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Review Article

Epidermal keratinocytes as the forefront of the sensory system

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Abstract Various sensors that respond to physical or chemical environmental factors have been identified in the peripheral nervous system. Some of them, which respond to mechanical stress, osmotic pressure, temperature and chemical stimuli (such as pH), are also expressed in epidermal keratinocytes. Neurotransmitters and their receptors, as well as receptors that regulate the neuroendocrine system of the skin, are also present in keratinocytes. Thus, broadly speaking, epidermal keratinocytes appear to be equipped with sensing systems similar to those of the peripheral and central nervous systems. It had long been considered that only nerve C-terminals in the epidermis play a role in skin surface perception. However, building on earlier work on skin receptors and new findings introduced here, we present in this review a novel hypothesis of skin sensory perception, i.e. first, keratinocytes recognize various environmental factors, and then the information is processed and conveyed to the nervous system.

Key words: transient receptor potential – P2X – nervous system – neurotransmitter – skin perception

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Sensory receptors in keratinocytes

The traditional view of the skin surface sensory system for environmental factors, such as temperature, humidity, mechanical stress and chemical stimuli, has been that the key sensors are the C fibres, which penetrate into the epidermis (1). However, the terminals of the nerve fibres are quite sparse. For example, the pressure points, which detect mechanical stimuli, are localized at distances of millimetres from each other (2). The skin can detect pattern on a much smaller scale (3–6) than would be expected on the basis of sampling theory if the nerve terminals were the only sensors (7). We previously suggested that epidermal keratinocytes might be at the forefront of skin surface perception (8). This idea has been supported by the recent cloning of a series of receptors, which are activated by temperature, mechanical stress, osmotic pressure and chemical stimuli. Many of these receptors are expressed in epidermal keratinocytes.

Abbreviations: TRP, transient receptor potential; P2X, ATP-activated purinergic receptor; 4α-PDD, 4α-phorbol 12,13-didecanone; 2-APB, 2-aminoethoxydiphenyl borate.

One of the most interesting receptor families is called transient receptor potential (TRP). The TRP receptor family members have been reported to act as sensors of temperature or other physical or chemical factors (9), and TRPV1, TRPV3 and TRPV4 were shown to be present in epidermal keratinocytes (Fig. 1a) (10–12). TRPV1 seems to be most strongly expressed in the upper and basal layers of the epidermis. High expression of the receptor at the surface of the epidermis would be consistent with a role in detecting external temperature. The reason for the high expression at the basal layer is not clear, but TRPV1 may also have some role in epidermal–dermal interaction. TPRV1 is activated by heat (>43°C), acidic conditions (pH < 6.6) and capsaicin (13). TRPV3 is activated by heat (>35°C), mechanical stress, camphor and 2-aminoethoxydiphenyl borate (2APB) (14,15). TRPV4 is activated by osmotic pressure (9). Previous reports suggest the existence of a water flux sensor in the epidermis (16,17). TRPV4 might play an important role as a sensor of water flux from the skin surface, although it is also activated by heat (>35°C) and 4α-phorbol 12,13-didecanone (4α-PDD) (9).

We recently demonstrated that TRPV1 and V4 are strongly associated with epidermal permeability barrier homeostasis (18). Activation of TRPV1 by heat (around

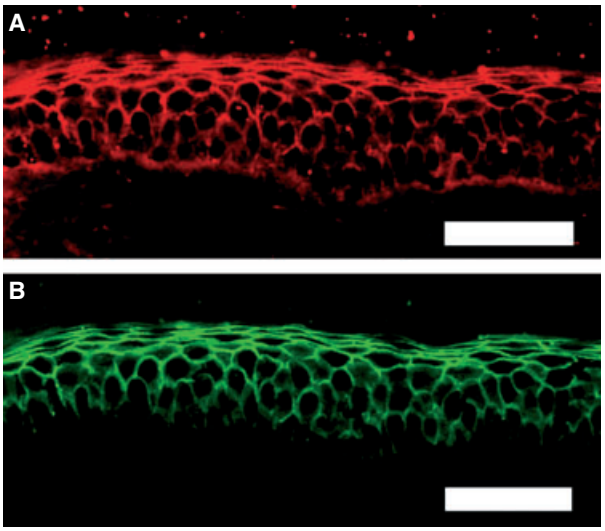

Figure 1 Immunostaining of human epidermis by anti-TRPV1 serum (a) and anti-P2X3 serum (b). Bars = 50 μm. Both pain receptors are expressed on keratinocyte cell membrane. TRPV1 is expressed strongly in the uppermost and basal layers of the epidermis. P2X3 expressed strongly in the upper area of the epidermis.

