Purinergic Signaling in Healthy and Diseased Skin

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Adenosine 5′-triphosphate and adenosine receptors have been identified in adult and fetal keratinocytes, fibroblasts, melanocytes, mast cells, Langerhans cells, and Meissner's corpuscles, as well as in hair follicles, sweat glands, and smooth muscle and endothelial cells of skin vessels. Purinergic signaling is involved in skin pathology, including inflammation, wound healing, pain, psoriasis, scleroderma, warts, and skin cancer.

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INTRODUCTION

Extracellular purine nucleotides and nucleosides have biological effects in a variety of cell and tissue types. The majority of studies have been concerned with short-term events that occur in neurotransmission and secretion (Burnstock, 2007a). Now, there is increasing evidence that purinergic signaling can have long-term, trophic effects on embryonic development, cell proliferation, differentiation, and apoptosis (Abbracchio and Burnstock, 1998; Burnstock, 2002a, b; Burnstock and Verkhratsky, 2010).

Purinergic receptors

Purinergic receptors are classified into two groups: P1 receptors (selective for adenosine) and P2 receptors (selective for adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), and uridine 5′-triphosphate (UTP), which act as extracellular signaling molecules) (Burnstock, 1978). Purinergic receptors are expressed in most neural and nonneural cells (see Burnstock and Knight, 2004).

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Abbreviations: ADP, adenosine 5′-diphosphate; AMP, adenosine monophosphate; ATP, adenosine 5′-triphosphate; BzATP, 2′,3′-O(benzoyl-4-benzoyl-ATP; cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; DMBA, 7,12-dimethyl-benz(a)anthracene; DRG, dorsal root ganglion; EC_{50}, half-maximal effective concentration; HPV, human papillomavirus; $InsP_3$, inositol 1,4,5-triphosphate; α,β-meATP, α,β-methyleneATP; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid; SSc, systemic sclerosis; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRPV, transient receptor potential V; UTP, uridine 5′-triphosphate

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P1 receptors. Four members of the adenosine/P1 receptor family have now been cloned and characterized from a variety of species: A_1, A_{2A}, A_{2B}, and A_3, and selective agonists and antagonists have been identified (Table 1). All P1 receptors couple to G-proteins (Figure 1a), and modulate adenylate cyclase activity in an inhibitory (A_1, A_3) or stimulatory (A_{2A}, A_{2B}) fashion, resulting in cyclic adenosine monophosphate (cAMP) changes.

P2 receptors. P2 receptors are divided into two families: P2X and P2Y (Figure 1b and c), based on molecular structure, transduction mechanisms, and pharmacological properties (Burnstock and Kennedy, 1985; Abbracchio and Burnstock, 1994).

P2X receptors are ligand-gated ion channels, and are activated by extracellular ATP to elicit a flow of cations (Na^+, K^+, and Ca^{2+}) across the plasma membrane. Seven subtypes of P2X receptors are recognized (Khakh *et al.*, 2001) (Table 2). All P2X receptors mediate fast signaling; however their localization, function, and pharmacological characteristics are different. Based on agonist efficacy and desensitization characteristics, P2X receptors have been grouped into three distinct classes. Group 1 includes $P2X_1$ and $P2X_3$ receptors with a high affinity for ATP (half-maximal effective concentration (EC_{50}) = 1 μM) that are rapidly activated and desensitized. Group 2 includes $P2X_2$, $P2X_4$, $P2X_5$, and $P2X_6$ receptors that have a lower affinity for ATP (EC_{50} = 10 μM), and show slow desensitization and sustained depolarizing currents. Group 3 is represented by the $P2X_7$ receptor that has very low affinity for ATP (EC_{50} = 300–400 μM), shows little or no desensitization, and, in addition to functioning as an ATP-gated ion channel, can also function as a nonselective ion pore (Di Virgilio *et al.*, 1999).

Functional P2X channels are homomultimers or heteromultimers formed by the association of at least three subunits. For a summary of possible P2X subunit combinations, see Burnstock and Kennedy (2011). It is not yet known how the properties of different P2X subunits influence the phenotype of the heteromultimeric receptors. Alternative splicing and species differences may increase heterogeneity of P2X receptors.

