REVISION

The Channel Physiology of the Skin

Attila Oláh, Attila Gábor Szöllősi, and Tamás Bíró

DE-MTA "Lendület" Cellular Physiology Research Group, Department of Physiology, University of Debrecen, Medical and Health Science Center, Research Center for Molecular Medicine, Debrecen, Hungary

Correspondence to:

Tamás Bíró MD, PhD, DSc

DE-MTA "Lendület" Cellular Physiology Research Group, Department of Physiology

University of Debrecen, Medical and Health Science Center

Research Center for Molecular Medicine

Nagyerdei krt. 98.

H-4032 Debrecen, Hungary

email: biro.tamas@med.unideb.hu; biro.lcmp@gmail.com

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1 Abstract

During embryonic development, the skin, the largest organ of the human body, and nervous system are both derived from the neuroectoderm. Consequently, several key factors and mechanisms that influence and control central or peripheral nervous system activities are also present and hence involved in various regulatory mechanisms of the skin. Apparently, this is the case for the ion and non-ion selective channels as well. Therefore, in this review, we shall focus on delineating the regulatory roles of the channels in skin physiology and pathophysiology. First, we introduce key cutaneous functions and major characteristics of the channels in question. Then, we systematically detail the involvement of a multitude of channels in such skin processes (e.g. skin barrier formation, maintenance, and repair, immune mechanisms, exocrine secretion) which are mostly defined by cutaneous non-neuronal cell populations. Finally, we close by summarizing data suggesting that selected channels are also involved in skin diseases such as e.g. atopic dermatitis, psoriasis, non-melanoma cancers and malignant melanoma, genetic and autoimmune diseases, etc., as well as in skin ageing.

2 The skin and its key functions

The skin is the largest barrier of the human body which protects the internal organs from various effects of the external environment, such as temperature changes, mechanic impacts, UV radiation and harmful pathogens. However, the skin is also our largest neuro-immuno-endocrine organ as it actively participates in the regulation of the body's water content, body temperature and possesses a multitude of sensory, endocrine, and immune functions. Below, we introduce key aspects of cutaneous physiology (for details see Bukowsky 2010; Draelos and Pugliese 2011).

2.1 The functional anatomy of the skin

The skin is the largest organ of the integumentary system (the organ system that protects the body from damage) and is composed of multiple layers and cell types.

Epidermis: The outermost layer of the skin is made of keratinocytes (providing the waterproofing and serving as key components of the "active" skin barrier); Merkel cells operating as mechanoreceptors; melanocytes which define skin color by the complex melanogenesis; and Langerhans cells which are professional antigen-presenting cells of the skin immune system. In addition, afferent nerve endings for the sensation of touch, pressure, temperature as well as pain and itch also reach the epidermis.

Dermis: The middle layer of the skin is a dense connective tissue composed of extracellular matrix components (collagens and elastic and reticular fibers) produced mainly by dermal fibroblasts. It is supplied by blood and lymphatic vessels and is densely innervated by both sensory afferent as well as motor efferent (which participate e.g. in vasoregulation) nerve fibers establishing a complex neuronal network. Of further importance, the pilosebaceous unit (hair follicles and sebaceous glands) and other appendages (sweat glands) are also located in this compartment.

Hypodermis (or subcutis): The lowermost layer of the skin is formed by adipocytes, fibroblasts, and macrophages. Similar to the dermis, it is also supplied by blood vessels and nerves.

2.2 Key functions of the skin

The various cell types of the skin layers form complex, multicellular communication networks, the proper function of which establishes the physiological skin homeostasis. These homeostatic mechanisms can be classified to 3 groups, i.e. barrier functions, neuroendocrine functions, and other functions (**Figure 1**).

2.2.1 Barrier functions

Possibly the most important function of the skin is the formation of the barrier (extensively reviewed in Elias and Feingold 2006). For a long time, it was believed that it is a "passive" function that originates from the unique structural features and the special anatomical properties of the skin. However, in the last few decades, it became increasingly accepted that the different types of cutaneous cells possess very important functions in generating a coordinated, "active" protection, thus forming a true first line of defense against the harmful impacts of the external environment such as e.g. physical environmental challenges (UV, temperature), microbial invasions, allergens, chemical irritants, etc.

The barrier exhibits a complex nature; hence, we can distinguish among different levels of protection (**Figure 2**). Yet, the different levels constantly communicate and coordinate their actions to be able to act according to the following "needs":

- "Keep the barrier intact"
- "Moisturize: attract and keep the water"
- "Should the barrier be destroyed, regenerate and repair it"
- "Let the valuable things penetrating the skin, both upward and downward"
- "Do not let the bad things invading the skin and the body"
- "Should the bad things penetrated, fight and destroy them"

2.2.1.1 The physical-chemical barrier

The key components of the outermost physical/mechanical barrier are the keratinocytes of the epidermis. During the course of their life-long, apoptosis-driven, physiological differentiation program, as they move "upward" from the deepest basal layer through the spinous (str. spinosum) and granular (str. granulosum) layers, their permeability to Ca^{2+} increases and the resulted elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) activates peptidases and convert pro-filaggrin into filaggrin. Filaggrin then aggregates various cytokeratins and other intermediate filaments in the

superficial cells which, after they have become anucleated (corneocytes), generate the solid mechanical/physical shield, i.e. the str. corneum, which is considered as the "real physical barrier" (Madison 2003; Proksch et al 2008; Jensen and Proksch 2009; Rawlings 2010).

In the str. corneum, each terminally differentiated corneocyte is surrounded by a protein shell called the cornified envelope. This highly insoluble structure – which is a product of (again) Ca^{2+}-dependent processes involving e.g. keratinocyte-specific transglutaminases – is composed of mainly loricrin and involucrin which form extensive links between each other and other filamentous structures of the cells (such as the above filaggrin and cytokeratins). In addition, a further stabilization of the corneocyte barrier is provided by corneodesmosomes, gap junctions, and other intercellular junctions formed by junctional proteins such as e.g. desmogleins, cadherins, envoplakin, etc. Importantly, the produced filaggrin (and possibly other proteins) will eventually be degraded in the corneocytes. The resulted amino acids will then be used to synthesize natural moisturizing factors (NMFs) which, due to their hygroscopic (water-holding) features, provide the proper hydration of the epidermis and hence, as a "mechanical shaping factor", establish another key component of the physical barrier (Madison 2003; Proksch et al 2008; Jensen and Proksch 2009; Rawlings 2010).

As a "morphological metaphor", corneocytes can therefore be imaged as "bricks in the wall" to form the physical/mechanical barrier. It is common knowledge, however, that "bricks cannot be stabilized without a proper mortar"; in the skin, the mortar is formed by lipids of the epidermis. Indeed, in the lower spinous and granular layers, lipid-containing lamellar bodies are formed in the keratinocytes. During the maturation of keratinocytes towards the str. corneum, various (again, Ca^{2+}-dependent) enzymes degrade the outer envelope of the lamellar bodies thereby releasing (via exocytosis) their content to the interstitial space at the border of the str. granulosum and corneum. This process results in the establishment of the physical/chemical "mortar", i.e. the continuous intercellular lipid layers of the epidermis. It should also be noted that involucrin and other cell-cell junction proteins of the corneocytes also serve as substrates for the covalent attachment of ceramide derivates resulting in the corneocyte-bound lipid envelope which binds both to the

cornified (protein) envelope and also to the intercellular lipid lamellae. Therefore, the constantly produced lipids (i.e. cholesterol, ceramides, and free fatty acids) – which are further supplemented by the high lipid content of the sebum, produced and released (to the skin surface) by the sebaceous glands – not only stabilize the "bricks", but also provide additional waterproofing and physical protection to the skin (Elias and Feingold 2006; Proksch et al 2008; Rawlings 2010).

Of great importance, the epidermal lipids also contribute to other "chemical" cutaneous homeostatic mechanisms such as e.g. setting the acidic pH. Furthermore, epidermal keratinocytes and sebocytes actively secrete additional factors exhibiting antimicrobial properties. These include 1) antimicrobial peptides (AMPs) such as e.g. the small cationic molecules defensins (which insert to bacterial walls and hence form "lethal" pores), LL-37 cathelicidin, cathepsins, etc.; and 2) antimicrobial lipids (AML) such as saturated (e.g., lauric acid, $C_{12:0}$) and unsaturated (e.g., monounsaturated MUFA sapienic acid, $C_{16:1\Delta6}$) fatty acids (Gallo and Huttner 1998; Bardan et al 2004; Braff and Gallo 2006; Niyonsaba et al 2009). The AMPs and AMLs not only strengthen the chemical defense of the skin but, as members of the innate immunity, contribute to the complex inflammatory/immune processes organized by the skin immune system (see also below)

2.2.1.2 The (micro)biological barrier

Similar to other barriers seen in various body parts, the skin also has a rich resident, commensal bacterial flora including e.g. *Propionibacterium acnes* and *Staphylococcus epidermidis* (Gallo and Nakatsuji 2011; Kranich et al 2011; Littman and Pamer 2011). Traditionally, it was suggested, that these microbes have a relatively passive function; they populate their niches and "use up" the available food sources hence making it more difficult (if not impossible) for the infection and colonization of pathogenic microbes (this process is referred to as competitive exclusion) (Rioux and Fedorak 2006). However, the "commensal" relationship (i.e. beneficial for the bacteria yet mostly neutral for the skin) has recently been revisited and a rather "symbiotic" (i.e. mutually beneficial) association has been suggested. Indeed, it was recently shown that bacteria of the normal skin flora (including e.g. *Propionibacterium acnes*) secrete factors (e.g. propionicins, jenseniin G, acneicin, lactic acid) that possess bacteriostatic or even antibacterial properties against certain

pathogenic strains (e.g. some Gram-negative bacteria, yeasts and molds) (Faye et al 2000; Miescher et al 2000; Cogen et al 2008). In addition, the skin commensal flora also seems to exert a continuous and dynamic action on the skin immune system; indeed, resident bacteria were shown to modulate AMP production of keratinocytes as well as cytokine production of other cutaneous immunocompetent cells (see also below) (Gallo and Nakatsuji 2011). Finally, it should also be noted that the constant physiological desquamation of the "dead" corneocytes not only strengthens the physical and biological barriers but also makes it difficult for the pathogenic microorganisms to establish permanent colonies.

2.2.1.3 The immunological barrier

Various immunocompetent cells and humoral factors establish the skin immune system (reviewed in Bos and Kapsenberg 1993; Kupper and Fuhlbrigge 2004). As immune cells, resident and infiltrating phagocytic cells, natural killer cells, mast cells, professional antigen-presenting cells (i.e. epidermal Langerhans cell, dermal dendritic cell) as well as T and B lymphocytes are localized in various skin compartments. In addition, a plethora of cytokines, chemokines, and other inflammatory mediators, as well as the aforementioned AMPs and AMLs, are synthesized in and hence released from practically all cell types of the skin. Therefore, upon infections, allergen exposure or barrier rupture, these innate and adaptive immunity components are co-activated to induce an orchestrated inflammatory and immune response (reviewed in Girardi 2007; Nestle et al 2009; Takeuchi and Akira 2010).

Of further importance, keratinocytes and sebaceous gland-derived sebocytes – which, as shown above, play key roles in the establishment of the physical-chemical barrier – were introduced as additional sentinels of the skin immune system. This immune role is attributed not only to their production of AMPs and AMLs and the antimicrobial sebum (see above), but also to their capability to recognize external pathogens via the functional expression of all sorts of pathogen recognition receptors, including various members of Toll-like receptor family (TLRs), i.e. TLR1-6 and 9 (Pivarcsi et al 2003; Miller 2008; Terhorst et al 2010). Activation of these receptors by various pathogenic microbes, via the release of numerous pro-inflammatory agents, leads to the initiation of active defense mechanisms, and as a

result, adaptive and innate immune events are launched (Pivarcsi et al 2004; Kurokawa et al 2009).

2.2.1.4 The barrier regeneration

As was shown above, the proper formation, maintenance, and function of the physical-chemical epidermal barrier depends on the constant proliferation–differentiation turnover of epidermal keratinocytes (and, via sebum production, of sebocytes). Upon disruption of the epidermal barrier – which, experimentally, can be performed by e.g. chemical agents (acetone, detergents), UV exposure, or mechanical tape stripping, which removes the corneocytes (Pinkus 1951) – the aforementioned processes are accelerated due to the active contribution of engaged keratinocytes and sebocytes in response to various agents released from the damaged cells (Proksch et al 2008; Rawlings 2010).

However, skin injuries very often reach the deeper skin layers resulting in a much more complex response which can be exemplified by the wound healing processes (Epstein 1999). Indeed, during the multiple phases of wound healing (e.g. coagulation, inflammation, proliferation, remodeling) numerous cutaneous cell types (including infiltrating macrophages and polymorphonuclear neutrophils, microvascular endothelial cells, dermal fibroblasts, epidermal keratinocytes) are activated and their cell-specific proliferation–migration–differentiation programs are initiated (Enoch and Leaper 2005; Reinke and Sorg 2012). Of further importance, wound healing is not possible without the active contribution of intracutaneous stem cells located in various cutaneous compartments including e.g. the epidermis, sebaceous and sweat glands, and, possibly most importantly, in the hair follicles (Tiede et al 2007; Lau et al 2009). It should also be noted that cellular regeneration programs and stem cell activities are orchestrated by a multitude of locally generated (by the above cell types), soluble mediators (e.g. growth and trophic factors, cytokines, chemokines, neuropeptides, neurotrophins, hormones) and concomitant changes in the expressions of cell surface molecules (e.g. receptors, adhesion molecules, integrins) recognizing these agents (Werner and Grose 2003; Gurtner et al 2008; Koh and DiPietro 2011).

Therefore, the delicate balance of cell/organ proliferation, survival, death, differentiation, and mediator production of practically all non-neuronal cell populations of the skin collectively establish the “life-long” regeneration and rejuvenation of the tissue and hence enables the skin barrier homeostasis (Gurtner et al 2008; Reinke and Sorg 2012).

2.2.1.5 Related cutaneous diseases

In light of the central role of barrier functions in skin biology, it is not surprising at all that impairment of the aforementioned balance results in pathological barrier formation and maintenance which eventually lead to the development of skin diseases. These conditions include e.g. irritant and allergic contact dermatitis, burns, ulcers, etc. On the other hand, several skin immune abnormalities may secondarily impair the epidermal skin barrier such as seen e.g. in Mycosis fungoides and in the autoimmune pemphigus vulgaris. However, the consequences of the very often co-existing impaired skin barrier and cutaneous inflammatory/immune responses may establish positive feed-back loops. These autocatalytic mechanisms, in turn, result in the development of such high-prevalence, chronic inflammatory “barrier diseases” as the atopic dermatitis (AD) and psoriasis (reviewed in Proksch et al 2008; Boguniewicz and Leung 2011).

Finally, skin tumors should also be mentioned. As in many organs, defective differentiation and/or uncontrolled proliferation of cutaneous cells may lead to the development of tumors. In the skin, non-melanoma skin cancers, i.e. basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), and malignant melanomas establish the major groups of tumors with increasingly growing incidence (Samarasinghe and Madan 2012; Tremante et al 2012). It is also noteworthy that stem cells which otherwise play key roles in wound healing are also implicated in skin tumor formation. Since wound repair and tumorigenesis both depend on intracutaneous communication networks of skin cells, the proper control of the inter- and intracellular signaling pathways are of key importance in successfully preventing tumor formation (Arwert et al 2012).

2.2.2 Neuroendocrine functions

Since this review will mainly focus on the roles of ion and non-ion selective channels expressed by non-neuronal cells, below, we only briefly summarize the neuroendocrine and other functions of the skin.

Sensory functions: Besides establishing the complex cutaneous barrier against constant environmental challenges, the skin simultaneously operates as the largest sensory organ of the vertebrate body (reviewed in Roosterman et al 2006; Slominski et al 2008). Indeed, all skin compartments are densely innervated by sensory afferent fibers specialized for the sensation and then neuronal processing of mechanical signals (touch, pressure, vibration), osmotic and thermal (heat, cold) challenges, chemical agents as well as noxious and pruritogenic stimuli inducing pain and itch, respectively (reviewed in Ansel et al 1997; Paus et al 2006a; Roosterman et al 2006; Fuchs and Horsley 2008). However, the activation of these sensory neurons not only induce the classical, ortho-dromic transmission of the signals (i.e. generation and propagation of action potentials) to the central nervous system, but may also result in the anti-dromic release of certain neuropeptides (such as substance P [SP] and calcitonin gene-related peptide [CGRP]) (Ansel et al 1997; Luger 2002). As the "efferent" functions of the sensory afferents, these neuropeptides may act on cutaneous non-neuronal cell types and exert local immuno-endocrine, vasoregulatory, and trophic actions. In addition, sensory stimuli as well as the released neuropeptides may induce the liberation of a plethora of mediators from non-neuronal cells which, vice versa, may act on the sensory nerve endings. Of further importance, the local, intracutaneous accumulation of these mediators may also act on other non-neuronal cell types of the skin and hence may alter their proliferation–differentiation status (Ansel et al 1997; Luger 2002; Paus et al 2006a and b; Peters et al 2007; Fuchs and Horsley 2008). Therefore, the established multi-directional, multi-cellular communication networks not only participate in the aforementioned formation and maintenance of the complex physical and immunological cutaneous barriers but also significantly modulate skin sensation processes ("sensory roles" of the non-neuronal cells) (Bíró et al 2007; Denda et al 2007a; Denda and Tsutsumi 2011; Fernandes et al 2012).

