receptors not only affect keratinocytes’ proliferation and differentiation but also their immune activities. Activation of β receptors on keratinocytes affects expression of β-defensin 3 (Martin-Ezquerra et al. 2011).

Studies on cultured melanoma cell lines have shown that catecholamines can be an additional factor affecting melanogenesis (Howe et al. 1991). Their role in the function of the pigmentary system has been well described in nonhuman systems (reviewed by Slominski et al. 2004c). Human melanocytes express α1 and β2 receptors (Gillbro et al. 2004; Hu 2000; Hu et al. 2000; Scarparo et al. 2000; Schallreuter et al. 1996). Activation of α1 receptors leads to the IP3-DAG signaling (Schallreuter et al. 1996) and β2 receptor activation leads to cAMP signaling (Gillbro et al. 2004). β2 but not α1 receptor activation induces pigmentation (Gillbro et al. 2004; Schallreuter et al. 1996). The expression of β2 receptors on human melanocytes increases in response to UV irradiation (Yang et al. 2006). UVB irradiation increases epinephrine release by cultured keratinocytes that in turn increases pigmentation in co-cultured melanocytes, which is an example of the interactions between these two cell types (Sivamani et al. 2009).
Adrenergic receptors are expressed also on immune cells of the dermis (Steinkraus et al. 1996). Binding of adrenergic agonists to their receptors on lymphocytes has immunostimulatory effect and affects their homing. On the contrary, stimulation of β receptor usually has immunosuppressive effects, but in other model systems can also cause immunostimulation, i.e., increase the number of lymphocytes (Bergmann and Sautner 2002).

Mouse Langerhans cells express α1, β1, and β2 adrenergic receptors (Seiffert et al. 2002), and it was shown that epinephrine and norepinephrine inhibit the ability of Langerhans cells to present antigens (Seiffert et al. 2002).

Agonists of β2 receptors on mast cells inhibit the release of preformed mediators such as histamine, and also newly synthesized mediators such as prostaglandin D2 from mast cells (Okayama and Church 1992). They also inhibit release of inflammatory cytokines from mast cells (Bissonnette and Befus 1997). β receptors are expressed on dermal fibroblasts (Pullar and Isseroff 2006; Pullar et al. 2008). Ligation of β2 receptors activates epidermal growth factor (EGF) receptor and extracellular signal-regulated kinase (ERK) signaling that in turn stimulates fibroblast migration. Binding of agonists to the β2 receptors can also activate protein A kinase (PKA) which can stimulate cell proliferation (Pullar and Isseroff 2006), attenuate collagen gel contraction, and alter actin cytoskeleton and focal adhesion distribution via PKA-dependent mechanisms (Pullar and Isseroff 2006). A link between body stress response system that results in the release of epinephrine and activation of intracellular signaling that leads to DNA damage has been shown recently (Hara et al. 2011). Specifically, in mouse and human fibroblasts binding of agonists to the β2 receptors led to Gs-protein-dependent activation of protein kinase A, followed by the recruitment of beta-arrestins. Then, β-arrestin 1 facilitated AKT-mediated activation of MDM2 and also promoted MDM2 protein binding to and degradation of p53 protein by acting as a molecular scaffold. The degradation of p53 resulted in the lack of protection and DNA damage (Hara et al. 2011).

### 2.2.2.3 Non-receptor-Mediated Effects of Catecholamines

In the skin there are several potential non-receptor-mediated effects, which are based on autoxidation of catecholamines in alkaline environment with a velocity increased by metal cations (Lassalle et al. 2003; Slominski et al. 2004c). The potential phenotypic implications are predominantly based on the well-documented activity of L-DOPA which through its oxidation products and active melanogenesis can affect functions of immune cells (Slominski and Goodman-Snitkoff 1992; Slominski et al. 2009b). The possible mechanisms of action were discussed previously (Slominski et al. 1998c, 2004c) and, therefore, have been shortly summarized below. L-DOPA dramatically inhibits an in vitro phosphorylation of glycoproteins dependent on the presence of Mn ions indicating action of quinones generated through oxidation of DOPA (Slominski and Friedrich 1992). It can also affect cellular metabolism in melanotic cells (Scislowski et al. 1984, 1985). Also, diffusible products of DOPA oxidation are potent inhibitors of lipid peroxidation.
(Memoli et al. 1997), and 5-S-cysteinyldopa inhibits hydroxylation/oxidation reactions induced by the Fenton reaction (Napolitano et al. 1996). The potential cycling from indole to quinone forms of l-DOPA and its derivatives may affect levels of reactive oxygen/nitrogen species or oxidation of intracellular proteins and lipids (Tsang and Chung 2009). Finally, both free and protein-bound l-DOPA can trigger expression of several antioxidant enzymes including superoxide dismutase or NAD(P)H:Quinone oxidoreductase (NQO1) (Nelson et al. 2007). Thus, taking into consideration similar chemical properties of DOPA and catecholamines (products of DOPA enzymatic metabolism), and that their oxidation leads to the production of neuromelanin, one can safely conclude that non-receptor-mediated effects and mechanisms will be similar to that described for DOPA (Slominski et al. 2011a). Taking into consideration the above chemical properties of dopamine, epinephrine, or norepinephrine, one can expect that at micromolar or higher concentrations the predominant effects will be non-receptor-mediated mainly through their oxidation products and neuromelanin polymers generated during this process. It is also possible that some of the phenotypic effects at lower concentrations could also be influenced by oxidative effects.