43°C) or capsaicin after barrier disruption delayed barrier repair. The delay was blocked by a TRPV1 antagonist, capsazepine. On the other hand, activation of TRPV4 by heat (36–40°C) accelerated barrier repair. These results suggest that the TRP receptors play an important role in epidermal homeostasis and indeed may act as temperature sensors not only for skin, but also for the whole body. Previous studies indicate that the optimum temperature for barrier repair (36–40°C) (18) is recognized as a pleasant temperature (19). Our emotional and sensory systems seem to 'know' the appropriate temperature range for skin barrier homeostasis; this could be related to the fact that dysfunction of barrier homeostasis can be fatal. The roles of the TRP receptors as components of the sensory system remain to be fully clarified. However, receptors, such as TRPs, expressed in the epidermal keratinocytes, which forms the interface between the environment and the body, could be expected to play a crucial role in maintaining our internal organ temperature constant at around 37°C under different environmental conditions. This may be the reason why we prefer 'comfortable' temperature and humidity ranges.

Another interesting receptor family is ATP receptor. The nervous system contains two distinct families of ATP receptors (20). One is the ATP-activated purinergic receptor (P2X) family, which is a ligand-gated ion channel, and the other is the P2Y family of metabotropic, heptahelical G-protein-coupled receptors. P2X3 was first identified as a pain receptor in the peripheral nervous system (21). In the case of tissue injury or inflammation, ATP is released and activates P2X3 receptors. We found that P2X3 is expressed in human epidermal keratinocytes (Fig. 1b) (22,23), and the expression was stronger in the upper area of the epidermis. We also showed that P2X3 is produced during terminal differentiation in a keratinocyte culture system (22). As ATP is released from epidermal keratinocytes by tape-stripping or exposure to air (23,24), itching of the skin in the case of dermatoses (e.g. atopic dermatitis), which are characterized by barrier dysfunction or skin surface dryness, might be transduced via the ATP system in the epidermis. TRPV4 might be a sensor of skin dryness, as noted above (17). Both TRP and ATP receptors on keratinocytes might play a role in a variety of skin sensations.

Slominski and co-workers found a variety of endocrinological receptors and their agonists in epidermal keratinocytes and melanocytes (25–28). They proposed that the epidermis and whole skin contain a neuroendocrine system (25), and they suggested an important role of corticotrophin-releasing hormone (CRH) in this system (26). We have demonstrated that CRH induced ion propagation in the epidermis (29) and this effect was blocked by an antagonist of the CRH receptor (29). Thus, the endocrinological system may play an important role in transmitting information from the sensory system of the skin.

Signal transduction

Not only P2X3, but also other P2X and P2Y receptors are expressed in epidermal keratinocytes (22,30). P2X3 in the epidermal keratinocytes is associated with epidermal barrier homeostasis (23). Koizumi et al. demonstrated that mechanical stimulation of a single keratinocyte induced intracellular calcium elevation not only in that cell, but also in its neighbours, and this response was prevented by application of an ATP receptor blocker (30). They obtained similar results in a co-culture system of keratinocytes and neurones, and found that mechanical stimulation of a single keratinocyte induced excitation of neurones (30). Thus, mechanical stress applied to a single keratinocyte can be signalled to surrounding cells and can also evoke excitation of the peripheral nervous system.

Recently, we also demonstrated that air-exposed keratinocytes released ATP, which induced intracellular calcium propagation in cultured human keratinocytes (24). A receptor activated by osmotic pressure, such as TRPV4 (9), might be the sensor of air exposure, leading to ATP release. The diffused ATP might then induce further elevation of intracellular calcium, and a calcium wave might be induced. In our study, the propagation of intracellular calcium was blocked by an ATP receptor blocker (24). Thus, ATP plays an important role in signal transduction between keratinocytes, and might also play a crucial role in signal transduction between keratinocytes and the nervous system.