Motor functions: The skin is also supplied by "truly" efferent fibers which belong to the somatomotor group. These sympathetic and parasympathetic nerves control e.g.

cutaneous vasoregulation (dilation or constriction of blood vessels), piloerection, skin metabolic activities, exocrine functions, etc. (see also below) (Hodges and Johnson 2009).

Endocrine functions: The skin is also our largest endocrine organ (Roosterman et al 2006; Slominski et al 2008). Indeed, the skin not only responds to the actions of circulating hormones but various cutaneous cells and tissues themselves produce a wide-array of hormones. Intriguingly, two peripheral equivalents of central hypothalamic – pituitary – target organ axes, i.e. Corticotropin Releasing Hormone (CRH) – Corticotropin (ACTH) – Cortisol; Thyrotropin Releasing Hornone (TRH) – Thyrotropin (TSH) – Thyroxine), are functionally expressed in the skin (Arck et al 2006; Slominski et al 2008; Bodó et al 2010; Poeggeler et al 2010; Ramot et al 2011; Knuever et al 2012). These, mostly locally released and acting hormones, on the one hand, provide additional humoral components to the multi-cellular networks regulating multiple skin functions. On the other hand, these hormones also act as active members of the intracutaneous “stress response system” which, via systemic neuro-endocrine mechanisms, keeps continuous contact with its central counterpart, thereby establishing the “brain-skin connection” (Arck et al 2006; Paus et al 2006b). In addition to the above hormones, certain skin cells express the full enzymatic machinery to synthesize e.g. vitamin D, testosterone, and estrogens which mostly control local events of growth, differentiation, and metabolism of non-neuronal skin cells (Schmuth et al 2007; Zouboulis et al 2007; Slominski et al 2008; Tóth et al 2011a).

2.2.3 Other functions

Transport functions: The proper barrier enables the up- and downward transport of respiratory gases, nutrients as well as topically applied products (pharmaceuticals, cosmeceuticals) between skin layers (Lademann et al 2011).

Thermoregulatory functions: The skin plays multiple roles in thermoregulation. With the subcuticular adipose tissue (which is cca. 50% of body fat), the skin is the major thermal insulator of the body. In animals, insulation is further supported by neuronal piloerection. In addition, the aforementioned neuronal and humoral vasoregulatory mechanisms (vasodilation, vasoconstriction) regulate the large cutaneous blood

supply and thereby precisely control direct heat losing mechanisms (i.e. radiation, convection and conduction). Finally, evaporation (both insensible via skin pores and sensible via sweating) and its control by neuronal and humoral actions are also related to the skin (Johnson 2010; Nakamura 2011; Pitoni et al 2011).

Exocrine functions: Skin appendages produce and release (to the skin surface) of sweat and sebum which exocrine products, as mentioned above, participate e.g. in thermoregulation, physical-chemical barrier formation, antimicrobial activity, etc.

3 A short introduction of ion and non-ion selective channels

The channels are pore proteins found in various (surface, intracellular) membranes of the cells. They are specialized for the passive transport of certain molecules between the cellular compartments separated by the membranes in which they are located.

Below we summarize the major channel groups and shortly introduce their key characteristics, with special emphasis on those which have regulatory roles in skin physiology. For the functional classification of channel proteins, we used the International Union of Basic and Clinical Pharmacology (IUPHAR) database. For details and references, please visit the IUPHAR website (http://www.iuphar-db.org) and corresponding textbooks of Physiology and Pharmacology.

3.1 Ion channels

Via these membrane pores, certain ions are transported (selectively or non-selectively) along their electrochemical gradients. The classification of the ion channels is mainly based on their gating characteristics (i.e. the energy form of the stimulus that opens or, rarely, closes the given channel) and other properties. Yet, it should be emphasized that certain channels exhibit “mixed” gating features; e.g. we will mention such channels whose opening could be equally regulated by binding of the respective ligands, certain voltages, and other factors.

3.1.1 Voltage-gated ion channels

Like most of the ion channels, voltage-gated pores – whose gating properties are mainly regulated by alterations in the membrane potentials – were originally described on excitable cells (i.e. various neurons and muscle types) as key molecules involved in the generation of action potentials. However, it also became apparent that, besides this electrogenic role, they additionally participate in a multitude of other cellular functions not only on excitable but also on non-excitable cells. These mechanisms (as will be detailed below) involve, among others, secretion of various mediators, regulation of intracellular ionic homeostasis, cellular growth and differentiation, immune response, etc. With respect to the skin, voltage-gated Na^+ channels (Catterall et al 2012a), Ca^{2+} channels (Catterall et al 2012b), K^+ channels (Gutman et al 2012a) as well as Ca^{2+} activated K^+ channels (Gutman et al 2012b),

two-pore domain K^+ channels (Plant et al 2012), and cyclic nucleotide-gated (CNG) non-selective cationic channels (Biel et al 2012) are of greatest importance.

3.1.2 Ligand-gated ion channels

The common feature of these channels is that they are gated by binding of (more or less) specific and/or selective ligands to the respective binding sites. Actually, they function as ionotropic receptors for neurotransmitters, neuromodulators, hormones, and other mediators participating in autocrine, paracrine and endocrine intercellular communication mechanisms. Similar to other ion channels, these receptors were first described on neurons and only lately on non-neuronal cells of the body. These channels "signal" mostly via modulating the intracellular ionic homeostasis of their host cells which, in turn, initiates various downstream signal transduction pathways including alterations of activities of e.g. kinase systems, enzymes and factors involved in the regulation of gene expression, cellular metabolic enzymes, etc.

Within this group, below, we review the cutaneous impact of the following ligand-gated channels:

Ionotropic cholinergic receptors: Nicotinic (nAChR) and muscarinic (mAChR) cholinergic receptors are specialized for mediating the cellular actions of acetylcholine (ACh), a key neurotransmitter and mediator. Among them nAChRs function as ligand-gated channels whereas mAChRs are seven-transmembrane (7-TM) G-protein-coupled receptors. Human nAChRs are composed of different subunits, i.e. α1–α10, β1–β4, γ, δ, and ε which can be combined to pharmacologically distinct, homo- or heteropentameric, non-selective cationic channels (Millar et al 2012).

Ionotropic glutamate receptors: Glutamate may act on metabotropic 7-TM (mGluR) or various ionotropic receptors. Within the latter group, the following non-selective cationic channels can be distinguished: N-methyl-D-aspartate receptors (NMDAR) exhibiting high permeability for Ca^{2+}; α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptors (AMPAR); and kainate receptors (Peters et al 2012).

Ionotropic purinergic receptors: Extracellular ATP may exert its cellular action by binding to P2Y 7-TM metabotropic and P2X ionotropic purinergic receptors. So far, seven P2X receptors are identified; all of them function as non-selective, mostly Ca^{2+}-permeable cationic channels (Khakh et al 2001; Evans et al 2012).

Ionotropic 5-hydroxytryptamine receptors: Among the multiple 5-hydroxytryptamine (5-HT) receptors, only 5-HT3 receptors operate as ligand-gated, cation-selective, pentameric ion channels (Lummis et al 2012).

Ionotropic gamma-aminobutyric acid receptors: Gamma-aminobutyric acid (GABA) signals via ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. $GABA_A$ receptors are Cl^- selective, heteropentameric channels derived from seven main receptor subunits (α, β, γ, δ, ε, π and θ) (Olsen and Sieghart 2008; Olsen et al 2012).

Glycine receptors: Similar to $GABA_A$ receptors, glycine receptors also function as pentameric Cl^- channels (Lynch 2012).

3.1.3 Transient receptor potential ion channels

Although the IUPHAR database classifies transient receptor potential (TRP) ion channels among the voltage-gated ones (Clapham et al 2012), due to their “mixed” gating properties and, moreover, to their key roles in cutaneous physiology (see below), we decided to detail their characteristics under a separate subheading.

TRP ion channels exhibit intriguing “mixed” gating properties as they function as broadly expressed polymodal “cellular sensors” (Clapham 2003). Indeed, they can be equally activated and/or modulated by e.g. alterations in temperature and pH, osmolarity, ionic concentrations, endogenous mediators, external chemical irritants, membrane potential changes, etc. (Ramsey et al 2006; Damann et al 2008; Vriens et al 2008; Vriens et al 2009). In addition, as we will see below, TRP channels not only act as “sensors”, but also as key “effectors” of various physiological (and often pathophysiological) processes such as e.g. cellular homeostasis of different ions, secretory mechanisms, sensory functions of the nervous system, inflammation, proliferation, differentiation, cell survival, etc. (Nilius and Owsianik 2010; Denda and Tsutsumi 2011; Moran et al 2011; Fernandes et al 2012).

Up to date, 28 mammalian members have been identified which can be further classified into the subfamilies of the canonical (or classical, TRPC), the vanilloid (TRPV), the melastatin (TRPM), the mucolipin (TRPML), the polycystin (TRPP), and the ankyrin (TRPA) groups (Clapham et al 2012). As detailed below, multiple TRPs participate in the regulation of skin functions.

3.1.4 Other ion channels

Within this group, we introduce the amiloride-sensitive, epithelial Na^+ channels (ENaC) which belong to the ENaC/degenerin ion channel family of genetically related glycoproteins. ENaC can be formed by different combinations of four homologous subunits, named ENaCα, β, δ, and γ (de la Rosa et al 2000; Kellenberger and Schildl 2002). The key unique feature of ENaC channels that they are mostly (if not exclusively) expressed on non-neuronal cells.

3.2 Non-ion selective channels

These membrane pores (which are, very often, also permeable for ions) enable the transport of various other molecules. Several members of the below families are involved in cutaneous functions.

3.2.1 Aquaporins

Aquaporins are a family of integral transmembrane proteins that facilitate osmotic fluid transport in numerous human tissues. They are involved in transepithelial and transcellular water movement, although more recent results point to their possible role in gas transport as well. Thirteen mammalian aquaporins have been identified to date, which can be classified into two groups; i) aquaporin molecules that only transport water (AQP-1, AQP-2, AQP-4, AQP-5 and AQP-8) and ii) aqua-glyceroporin molecules that also transport glycerol and other small molecules such as lactic acid (AQP-3, AQP-7, AQP-9 and AQP-10) (Hara-Chikuma and Verkman 2008a).

3.2.2 Connexins

Connexins (Cx) are transmembrane proteins that homo- or heterosextamerize on the plasma membrane to form the hemi-channel connexons. Connexons on adjoining cells associate to form gap junctional channels, and allow the direct passage and exchange of ions, secondary messenger molecules (cAMP, IP_3), energy sources

(ATP, GTP), reducing/oxidizing agents (glutathione) and nutrients (glucose, amino acids) between cells. Therefore, gap junctions are key molecules of cell–cell communications (Proksch et al 2008; Xu and Nickolson 2012).

3.2.3 Pannexins

Pannexins (Panx) are mammalian orthologs of the invertebrate gap junction proteins innexins (Panchin et al 2000). However, pannexins do not take part in the formation of gap junctions; rather they form single membrane channels in cellular communication with the environment (Sosinky et al 2011). To date, three Panxs have been described: Panx1 appears to be ubiquitously expressed whereas Panx2 was mostly found in the adult brain. Panx3 expression was identified in osteoblasts, synovial fibroblasts, whole joints of mouse paws, and cartilage from the inner ear (Baranova et al 2004) as well as in cartilage, the heart, and, of great importance, human skin (Penuela et al 2007). Panx1 has been implicated in numerous cellular functions such as immune response, tumorigenesis, apoptosis, and ischemic cell death. In addition, Panx2 and Panx3 have shown to take part in the differentiation and development of tissues which express these channels (reviewed in Penuela et al 2012a).

4 Roles of channels in skin physiology and in certain dermatoses

In this chapter, we provide an extensive review on the roles of various ion and non-ion selective channels in the regulation of certain functions of the skin. Since the involvement of a multitude of (mostly voltage-gated and TRP) ion channels in sensory neuron-coupled functions (such as e.g. thermosensation, pain, itch) are extensively detailed in numerous comprehensive reviews, below, we focus on defining the roles of the channels on non-neuronal cells (summarized in **Table 1**). In addition, we present data on the potential impact of these molecules in certain skin diseases (summarized in **Table 2**).

4.1 Roles of channels in epidermal physical-chemical barrier functions and barrier recovery

As we introduced above (under 2.2.1.), the formation, maintenance, and recovery of the epidermal physical-chemical barrier are mainly determined by the proper, $[Ca^{2+}]_i$-dependent differentiation program of the epidermal keratinocytes resulting in the lipid-embedded layers of corneocytes. Therefore, in this chapter, we introduce roles of channels (which regulate intracellular ionic homeostasis) in controlling growth, differentiation, and survival of keratinocytes. Moreover, we present findings of animal experiments aimed at defining rate of recovery after barrier insults. Finally, since sebaceous gland-derived sebum production is an additional factor of the chemical epidermal barrier, we also detail the related channel physiology of sebocytes.

4.1.1 Voltage-gated channels

4.1.1.1 Voltage-gated Ca^{2+}-channels

The main subunit of the L-type voltage-gated Ca^{2+} channels, $Ca_v\alpha_{1C}$, was identified in mouse and human epidermis *in situ* (Denda et al 2006). In addition, functional Ca_v channels were found on cell cultures of normal human epidermal keratinocytes (NHEKs) (Denda et al 2003b; Denda et al 2006). Of great importance, in hairless mice, topical application of Ca_v channel antagonists (nifedipine and R-(+)-BAY K8644) to mechanically injured skin (tape stripping) accelerated barrier recovery whereas treatment with a Ca_v channel agonist (S-(-)-BAY K8644) delayed barrier repair (Denda et al 2006). Likewise, topical application of Ca^{2+} on the skin after str. corneum barrier disruption delayed the recovery of the barrier which effect was prevented by the co-administration of the Ca_v channel antagonists nifedipine and

verapamil (Lee et al 1991). In good agreement with these findings, Ca_v channels were shown to mediate the effects of adrenergic β_2 receptor agonists to inhibit barrier repair (Denda et al 2003b; Denda et al 2006).

Interestingly, in a retrospective case-control study, chronic (>2 years) intake of Ca_v channel blockers (nifedipine, felodipine, and amlodipine) was found to be significantly associated with both the exacerbation as well as the precipitation of new-onset psoriasis (Cohen et al 2001), a skin diseases with altered keratinocyte functions and impaired epidermal skin barrier (reviewed in Proksch et al 2008; Boguniewicz and Leung 2011).

Collectively, these findings suggest that proper Ca_v channel activation is a key factor in the $[Ca^{2+}]_i$-dependent events of keratinocyte differentiation and hence epidermal mechanical barrier formation. However, the above results with the application of Ca_v channel agonists/antagonist and of Ca^{2+} to mechanically injured skin implies that extreme accumulation of Ca^{2+} in the keratinocytes may lead to impaired keratinocyte differentiation and hence barrier recovery (see also under 4.1.5.)

4.1.1.2 Ca^{2+}-activated K^+-channels

Various Ca^{2+}-activated K^+-channels (K_{Ca}) were implicated in the regulation of growth and differentiation of epidermal keratinocytes. Indeed, K_{Ca} channels were identified on both human and mouse epidermis *in situ* and also on NHEKs. The activation of this 70 pS conductance K_{Ca} channel was shown to be indispensible for the effect of elevated extracellular Ca^{2+}-concentration ($[Ca^{2+}]_e$) to induce keratinocyte differentiation (Mauro et al 1997). In addition, on cultured human immortalized HaCaT keratinocytes, a large-conductance (170 pS) K_{Ca} channel (BK) (IUPHAR nomenclature: $K_{Ca}1.1$) was detected and was implicated in the establishment of resting membrane potential; therefore, these channels may also control Ca^+-influx and differentiation (Nguyen and Markwardt 2002). Furthermore, $[Ca^{2+}]_e$ or vitamin D induced differentiation of NHEKs were shown to upregulate mRNA levels of the intermediate-conductance K_{Ca} (IK1) (IUPHAR nomenclature: $K_{Ca}3.1$) channel (Manaves et al 2004) which were suggested to play a central role in linking changes in membrane potential to the growth and differentiation of HaCaT keratinocytes (Koegel and Alzheimer 2001).