The P2Y family of receptors is a subclass of the superfamily of G-protein-coupled receptors, each having seven transmembrane domains. The third intracellular loop and the C-terminus are thought to be involved in G-protein coupling, whereas the third, sixth, and seventh transmembrane domains have been implicated in nucleotide binding (Burnstock and Kennedy, 2011). The principal signal transduction pathway of P2Y receptors involves phospholipase C, which

Table 1. Summary of properties of P1 receptor subtypes in the skin

Receptor	Site	Agonist	Antagonist	Receptor properties
A_1	Endothelial cells	CCPA adenosine	DPCPX, theophylline	Reduces bradykinin and histamine-induced vascular leakage
A_{2A}	Endothelial cells; neutrophils; fibroblasts	CGS21680 adenosine	Theophylline	Endothelial cell proliferation; reduces bradykinin and histamine-induced vascular leakage; decreases oxygen free radical production of neutrophils; increases angiogenesis in wound healing; promotes dermal collagen production by fibroblasts
A_{2B}	Keratinocytes; vascular smooth muscle cells; human retinal endothelial cells	Adenosine, NECA	MRS1751, theophylline	Antiproliferative in keratinocytes and vascular smooth muscle cells; mediates VEGF expression in retinal endothelial cells; promotes wound healing
A_3		Cl-IB-MECA	MRE 3008F20	Not established

Abbreviations: CCPA, 2-chloro-N6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; NECA, 5′-N-ethylcarboxamidoadenosine; VEGF, vascular endothelial growth factor.

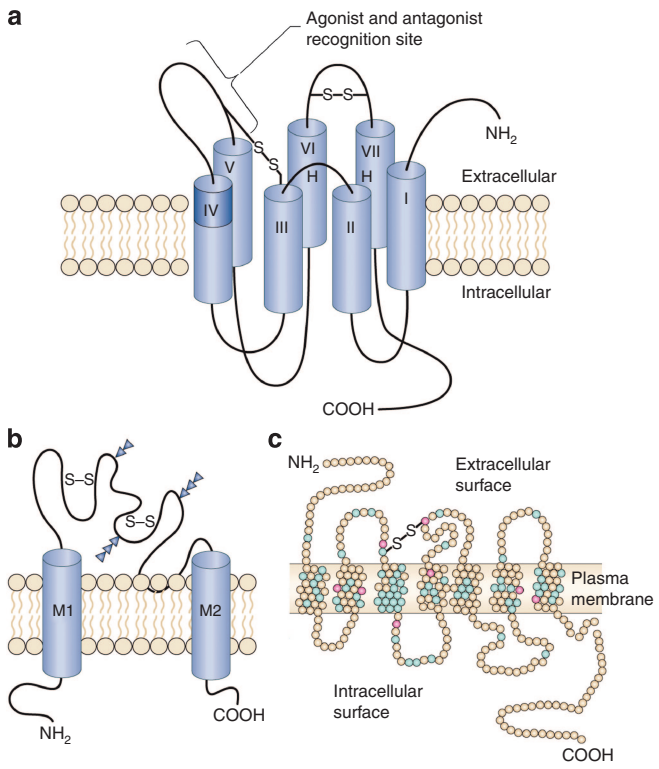

Figure 1. Membrane receptors for extracellular adenosine and adenosine 5′-triphosphate (ATP). (**a**) The P1 family of receptors for extracellular adenosine are G-protein-coupled receptors (S–S; disulfide bond). (**b**) The P2X family of receptors are ligand-gated ion channels (S–S; disulfide bond; M1 and M2, transmembrane domains), and (**c**) the P2Y family are G-protein-coupled receptors (S–S; disulfide bond; green circles represent amino-acid residues that are conserved between $P2Y_1$, $P2Y_2$, and $P2Y_3$ receptors; fawn circles represent residues that are not conserved; and red circles represent residues that are known to be functionally important in other G-protein-coupled receptors). Panel **a** is from Ralevic and Burnstock (1998); reproduced with permission from the American Society for Pharmacology and Experimental Therapeutics. Panel **b** is from Brake *et al*. (1994); reproduced with permission from Nature. Panel **c** is modified from Barnard *et al*. (1994); reproduced with

and mobilization of intracellular calcium. $InsP_3$ regulates cell growth and DNA replication (Berridge, 1987). Eight subtypes of P2Y receptors have been described so far (Burnstock, 2007b) (Table 3).

Nucleotide ligands

ATP acts as a cotransmitter in many nerves of both peripheral and central nervous systems. ATP is released together with noradrenaline and neuropeptide Y from sympathetic nerves. It is released as a cotransmitter with acetylcholine from parasympathetic nerves supplying the bladder, developing skeletal neuromuscular junctions and some neurons in the brain. It is released with nitric oxide and vasoactive intestinal polypeptide from nonadrenergic inhibitory enteric nerves; with glutamate from primary afferent sensory nerves and in different subpopulations of neurons in the brain; with dopamine, noradrenaline, acetylcholine, glutamate, γ-aminobutyric acid, and 5-hydroxytryptamine. Cotransmission offers subtle, local variations in neurotransmission and neuromodulation mechanisms (Burnstock, 2009a).