Purinergic receptors are also related to the function of TRP receptors. For example, activation of P2Y receptor reduced the threshold temperature of TRPV1 (31). Such interaction between receptors could play a regulatory role in complex signalling systems. Further study is needed to reach a better understanding of the role of each receptor in keratinocytes in relation to the skin sensory system.

Not only ATP, but also several neurotransmitters, such as glutamate and dopamine are secreted from the epidermis after barrier injury (32,33). The receptors of these transmitters also exist in epidermal keratinocytes (22,23,32,33). These transmitters might influence not only epidermal homeostasis, but also the nervous system, as well as the endocrinological and immunological status of the body.

We have recently demonstrated the expression of a functional voltage-gated calcium channel in epidermal keratinocytes (34). The existence of the receptor was previously suggested by Lee et al. (35), and they also proposed a relationship between the channel and epidermal barrier homeostasis (35). Thus, an electrochemical communication between adjacent cells (keratinocyte–keratinocyte or keratinocyte–neurone) might play a crucial role in both epidermis–nervous system communication and epidermal homeostasis. A synapse-like connection between keratinocytes and nerve endings has been identified (36), but no functional study was carried out. The normal structure of the epidermis may be important not only for the permeability barrier, but also for signal transduction. As the other side of the same coin, abnormality of the epidermis might affect the sensory system.

The skin neuroendocrine system proposed by Slominski and co-workers should be important not only for signal transduction within the skin, but also in skin–nerve and skin–endocrine communication. The operation of the skin–neuroendocrine system has been suggested to be important for the proper functioning of the immune system, the vascular system, the metabolic system, thermoregulation and the sensory system (25–28). Further studies of the interaction of keratinocyte sensors, such as TRP and purinergic receptors, with the endocrine system are needed to understand the role of the epidermis in relation to the whole body.

Information processing by epidermal keratinocytes

Epidermal keratinocytes also contain a series of receptors, which were originally found in the central nervous system as neurotransmitter receptors. These receptors can be categorized into two groups, i.e. ionotropic receptors and G-protein-coupled receptors. Among the former group, calcium ion or chloride ion permeable channels play a crucial role in epidermal permeability barrier homeostasis. Topical application of calcium channel agonists delays barrier recovery, and antagonists accelerate barrier repair (23,32,37). Topical application of chloride ion channel agonists accelerates the barrier recovery (37,38).

Intracellular cAMP level, which is influenced by G-protein-coupled receptors, plays a crucial role in epidermal barrier homeostasis (39). Activation of dopamine-2-like receptors, melatonin receptors and serotonin receptor (type 5-HT1) decreases intracellular cAMP, and consequently accelerates barrier recovery, while activation of adrenergic β2 receptors increases the intracellular cAMP and delays barrier repair (40,41). Barrier disruption induces an increase of intracellular cAMP. Thus, topical application of agonists of receptors that reduce the intracellular cAMP level accelerates barrier repair. The results of our studies are included in Table 1.

Grando suggested the importance of nicotine type and muscarine type cholinergic receptors in skin (42), and proposed that the distribution of each receptor is important for epidermal homeostasis. We have observed different localizations of dopamine receptors, type 2 and 4 (33). The role of each receptor might be dependent upon its localization in the epidermis.

We have focused here on the role of these receptors in epidermal barrier homeostasis. However, these receptors also play crucial roles in the brain. For example, the *N*-methyl-d-aspartate (NMDA) receptor in the hippocampus is important for learning and memory (43,44). Conditional NMDA receptor knockout mice showed a reduced ability to learn (43), while transgenic mice expressing an increased level of the receptor showed significantly higher ability to learn (44). We found NMDA receptor in epidermal

Table 1 Effects of neurotransmitter receptor agonists and antagonists on skin permeability barrier recovery