4.1.2 Ligand-gated channels

4.1.2.1 nAChRs

Practically all cell types of the skin express nAChRs which control a plethora of cutaneous functions. These were detailed in comprehensive reviews (Kurzen et al 2004; Grando et al 2006; Curtis and Radek 2012); therefore, below, we only highlight the most important nAChR-coupled functions.

Keratinocytes, as one of the major extra-neuronal sources, were shown to produce and release ACh (Grando et al 1993), similar to a multitude of cutaneous cells which express the ACh-synthesizing enzyme, choline-acetyltransferase (ChAT) (Wessler et al 2003). In addition, an upward (i.e. towards the str. corneum) concentration gradient of free ACh was detected in the epidermis (Nguyen et al 2001), in parallel with the also upward epidermal Ca^{2+} gradient (Hennings et al 1980; Lansdown 2002). Of further importance, *in situ* expressions of multiple nAChR subunits were identified in the human epidermis; α3, α5, α9, and β2 subunits were localized mainly to the basal layers whereas α7, α10, β1, and β4 subunits were found in the str. spinosum and granulosum (Nguyen et al 2001; Kurzen et al 2004).

That the above extra-neuronal nAChR-coupled cholinergic system is indeed functional in keratinocytes was shown in numerous studies. In NHEKs, nicotine increased Ca^{2+} influx and increased cellular differentiation (upregulation of expression of keratin 10, transglutaminase type I, involucrin, and filaggrin as well as induction of cornified envelop formation) (Grando et al 1996). As a possible mechanism of action of nicotine, in organotypic keratinocyte cultures, inhibition of α9 subunit was shown to markedly inhibit epidermal differentiation, suppress expressions of proteins involved in epidermal cell-cell contacts, and induce lipid accumulation in the basal layers suggesting barrier disruption (Kurzen et al 2005; Kurzen et al 2007). In line with these findings, lower levels of cell adhesion molecules (cadherins, catenins) were detected in epidermis of α9 (as well as α3) knockout mice (Nguyen et al 2004). Consequently, stimulation of nAChRs resulted epidermal thickening and higher lipid content of the corneal layer (Kurzen et al 2005; Kurzen et al 2007).

Furthermore, as was shown in cultured keratinocytes and knockout animals, elimination of α7 receptor activities or levels also inhibited differentiation (suppression of levels of filaggrin, loricrin, and cytokeratins). In addition, decreased levels of apoptosis markers (caspase-3), but increased expressions of proliferation markers (Ki-67, proliferation cell nuclear antigen [PCNA]) were detected in epidermis of α7 knockout mice (Arredondo et al 2002). It is concluded therefore that cutaneous ACh signaling, most probably by inducing Ca^{2+} influx to keratinocytes via multiple nAChR channels, plays a key role in inducing terminal epidermal differentiation and hence barrier formation.

However, topical application of the nAChR agonist nicotine to the skin hairless mice delayed the barrier repair after tape stripping (Denda et al 2003a). Furthermore, topical administration of nicotine to mouse skin also resulted in a marked suppression of AMP production (Radek et al 2010; Curtis and Radek 2012) which, in turn, may lead to barrier impairment (see also below). The possible explanation(s) for these quite “unexpected” findings will be provided under 4.1.5.

4.1.2.2 Ionotropic glutamate receptors

Among these ion channels, certain NMDARs and AMPARs are expressed in epidermal keratinocytes. Indeed, in human skin, the NMDAR1 subunit (IUPHAR nomenclature: GluN1) was found in all layers of the epidermis; the greatest expression was located to the granular layer (Fischer et al 2004a; Fischer et al 2004b). NMDAR1 was also identified on cultured NHEKs and HaCaT keratinocytes (Morhenn et al 1994; Fischer et al 2004a), especially at the site of cell-cell contacts (Nahm et al 2004). NMDAR1 expressed by cultured keratinocytes is functional since the application of NMDA resulted in elevation of $[Ca^{2+}]_i$ which was suppressed by MK-801, an NMDAR inhibitor (Fuziwara et al 2003; Nahm et al 2004). Moreover, it appears that the physiological NMDAR-coupled signaling mechanisms are indispensible for proper growth and differentiation of keratinocytes. Indeed, treatment of NHEKs with MK-801 markedly suppressed the expression of differentiation markers cytokeratin 10 and filaggrin (Fischer et al 2004a; Fischer et al 2004b).

Interestingly, in hairless mice, topical application of glutamate (Denda et al 2003a), aspartate (non-specific glutamate receptor agonists), and NMDA (Fuziwara et al

2003), unlike AMPA, delayed the barrier recovery after disruption with tape stripping which effect was effectively abrogated by the co-administration of MK 801 and D-AP5 (another NMDAR antagonist). Of further importance, topical administration of NMDAR antagonists alone accelerated the barrier repair (Fuziwara et al 2003). Since epidermal keratinocytes are able to synthesize and release glutamate (Fischer et al 2009) and, furthermore, barrier injury markedly increased the release glutamate from mouse skin (Fuziwara et al 2003), it is proposed that the ionotropic glutamatergic signaling of keratinocytes plays a key role in the processes of barrier damage. This idea is further strengthened by presenting that NMDAR antagonists specifically inhibited the actions of oleic acid to pathologically increase transepidermal water loss (indicator of barrier impairment) and to induce keratinocyte hyperproliferation in mice. Furthermore, in cultured NHEKs, NMDAR inhibitors likewise inhibited the effects of oleic acid to elevate $[Ca^{2+}]_i$ and to stimulate production of IL1α (Katsuta et al 2009) which cytokine, along with ATP, is regarded as a "mediator" of barrier disruption (Wood et al 1996). The complex role of NMDAR-coupled mechanisms in barrier formation and repair will be discussed under 4.1.5.).

4.1.2.3 P2X receptors

Multiple ionotropic P2X receptors were identified in the skin and were implicated in various skin functions. Since a recent paper (Burnstock et al 2012) reviewed characteristics of the cutaneous purinergic system, we highlight only major components of it.

Several P2X receptors were detected in human epidermis and cultured NHEKs. The expression of mRNA specific for P2X2, P2X3, P2X5, and P2X7 receptors were increased in differentiated cells. Since P2X agonists elevated $[Ca^{2+}]_i$, it is proposed that multiple P2X receptors might be involved in the regulation of epidermal differentiation (Inoue et al 2005).

Indeed, in normal rat epidermis, P2X5 receptors were found to be highly expressed in proliferating and differentiating epidermal keratinocytes in basal and suprabasal layers whereas P2X7 receptors were associated with terminally differentiated keratinocytes in the str. corneum. In addition, expressions of P2X5 receptors were found to be increased in the regenerating epidermis (Greig et al 2003)

Of further importance, similar to the above ACh and glutamate induced mechanisms, ATP was also shown to delay barrier recovery in hairless mice via the stimulation of another purinergic receptor, P2X3, also functionally expressed by epidermal keratinocytes. Consequently, inhibitors of P2X3 receptors accelerated skin barrier repair and prevented epidermal hyperplasia induced by skin barrier disruption (Denda et al 2002a). The significance of these data will be discussed under 4.1.5.

4.1.2.4 5-HT3 receptors

5-HT3 receptors were localized to basal epidermal keratinocytes in human skin *in situ* (Lundeberg et al 2002; Nordlind et al 2006), yet, as of today, we lack information about the functional role of these receptors in epidermal biology. However, as shown below (under 4.6.2.2.), altered expression patterns were observed in psoriatic (but not in AD) skin.

4.1.2.5 $GABA_A$ receptors

As we detailed above, the modulation of $[Ca^{2+}]_i$ homeostasis of epidermal keratinocytes via various ion channels is a key factor in regulating the physical epidermal barrier. It appears, however, that the control of Cl^- influx to keratinocytes establishes an additional mechanism. Indeed, $GABA_A$ receptors were identified in mouse epidermis (Denda et al 2002b). In addition, in the aforementioned hairless mouse model, topical application of GABA accelerated barrier repair and prevented epidermal hyperplasia via the stimulation of epidermal $GABA_A$ receptors (Denda et al 2002b; Denda et al 2003a). In line with these findings, GABA induced Cl^- influx to NHEKs which was blocked by the $GABA_A$ receptor antagonist bicucullin. Since GABA can be synthesized by human keratinocytes and dermal fibroblasts (Canellakis et al 1983; Ito et al 2007) and hence can be released upon skin barrier injury, it can be postulated that cutaneous non-neuronal GABA-ergic signaling acts as a key autocrine regulator of epidermal barrier homeostasis – just as described for locally produced and released ACh, glutamate, and ATP and their ionotropic receptor-coupled signal transduction mechanisms (for details, see also 4.1.5.).

4.1.2.6 Glycine receptors

Glycine receptors, another group of ligand-gated Cl^- channels, are also involved in barrier regeneration. In hairless mice, topical application of glycine, similar to the effect of $GABA_A$ receptor stimulation, accelerated the barrier repair after tape stripping which effect was completely prevented by the glycine receptor antagonist strychnine (Denda et al 2003a).

4.1.3 TRP channels

Numerous TRP channels exhibit permeability for Ca^{2+}, hence significantly modulate cellular Ca^{2+} homeostasis (Holzer 1991; Szallasi and Blumberg 1999; Caterina and Julius 2001; Clapham 2003; Dhaka et al 2006; Nilius and Mahieu 2006; Ramsey et al 2006; Nilius et al 2007; Vriens et al 2009). As detailed above, alterations in the $[Ca^{2+}]_i$ markedly affect proliferation and differentiation programs as well as of survival and mediator production of various skin cells (Hennings et al 1980; Lansdown 2002; Proksch et al 2008; Tóth et al 2009b). Therefore, besides the well-appreciated contribution to sensory neuron-coupled sensory processes (e.g. pain, itch) detailed in numerous comprehensive reviews, the functional expression of Ca^{2+}-permeable TRP channels on several non-neuronal skin cell types implicate their roles in controlling cutaneous growth and differentiation.

4.1.3.1 TRPV1

TRPV1, the heat-sensitive (>43 ^oC) “capsaicin receptor”, was originally described on nociceptive sensory neurons (Caterina et al 1997, 2000) and was implicated in a multitude of sensory-neuron coupled processes including sensation of e.g. pain, itch, warm, chemical agents, etc. Moreover, TRPV1 was shown to be involved in neurogenic inflammation and inflammation-related thermal hyperalgesia (reviewed in Szallasi and Blumberg 1999; Caterina and Julius 2001; Clapham 2003; Dhaka et al 2006; Vriens et al 2008, 2009). However, besides sensory neurons, an emerging body of evidence indicates that TRPV1 is widely expressed on several non-neuronal cell-types, including those of the skin. Indeed, expression of TRPV1 was demonstrated on epidermal and hair follicle keratinocytes, mast cells, Langerhans cells, sebocytes and endothelial cells (Bíró et al 1998a; Bíró et al 1998b; Birder et al 2001; Denda et al 2001; Inoue et al 2002; Southall et al 2003; Amantini et al 2004; Bodó et al 2004; Bodó et al 2005; Stander et al 2004; Basu and Srivastava 2005; Tóth et al 2009a; Tóth et al 2009b; Tóth et al 2011a).

Functional TRPV1 channels were identified on cultured keratinocytes as well, where their stimulation by either capsaicin or heat induced membrane currents and the influx of Ca^{2+} resulting in the concomitant elevation of $[Ca^{2+}]_i$. These cellular actions were effectively inhibited by capsazepine, a TRPV1 antagonist suggesting the specific involvement of the channel (Inoue et al 2002; Southall et al 2003; Bodó et al 2004; Bodó et al 2005; Radtke et al 2011). Furthermore, just as has been described on numerous extra-cutaneous cell types (Sanchez et al 2006; Prevarskaya et al 2007), activation of TRPV1 (most probably via the resulting Ca^{2+}-influx) on NHEKs decreased proliferation and increased apoptosis (Tóth et al 2011a) suggesting that these effects may all contribute to altered barrier functions. Indeed, activation of TRPV1 delayed the barrier recovery after tape stripping which effect was blocked by the topical application of capsazepine (Denda et al 2007b). Likewise, oral administration another TRPV1 antagonist, PAC-14028, also accelerated barrier recovery after mechanical and dermatitis-associated barrier injuries (Yun et al 2011).

Currently, we lack information on the possible roles of TRP channels in the production of those structural lipids, which constitute the major portion of the epidermal chemical barrier. However, TRPV1 channels (and, as suggested by our preliminary observations, TRPV3 and TRPV4 as well) (Oláh et al 2009; Oláh et al 2010; Ambrus et al 2011) are involved in the regulation of lipid-rich sebum production of the sebaceous glands. Indeed, TRPV1 was identified in the human sebaceous gland *in situ* (Bodó et al 2004; Stander et al 2004; Roosterman et al 2006; Zouboulis et al 2008; Tóth et al 2009b). In addition, stimulation of TRPV1 expressed on human sebaceous gland-derived immortalized SZ95 sebocytes (Zouboulis et al 1999) by capsaicin inhibited basal and arachidonic acid-induced lipid synthesis and suppressed expressions of multiple genes involved in cellular lipid homeostasis (Tóth et al 2009b). These data collectively argue for that TRPV1 inhibits the formation and the recovery of the physical-chemical skin barrier.

4.1.3.2 TRPV3 and TRPV4

TRPV3 is most abundantly expressed on epidermal keratinocytes; yet, it was also found on sensory neurons in co-expression with TRPV1 (Peier et al 2002b; Smith et al 2002; Xu et al 2002; Eid and Cortright 2009). TRPV4 was originally described as

an osmoreceptor expressed in various tissues including sensory neurons (Liedtke et al 2000; Strotmann et al 2000; Wissenbach et al 2000; Delany et al 2001) and keratinocytes (Suzuki et al 2003). Both TRPV3 and TRPV4 are activated by physiological, innocuous warm temperature ranges (>33°C for TRPV3 and cca. >30 °C for TRPV4) (Guler et al 2002; Peier et al 2002b; Smith et al 2002; Watanabe et al 2002; Xu et al 2002; Benham et al 2003; Eid and Cortright 2009) and their genetic deletion results in altered sensation of thermal stimuli (Todaka et al 2004; Lee et al 2005; Moqrich et al 2005).

TRPV3 and TRPV4 are implicated in the regulation of the physical-chemical epidermal barrier. Indeed, TRPV3 was found to form a functional complex with the receptor of epidermal growth factor (EGF), which is indispensable for the physiological formation of the barrier. Moreover, deletion of TRPV3 resulted in impaired epidermal barrier formation (e.g. thinner cornified envelope, decreased transglutaminase activity) (Cheng et al 2010). In addition, temperature ranges activating TRPV3 and TRPV4 as well as agonists of TRPV4 (but, interestingly, not of TRPV3) accelerated barrier recovery after tape striping (Denda et al 2007b). The barrier promoting role of TRPV4 was also verified by employing temperature challenges and specific agonists on cultured NHEKs and human skin cultures (Kida et al 2011).

Of further importance, TRPV4 was found to co-localize and interact with junctional proteins (β-catenin and E-cadherin) which further suggest its role in the formation of the epidermal barrier (Kida et al 2011). In support of this proposal, in TRPV4 KO mice, leaky cell-cell junctions and delayed actin rearrangement and stratification were observed which were associated with reduced $[Ca^{2+}]_i$ levels and suppressed Rho activation (Sokabe et al 2010; Sokabe and Tominaga 2010).

4.1.3.3 TRPV6

The Ca^{2+}-selective TRPV6, a non-thermosensitive member of the TRPV family, was also shown to promote epidermal differentiation and, most probably, barrier formation. Indeed, silencing of TRPV6 impaired keratinocyte differentiation (decreased expression of cytokeratin 10, involucrin and transglutaminase 1; impaired formation of intercellular contacts and stratification) induced by the elevation of

$[Ca^{2+}]_e$ (Lehen'kyi et al 2007). Moreover, TRPV6-mediated Ca^{2+}-influx was shown to be involved in mediating the differentiation-stimulatory effects of vitamin D3 (Bouillon et al 2006; Lehen'kyi et al 2007). Interestingly, treatment of NHEKs with Avène Thermal Spring water (TSW), which was shown to be beneficial in various human dermatoses, increased TRPV6 channel expression and initiated a TRPV6-mediated Ca^{2+}-entry that resulted in differentiation (increased expression of involucrin and cytokeratins 1 and 10) (Lehen'kyi et al 2011). In accordance with these findings, the skin of TRPV6 KO mice displays fewer and thinner layers of str. corneum, decreased total Ca^{2+}-content, and loss of the normal Ca^{2+}-gradient in the skin (Bianco et al 2007).