2.2.2.4 Conclusions

Dopamine, epinephrine, and norepinephrine are produced in the skin resident and nonresident cells. Their phenotypic effects are mediated through activation of dopaminergic and adrenergic receptors, the expression of which is cell-type and cell-context dependent. Their roles in epidermal, dermal, and adnexal as well as skin immune functions remain to be further investigated. There are also non-receptor-mediated mechanisms shared by their precursor, l-DOPA. It is likely that cutaneous catecholaminergic system will communicate with brain by activating sensory nerves, or, with other tissues, via entry into systemic circulation and by affecting immune cells circulating from the skin to other organs (Fig. 1.1).

2.3 Histamine

2.3.1 Production and Metabolism of Histamine

Histamine is derived from the decarboxylation of histidine by the l-histidine decarboxylase. After release, histamine is degraded by histamine-N-methyltransferase (in brain and at periphery) or diamine oxidase (in the periphery) (Fitzpatrick et al. 1993; Zhang et al. 2007). Histamine is produced mainly by mast cells and basophils. Cross-linking of IgE antibodies attached to the cell membrane represents a main mechanism for histamine release. Histamine binds to four different types of seven-transmembrane receptors that signal through G-proteins.
The H₁ receptor is found on smooth muscle and endothelial cells and is responsible for smooth muscle contraction and decreased adhesion of endothelial cells. H₂ receptor is located on vascular smooth muscles and parietal cells in the stomach and is responsible for vasodilatation and gastric acid secretion. H₃ receptor is found in the central and peripheral nervous systems and is responsible for decreased secretion of several neurotransmitters including histamine, acetylcholine, serotonin, and norepinephrine. H₄ receptor is found primarily on basophils and has a role in chemotaxis (Fitzpatrick et al. 1993; Zhang et al. 2007).

2.3 Histamine

2.3.2 Bioregulatory Role of Histamine in the Skin

In the epidermis, H₁ and H₂ receptors are expressed on keratinocytes (Albanesi et al. 1998; Koizumi and Ohkawara 1999; Koizumi et al. 1998; Shinoda et al. 1998) and H₂ receptors on epidermal melanocytes (Yoshida et al. 2000). Mediators released from mast cells inhibit keratinocyte growth in culture (Huttunen et al. 2001). Activation of keratinocyte H₂ receptors affects proliferation and differentiation via activation of the cyclic AMP pathway and also phospholipase C pathway with associated increase in intracellular calcium levels (Koizumi and Ohkawara 1999). In mouse keratinocytes, H₂ receptor signaling through the PLC second messenger system is inhibited during calcium-induced keratinocyte differentiation by an autocrine loop which involves downregulation of H₂ receptor expression and inhibition of histamine metabolism (Fitzsimons et al. 2002). In keratinocytes, activation of the H₁ receptor enhances UVB-induced IL-6 production (Koizumi and Ohkawara 1999; Koizumi et al. 1998), whereas H₁ receptor antagonists inhibit ICAM-1 expression (Ling et al. 2004). Histamine upregulates keratinocyte MMP-9 production via the H₁ receptor (Gschwandtner et al. 2008). H₂, however, not H₁, agonists stimulate intracellular calcium signaling in keratinocytes (Koizumi and Ohkawara 1999). In these cells, histamine acting on H₁ receptors increases the expression of IFN-γ-induced intercellular adhesion molecule 1 (ICAM-1) and MHC class I molecules. It also augments IFN-γ-induced release of chemokines such as CXCL10, as well as the release of GM-CSF via protein kinase Cz and extracellular signal-regulated (ERK) kinase (Giustizieri et al. 2004; Kanda and Watanabe 2004). In cultured keratinocytes, histamine through the activation of H₁ receptor inhibits CCL17 production by suppressing p38 MAP kinase and NF-κB activities. Histamine acts as a negative feedback signal for existing Th2-dominant inflammation by suppressing CCL17 and enhancing CXCL10 production (Fujimoto et al. 2011). The effect of histamine acting through H₂ receptor appears to be the opposite. Histamine, via H₂ receptor, increases survival of keratinocytes acting by NF-κB activation (Kim and Lee 2010). IL-17, produced by Th17 cells infiltrating into the dermis (a cytokine involved in various inflammatory skin diseases including psoriasis), stimulates keratinocytes to produce inflammatory mediators such as IL-36, TNF-α, IL-6, and IL-8 (Carrier et al. 2011). Histamine markedly augments the production of IL-8 and GM-CSF in the presence of IL-17 and TNF-α in...
keratinocytes (Moniaga et al. 2011). Moreover, histamine induces human β-defensin 2 and 3 production in keratinocytes acting via H₁ receptors by activating NF-κB, AP-1 pathway, or STAT1, STAT3, and AP-1 as well as JAK2 and MEK/ERK signaling pathways (Ishikawa et al. 2009; Kanda and Watanabe 2007). Histamine promotes cutaneous antimicrobial defenses and wound repair by stimulating secretion of defensins (Ishikawa et al. 2009; Kanda and Watanabe 2007). Histamine also enhances nerve growth factor production by inducing c-Fos expression in keratinocytes (Kanda and Watanabe 2003).