	Accelerate barrier recovery	Delay barrier recovery
Ionotropic receptor		
P2X receptor (23)	Antagonist	Agonist
NMDA receptor (32)	Antagonist	Agonist
Cholinergic receptor (Nicotinic) (37)	Antagonist	Agonist
GABA(A) receptor (38)	Agonist	–[1]
Glycine receptor (37)	Agonist	–
G-protein-coupled receptor		
Adrenergic β2 receptor (40)	Antagonist	Agonist
Dopamine-2-like receptor (33)	Agonist	Antagonist
Serotonin receptor (41)	Agonist	–
Melatonin receptor (41)	Agonist	–

[1]No effect or no result available.

keratinocytes and demonstrated a role of the receptor in barrier homeostasis and epidermal proliferation (32). It remains an interesting possibility that the neurotransmitter receptors in the epidermis also have a much more sophisticated role.

We have reported the presence of several sensory receptors in epidermal keratinocytes as described above (10,11,23). Various environmental factors might be initially sensed by these receptors, but it is not yet clear whether signalling occurs from the receptors, and if so, how it is mediated. As we show in Table 1, epidermal keratinocytes contain most of the representative neurotransmitter receptors originally found in the central nervous system. They are key components of information processing in the brain. Thus, we speculate that environmental signals are sensed by keratinocytes, then processed before being passed to C-fibres in the epidermis.

As previously noted, human skin can detect patterning at the micron scale (3–6). Recognition of such a pattern would require processing of information from several keratinocytes. The structures of epidermis and brain are very different, so the signal processing systems in the two are not necessarily similar. In this connection, it is interesting to note that a specific electrochemical information processing system might exist in the epidermis. We have observed oscillation of intracellular calcium levels in cultured human keratinocytes (24), with a stable frequency in each keratinocyte. The mechanism and role of the oscillation are not yet known, but in the absence of extracellular calcium, the oscillation was not observed (24). This might suggest that a calcium permeable channel contributes to the generation of the oscillation. Such a stable frequency oscillation can transfer information, and could play a role in information processing by epidermal keratinocytes. We are planning to investigate this possibility by observing calcium ion propagation in skin slices.

It will also be interesting to examine whether this system is linked with the various neuropeptides, neurohormones and their receptors of Slominski et al.'s skin neuroendocrine system (25–28). We may be approaching an understanding of the way in which environmental signals can be detected, processed and transferred from keratinocytes to the immune system, physiological system and endocrinological system.

Conclusion

It has been established that epidermal keratinocytes contain a variety of sensory and information transmission systems, and the traditional view of the sensory system in skin, focusing on C-fibres as the sensor, can no longer be regarded as sustainable. Building upon the work of Slominski et al. on the cutaneous neuroendocrine system (25), as well as our own and other researchers' findings on keratinocyte receptors, we propose that epidermal keratinocytes are at the forefront of the body's sensory system, serving to sense a variety of environmental factors (Fig. 2). We suggest that the signals generated are processed in the epidermis, and may influence emotional state, as well as endocrinological, immunological and physiological status and a variety of physiological functions.

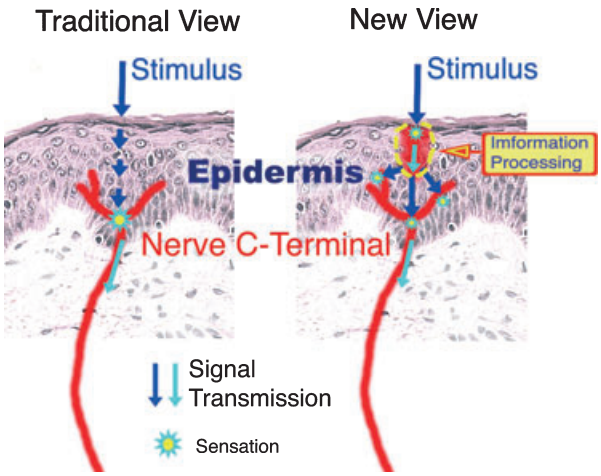

Figure 2 Schematic illustration of the hypothetical skin surface sensory system. (a) Traditional view, with C-fibres at the forefront of the sensory system. (b) Proposed new view, with epidermal keratinocytes at the forefront of the sensory system. A signal is sensed by keratinocytes, processed and transferred to C-fibre terminals.

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