4.1.3.4 TRPC channels

Various TRPC channels (TRPC1, TRPC4-7) were found to be expressed in keratinocytes (Bezzerides et al 2004; Cai et al 2005; Fatherazi et al 2007), where their expression levels showed marked dependence on differentiation status of the cells (Cai et al 2005; Cai et al 2006; Fatherazi et al 2007). Among them, TRPC1 (Cai et al 2006; Beck et al 2008), TRPC4 (Beck et al 2008) and TRPC6 (Müller et al 2008) were shown to promote the differentiation of epidermal keratinocytes. Indeed, silencing of TRPC1 or TRPC4 prevented $[Ca^{2+}]_e$-induced differentiation (Beck et al 2008). Moreover, TRPC6 activation by hyperforin induced NHEK differentiation and inhibition of cell proliferation (Müller et al 2008). Likewise, TRPC6 was shown to mediate (at least in part) the epidermal differentiation-promoting effects of triterpenes, which inhibit cancer cell growth of various cell types (reviewed in Shanmugam et al 2012). Triterpenes increased Ca^{2+}-influx and upregulated various differentiation markers in a TRPC6-dependent manner, and also elevated the expression of TRPC6 in keratinocytes and in human skin explants (Woelfle et al 2010).

4.1.3.5 TRPA1

Like many other TRP channels, TRPA1 was first identified on sensory neurons (Story et al 2003; Kobayashi et al 2005). The channel can be activated by noxious cold (<17 °C) and other agents (e.g. mustard oil, allyl isothiocyanate, cinnamaldehyde, formalin, and nicotine) (Bandell et al 2004; Jordt et al 2004; McNamara et al 2007; Karashima et al 2009; Talavera et al 2009). Similar to its closest “functional relative”, i.e. TRPV1, TRPA1 was also shown to be involved in numerous sensory neuron-

coupled processes such as e.g. thermosensation, pain, itch, neurogenic inflammation, etc. (Dhaka et al 2006; Nilius and Mahieu 2006; Ramsey et al 2006; Nilius et al 2007).

Importantly, TRPA1 expression was also reported on epidermal keratinocytes. Exposure of NHEKs to low temperature (13-15 ^{o}C) or to TRPA1 agonists (allyl isothiocyanate or cinnamaldehyde) induced elevation of $[Ca^{2+}]_e$, which was prevented by the co-application of the TRPA1 antagonist HC030031; interestingly, these effects were more prominent on undifferentiated cells (Tsutsumi et al 2010). Moreover, treatment of NHEKs with icilin (activator of both TRPA1 and TRPM8, another cold-sensitive channel, see below) caused alterations in the expressions of adhesion and extracellular matrix components as well as molecules regulating cell cycle, apoptosis, and differentiation (Atoyan et al 2009; Bíró and Kovács 2009).

These data suggest that TRPA1 on keratinocytes may regulate the epidermal barrier. Indeed, following tape striping to mice, topical application of the above TRPA1 agonists accelerated barrier recovery, which effect was prevented by pretreatment with HC030031. Interestingly, HC030031 alone delayed the barrier recovery which argues for the “constitutive” role of TRPA1 in epidermal barrier homeostasis. Local cooling of the skin (10-15 °C for 1 min) evoked similar effects, most probably via accelerated secretion of (barrier-forming) lamellar bodies at the interface of stratum granulosum and corneum; this action was also inhibited by the TRPA1 antagonist (Denda et al 2010b).

4.1.3.6 TRPM channels

TRPM8 is another cold sensitive (<25 °C) channel, originally found on a specific subset of sensory neurons which usually do not express TRPV1. The channel is considered as a major sensor of environmental cold stimuli and it can also be activated by menthol, eucalyptol or the synthetic “supercooling” agent icilin (McKemy et al 2002; Peier et al 2002a; Bautista et al 2007; Colburn et al 2007).

Importantly, topical application of menthol or the TRPM8 agonist WS12 to mice potentiated the barrier recovery after tape stripping, which effect was blocked by the TRPM8 specific antagonist BTCT (Denda et al 2010a). Since TRPM8 was identified

on epidermal keratinocytes (Denda et al 2010a), these results argue for that (similar to the other cold receptor, TRPA1) TRPM8 is also involved in skin homeostasis.

4.1.4 Non-ion selective channels

4.1.4.1 Aquaporins

Numerous APQs were shown to play key roles in various cutaneous functions. AQP3, the key aquaglyceroporin, regulate hydration of the skin, a major determinant of the physical properties of the epidermis (see reviewed in Hara-Chikuma and Verkman 2008a; Qin et al 2011). APQ3 is abundantly expressed and functionally localized in cultured keratinocytes (Sugiyama et al 2001) and to the basal and spinous layers (but, importantly, not in the str. corneum) of human and rat epidermis *in situ* (Frigeri et al 1995; Matsuzaki et al 1999; Sougrat et al 2002). In addition, AQP3 levels (as well as water and glycerol contents) were higher in proliferating mouse keratinocytes but were reduced upon $[Ca^{2+}]_e$ or vitamin D3 induced differentiation (Zheng and Bollag 2003; Hara-Chikuma et al 2009).

In perfect agreement with these findings, AQP3-deficient mice exhibit a characteristic skin phenotype such as dry, rough and aged skin appearance, reduced glycerol content and hydration of the epidermis (Ma et al 2002), impaired elasticity, and delayed barrier recovery after tape stripping (Hara et al 2002). Interestingly, expressions of differentiation markers and the differentiation process of keratinocytes as well as epidermal structure and lipid, amino acid, and ionic contents were not different from those of the wildtype animals (Hara et al 2002; Hara-Chikuma et al 2009). Of further importance, the cutaneous malfunctions of AQP3-deficient mice could be corrected by oral administration of glycerol which points to an intrinsic defect in water-holding capacity of the skin due to the lack of glycerol transport (Hara and Verkman 2003).

It is noteworthy that AQP3 was also identified in sebaceous glands (Frigeri et al 1995). Since epidermal glycerol, mostly located to the str. corneum, is also derived from sebaceous glands (Fluhr et al 2003), further studies are invited to determine the relative contribution of AQP3 localized to sebaceous glands in the regulation of the glycerol homeostasis of the skin.

It should also be mentioned that other AQPs (e.g. AQP1, 9, and 10) were also identified in human and murine keratinocytes and epidermis (Sugiyama et al 2001; Boury-Jamot et al 2006; Rojek et al 2007). Yet, their functional role is not known.

4.1.4.2 Connexins

In animal models (and in different human skin conditions, see below), the central role of certain gap-junction-forming connexins in the establishment of the epidermal barrier was suggested (reviewed in Proksch et al 2008). Indeed, mice lacking the C-terminal region of Cx43, the most abundantly expressed connexin form in the human epidermis, show a highly defective epidermal barrier, most probably due to suppressed filaggrin expression and hence impaired terminal differentiation of the epidermal keratinocytes (Maass et al 2004). On the other hand, downregulation of another connexin, Cx26, is required for barrier acquisition during development. Indeed, epidermal overexpression of Cx26 (which is hardly detectable in the healthy, adult epidermis) resulted in the development of psoriasiform hyperproliferation and infiltration of immune cells. Moreover, overexpression of Cx26 induced ATP release from keratinocytes which, in turn, delayed epidermal barrier recovery (Djalilian et al 2006).

4.1.4.3 Pannexins

Expressions of Panx1 and 3 have been described both in human (Penuela et al 2007) and murine (Celetti et al 2010) epidermal keratinocytes. Interestingly, the expression pattern of Panx1 changed in embryonic and newborn skin, with a higher expression found after birth (Panx3 expression showed no such alteration). Functionally, when overexpressed in organotypic rat keratinocytes, both Panxs decreased cell proliferation, whereas Panx1 also disrupted the architecture of organotypic epidermis and dysregulated the expression and cellular localization of cytokeratin 14 (Celetti et al 2010). Taken together, these findings suggest that certain Panxs (especially Panx1) are important factors in keratinocyte differentiation.

4.1.5 The complex "channel" regulation of the epidermal barrier – Controversies, explanations, theories

As detailed above, the delicate regulation of $[Ca^{2+}]_i$ and the coupled Ca^{2+}-dependent processes is the key event in controlling the physiological growth and differentiation

of keratinocytes. Correspondingly, numerous Ca^{2+}-permeable channels were shown to promote the (terminal) differentiation of keratinocytes. These include voltage-gated Ca_v channels; K_{Ca} K^+-channels; nAChRs subtypes α3, α7, and α9; NMDAR glutamate receptors; P2X5 and P2X7 purinergic receptors; and TRPC1, C4, C6 as well as TRPV6 (and possibly TRPV3, TRPA1, and TRPM8) TRP channels.

However, although the formation and maintenance of the epidermal physical-chemical barrier is based on the proper keratinocyte differentiation program, the activation of some of these channels (Ca_v channels, nAChRs, NMDARs, purinergic receptors) was shown to delay, whereas stimulation of others (TRPV4, TRPA1, TRPM8, and possibly TRPV3 and V6) accelerated the recovery of the barrier after mechanical disruption (**Figure 3**).

As possible explanations for these quite unexpected findings, the followings could be listed:

- As we presented above, keratinocytes are able to synthesize and release ACh, ATP, and glutamate. Moreover, an "upward" Ca^{2+}-gradient was described in the epidermis. Therefore, these locally produced, autocrine/paracrine mediators constitutively promote the differentiation of keratinocytes, via the activation of their respective ionotropic, Ca^{2+}-permeable receptors/channels and the concomitant increase in $[Ca^{2+}]_i$.
- However, during mechanical disruption of the epidermis, the release of these endogenous agents from keratinocytes is markedly increased and the high-level activation of their receptors may result in an excessive Ca^{2+} influx which impairs keratinocyte differentiation. It appears, therefore, that the intracutaneous "ACh-ATP-glutamate-Ca^{2+} tone" is indispensible for the maintenance of the healthy barrier; however, when this "fine-tuned tone" is pathologically augmented, these agents may start functioning as mediators of the barrier injury itself.
- The validity of this theory is supported by experimental data obtained after topical administration of these agents as well as their agonists and antagonists to mechanically injured barrier (tape stripping) in mice. Indeed, topical agonists (by further increasing the already highly augmented "tone") delayed barrier

recovery whereas antagonists (by normalizing the “tone”) accelerated the rate of recovery.

- However, experimental data about the role of TRPV4, TRPA1, and TRPM8 channels do not really fit to this theory. Namely, as detailed before, the activation of these (likewise) Ca^{2+}-permeable channels also stimulated epidermal differentiation. Interestingly, topical application of their agonists after tape stripping – in contrast to the effects of ionotropic cholinergic, glutamatergic, and purinergic stimulations – accelerated barrier repair.
- With respect to the promoting role of TRPV4 it was suggested that, due to its sensitivity to not only to moderate heat but also to osmotic challenges, it may act as part of the “keratinocytes sensory system” that recognizes water flux (Sokabe and Tominaga 2010). A possible support for this hypothesis could be that AQP3 water (and glycerol) channels are actively participating in the barrier regeneration processes by increasing water flux and water content within the epidermis. Nevertheless, the direct or indirect connection between TRPV4 and AQP3 has not yet revealed.
- Also, further studies are needed to clarify the (most probably distinct) signaling pathways which are initiated after induction of TRPA1 or TRPM8 activities by agonists or by cooling of the skin surface.

Finally, it should be mentioned that the induction of Cl^--influx to keratinocytes by topical activation of GABA-ergic and glycinergic signaling mechanisms also accelerated barrier regeneration. As possible mechanisms of action, it can be postulated that the Cl^--influx results in hyperpolarization of the cell membrane which (as a pro-proliferating factor) speeds up the turn-over of keratinocytes to “heal” the barrier. In addition, the Cl^--mediated hyperpolarization may counterbalance the effect of the augmented “ACh-ATP-glutamate-Ca^{2+} tone” thereby preventing the excessive Ca^{2+} influx and its damaging consequences.

Nevertheless, since modulation of the activities of these, rather complex, mechanisms may represent novel therapeutic approaches; further studies are urgently invited to (i) dissect the exact mechanistic details of their modes of action; and ii) explore their impact in such high-prevalence “barrier diseases” as AD or psoriasis.

4.2 Roles of channels in wound healing

Like in the formation and regeneration of the epidermal physical-chemical barrier, multiple channels participate in the complex multi-cellular events of wound healing.

4.2.1 Voltage-gated channels

4.2.1.1 Voltage-gated and Ca^{2+}-activated K^+-channels

All three members of the K_{Ca} channel family – i.e. large-conductance BK, intermediate-conductance IK, and small-conductance K_{Ca} – were identified on cultured human dermal fibroblasts. Moreover, activation of BK or IK K_{Ca} channels decreased the proliferation of fibroblasts and induced apoptotic changes by mitochondrial membrane potential disruption (but without the involvement of the caspase-dependent apoptotic pathways) (Yun et al 2010). In addition, nitric oxide (NO), which plays an important promoting role in wound healing (Shi et al 2003), was shown to stimulate BK K_{Ca} channel activity via the engagement of protein kinase A and G coupled signaling pathways in human dermal fibroblast (Lim et al 2005; Roh et al 2007) and via increasing cyclic-GMP in human hair follicle-derived dermal papilla fibroblasts (Nameda et al 1996). It appears, therefore, that K_{Ca} are involved in fibroblast-driven cutaneous wound healing.

Other voltage-gated K^+-channels (fast-inactivating A-type K^+-channels; inward rectifier (K_{ir}) K^+-channels; cell-to-cell contact-associated K^+-channels) as well as Na^+ channels (tetrodotoxin-sensitive Na^+-channels) were also identified on human dermal fibroblasts; yet their functional roles were not revealed (Estacion 1991).

4.2.2 Ligand-gated channels

4.2.2.1 nAChRs

Keratinocyte migration events, such as chemokinesis and chemotaxis, are key events of epithelial re-epithelialization during wound healing (Epstein 1999, Enoch and Leaper 2005; Reinke and Sorg 2012). Since locally produced ACh, which could be released during injury of the skin, may act as both a chemokine and a chemoattractant for cell migration (reviewed in Gando et al 2006), involvement of non-neuronal nicotinergic signaling in wound healing is also proposed. Indeed, α3β2 nAChR channels were shown to play central roles in mediating ACh-dependent

chemokinesis whereas α7 nAChRs were found to be involved in chemotaxis (Chernyavsky et al 2004). In addition, α7 channels (and a complex intracellular signaling pathway involving Ras/Raf-1/MEK1/ERK-mediated upregulation of integrins) were described to control directional migration of keratinocytes (Chernyavsky et al 2005). Finally, α9 nAChRs seem to be indispensable for the initial phase of epithelialization as the coupled signaling controls the dynamics and strength of cell-cell cohesion as well as the disassembly and reassembly of focal adhesions (Chernyavskyet al 2007).

Another key cell type of wound healing is the dermal fibroblasts (see above, 2.2.1.4) which also possess a functional cholinergic system. Indeed, α3, α5, α7, β2, and β4 nAChRs were described in cultured human dermal fibroblast. Among these, as reveled by gene silencing, mainly α3 nAChR channels were implicated in mediating the effects of nicotine to significantly modulate cell growth, cycling, and survival (upregulation of p21, cyclin D1, PCNA, Ki-67, caspase 3 and bcl-2 mRNA transcripts) as well as production of extracellular matrix components (upregulation of collagen type Iα_1, elastin, and matrix metalloproteinase-1, MMP-1). Therefore, the cholinergic system may play a key role in controlling proper dermal fibroblast functions involved in tissue remodeling and wound healing (Arredondo et al 2003).

4.2.2.2 $GABA_A$ receptors

Of further importance, non-neuronal GABA-ergic mechanisms seem to not only stimulate epidermal barrier recovery, but also cutaneous wound healing. In a rat excisional open wound model, topical GABA, treatment, most probably via activation of $GABA_A$ receptors, was shown to effectively accelerate the healing process (especially its early phase) by stimulating keratinocyte reepithelialization and fibroblast organization as well as by upregulating fibroblast growth factor and platelet-derived growth factor, implying extracellular matrix synthesis and remodeling of the skin (Han et al 2007). Further supporting its promoting role, GABA was shown to stimulate the synthesis of hyaluronic acid, a key component of the extracellular matrix, and to enhance the survival rate of the human dermal fibroblasts against oxidative stress (Ito et al 2007).