The activation of the H₂ receptors on melanocytes stimulates melanogenesis (Yoshida et al. 2000). Histamine, similarly to α-MSH, contributes to hyperpigmentation by enhancing eumelanin/pheomelanin ratio (Lassalle et al. 2003). Acting at the H₂ receptor histamine stimulates melanocyte migration in culture via signaling through ERK, CREB, and Akt (Kim and Lee 2010). Histaminergic system is upregulated in the B16F10 melanoma cells when compared to noncancerous melanocytes, which indicates that it might have a role in tumorigenesis (Davis et al. 2011). Both Western blot and immunohistochemical studies showed much stronger histidine decarboxylase expression in melanoma cells as compared to normal melanocytes (Haak-Frendscho et al. 2000). Moreover, H₁ histamine receptor antagonists were shown to induce genotoxic and caspase-2-dependent apoptosis in human melanoma cells, but not normal melanocytes (Jangi et al. 2006).

In the dermis, histamine receptors are expressed on fibroblasts, immunocytes, endothelial cells, blood vessels, smooth muscle, and nerve endings (Fitzpatrick et al. 1993). In Th2 lymphocytes stimulation of H₄ receptor led to the activation of transcription factor AP-1 followed by the release of IL-31, which is involved in the development of pruritus (Gutzmer et al. 2009). On the other hand, activation of H₄ histamine receptors expressed on monocytes activated intracellular calcium mobilization and inhibited the CCL2 chemokine production which reduced recruitment of monocytes (Dijkstra et al. 2007). Histamine acts on H₄ receptors of eosinophils and mediates their chemotaxis, induces cell shape change, and upregulates adhesion molecules CD11b/CD18 (Mac-1) and CD54 (ICAM-1). This effect, while observed in cultured eosinophils, may be of paramount importance in the skin (Ling et al. 2004).

Histamine also acts on H₂ and H₄ receptors of plasmacytoid dendritic cells and downregulates production of TNF-α, IFN-α, and CXCL8 (Mazzoni et al. 2003). Plasmacytoid dendritic cells migrate in response to H₄ receptor agonist stimulation. Of note, H₄ receptor is present in high levels on plasmacytoid dendritic cells in the lesional psoriatic skin (Gschwandtner et al. 2011).

### 2.3.3 Conclusions

Histamine is produced not only by mast cells but also by other cells of epidermis and dermis and acts locally in the epidermis and dermis by binding to H₁-H₄ receptors. Histamine targets not only endothelium and smooth muscles of blood
vessels but also modulates function of keratinocytes, melanocytes, and cells of skin immune system. It affects intracellular signaling cascades, cell proliferation, and melanogenesis. Histamine is upregulated in melanoma cells. It signals mainly via H4 receptor on the cells of the immune system and affects their migration and cytokine secretion patterns. Moreover, it modulates Th2-type immune responses and antimicrobial peptide expression. Thus, histamine is an important part of the neuro-immuno-endocrine system of the skin (Slominski and Wortsman 2000) with local and systemic effects (Figs. 1.1 and 1.2).