4.2.3 TRP channels

4.2.3.1 TRPV1

Although the expression of functional TRPV1 channels was described on human dermal fibroblasts (Kim et al 2006), the role of the channels in fibroblast-specific functions was not assessed. However, activation of TRPV1 by capsaicin in organ-cultured human scalp hair follicles (HF) inhibited hair shaft elongation and induced premature follicular regression (catagen transformation) (Bodó et al 2005). In line with these data, stimulation of TRPV1 in HF-derived cultured outer root sheath (ORS) keratinocytes, which showed the greatest TRPV1 expression in the HF (Bodó et al 2004; Stander et al 2004; Bodó et al 2005), resulted in suppression of proliferation and the onset of apoptosis. The growth inhibitory role of TRPV1 was further verified in TRPV1 knockout mice; i.e. a remarkable delay in the onset of the apoptosis-driven catagen retardation was observed when compared to the HF cycle of wildtype animals (Bíró et al 2006).

As mentioned above, the HF (and especially the ORS compartment) is a rich source of stem cells activated during wound healing and tissue regeneration in general. Therefore, these data collectively suggest that TRPV1-coupled signaling rather inhibits wound healing, just as seen for the formation and regeneration of the epidermal barrier (see above).

4.2.3.2 TRPV3

As will be discussed below (see under 4.4.2.1.), activation of TRPV3 on keratinocytes by heat or various agonists results in the release of various mediators. Among these, NO, released upon TRPV3 stimulation of keratinocytes, was shown to promote keratinocyte migration *in vitro* and, as expected, wound healing *in vivo* (Miyamoto et al 2011).

Furthermore, using the above HF organ-culture and ORS cell culture models, we have recently shown that activation of TRPV3, identical to the above action of TRPV1, inhibited hair shaft elongation and cellular proliferation and induced apoptosis (Borbíró et al 2011). Interestingly, TRPV3 KO mice exhibited only subtle and irregular hair abnormalities (wavy hair coat and curly whiskers) (Moqrich et al 2005). However, a gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a spontaneous hairless phenotype in DS-*Nh* mice (Yoshioka et al 2009) as well as in

hairless WBN/Kob-Ht rats (Asakawa et al 2006; Imura et al 2007). These results collective argue for the negative regulatory role of TRPV3 in the HF.

4.2.4 Non-ion selective channels

4.2.4.1 Aquaporins

In addition to impaired epidermal differentiation and barrier functions, a delayed wound healing process was also seen in AQP3-deficient mice (Hara et al 2002; Hara-Chikuma and Verkman 2008a), which suggests the role of AQP3 in promoting epidermal cell migration and proliferation. Indeed, by using AQP3-knockdown (small interfering RNA) NHEK cultures and AQP3-knockout mouse keratinocyte cultures (Hara-Chikuma and Verkman 2008c), it was proposed that the water, transported via AQP3, is likely to play a role in epidermal hydration and hence migration controlled by changes in the hydrostatic pressure. On the other hand, the transported glycerol may equally act as an energy source for ATP production; a precursor for fat and phospholipid synthesis (such as phosphatidylglycerol, a phospholipase D product, which is known to regulate keratinocyte proliferation and differentiation (Qin et al 2011); and an osmotically active agent (Hara-Chikuma and Verkman 2008b).

4.2.4.2 Connexins

As shown above, Cx26 promotes proliferation (and inhibits differentiation) of keratinocytes. In line with these data, expression of Cx26 was found to be increased during the epidermal regeneration phase of wound healing (Brandner et al 2004), with an additional suppression of the level of the pro-differentiating Cx43 (Wang et al 2007).

4.3 Roles of channels in cutaneous immune functions and in the “formation” of the immunological barrier

The skin possesses its own immune system which involves numerous cellular and humoral components of the innate and adaptive immunity. Immuno-competent cells express various ion channels which, as shown below, play significant roles in the regulation of cutaneous inflammatory and immune responses.

4.3.1 Voltage-gated channels

Although expressions of genes encoding various voltage-gated K^+-channels ($K_{ir}2.1$, $K_{ir}2.4$, and BK K_{Ca} α and β4 channel subunits), Na^+-channels ($Na_v1.8$ and $Na_v1.9$ channels as well as the auxiliary subunit $Na_v\beta1.1$), and Ca^{2+}-channels (the auxiliary subunit $Ca_v\alpha_2\delta_2$) were identified on human skin-derived mast cells (Bradding et al 2003), their functional role is not known. Likewise, we lack data on whether the functional voltage-gated channels expressed by keratinocytes and fibroblasts may participate in the immune responses of these cells.

4.3.2 Ligand-gated channels

4.3.2.1 nAChRs

Nicotinic AChR-coupled signaling was suggested to be involved in cutaneous inflammatory responses. As we mentioned above, topical administration of nicotine to mouse skin resulted in a marked suppression of AMP production which affect was reversed by the nAChR antagonist α-bungarotoxin (Radek et al 2010). Likewise, stimulated production of AMPs (LL-27 cathelicidin, β-defensin) in NHEKs was suppressed by ACh which effect was reversed by α-bungarotoxin. Of further importance, Chga knockout mice, which exhibit unopposed nAChR activation due to genetic deletion of the endogenous nAChR inhibitor, catestatin (Mahapatra et al 2005), showed increased susceptibility to bacterial infections. These data propose that the proper intracutaneous cholinergic ACh-nAChR signaling not only regulates skin barrier formation and wound healing (see above, 4.1.2.1. and 4.2.2.1.), but also the innate host defense of the skin. Moreover, since activity of the neuronal and the non-neuronal, cholinergic systems is markedly increased during chronic stress, the pathological augmentation of the above mechanism may contribute to the highly elevated susceptibility to infection following prolonged stress (Radek et al 2010; Curtis and Radek 2012).

4.3.2.2 P2X receptors

Certain P2X receptors were also implicated in skin immune functions and inflammation. Indeed, in human skin vascular endothelial cells, among the several ionotropic purinergic receptors expressed by these cells, P2X4 was described in mediating the effect of ATP to increase Ca^{2+}-influx and to induce the release of the pro-inflammatory and vasoactive NO and prostaglandin PGI_2 (Yamamoto et al 2000). In addition, among P2X receptors, the human dermal microvascular endothelial cell-1

(HMEC-1) cell line was shown to strongly express P2X4, P2X5, and P2X7 receptors and weakly express P2X1 and P2X3 receptors (Seiffert et al 2006). Administration of ATPγS, a hydrolysis-resistant purinergic agonist, to HMEC-1 cells increased the release of numerous pro-inflammatory mediators (IL-6, IL-8, monocyte chemoattractant protein-1, growth-regulated oncogene-α) and upregulated the expression of intercellular adhesion molecule-1 (ICAM-1); these events were effectively prevented by various purinergic antagonists.

Of further importance, intradermal administration of ATPγS in mice resulted in an enhanced contact hypersensitivity response and the induction of delayed-type hypersensitivity. Moreover, in cultured mouse Langerhans cells, ATPγS (in the presence of bacterial lipopolysaccharide [LPS] and granulocyte-macrophage colony-stimulating factor) enhanced the antigen-presenting functions of the cells (Granstein et al 2005). In perfect line with these data, mice lacking the P2X7 receptor were shown to be resistant to contact hypersensitivity. Dendritic cells from P2X7-deficient mice failed to induce sensitization to contact allergens and did not release IL-1β, a key molecule in the sensitization process, in response to LPS and ATP (Weber et al 2010).

Expression of functional P2X7 receptors was also demonstrated both on human and mouse epidermal Langerhans cells (Georgiou et al 2005; Tran et al 2010). Activation of P2X7 on human Langerhans cells induced downstream signaling events, i.e. shedding of the low-affinity receptor for IgE (CD23), which effect was impaired in Langerhans cells obtained from subjects homozygous for the loss-of-function polymorphism in the P2X7 receptor (Georgiou et al 2005).

On cultured NHEKs, extracellular ATP displayed a complex regulation of interferon-γ stimulated chemokine expression, with upregulation of chemokine ligand 2 (CCL2), CCL5 and CXC chemokine ligand 8 (CXCL8), and suppression of the receptor CXC chemokine receptor 3 (CXCR3), CXCL9, CXCL10, and CXCL11. It is suggested that P2X7 receptors are involved in this complex process (Pastore et al 2007). Of further importance, P2X7 receptors expressed by human keratinocytes were also implicated as key components of the signaling pathway (P2X7-SFK-Akt-CREB/ATF1) activated by LL-37 cathelicidin, a multifunctional immunomodulatory AMP, to augment the

production of immune mediators in response to microbial compounds (Nijnik et al 2012).

Stimulation of functional P2X7 receptors was also found to induce the release of the pro-inflammatory cytokine IL-6 on human skin fibroblasts (Solini et al 1999). In addition, augmented ATP release and enhanced P2X7 receptor-mediated cellular responses (including microvesiculation, enhanced fibronectin and IL-6 secretion, accelerated apoptosis) were demonstrated on dermal fibroblasts of type 2 diabetic subjects (Solini et al 2004).

Collectively, it is proposed that ATP, when released after trauma, infection or exposure to contact allergens, may act as an endogenous adjuvant to enhance the immune response, most probably via P2X7-coupled signaling found on immunocompetent keratinocytes, Langerhans cells, microvascular endothelial cells, and fibroblasts. Interference with P2X7 receptors may therefore be a promising strategy for the prevention of allergic contact dermatitis and possibly other inflammatory skin conditions.

4.3.2.3 $GABA_A$ receptors

In NC/Nga mice, a murine model of AD, oral administration GABA reduced the development of AD-like skin lesions, most probably by suppressing serum immunoglobulin E and splenocyte IL-4 production (Hokazono et al 2010). Although it cannot be excluded that the above beneficial effects were due to the aforementioned effects of GABA to promote barrier formation and repair (which processes are highly impaired in AD), these results also propose the anti-inflammatory functions of the non-neuronal GABA-ergic signaling of the skin.

4.3.3 TRP channels

As mentioned above (2.2.2.), activation of sensory afferents in the skin results in the release of various neuropeptides (SP, CGRP) which – via the stimulation of immunocompetent cells of the skin (e.g. keratinocytes, sebocytes, mast cells, etc.) and the concomitant induction of liberation of various inflammatory mediators (cytokines, chemokines, vasoactive agents) from these cells – induces neurogenic inflammation (Ansel et al 1997; Luger 2002; Paus et al 2006a and b; Peters et al

2007; Fuchs and Horsley 2008). With respect to TRP channels, TRPV1 and TRPA1 were implicated in this process. However, the identification of various functional TRPs on non-neuronal cell types of the skin suggests that these molecules are also involved in non-neurogenic skin inflammation.

4.3.3.1 TRPV1

As we have detailed above (4.1.3.1.), the TRPV1 inhibitor PAC-14028, when applied orally, accelerated barrier recovery after tape stripping. However, PAC-14028 seems to be beneficial against experimentally induced AD as well (Yun et al 2011). Indeed, in a mouse model of AD (induced by *Dermatophagoides farina* and oxazolone), the orally administered TRPV1 antagonist was able to efficiently prevent the dermatitis-associated barrier damages (by suppressing of trans-epidermal water loss, inducing reconstruction of epidermal lipid layers, and normalizing of altered expressions of epidermal differentiation markers) and, at the same time, improved the AD-like symptoms (clinical severity, skin score, serum IgE levels, mast cell degranulation status, etc.).

In good agreement with these *in vivo* data, TRPV1 activation on cultured human keratinocytes by capsaicin resulted in the induction of cyclooxygenase-2 (COX-2) and the release of pro-inflammatory IL-8 and PGE_2 (Southall et al 2003). Importantly, stimulation of TRPV1 by heat on NHEKs not only altered proliferation and cellular survival, but also induced MMP-1 production (Li et al 2007; Lee et al 2008). Likewise, TRPV1-coupled Ca^{2+}-dependent signaling was shown to be involved in mediating the effects of UV irradiation to upregulate MMP-1 in cultured keratinocytes (Lee et al 2009b). Furthermore, in a mouse model, the TRPV1 inhibitor 5′-iodoresiniferatoxin (I-RTX), when applied topically, was shown effectively prevent the UV-induced reactions (skin thickening, inflammation, upregulation of MMPs, COX-2, and pro-inflammatory cytokines such as IL-1β, IL-2, IL-4, tumor necrosis factor-α, TNFα) (Lee et al 2011).

Finally, it should be mentioned that activation of TRPV1 by capsaicin on cultured HF-derived ORS keratinocytes (besides inducing cellular arrest and apoptosis, see above under 4.2.3.1.) stimulated the synthesis of the pro-inflammatory IL-1β and

transforming growth factor-β_2 (Bodó et al 2005). These results collectively argue for the pro-inflammatory role of TRPV1 in non-neurogenic cutaneous inflammation.

4.3.3.2 TRPV3

As we have detailed above (4.2.3.2.), the gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a hairless phenotype in mice and rats. However, of great importance, this mutation is also accompanied by a spontaneously developing AD-like dermatitis (Asakawa et al 2006; Xiao et al 2008). Moreover, keratinocyte-targeted transgenic overexpression of the mutant $TRPV3^{Gly573Ser}$ channels in mice also led to the development of AD-like cutaneous (dermatitis, hyperkeratosis, itch, infiltration of mast cells and CD4+ lymphocytes, increased skin nerve growth factor [NGF] levels) and systemic (increased serum levels of IgE and pro-inflammatory cytokines) symptoms (Yoshioka et al 2009). As a further support for its pro-inflammatory role, TRPV3 stimulation in cultured keratinocytes by agonists (eugenol, 2-aminoethoxydiphenyl borate) or heat was shown to induce the release of the pro-inflammatory IL-1α and PGE_2 (Xu et al 2006; Huang et al 2008).

4.3.3.3 TRPA1

TRPA1, similar to TRPV1 and TRPV3, also seems to act as a pro-inflammatory channel. Stimulation of TRPA1 on NHEKs induced the synthesis of the pro-inflammatory IL-1α and IL-1β (Atoyan et al 2009). Moreover, as expected, topical application of the TRPA1 agonist cinnamaldehyde induced skin inflammation. Interestingly, however, whereas the edema component was prevented by aprepitant, an antagonist of the tachykinin NK1 receptor recognizing SP released from sensory afferent upon TRPA1 stimulation, it was not affected by HC030031, a TRPA1 antagonist. On the contrary, the cinnamaldehyde-induced leukocyte infiltration was effectively suppressed by the TRPA1 inhibitor whilst the NK1 antagonist was ineffective (Silva et al 2011).

These intriguing data suggest that the TRPA1-coupled signaling on sensory neurons and non-neuronal skin cells, when co-activated e.g. by topical or intracutaneous administrations of agonists, act in concert to equally induce neurogenic and non-neurogenic skin inflammation. We propose that this is the case for TRPV1 and possibly for TRPV3 as well.

4.3.4 Non-ion selective channels

4.3.4.1 Aquaporins

The aquaglyceroporin AQP7 was identified on mouse dermal and epidermal dendritic cells. In dendritic cells isolated from AQP7 deficient mice, significantly decreased antigen uptake and reduced chemokine-dependent cell migration were identified in comparison to wild-type cells. Moreover, AQP7-deficient mice exhibited a suppressed accumulation of antigen-retaining dendritic cells in the lymph node after antigen application to the skin. These results suggest that AQP7 in skin dendritic cells is primarily involved in antigen uptake and in the subsequent migration of the cells which suggest their role in the initiation of the concomitant immune responses (Hara-Chikuma et al 2012). AQP3 and AQP9 were also found in monocyte-derived Langerhans cells but their role is still unclear (Boury-Jamot et al 2006).

In addition, TNFα coupled signaling (involving p38 and Erk kinase cascades) was shown to suppress AQP3 expression in cultured keratinocytes which effect may contribute to the pro-inflammatory effects of this cytokine (Horie et al 2009).

4.4 "Sensory roles" of epidermal keratinocytes

As we detailed above (2.2.2.), various stimuli that reach the skin may not only activate sensory afferent fibers, but also non-neuronal skin-derived cells. Among these cells, direct activation of epidermal keratinocytes, which establish the very first line of defense, results in the release of various mediators. These agents, in turn, act on the sensory afferents and induce their excitation. Therefore, keratinocytes and, via the established multi-cellular neuronal – non-neuronal cell networks, possibly other skin-derived cells significantly contribute to skin sensory physiology.