2.4 Serotoninergic System

2.4.1 Production and Metabolism of Serotonin

2.4.1.1 An Overview

Serotonin (5-hydroxytryptamine, 5-TH) is widely synthesized throughout the animal kingdom, plants, and unicellular organisms (Azmitia 2001, 2007). In plants, serotonin serves as a trophic factor and an antioxidant which is similar to the animal kingdom (Azmitia 2001). In humans, serotonin was shown to be synthesized predominantly by intestinal enterochromafin cells with other sites of production represented by the central nervous system, pineal gland, retina, ovaries, placenta, thymus, pancreas, skin, breast, gestational tissues, blood vessels, rectal epithelium, bronchial epithelial cells, thyroid parafollicular cells, mast cells, and lymphocytes (Nordlind et al. 2008).

The first obligatory step in the synthesis of serotonin is the hydroxylation of L-tryptophan to produce 5-hydroxytryptophan (TrpOH) in a reaction catalyzed by tryptophan hydroxylase (TPH) (Mockus and Vrana 1998), a protein encoded by either TPH1 gene expressed ubiquitously (Mockus and Vrana 1998) or TPH2 gene expressed predominantly in the brain (Zhang et al. 2004). This reaction requires oxygen and cofactor 6BH4. TrpOH is further decarboxylated by AAD to produce 5-HT. In humans, L-tryptophan is present in blood plasma at steady-state level both in the free form (approximately 1.2 \times 10^{-5} M) and bound to serum albumins (ca. 6 \times 10^{-5} M), with TPH having a Kda for tryptophan of approximately 10^{-8} M. Thus, fluctuations in free pool of tryptophan directly and immediately alter the level of serotonin synthesis (Nordlind et al. 2008). Catabolism of serotonin is initiated by MAO with the production of 5-hydroxyindoleacetaldehyde, oxidized further by aldehyde dehydrogenase (E.C. 1.2.1.3) to 5-hydroxyindole-3-acetic acid (5-HIAA), which is the main product of metabolism, or reduced to 5-hydroxytryptophol (HTOL) by alcohol dehydrogenase (E.C. 1.1.1.1) (Fig. 2.3). 5-HT can also be methylated to 5-methoxytryptamine (5MTT) and catabolized as shown in Fig. 2.3. Additional pathway involves serotonin acetylation by aryalkylamine N-acetyltransferase (AANAT) or aryamine N-acetyltransferase isoenzyme showing substrate specificity toward both aryamines and aryalkylamines to produce N-acetylserotonin (NAS)
NAS can also be further metabolized to melatonin (Reiter 1991). In the skin, a number of NAS metabolites unrelated to melatonin were found, the nature and mechanism of generation of which remain to be defined (Slominski et al. 2003b, c). After release into blood, serotonin is actively taken up into platelets and stored in solid granules with the help of a serotonin transporter (5-HTT), an e member of the Na+/Cl−/CO2-dependent transporter superfamily, which actively regulates serotonin transport. Serotonin can be transported through the plasma membrane in either direction; however, under most conditions, its reuptake is favored (Nordlind et al. 2008). Plasma serotonin is also cleared by the liver and lung endothelial cells and further catabolized to 5-HIAA.
2.4.1.2 Production and Metabolism of Serotonin in the Skin

Mammalian skin cells can produce serotonin via the sequential transformation of L-tryptophan by TPH and AAD (Slominski et al. 2005c) (Fig. 2.3). Thus, the TPH1 gene is expressed in human skin under normal and pathological conditions as well as in a wide array of normal and transformed human epidermal, dermal, and adnexal skin cells with some cells expressing the aberrant TPH1 transcript (Slominski et al. 2002c, 2003b, c). As to the TPH2 gene, it is expressed in the retinal pigment epithelium (Zmijewski et al. 2009b) and normal and malignant melanocytes (Zmijewski and Slominski, unpublished). Although the TPH gene is expressed almost in all types of human skin cells, the highest expression was found in normal and malignant melanocytes that also accumulated significant amounts of serotonin (Figs. 2.4 and 2.5) (Slominski et al. 2003a, 2005c). Interestingly, the enzymatic conversion of tryptophan to TrpOH in melanoma cells occurs at high levels, comparable to those in the brain (Slominski et al. 2002a, c). TPH and TPH1 were also detected in the mouse and hamster skin, and in cultured mouse follicular melanocytes and melanoma cells (Slominski et al. 2002a, 2003b, c). Interestingly, the TPH1 gene expression changes during murine hair cycle (Slominski et al. 2003b, c). In addition, TPH and serotonin are strongly expressed in rodent masts cells. It is also important to notice that the skin has a capability for de novo synthesis/recycling of the 6BH4 (Schallreuter et al. 1997, 1998, 2008a) and of pyridoxal 5'-phosphate (PLP) (Coburn et al. 2003) both serving as important cofactors necessary for the production of TrpOH and serotonin. Interestingly, nonenzymatic production of TrpOH through H2O2 and UVA radiation indicates that a free-radical-mediated oxidation of L-tryptophan is also possible in the skin (Schallreuter et al. 2008a).

In human skin biopsies immunoreactivity of TPH and serotonin was found in normal epidermal melanocytes and malignant melanomas (Figs. 2.4 and 2.5) (Slominski et al. 2003a) with additional detection by immunofluorescence techniques in epidermal keratinocytes, hair follicles, eccrine glands, blood vessels, and skin mast cells (Slominski et al. 2005c). These findings are consistent with the immunodetection of serotonin in perivascular human mast cells of adrenal cortex (Lefebvre et al. 2001) and breast epithelial cells (Matsuda et al. 2004). Serotonin was also detected by immunocytochemistry in dermal Merkel cells in rat and pig skin at the epidermal rete ridges and upper hair follicles adjacent to nerve terminals (Nordlind et al. 2008). Cutaneous serotonin content can be affected by inflammatory processes (Lonne-Rahm et al. 2008; Nordlind et al. 2008; Rasul et al. 2011; Thorslund et al. 2009). For example, human skin affected by psoriasis or chronic eczema showed elevated expression of serotonin in the epidermal and adnexal structures (Nordlind et al. 2008).

The catabolism of serotonin in mouse skin is initiated by its deamination by MAO, followed by the oxidation or reduction of the resultant 5-hydroxyindole acetaldehyde to 5-HIAA and/or 5-HTPOL (Slominski et al. 2003b, c). Similar metabolism was uncovered in rat skin, although in this species 5-HIAA was the
main degradation product and 5-HPDOL remained below the limit of detectability (Semak et al. 2004). MAO metabolism of serotonin was also detected in guinea pig skin (Tachibana et al. 1990) and production of 5-HIAA was documented in human epidermal keratinocytes and melanoma cells (Slominski et al. 2002c).

The alternative serotonin metabolism pathway in the skin is represented by its acetylation to N-acetylserotonin, which in human and rodent skin and cultured skin cells is mediated via the action of either AANAT or NAT with mixed arylamine/arylatedylamine substrate specificity (Slominski et al. 2005c). In hamster skin, we characterized two N-acetyltransferase activities including NAT-1 with substrate specificity toward arylamines, and NAT-2 showing substrate specificity toward both arylamines and arylalkylamines such as serotonin, tryptamine, and methoxytryptamine (Gaudet et al. 1993; Slominski et al. 2002a). Furthermore, we demonstrated that at least part of this activity in hamster, rat, and human skin represented native AANAT (Slominski et al. 2002a). In accordance, serotonin N-acetyltransferase activity was significantly inhibited by low concentrations of...
coenzyme A-S-N-acetyltryptamine [Cole bisubstrate; BSI, see (Hickman et al. 1999; Khalil et al. 1998)], indicating true AANAT activity. However, significant enzymatic activity generating NAS was resistant to BSI suppression, showing that in rodents arylamine activity (NAT-2) resistant to BSI can also participate in the acetylation of serotonin (Semak et al. 2004; Slominski et al. 2002a). Rodent NAT-2 is a homologue of human NAT-1; thus, it is likely that NAT-1 may contribute to NAS production also in the human skin. Interestingly, in the C57BL/6 mouse

Fig. 2.5 Melatoninegic system in the skin. TPH1 Western blot insert in the Panel a is of approximately 50 kDa (arrowhead) that is processed and/or degraded to lower molecular weight species (asterisk). It was immunolocalized in the epidermis (ES), hair follicle (ORS), eccrine glands (EG), showing the highest expression in melanocytes (arrows) (Panels a and b). 5-hydroxytryptophan is further decarboxylated by aromatic amino acid decarboxylase (AAD). AANAT (enzyme acetylating serotonin) is expressed in cells of epidermal, dermal, and adnexal compartments (E, BV, EG, and hair follicle structures in Panel c on the left). Immunocytochemical localization of melatonin-like immunoreactivity is shown in Panel d on the right (upper E, BV, and MC). Immunocytochemistry was performed on human skin biopsies: E epidermis, D dermis, BV blood vessel, EG eccrine gland, HF ORS hair follicle outer root sheath, FP hair follicle papilla; MX hair follicle matrix, MC mast cells. For technical details, see Slominski et al. (2005d). Reproduced with permission from the publisher (Slominski et al. 2008a)
producing inactive AANAT (Roseboom et al. 1998), we detected cutaneous trans-
formation of serotonin to NAS and, to a lesser extent, acetylation of tryptamine
(Slominski et al. 2003b, c). Most interestingly, acetylation of serotonin, but not of
tryptamine, was dependent on the phase of hair cycle, skin anatomic location, and
the presence of pathology (melanoma). NAS was further metabolized to several
products (the chemical nature of which remains to be defined) in a hair cycle-
dependent fashion (Slominski et al. 2003b, c). In humans, both skin racial pigmen-
tation and cutaneous pathology determine the reaction rate and specificity of
serotonin acetylation (Slominski et al. 2002c).