4.4.1 Voltage-gated ion channels

4.4.1.1 Voltage-gated Na^+-channels

Various voltage-gated Na^+-channels were identified on epidermal keratinocytes. $Na_v1.1$, $Na_v1.6$, and $Na_v1.8$, expressed on rat cultured keratinocytes, were shown to contribute to the release of ATP from these cells (Zhao et al 2008). It was suggested that the release ATP, in turn, may stimulate nociceptive sensory afferents (located in a close vicinity of epidermal keratinocytes in the epidermis) (Ansel et al, 1997) and

hence may initiate pain. In addition, *in situ* epidermal expressions of $Na_v1.5$, $Na_v1.6$, and $Na_v1.7$ were identified on histological skin sections from healthy human subjects. Interestingly, levels of these channels were shown to be markedly increased in skin samples obtained from patients with various pain syndromes (complex regional pain syndrome type 1 and post-herpetic neuralgia), with additional appearances of $Na_v1.1$, $Na_v1.2$, and $Na_v1.8$. Although it is not known whether or not these channels contribute to the regulation of keratinocyte growth control, the “sensory roles” of the increased Na^+-channel expression in the pathogenesis of the above pain syndromes is suggested (Zhao et al 2008).

4.4.1.2 Two-pore K^+ channels (K_{2P})

Six two-pore K^+ channels (TASK-1, TASK-2, TASK-3, TREK-1, TREK-2 and TRAAK) were described in human epidermal HaCaT keratinocytes as well as in rat skin. Since K^+ currents were induced by different activators of these channels (arachidonic acid and heat), these results suggest that K_{2P} channels could act as thermosensors in human keratinocytes (Kang et al 2007).

4.4.2 TRP channels

4.4.2.1 TRPV3

Similar to the above, TRPV3 (and most probably of TRPV4) expressed by keratinocytes may also provide thermo-sensory functions these cells. Namely, moderate heat-activation of TRPV3 expressed by keratinocytes resulted in the release of ATP which, in turn, may stimulate sensory neurons (Chung et al 2003; Chung et al 2004; Lee et al 2005; Mandadi et al 2009). Likewise, overexpression of TRPV3 in keratinocytes was shown to modulate sensory processes by the TRPV3-mediated release of PGE2 (Huang et al 2008). Finally, NO, which is released from keratinocytes upon TRPV3 stimulation, not only promotes keratinocyte migration and wound healing (see above, 4.2.3.2.), but also regulates thermosensory behavior, most probably by acting on and hence stimulating thermosensitive sensory afferents (Miyamoto et al 2011).

4.4.3 Other ion channels

4.4.3.1 Amiloride-sensitive Na^+ channels

Amiloride-sensitive epithelial Na^+ channels (ENaCδ) were also found in human epidermis. In cultured NHEKs, acidic stress, activator of these channels, evoked ATP release which was inhibited by amiloride. Interestingly, ENaCβ and γ were also identified in human keratinocytes; yet, their physiological functions are not known. These data suggest that ENaCδ expressed by keratinocytes may be involved in pH sensing of the skin (Yamamura et al 2008b).

4.5 Other skin functions

Here, we mostly detail the roles of various channels in the control of sweat production and skin metabolism.

4.5.1 Voltage-gated channels

4.5.1.1 Ca^{2+}-activated K^+-channels

K_{Ca} channels also play key roles in regulating exocrine gland functions of the skin. Indeed, BK K_{Ca} channels were identified on primary cultures of human (Henderson and Cuthbert 1991a) and equine (Huang et al 1999) eccrine sweat gland cells as well as on exocrine gland cells in frog skin (Andersen et al 1995; Sørensen et al 2001). In human cell cultures (especially in the younger, dividing ones), these BK K_{Ca} channels, located on the basolateral membrane of the cell, were implicated in the Ca^{2+}-dependent secretory and absorptive events seen in the intact sweat gland.

In cultured human eccrine sweat gland cells, intermediate-conductance IK K_{Ca} channels were also identified. Interestingly, estradiol rapidly activated these channels in an estrogen receptor-independent manner. In addition, estradiol was shown to induce the translocation of IK K_{Ca} both to the apical and basolateral cell membranes in a calmodulin-dependent manner. This mechanism was suggested as a new mode of estrogen action in human sweat gland epithelial cells (Muchekehu and Harvey 2009).

4.5.2 Ligand-gated channels

4.5.2.1 nAChRs

It is a common knowledge in physiology that sweating can be induced by efferent neuronal cholinergic stimulation, mediated by the binding of the released ACh to muscarinic mAChRs expressed by the sweat glands. Likewise, sweating can be

induced by intradermal injection of cholinomimetic compounds, which can be efficiently prevented by the mAChR antagonist atropine (Longmore et al 1985; Smith et al 1992). However, application of ACh to primary human sweat gland-derived epithelial cells was shown to also induce Ca^{2+} influx which may argue for the existence of functional nAChR channels (Lei et al 2008). Indeed, various nAChR channels (including α3 and α7) were described in the ductal and acinar compartments of sweat glands. Moreover, the enzymatic apparatus for the synthesis and degradation of ACh is also expressed by sweat glands (Kurzen et al 2004; Hagforsen 2007). Therefore, further studies are invited to define the relative contribution of nAChRs and mAChRs to sweating induced by neuronal and non-neuronal ACh.

4.5.3 Non-ion selective channels

4.5.3.1 Aquaporins

AQP5 was found to be expressed in secretory cells and ductal parts of sweat glands in humans, rat, and mice (Nejsum et al 2002; Song et al 2002). Using various methods, Song et al concluded that AQP5 deletion in mice did not affect intensity and composition of sweat secretion (Song et al 2002). However, others have shown that genetic depletion of AQP5 in mice greatly decreased the response of sweat glands to pilocarpine, a known inducer a sweat production (Nejsum et al 2002). In light of these data, further studies are needed to unambiguously define the role is AQP5 in human sweat secretion.

AQP7 is also expressed by subcutaneous adipocytes and seems to be involved in cutaneous fat metabolism. Indeed, in AQP7 knockout mice, a progressive adipocyte hypertrophy was observed which effect was most probably due to the reduced AQP7-facilitated plasma membrane glycerol exit from adipocytes (Hara-Chikuma et al 2005; Hibuse et al 2005).

4.6 Skin diseases

So far, we have presented a plethora of evidence about the active contribution of numerous channels in various skin functions. Therefore, it is not surprising at all that multiple channels are reportedly associated with multiple skin conditions (summarized in **Table 2**). However, it should be mentioned that most of the below

data only indirectly link the given channel to the given disease, and that only very few "real", pathogenetic correlations could be identified. Therefore, further studies are invited to explore the causative roles of the identified alterations in the expressions/functions of the channels.

Below, we start by listing the available literature data in relation to the most prevalent "barrier diseases", AD and psoriasis. Then we continue with describing the roles of the channels in various skin tumors and in other dermatoses. Finally, although skin ageing *per se* cannot be considered as a disease, the related, quite exciting findings prompted us to close this section with mentioning the possible involvement of certain channels in the ageing process.

4.6.1 AD

4.6.1.1 Ligand-gated channels

The expression level of ChAT (which is a key enzyme of ACh biosynthesis) was found to be highly elevated in the epidermis (14-fold) and in the upper dermis (3-fold) of AD patients when compared to healthy skin compartments (Wessler et al 2003). Moreover, irregular nAChR subtype expression patterns were described in AD lesions (Curtis and Radek 2012). Likewise, in lesional skin of AD (and psoriasis) patients, intense P2X7 reactivity was confined to the cell membrane of the basal layer, with an additional, diffuse P2Y1 metabotropic purinergic receptor immunostaining throughout the epidermis (Pastore et al 2007). Unfortunately, the pathogenetic roles of these phenomena are not clarified. Also, the human clinical relevance of those intriguing findings (detailed under 4.3.2.3.) that orally administered GABA was beneficial against experimentally induced AD in mice should also be carefully investigated.

4.6.1.2 TRP channels

As we have shown (4.3.3.1. and 4.3.3.2.), TRPV1 and TRPV3 activities promoted the development of AD-like dermatitis in mice. However, further studies are required to define the roles of these (and possibly other) TRP channels in the pathogenesis of human AD.

4.6.1.3 Non-ion selective channels

Elevated AQP3 expression was found in AD skin (Olsson et al 2006; Nakahigashi et al 2011). In addition, CCL17, which is highly expressed in AD, was found to be a strong inducer of AQP3 expression and enhanced keratinocyte proliferation. In a mouse model of AD, the induced epidermal hyperplasia, a characteristic symptom of the disease, was reduced in AQP3-deficient mice, with a decreased number of proliferating keratinocytes (Nakahigashi et al 2011). These results suggest the possible involvement of AQP3 in the development of AD.

It should be mentioned that although altered levels of AQP3 were also described in the closely related epidermal spongiosis associated with eczema (Boury-Jamot et al 2006) and erythema toxicum neonatorum (Marchini et al 2003), the functional role of AQP3 in these diseases is not yet known.

4.6.2 Psoriasis vulgaris

4.6.2.1 Voltage-gated channels

Keratinocytes and skin from psoriatic individuals were found to express higher levels of mRNA encoding the non-functional splice-variant of cyclic guanosine monophosphate-gated (CNG), Ca^{2+}-permeable, non-selective cationic channels. Since overexpression of the splice variant by transfection of HEK293 in culture leads to loss of protein expression for the functional CNG channels (McKenzie et al 2003); and, furthermore, since Ca^{2+} influx to human keratinocytes may occur, among others, via CNG channels, these data may suggest the potential role of this CNG isoform shift in psoriasis.

4.6.2.2 Ligand-gated channels

As was shown above, NMDAR-coupled signaling was implicated in the proper growth and differentiation of keratinocytes. In support of this proposal, in parakeratotic skin lesions of psoriasis patients, a significant reduction in the expression of NMDAR1 in the upper epidermis was identified (Fischer et al 2004b). This alteration was suggested to result in a suppressed Ca^{2+} influx to the diseased keratinocytes leading to impaired differentiation and barrier formation, hallmarks of the disease.

As mentioned above, 5-HT3 receptor was localized to basal epidermal keratinocytes in human skin *in situ*. This expression pattern was not altered in skin samples of AD

patients or in non-involved psoriatic skin; however, 5-HT3 receptor was identified in the acrosyringium, but not in basal keratinocytes, in involved psoriatic skin (Lundeberg et al 2002; Nordlind et al 2006). Therefore, it can be hypothesized (and to be investigated in future trials) that epidermal 5-HT3 receptors may contribute to the development of psoriasis.

Expressions of GABA ligand and $GABA_A$ receptor were found to be increased in inflammatory cells located in lesional psoriatic skin when compared to non-lesional skin parts. GABA ligand was mostly expressed in macrophages whereas $GABA_A$ receptor was localized in macrophages, neutrophils and lymphocytes. Moreover, a positive correlation was identified between the inflammatory cell GABA release and $GABA_A$ receptor expression, and the severity of pruritus, a characteristic symptom of the disease (Nigam et al 2010).

4.6.2.3 TRP channels

Decreased expressions of the pro-differentiating TRPC1/4/5/6/7 were reported in the epidermis and isolated keratinocytes of psoriatic patients. In addition, cultured psoriatic keratinocytes exhibited substantial defects in Ca^{2+} influx in response to high extracellular Ca^{2+} levels (Leuner et al 2011), which may be explained by the suppressed TRPC channel expressions.

4.6.2.4 Non-ion selective channels

Elevated levels of AQP9 were described in lesional skin of psoriatic patients (Suárez-Fariñas et al 2011). Likewise, highly upregulated levels of Cx26, which was shown to inhibit epidermal keratinocyte differentiation and hence barrier formation, were identified in human psoriatic plaques and in hyperplastic warts (Lucke et al 1999). Of clinical importance, the highly elevated Cx26 levels in psoriatic lesions were significantly suppressed after treatment of psoriasis with methotrexate and PUVA (Shaker and Abdel-Halim 2012) which suggest the role of Cx26 in the pathogenesis of the disease.

4.6.3 Non-melanoma skin cancers

4.6.3.1 Voltage-gated channels

Expression of mRNA of Kv3.4 K^+ channel was found to be increased in SCC. In addition, inhibition of Kv3.4 suppressed growth of oral SCC cells (Chang et al 2003) which argues for that K^+ channel activities support malignant cell growth.

4.6.3.2 Ligand-gated channels

Expression of NMDAR1 in cutaneous SCC was found to inversely correlate with the degree of malignancy. Namely, very low (if any) expression was identified in un-differentiated SCC samples (Kang et al 2009) whereas the reactive (non-neoplastic) epithelium surrounding the SCC showed strong NMDAR1 levels (Nahm et al 2004). These data, on the one hand, further support the pro-differentiating role of NMDAR1-coupled signaling in keratinocytes; on the other hand, they also argue for that NMDAR1 may be a prognostic indicator for cutaneous SCC.

Human papillomaviruses are recognized as important human tumor promoters in the development of non-melanoma skin cancers (Biliris et al 2000; Greig et al 2006). Interestingly, in human skin warts as well as in raft cultures of CIN 612 cells, a model of keratinocytes infected with human papillomavirus type 31 (Ozbun 2002), up-regulation of the expression of P2X5 receptors was detected. In addition, P2X5 and P2X7 receptors were found in the nuclei of koilocytes, the abnormal keratinocytes characteristic of human papillomavirus infection (Greig et al 2006). Based on these findings, as well as on the expression pattern of P2X receptor in the epidermis, it is therefore proposed that P2X5 receptors are likely to be involved in keratinocyte differentiation and P2X7 receptors are likely to be part of the machinery of end stage terminal differentiation/apoptosis of keratinocytes (Burnstock 2006; Gorodeskin 2009; Burnstock et al 2012). As an additional factor, the promoting role of these receptors in the anti-viral immune response may also be involved (see under 4.3.1.2.).

Indeed, the pro-apoptotic role of P2X7 was also demonstrated in a two-stage (DMBA/TPA) mouse model of skin papilloma and SCC. In this model, the P2X7 specific agonist BzATP inhibited formation of tumors. Moreover, in cultured mouse keratinocytes BzATP induced prolonged Ca^{2+} influx and caspase-9 coupled apoptosis. Importantly, apoptosis was much less efficient in SCC keratinocytes which exhibited 4-5 fold lower levels of P2X7 in cancer tissues. Therefore, activation of P2X7-dependent apoptosis (and possibly of the pro-differentiating P2X5 receptors) in

skin papillomas and cancers as well as in melanomas may represent novel therapeutic tools.

4.6.3.3 TRP channels

In BCC samples, the lack of epidermal expression of the pro-differentiating TRPC1/TRPC4 was observed (Beck et al 2008) which was correlated with the impaired differentiation and enhanced proliferation of tumor cells. In addition, topical treatment with triterpenes of actinic keratosis, an *in situ* form of SCC, promoted cellular differentiation, most probably via the stimulation of TRPC6-mediated Ca^{2+}-influx to the cells (Woelfle et al 2010).

In addition, TRPV1 knockout mice were shown to exhibit a highly increased susceptibility to induction of skin carcinogenesis (Bode et al 2009). Since TRPV1 was described to inhibit proliferation and induce apoptosis in keratinocytes (see above under 4.1.3.1.), it is proposed that TRPV1 (just as the above TRPCs) may be protective against skin tumor formation.

4.6.3.4 Non-ion selective channels

Further supporting the promoting role of AQP3 in epidermal proliferation, highly increased levels of AQP3 were identified in human SCC when compared to control skin (Hara-Chikuma and Verkman 2008c). In addition, in a multistage murine carcinogenesis model, AQP3 knockouts were found to be resistant to induction of tumorigenesis, also arguing for the pro-mitogenic role of AQP3 (Hara-Chikuma and Verkman 2008c). As tumor cells generally exhibit an aggressive energy metabolic profile (Gatenby and Gillies 2007), the glycerol transport mediated by AQP3 and the concomitant accumulation of cellular ATP may act as an important determinant of skin tumorigenesis. Hence, inhibition of AQP3 activity may provide a rational basis for the therapy of skin (and possibly other) cancers associated with overexpression of aquaglyceroporins.

4.6.4 Malignant melanoma

4.6.4.1 Voltage-gated channels

The tumor-promoting roles of various K^+ channels are suggested in malignant melanoma. Indeed, on cell cultures of the human melanoma cell line SK MEL 28,

inhibition of the expressed inwardly rectifying (K_{ir}) K^+ channels or K_{Ca} channels inhibited cell-cycle progression (Lepple-Wienhues et al 1996). Likewise, in metastatic human melanoma cell lines, activation of $K_{Ca}3.1$ channels was shown to promote the secretion of melanoma inhibitory activity, a soluble melanoma-derived factor which, by interacting with cell adhesion molecules and hence facilitating cell detachment, stimulates the formation of metastases (Schmidt et al 2010).