2.4.2 Bioregulatory Role of Serotonin in the Skin

Serotonin regulates a wide range of physiological processes at the central and
peripheral levels acting as a neurotransmitter, hormone, cytokine, biological modi-
fier, growth factor, morphogen, and antioxidant or pro-oxidant (Azmitia 2007,
2010). The above functions are mediated through receptor-dependent and
receptor-independent mechanisms (Hoyer et al. 2002).

Serotonin acts via multiple receptor subtypes labeled as 5-HT1 through 5-HT7
(Hoyer et al. 2002). Most of these receptors are metabotropic, with the exception of
5-HT3, which is ionotropic and primarily gates sodium and potassium ions. 5-HT1
receptors (1A, 1B, 1D, 1E, and 1F) couple via Giα to inhibit cAMP formation while
5-HT4, 5-HT6, and 5-HT7 all couple via Gsα to stimulate cAMP production (Hoyer
et al. 2002). In addition, 5-HT1A receptors produce membrane hyperpolarization
by coupling to K+-channels. 5-HT2 (2A, 2B, and 2C) receptors couple via Gqα to
phosphatidylinositol hydrolysis and the formation of inositol trisphosphate and
diacylglycerol (Hoyer et al. 2002). The 5-HT5 receptor (5A and 5B) is considered
to be an orphan receptor. Serotonin receptor function can be modulated by RNA
editing, endogenous lipids that act as allosteric modulators, and serotonin moduline
(tetrapeptide, 5-HT-moduline) that is produced by proteolytic modification of
chromogranin. 5-TH moduline is an allosteric modulator which regulates 5-HT5
receptor dimerization and formation of either homodimers or heterodimers. The
receptors’ heterogeneity and functional diversity are also amplified by the process
of alternative splicing and differential subunit incorporation into the receptor
complex. The regulation of 5-HT receptor activity is also affected by serotonin
transporters, which remove serotonin from the extracellular environment or, under
certain conditions, pump it out of the cell.

In human skin and skin cells, we identified expression of genes coding 5-HT
receptors, including HTR1A, 1B, 2A, 2B, 2C, and 7 genes, and it was shown that the
pattern of expression was cell specific and modified by skin pathology
(Slominski et al. 2003d). Interestingly, alternatively spliced form of HTR2C with
a deletion of exon 2, a fragment of exon 3, and an insertion of cryptic exon
containing termination codon was found in human melanoma, while the HTR2B
isoform with a deletion of exon 2, but with a preserved reading frame coding for a
receptor protein without transmembrane domains 3 and 4 was found in normal
human skin and skin affected by basal cell carcinoma (Slominski et al. 2003a). We also found RNA editing (A to G substitution) in human HTR7 gene (Slominski et al. 2003a), which may be connected to the local expression of adenosine deaminases. In mouse and hamster skin, expression of the HTR2B and HTR7 genes was demonstrated, which was dependent on the phase of hair cycle (mouse) and type of tissue or cells (Slominski et al. 2004b).

We should also mention that Kaneko et al. have failed to detect 5-HT2A gene in epidermal keratinocytes (Kaneko et al. 2009). However, these findings have to be considered with caution, since other researchers demonstrated that 5-HT2A antagonists inhibited UVR-induced skin carcinogenesis (Sreevidya et al. 2008, 2010) and that sunlight-induced immunosuppression could be mediated via the activation of 5-HT2A by cis-urocanic acid (Walterscheid et al. 2006). Furthermore, 5-HT2A protein was detected by immunocytochemistry in dermal lymphocytes, fibrocytes, vasculature, and sensory nerve endings, abating the epidermis (Nordlind et al. 2008), while 5-HT1A receptor was localized to keratinocytes of the upper epidermis, epidermal melanocytes, mast cells, and dermal vasculature (Nordlind et al. 2008). Furthermore, 5-HT1A and 5-HT2A were detected in the majority of benign tumors such as compound nevi, dysplastic nevi, and also in malignant melanomas (Nordlind et al. 2008). By the use of immunocytochemistry, 5-HT2C was detected in epidermal Langerhans cells and melanocytes, 5-HT3 in the basal epidermal keratinocytes, and 5-HT7 in dermal vasculature (Nordlind et al. 2008). 5-HT1A, 2A, and 2C were also detected in rodent skin dermal and epidermal immune cells (Nordlind et al. 2008). Diverse expression of 5-HT receptors was also found in immune cells that were dependent on cell type and their level of activation.

Also Merkel, Langerhans and mast cells, lymphocytes and macrophages (Nordlind et al. 2008), and immortalized human epidermal keratinocytes and melanoma cells express 5-HTT (Fig. 2.4). Their role is substantiated by observations which showed that serotonin uptake inhibitors could induce spontaneous bruising, pruritus, urticaria, angioedema, erythema multiforme, the Steven–Johnson syndrome, toxic epidermal necrolysis, erythema nodosum, alopecia, hypertrichosis, leukocytoclastic vasculitis, and acneiform eruption (reviewed by Nordlind et al. 2008; Slominski et al. 2005c). This can also be associated with flares of psoriasis vulgaris and development of delayed hypersensitivity.

Under in vitro conditions, serotonin exerted variable effects on skin cells depending on the context (Nordlind et al. 2008; Salim and Ali 2011; Slominski et al. 2005c). It stimulated proliferation of dermal fibroblasts (Slominski et al. 2005c), similarly to non-skin fibroblasts (Seuwen and Pouyssegur 1990). Serotonin also stimulated growth of epidermal melanocytes in the absence of growth factors, while inhibiting their proliferation in media supplemented with serum (Slominski et al. 2003a). The former effect could be linked with the stimulation of intracellular cAMP accumulation, while the latter could represent serotonin antagonism with serum growth factors (Slominski et al. 2005c). NAS, the product of serotonin metabolism, showed no effect on the proliferation of fibroblasts and melanocytes (Slominski et al. 2003a) and serotonin or inhibitors of its uptake inhibited melanogenesis (reviewed by Slominski et al. 2004b; Slominski et al. 2005c). In addition,
serotonin modulated proliferation of cultured murine keratinocytes (Maurer et al. 1997). Interestingly, serotonin content within mast cell granules steadily decreased throughout anagen and increased during catagen and telogen phases of hair cycle (Hasse et al. 2007).

Serotonin shows vasoactive and immunomodulatory effects. For example, it plays a role in the Arthus reaction (Tachibana et al. 1990; Yuasa et al. 2001), induces sustained vascular permeability (Fujii et al. 1994), and also modulates the inflammatory response to substance P (SP) via capsaicin-sensitive sensory fibers (Khalil and Helme 1990). Serotonin participates in the activation of T cells and natural killer cells by macrophages, initiation of delayed-type hypersensitivity responses, production of chemotactic factors, and the modification of innate immune responses (Benton et al. 2010; Betten et al. 2001; Cloez-Tayarani and Changeux 2007; Hsueh et al. 2002; Mossner and Lesch 1998). In allergic contact dermatitis and psoriasis, the number of cells expressing both 5-HT1A and tryptase diminishes, whereas the number of dermal cells expressing 5-HT2A and CD3 increases, including atopic dermatitis (Lonne-Rahm et al. 2008; Nordlind et al. 2008; Rasul et al. 2011; Thorslund et al. 2009). Similar pattern is found in the murine epidermis affected by contact eczema. Furthermore, both eczematous and psoriatic human skin shows increased number of mononuclear cells expressing 5-HTT (reviewed by Nordlind et al. 2008). In addition, serotonin can act as a chemoattractant for eosinophils, probably by binding to 5-HT2A receptors. It is involved in the mast cell recruitment to the site of tissue injury through the activation of 5-HT1A, however, without inducing their degranulation (Nordlind et al. 2008). Regulatory function of 5-HT1A in inflammatory responses is emphasized by the suppression of the severity of contact allergy in rats, after topical or oral administration of its agonist, buspirone (Nordlind et al. 2008). Another 5-HT1A agonist, tandospirone, attenuates itching in patients with atopic dermatitis (Nordlind et al. 2008). On the other hand, treatment with 5-HT2A antagonists reduced the severity of contact allergic reactions in mice and one of them, spiperone, was effective when applied either systemically or topically. Furthermore, 5-HT2 receptor antagonist, ketanserin, inhibited the established but not challenge-induced phases of allergic contact dermatitis (Nordlind et al. 2008).