Based on these results, it is proposed that membrane depolarization following the inhibition of these voltage-gated K^+ channels most probably reduces the driving force for the influx of Ca^{2+}, a key messenger in the mitogenic signal cascade of human malignant cells, which eventually results in cell cycle arrest. Therefore, voltage-gated K^+ channel inhibitors may represent novel therapeutic tools in the treatment of malignant melanoma.

Finally, it should be mentioned that silencing of the two-pore K^+ channel TASK-3, which is predominantly localized in the mitochondria in malignant melanoma cells (Rusznák et al 2008), impaired cellular integrity and viability as well as proliferation of these cells (Kosztka et al 2011).

4.6.4.2 Ligand-gated channels

Cultured melanocytes were shown to express the AMPARs GluA2 and 4 and the NMDAR2A and 2C whose activation by AMPA and NMDA resulted in elevation of intracellular Ca^{2+} concentration (Hoogduijn et al 2006). Melanocytes also express specific glutamate transporters and decarboxylases; yet, glutamate production or release was not found. Glutamate treatment of human melanocytes did not affect melanin production and cell survival. However, application of AMPARs and NMDARs inhibitors induced disorganization of actin and tubulin microfilaments. In addition, the AMPA receptor inhibitor CFM-2 markedly suppressed the expression of microphthalmia-associated transcription factor, a key regulator of melanocyte differentiation and proliferation. Therefore, further studies are invited to define the potential role of ionotropic glutamatergic signaling in malignant melanoma.

In addition, increased expression of P2X7 receptors were identified in malignant melanomas (Slater et al 2003) whose stimulation resulted in a Ca^{2+} influx-dependent

induction of apoptosis (Deli et al 2007). Therefore, just as described under 4.6.3.2., P2X7-targeted approaches may be beneficial not only in non-melanoma skin cancers, but also in malignant melanomas.

4.6.4.3 TRP channels

Human epidermal melanocytes also express TRPM1 whose level was shown to correlate with melanin content of the cells indicating that functional TRPM1 channels are critical for normal melanocyte pigmentation (Devi et al 2009; Oancea et al 2009). Indeed, decreased expression of the *trpm1* gene was found to be associated with the coat spotting patterns of the Appaloosa horse (*Equus caballus*) (Bellone et al 2008). In part similar to these findings, two mutations in the gene encoding TRPML3 were found to be correlated with the diluted coat color of the varitint-waddler mouse (Di Palma et al 2002; Cuajungco and Samie 2008).

Certain TRPM channels also seem to be involved in the development of cutaneous melanoma. Namely, expression of the *trpm1* gene, which encodes the pro-apoptotic TRPM1, was found to exhibit an inverse correlation of with the *in vivo* metastatic potential of skin melanoma (Deeds et al 2000; Duncan et al 2001; Miller et al 2004; Zhiqi et al 2004; Lu et al 2010). Therefore, down-regulation of TRPM1 in the tumor was proposed as a prognostic marker for metastasis (Deeds et al 2000; Duncan et al 2001; Miller et al 2001; Zhiqi et al 2004). Likewise, upregulation of antisense, tumor-enriched (TE) transcripts of TRPM2 (another growth-inhibitory TRPM channel) was identified in human cutaneous melanoma (Orfanelli et al 2008). Accordingly, functional knockout of TRPM2-TE or overexpression of wild-type TRPM2 in melanoma-derived cells augmented susceptibility to apoptosis (Orfanelli et al 2008). Interestingly, an increased (and not decreased) level of TRPM8-specific transcripts, were also demonstrated in malignant melanoma (Tsavaler et al 2001). Since activation of TRPM8 in human cultured melanoma cells induced Ca^{2+}-dependent cell death (Slominski 2008; Yamamura et al 2008a), the functional significance of these findings are not currently understood.

4.6.4.4 Non-ion selective channels

Panx1 expression, which was found to be low in normal mouse melanocytes, increased in tandem with tumor cell aggressiveness in mouse malignant melanoma

cell lines (Penuela et al 2012b). In addition, gene silencing of Panx1 in BL6 mouse melanoma cell lines resulted in a marked suppression of *in vitro* cellular growth and migration and the down-regulation of the malignant melanoma markers vimentin and β-catenin. Likewise, the growth rate and metastasis-forming capacity of Panx1 knock-down cells also significantly decreased in a xenograft model (Penuela et al 2012b). Although we lack human data, these findings collectively argue for the putative pathogenetic role of Panx1 (at least in murine) melanoma. In addition, these results also raise the possibility of a future management of the malignancy by inhibiting and/or down-regulating Panx1.

AQP1 channels are also expressed on human cultured melanocytes; however, their role in melanogenesis and melanocyte/melanoma growth is not known (Boury-Jamot et al 2006). In addition, the elevated expressions of pro-proliferating Cx26 and Cx30 (but not of the pro-differentiating Cx43) were identified in the epidermal tumor microenvironment of malignant melanoma which correlated to the degree of malignancy (Haass et al 2010).

4.6.5 Other skin diseases

4.6.5.1 Olmsted syndrome

As we have shown above (under 4.2.3.2. and 4.3.3.2.), a gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a spontaneous hairless phenotype and the development of AD-like itchy dermatitis in mice. Of greatest importance, most recently, similar gain-of-function mutations of *trpv3* were identified in Olmsted syndrome, a rare congenital disorder characterized by palmoplantar and periorificial keratoderma, alopecia, and severe itching (Lin et al 2012). In heterologous systems, mutant TRPV3 channels conveyed increased membrane currents and mediated augmented apoptosis which was also detected in the epidermis of the diseased patients. Therefore, Olmsted syndrome can be regarded as the first “truly cutaneous TRPpathy”.

4.6.5.2 Smith–Lemli–Opitz syndrome

Smith–Lemli–Opitz syndrome (SLOS) is an inherited disorder of cholesterol synthesis caused by mutations of the *dhcr7* gene which encodes the final enzyme in the cholesterol synthesis pathway (Tint et al 1994). In this disease, 7-dehydrocholesterol

accumulates in various cells and impairs key cells functions including those of skin fibroblasts (Honda et al 1997; Wassif et al 2002). In membrane caveolae of dermal fibroblasts of SLOS patients, impaired activity and markedly suppressed protein levels of BK K_{Ca} channel were observed (Ren et al 2011). Since BK K_{Ca} channels were shown to co-migrate with caveolin-1, a key component of lipid rafts and hence regulator of a multitude of cell membrane-localized proteins (channels, receptors, transporters, etc.) and their signaling (Rothberg et al 1992; Simons and Ikonen 1997; Ren et al 2011), alterations in BK K_{Ca} channel functions may contribute to the pathophysiology of SLOS.

4.6.5.3 Pemphigus vulgaris

Pemphigus vulgaris (PV) is a severe autoimmune blistering disease. In the pathogenesis of the dermatosis, the role of autoantibodies targeting (and then destroying) desmoglein-3, a key cell adhesion molecule of the epidermis, are suggested (Amagai and Stanley 2012). Intriguingly, α9 nAChRs were also found to be targeted by PV. Of further importance, inhibition of α9 nAChRs activity in keratinocyte cultures resulted in the development of PV-like morphology (acantholysis) which findings, besides further supporting the role of the ion channel in keratinocytes adhesion processes (see above under 4.1.2.1), argue for a potential pathogenetic role of α9 nAChRs in PV (Nguyen et al 2000).

4.6.5.4 Mal de Meleda

Mal de Meleda is an autosomal recessive inflammatory and keratotic palmoplantar skin disorder due to mutations in the gene encoding SLURP-1 (secreted mammalian Ly-6/uPAR-related protein 1) (Fischer et al 2001). Interestingly, SLURP-1 was shown to potentiate the effect of ACh on α7 nAChR channels (Chimienti et al 2003; Chernyavsky et al 2012). Since, as was detailed above, α7 nAChR receptors play multiple roles in skin function, the authors hypothesized that the lack of this potentiation by SLURP-1 downregulation may contribute to the development of the characteristic skin symptoms of the disease.

4.6.5.5 Darier's disease

TRPC1 is overexpressed in keratinocytes of patients with Darier's disease (DD) (or keratosis follicularis), a genetic disorder with loss-of-function mutations in the

SERCA2b gene encoding endoplasmic reticulum Ca^{2+}-pumps, which is characterized by abnormal keratinization of the epidermis (Barfield et al 2002; Pani et al 2006). Importantly, cultured DD keratinocytes exhibited a greatly enhanced TRPC1-mediated (store-operated) Ca^{2+} influx, proliferation, and apoptosis resistance suggesting that TRPC1 may be involved in the pathological differentiation program (Pani et al 2006).

4.6.5.6 Prurigo nodularis

Markedly elevated TRPV1 levels were detected in the highly hyperkeratotic lesions of skin samples of prurigo nodularis patients (Stander et al 2004). Furthermore, chronic (for several months) topical capsaicin treatment of the prurigo lesions (and hence the prolonged activation of the apoptosis-promoting TRPV1 expressed on keratinocytes) not only mitigated the intense pruritus, but also markedly reduced the epidermal hyperplasia and the hyper-orthokeratosis of the skin (Stander et al 2001).

4.6.5.7 Diseases of the adnexal structures

K_{Ca} channels on normal sweat gland-derived cells exhibited similar functional properties to those expressed by cells from patients with cystic fibrosis (Henderson and Cuthbert 1991a), a common genetic disease characterized by, among others, defective sweat gland functions (Quinton 2007). However, eccrine sweat gland cells from these patients additionally expressed Ca^{2+}-independent, small-conductance, outwardly rectifying K^+ channels which were practically absent on cells from healthy donors (Henderson and Cuthbert 1991b). The impact of these findings is not yet known.

Although expressions of a huge variety of nAChR subunits were described in various compartments of the HF and sebaceous glands (summarized in Kurzen 2004; Kurzen et al 2004; Kurzen and Schallreuter 2004; Grando et al 2006; Kurzen et al 2007), the functional role of these channels in pilosebaceous unit biology is not revealed. Likewise, it is also unknown whether ACh and the cutaneous cholinergic system is involved in mediating the effect of smoking to significantly increase the prevalence and disease severity of acne vulgaris (Schäfer et al 2001).

However, with respect to the pathology of the pilosebaceous unit and the relationship with smoking, intriguing observations were made during the assessment of skin samples of hidradenitis suppurativa (HS) patients. Clinically, HS (a.k.a. acne inversa) is a chronic inflammatory skin disease emerging from the pilosebaceous units of the intertriginous areas (e.g. underarms, thighs, groin). Importantly, HS is considered as a nicotine-dependent dermatosis as more than 80% of patients are active smokers (Jansen et al 2001). In organotypic epidermal culture system, chronic nicotine exposure (12 days) resulted in epidermal thickening which was very similar to the hyperplastic epidermis seen in HS. In addition, highly elevated α7 nAChR levels were identified in HS epidermis, especially in the follicular infundibulum. These results propose that the cutaneous cholinergic system, most probably by promoting infundibular epithelial hyperplasia and thus follicular occlusion, may have a pathogenetic role in HS (Hana et al 2007).

Another smoking-associated inflammatory skin disease, which is related to adnexal skin structures, is palmoplantar pustulosis (PPP) characterized by pustules, erythema and scaling on the soles and palms. Apparently, sweat glands are involved in the pathogenesis of PPP (impaired structure of the acrosyringium, outward migration of granulocytes from the acrosyringium to the str. corneum to form pustules). Importantly, in involved PPP skin, significant expressions of ChAT and α3 nAChRs were observed in the infiltrating granulocytes indicating a role for ACh in inflammation. Moreover, irregular expression patterns of α3 and α7 nAChRs were found throughout the epidermis. Currently, the exact mechanisms for the effect of nicotine/smoking in PPP is still unknown; yet, it is noteworthy that cessation of smoking improved all skin symptoms characteristics for PPP (Hangforsen 2007).

Finally, it might be of clinical importance that decreased AQP5 levels were detected in the secretory part of eccrine sweat glands of patients with Sjögren's syndrome, but not in skin affected by idiopathic segmental anhidrosis or idiopathic pure sudomotor failure (Iizuka et al 2012).

4.6.5.8 Chronic venous insufficiency

The expression patterns of P2X5 and P2X7 receptors were found to be altered in the epidermis of patients with chronic venous insufficiency (CVI). In CVI, elevated P2X5

receptor levels were found mainly in the spinosal layer and extending further into the str. granulosum whereas expressions of P2X7 receptors were reduced in the str. corneum. It is proposed that the above alterations may contribute to the appearance of the thinner epidermis seen in CVI (Metcalfe et al 2006).

4.6.5.9 "Connexin diseases"

Further supporting its potential role in epidermal biology, Cx26 mutations were found to be associated with numerous hyperkeratotic skin disorders (palmoplantar keratoderma, keratitis-ichthyosis deafness syndrome, Vohwinkel syndrome, hystrix-ichthyosis deafness syndrome, and Bart-Pumphrey syndrome) which are characterized by pathologically altered epidermal growth and differentiation (reviewed in Lee and White 2009).

4.6.6 Skin ageing

As we have presented above (4.3.3.1), TRPV1 was shown to mediate the effect of UV exposure and heat to induce skin inflammation and to upregulate MMP-1. Since chronic UV exposure is suggested to promote skin ageing, the role of TRPV1 in these processes is suggested. Indeed, applications of both heat and UV elevated expression of TRPV1 proteins in human skin *in vivo*. Moreover, as further support for its pro-ageing role, increased TRPV1 levels were found both in photoaged and intrinsically aged skin samples when compared to the expressions of the channel found in skins obtained from sunprotected areas and from young individuals, respectively (Lee et al 2009a ; Lee et al 2012).

Interestingly, AQP3 expression was lower in aged than in young skin (Li et al 2010), which is one of the key factors resulting in lower epidermal/skin water content and dry skin conditions seen in the elderly. Furthermore, UVB irradiation of NHEKs upregulated the expression of the pro-apoptotic P2X7 receptors (Inoue et al 2005), which may lead to premature cell death.

Of further importance, comparison of dermal fibroblasts obtained from young, elderly and centenarian donors revealed age-dependent changes in K^+ channel expression and function. K^+ current amplitude was significantly smaller in fibroblasts from elderly than from young donors. In addition, expression of voltage-gated (K_v) shaker $K_v1.1$

channels was found to be higher in fibroblasts of elderly and centenarians whereas the BK K_{Ca} channel β1 subunit showed lower expression levels in fibroblasts of centenarians. It is possible, therefore, that the age-related remodeling of dermal fibroblast K^+ channel subtypes in centenarians might be associated with “successful” aging and hence provide a “predictive marker of longevity” (Zironi et al 2010).

Finally, it should be noted that chronic nicotine exposure of cultured human dermal fibroblasts markedly altered the expression patterns of α3, α5, α7, β2, and β4 nAChR subunits (Arredondo et al 2003). Therefore, it can also be postulated that premature ageing of the skin and impaired wound healing seen in chronic smokers may be related to pathological alterations of the fibroblast cholinergic system.

5 Concluding remarks

In this paper, we have attempted to review the roles of ion and non-ion selective channels on non-neuronal cell types of the skin. Moreover, we have detailed recent evidence suggesting the involvement of certain channels in various skin diseases.

The major messages of this review are the followings:

- A plethora of ion and non-ion selective channels are expressed by various non-neuronal cell populations of the skin.
- On these cells, numerous channels, their endogenous activators/inhibitors, and their related signaling mechanisms were shown to play central roles in such key cutaneous processes as e.g. cellular growth, differentiation, and survival; formation, maintenance, and regeneration of the epidermal physical-chemical barrier; wound healing; inflammatory and immune responses, exocrine functions, etc.
- In addition, certain channels expressed by epidermal keratinocytes also contribute to the sensory functions of the skin (e.g. thermo-, osmo-, and pH sensation), via the release of soluble intercellular mediators which stimulate cutaneous sensory afferents.
- It is important to note, that the involved channels may exert both synergistic as well as antagonistic effects in the regulation of the above processes. This is especially remarkable during the recovery of the epidermal barrier following its disruption.
- Finally, pathological alterations in the homeostatic channel-coupled mechanisms are implicated in various dermatoses (e.g. AD, psoriasis, skin cancers, autoimmune and genetic diseases, etc.) and in skin ageing.

Evidently, more extensive *in vitro* and *in vivo* studies are urgently needed to reveal the exact molecular roles of these channels in skin physiology and pathophysiology. Yet, we strongly believe that the presented intriguing findings will encourage future, highly sophisticated pre-clinical and clinical trials to explore the seemingly rich potential of channel-targeted management of various skin diseases.