Serotonin is also involved in the pathogenesis of cholestatic and uremic pruritus, urticaria, and itch reaction (reviewed by Slominski et al. 2005c).

### 2.4.3 Serotonin Receptors on Sensory Nerves

5-HT receptors were widely detected on cutaneous sensory nerve endings (reviewed by Nordlind et al. 2008; Slominski et al. 2005c). Intradermal injection of serotonin into rat elicited enhanced c-fos-like immunoreactivity in superficial lamina at the lateral aspect of the dorsal horn, in a manner similar to the immunoreactivity evoked by capsaicin. The 5-HT receptor was detected in unmyelinated sensory axons at the dermal–epidermal junction and the nerve endings of Pacinian
corpuscles of rat glabrous skin (Carlton and Coggeshall 1997) and rat sinus hair follicle (Tachibana et al. 2005). 5-HT1 receptors are present in the dermis of rabbits on afferent nerve fibers around hair follicles and sebaceous glands (Branchek et al. 1988). 5-HT2A receptors are partially responsible for mediating scratching in mice (Tachibana et al. 1990). Although neither 5-HT2 nor 5-HT3 appears to be involved in itch responses caused by chronic allergic skin dermatitis in rats, acute scratching is mediated by skin 5-HT2 receptors, and intradermal injection of serotonin induced itching in normal, but not inflamed skin (reviewed by Nordlind et al. 2008; Slominski et al. 2005c). In human skin, 5-HT2A and 5-HT3 are localized on sensory nerve ending in the dermis or located close to or entering the epidermis, and their activation may explain pruritic responses to intradermally injected serotonin (Nordlind et al. 2008; Slominski et al. 2005c). Specifically, an antagonist of 5-HT3, ondansetron, can reduce the severity of pruritus, while paroxetine is used in the treatment of pruritus and its antipruritic action is connected with downregulation of 5-HT3 expression (Nordlind et al. 2008; Slominski et al. 2005c).

2.4.4 Reception of Ultraviolet Light

The cutaneous serotoninergic system may play a role in body reception of and reaction to light (Slominski et al. 2005c). For example, it has been reported that UVA-induced well-being can be linked to increased serum serotonin and decreased melatonin levels after a single radiation exposure (Gambichler et al. 2002). It has also been proposed that 5-HT2A plays a role in the transduction of UVR energy into biological responses by serving as the receptor for cis-uurocanic acid (cis-UCA), generated through photoisomerization of the trans-UCA in the stratum corneum after absorption of UVR (Walterscheid et al. 2006). Cis-UCA acts as a powerful local and systemic immunosuppressor (Garssen et al. 2001), and it was proposed that 5-HT2A mediates immunosuppressive effects of UVR after binding of cis-UCA (Walterscheid et al. 2006). A role for 5-HT2A in UVB-induced skin photocarcinogenesis was also suggested (Sreevidya et al. 2008, 2010). Other authors proposed that cis-UCA and serotonin mediate UVB-induced immunomodulation, however, via independent pathways in which cis-UCA does not act through 5-HT2A (Kaneko et al. 2009). Thus, there is sufficient information to support involvement of the local serotoninergic system in cutaneous responses to the UV light; however, the mechanism may be more complex than originally anticipated. It may include activation of 5-HT receptor signaling on either nerve ending or skin cells secondary to UVR-induced local production of serotonin or alternative ligands for HT receptors with a consequent regulation of local homeostasis and immune system. Such signals will be projected to the brain via the ascending nerve routes. Furthermore, release of serotonin into circulation may generate endocrine effects.
2.4.5 Conclusions

The mammalian skin cells have the capability to produce and metabolize serotonin. The cutaneous phenotypic effects are mediated by its interactions with 5-HT receptors including 5-HT1A, 1B, 2A, 2B, 2C, 3 and 7, and 5-HTT receptors, which are expressed in a cell type-dependent manner. The serotonin receptors are also expressed on sensory nerve endings, which transmit to the brain information on changes in skin homeostasis induced by either intrinsic or environmental factors (Slominski 2005; Slominski and Wortsman 2000). The topical application of specific receptors agonists or antagonists, serotonin uptake inhibitors or modulation of local serotonin production/degradation may represent future novel therapies of skin diseases including neurodermatoses and itching disorders. Finally, the cutaneous serotoninergic system may be involved in the transformation of light energy of solar radiation into local and systemic biological responses, with the latter mediated via transmission to brain, endocrine effects, or regulation of systemic responses as shown on Figs. 1.1 and 1.2.
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