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7 Figures and legends

Figure 1. *Key functions of the skin*

The multiple homeostatic functions of the skin can be classified into the groups of barrier, neuroendocrine, and other functions. For further details, see text under 2.2.

Figure 2. *Key components of the complex skin barrier*

The highly complex skin barrier provides multiple levels of protection for the organism. These include the physical-chemical barrier, the (micro)biological barrier, and the immunological barrier as well as the life-long regeneration of these components. For further details, see text under 2.2.1.

Figure 3. *The complex "channel" regulation of the epidermal barrier recovery*

Numerous channels play central, yet partly antagonistic, roles in the regulation of the recovery processes following the mechanical disruption of the epidermal barrier. Therefore, targeted modulations of channel activities represent exciting novel future therapeutic possibilities. For further details, see text under 4.1.

8 Tables

Table 1. *Highly selected skin-related functions of certain designated channels*

Channels	Putative function(s)	Reference(s)
Ca_v channels	Promotion of keratinocyte differentiation; delay of barrier recovery	Denda et al 2003b; Denda et al 2006
nAChRs	Promotion of keratinocyte differentiation and wound healing; delay of barrier recovery	Grando et al 1996; Arredondo et al 2003; Denda et al 2003a; Kurzen et al 2005; Chernyavskyet al 2007; Kurzen et al 2007; Radek et al 2010; Curtis and Radek 2012
P2X7	Promotion of keratinocyte differentiation and antitumor effects	Slater et al 2003; Inoue et al 2005; Deli et al 2007
NMDAR	Promotion of keratinocyte differentiation, delay of barrier recovery	Denda et al 2003a; Fuziwara et al 2003; Fischer et al 2004a; Fischer et al 2004b
$GABA_A$	Promotion of barrier recovery and wound healing	Denda et al 2002b; Denda et al 2003a; Han et al 2007; Ito et al 2007
Glycine receptor	Promotion of barrier recovery	Denda et al 2003a
TRPV1	Delay of barrier recovery and wound healing	Bodó et al 2005; Bíró et al 2006; Denda et al 2007b; Tóth et al 2011a; Yun et al 2011
	Antitumor effects	Bode et al 2009
TRPV3	Promotion of barrier recovery and wound healing	Denda et al 2007b; Cheng et al 2010; Miyamoto et al 2011
TRPV4	Promotion of barrier recovery	Denda et al 2007b; Kida et al 2011; Sokabe et al 2010; Sokabe and Tominaga 2010
TRPA1	Promotion of barrier recovery	Denda et al 2010b
TRPC1, 4, 5, 6, and 7	Promotion of keratinocyte differentiation and possible antitumor effects	Cai et al 2006; Beck et al 2008; Muller et al 2008; Woelfle et al 2010; Shanmugam et al 2012
TRPM1, 2 and 8	Antitumor effects	Deeds et al 2000; Duncan et al 2001; Miller et al 2004; Zhiqi et al 2004; Orfanelli et al 2008; Slominski 2008; Yamamura et al 2008a; Lu et al 2010
AQP3	Promotion of barrier recovery	Hara et al 2002; Hara and Verkman 2003

Table 2. *Putative roles of key channels in the pathogenesis of selected human skin diseases and skin-related processes*

Diseases or skin-related conditions	Putative pathomechanism(s) and Related channel(s)	Reference(s)
Psoriasis	Chronic use of Ca_v channels is associated with the disease	Cohen et al 2001
	Upregulation of non-functional CNG channels	McKenzie et al 2003
	Altered expression profile of 5-HT3	Lundeberg et al 2002; Nordlind et al 2006
	Downregulation of NMDAR1	Fischer et al 2004b
	Upregulation of GABA ligand and $GABA_A$ receptor	Nigam et al 2010
	Downregulation of TRPC1, 4, 5, 6, and 7 and the coupled Ca^{2+}-influx	Leuner et al 2011
	Upregulation of AQP9	Suárez-Fariñas et al 2011
	Upregulation of Cx26 which can be suppressed by anti-psoriasis therapy	Lucke et al 1999; Shaker and Abdel-Halim 2012
	Irregular nAChR expression pattern	Curtis and Radek 2012
Atopic dermatitis	Altered P2X7 expression profile	Pastore et al 2007
	Irregular nAChR subtype expression pattern	Curtis and Radek 2012
	Downregulation of NMDAR1	Kang et al 2009
	Decreased GABA-ergic signaling	Hokazono et al 2010
	Activation of TRPV1 and 3 results in AD-like syndromes in mice	Asakawa et al 2006; Xiao et al 2008; Yun et al 2011
	Upregulation of AQP3	Olsson et al 2006; Nakahigashi et al 2011
Non-melanoma skin cancers	Upregulation of $K_v3.4$; inhibition of $K_v3.4$ suppressed tumor cell growth	Chang et al 2003
	NMDAR1 expression inversely correlates with the degree of malignancy	Nahm et al 2004; Kang et al 2009
	Downregulation of P2X7-coupled signaling; altered P2X receptor expression pattern	Burnstock 2006;Greig et al 2006; Gorodeskin 2009; Burnstock et al 2012
	Decreased TRPV1 and TRPC6-coupled signaling; TRPV1-KO mice exhibit increased tumorigenesis	Bode et al 2009; Woelfle et al 2010
	Downregulation of TRPC1 and 4	Beck et al 2008
	Upregulation of AQP3; AQP3-KO mice exhibit decreased tumorigenesis	Hara-Chikuma and Verkman 2008c
Malignant melanoma	Increased activity of $K_{Ca}3.1$, K_{ir} and TASK-3	Lepple-Wienhues et al 1996; Schmidt et al 2010; Kosztka et al 2011
	Upregulation of P2X7	Slater et al 2003
	TRPM1 expression inversely correlates with the degree of metastatic potential; Upregulation of antisense TRPM2; Downregulation of TRPM8; Activation of TRPM8 inhibit tumor cell growth	Deeds et al 2000; Duncan et al 2001; Tsavaler et al 2001; Miller et al 2004; Zhiqi et al 2004; Orfanelli et al 2008; Lu et al 2010

	Upregulation of Panx1 in mouse melanoma; Silencing of Panx1 inhibits tumor growth	Penuela et al 2012
Olmsted syndrome	The main pathogenetic cause is the gain-of-function mutation of TRPV3	Lin et al 2012
Skin ageing	Altered K^+-channel expression profile (fibroblasts)	Zironi et al 2010
	Altered nAChR expression profile (fibroblasts)	Arredondo et al 2003
	Upregulation of P2X7 (keratinocytes)	Inoue et al 2005
	Upregulation of TRPV1 and the coupled signaling (keratinocytes)	Lee et al 2009a ; Lee et al 2012
	Downregulation of AQP3 (keratinocytes)	Li et al 2010

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10 List of abbreviations

5-HT: 5-hydroxytryptamine

ACh: acethylcholine

ACTH: corticotropin

AD: atopic dermatitis

AML: antimicrobial lipid

AMP: antimicrobial peptide

AMPA(R): α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (receptor)

ATP: adenosine-5’- triphosphate

AQP: aquaporin

BCC: basal cell carcinoma

BK: large conductance Ca^{2+}-activated K^{+}-channel

$[Ca^{2+}]_e$: extracellular Ca^{2+}-concentration

$[Ca^{2+}]_i$: intracellular Ca^{2+}-concentration

Ca_v: L-type voltage-gated Ca^{2+}-channels

CCL: chemokine ligand

ChAT: choline-acetyltransferase

CNG: cyclic guanosine monophosphate-gated channels

COX: cyclooxygenase

CXCL: CXC chemokine ligand

CXCR: CXC chemokine ligand receptor

CGRP: calcitonin gene-related peptide

CNG channels: cyclic nucleotide-gated channels

CVI: chronic venous insufficiency

Cx: connexin

CRH: corticotrophin releasing hormone

DD: Darier's disease (keratosis follicularis)

DMBA: dimethylbenz[a]anthracene

EGF: epidermal growth factor

ENaC: amiloride-sensitive Na^+ channels

GABA: gamma-aminobutyric acid

HS: hidradenitis suppurativa (acne inversa)

ICAM-1: intercellular adhesion molecule-1

IK1: intermediate conductance K_{Ca}

IL: interleukin

I-RTX: 5′-iodoresiniferatoxin

K_{2P}: two-pore K^+-channels

K_{Ca}: Ca^{2+}-activated K^+-channels

K_{ir}: inward rectifier K^+-channels

LPS: bacterial lipopolysaccharide

mAChR: muscarinic acethylcholine receptor

mGluR: metabotropic glutamate receptor

MMP-1: matrix metalloproteinase-1

MUFA: monounsaturated fatty acid

nAChR: nicotinic acethylcholine receptor

Na_v: voltage-gated Na^+-channels

NHEK: normal human epidermal keratincyte

NGF: nerve growth factor

NK-1 (receptor): neurokinin-1 (receptor)

NMDA(R): N-methyl-D-aspartate (receptor)

NMF(s): natural moisturizing factor(s)

NO: nitric oxide

PCNA: proliferation cell nuclear antigen

PG: prostaglandin

PPP: palmoplantar pustulosis

SCC: squamous cell carcinoma

SLURP-1: secreted mammalian Ly-6/uPAR-related protein 1

SP: substance P

str.: stratum (layer)

TLR: Toll-like receptor

TM: transmembrane (domain)

TPA: 12-O-tetradecanoylphorbol-13-acetate

TRH: thyreotropin releasing hormone

TRP: transient receptor potential

TRPA: “ankyrin” subfamily of the TRP channels

TRPC: “canonical” or “classical” subfamily of the TRP channels

TRPM: “melastatin” subfamily of the TRP channels

TRPML: “mucolipin” subfamily of the TRP channels

TRPP: “polycystin” subfamily of the TRP channels

TRPV: “vanilloid” subfamily of the TRP channels

TSH: thyreotropin

TSW: Avène Thermal Spring water

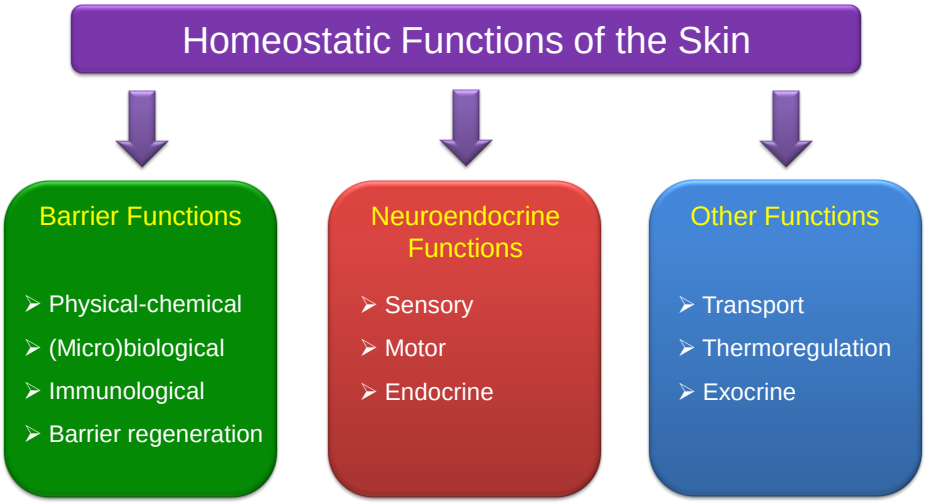
Homeostatic Functions of the Skin
Barrier Functions
Physical-chemical
(Micro)biological
Immunological
Barrier regeneration
Neuroendocrine Functions
Sensory
Motor
Endocrine
Other Functions
Transport
Thermoregulation
Exocrine

Figure 1.

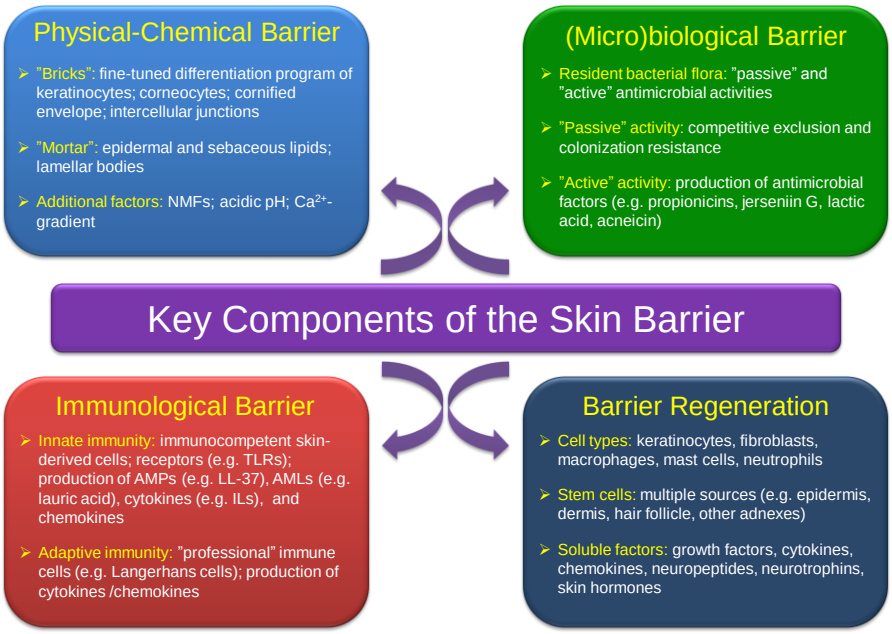
Physical-Chemical Barrier
"Bricks": fine-tuned differentiation program of keratinocytes; corneocytes; cornified envelope; intercellular junctions
"Mortar": epidermal and sebaceous lipids; lamellar bodies
Additional factors: NMFs; acidic pH; Ca^{2+}-gradient
(Micro)biological Barrier
Resident bacterial flora: "passive" and "active" antimicrobial activities
"Passive" activity: competitive exclusion and colonization resistance
"Active" activity: production of antimicrobial factors (e.g. propionicins, jerseniin G, lactic acid, acneicin)
Key Components of the Skin Barrier
Immunological Barrier
Innate immunity: immunocompetent skin-derived cells; receptors (e.g. TLRs); production of AMPs (e.g. LL-37), AMLs (e.g. lauric acid), cytokines (e.g. ILs), and chemokines
Adaptive immunity: "professional" immune cells (e.g. Langerhans cells); production of cytokines /chemokines
Barrier Regeneration
Cell types: keratinocytes, fibroblasts, macrophages, mast cells, neutrophils
Stem cells: multiple sources (e.g. epidermis, dermis, hair follicle, other adnexes)
Soluble factors: growth factors, cytokines, chemokines, neuropeptides, neurotrophins, skin hormones

Figure 2.

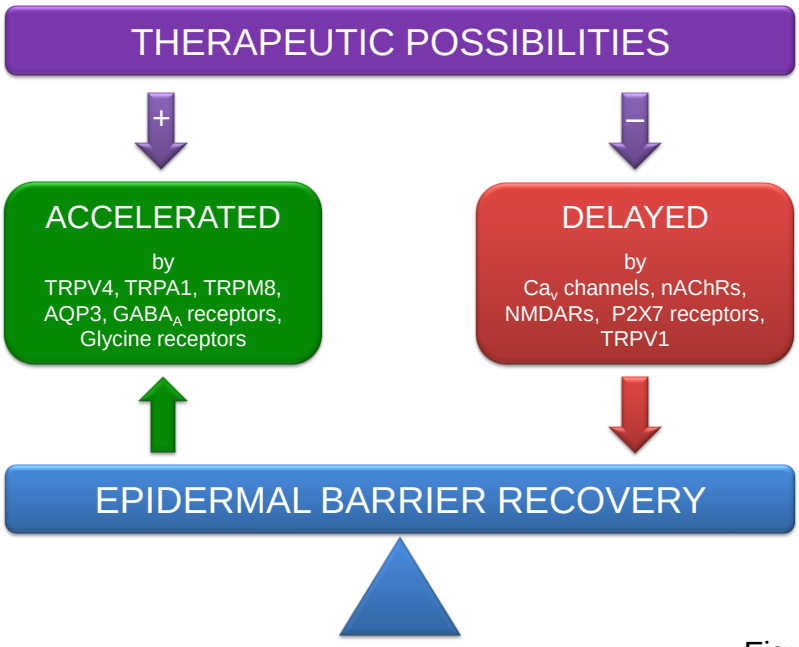
THERAPEUTIC POSSIBILITIES
+
–
ACCELERATED
by
TRPV4, TRPA1, TRPM8,
AQP3, GABA$_A$ receptors,
Glycine receptors
DELAYED
by
Ca$_v$ channels, nAChRs,
NMDARs, P2X7 receptors,
TRPV1
EPIDERMAL BARRIER RECOVERY

Figure 3.