Adenosine, AMP, ADP, and ATP can be released into the extracellular environment during inflammation, wounding, hypoxia, and other pathological states. There is a complex relationship between these nucleotides. Extracellular ATP has a very short half-life before it is degraded to adenosine (from milliseconds to seconds depending on the site of release and the level of activity of ectonucleotidases). AMP, ADP, and ATP can be converted to adenosine through spontaneous hydrolysis and/or through the activity of 5′-ectonucleotidases, ecto-ADPases, or ecto-ATPases. Adenosine can be converted to inosine via the action of adenosine deaminase, or by uptake into cells through nucleoside transporters. When externalized, adenosine and its nucleotides can participate in physiological processes. The rapid breakdown of ATP to adenosine results in a multiplicity of different receptor subtypes being activated, which can mediate different physiolog-

Table 2. Summary of properties of P2X receptor subtypes in the skin

Receptor	Site	Agonist	Antagonist	Receptor properties
$P2X_1$	Smooth muscle; platelets	α,β-meATP	TNP-ATP, isoPPADS	Rapidly desensitizing
$P2X_2$	Smooth muscle; autonomic and sensory ganglia	Weak agonists: 2-MeSATP, β,γ-meATP, ATPγS	isoPPADS, Reactive Blue 2	Significant permeability to Ca^{2+}; sensitive to extracellular acidification
$P2X_3$	Nociceptive sensory neurons (trigeminal, nodose, and dorsal root ganglia); endothelial and epithelial cells	α,β-MeATP	TNP-ATP, isoPPADS	Rapidly desensitizing
$P2X_4$	CNS; testis; colon	ATP	TNP-ATP	Slowly desensitizing
$P2X_5$	Keratinocytes; growing hair follicles	ATPγS	PPADS, suramin (nonselective)	Proliferating and differentiating cells in keratinized and nonkeratinized epithelia
$P2X_6$	CNS; motor neurons in spinal cord	2-MeSATP	None	Minimal desensitizing
$P2X_7$	Apoptotic cells—e.g., keratinized epithelium, macrophages, monocytes, lymphocytes, granulocytes	BzATP	KN-62, Coomassie Brilliant Blue G	Bifunctional: either acts as cation channel or forms a large pore and allows calcium entry and cell death

Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; BzATP, 2′,3′-O-(4-benzoyl-benzoyl)-ATP; CNS, central nervous system; α,β-meATP, α,β-methyleneATP; isoPPADS, pyridoxal-phosphate-6-azophenyl-2′,5′-disulfonic acid; 2-MeSADP, 2-methylthio ADP; 2-MeSATP, 2-methylthio ATP; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid; TNP-ATP, 2′,3′-O-(2,4,6-trinitrophenyl)-ATP.

Table 3. Summary of properties of P2Y receptor subtypes in the skin

Receptor	Site	Agonist	Antagonist	Receptor properties
$P2Y_1$	Epithelial and endothelial cells; platelets; immune cells	ADP, ATP, 2-MeSADP	MRS2179	Platelet aggregation; vascular relaxation; endothelial cell proliferation
$P2Y_2$	Epithelial cells; endothelial vascular smooth muscle cells; immune cells	UTPγS, UTP, ATP	PPADS, suramin	Epithelial Cl^- secretion, vascular relaxation; keratinocyte proliferation
$P2Y_4$	Endothelial cells	UTP, ATP	PPADS	Not established
$P2Y_6$	Some epithelial cells; T cells	UDP, UTP	PPADS, suramin	Not established
$P2Y_{11}$	Granulocytes	ATP	Suramin	Not established
$P2Y_{12}$	Platelets	ADP	Suramin	Platelet aggregation
$P2Y_{13}$	Lymph nodes	ADP, 2-MeSADP	MRS2211, 2-MeSAMP	Stimulation of MAPK; inhibition of adenylate cyclase
$P2Y_{14}$	Adipose tissue	UDP glucose, UDP galactose	Not known	Chemoattractant and neuroimmune functions

Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; MAPK, mitogen-activated protein kinase; 2-MeSADP, 2-methylthio ADP; 2-MeSAMP, 2-methylthio AMP; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid; UTP, uridine 5′-triphosphate.

and cell death), and thus purines may act as important local messengers in the skin.

This review article aims to give an overview of purinergic signaling in cells in the skin, skin appendages, and then in pathological processes affecting the skin.

KERATINOCYTES

P1 receptors

P1 receptors were first hypothesized to be present in the epidermis in the 1970s. Adenosine, AMP, ADP, and ATP activated adenylate cyclase in pig epidermis, resulting in accumulation of cAMP. Theophylline, an adenosine receptor antagonist, blocked the response to adenosine as well as to likely that responses to nucleotides were mediated by P1 receptors after enzymatic breakdown to adenosine.

Human keratinocytes express A_{2B} receptor mRNA, but not significant levels of A_1, A_{2A}, or A_3 receptor mRNA. A_{2A} receptor stimulation promotes both proliferation and apoptosis, whereas A_3 stimulation arrests proliferation and apoptosis (Merighi *et al.*, 2002). Both primary cultures of mouse keratinocytes and the murine keratinocyte cell line, MSC-P5, express A_{2A}, A_{2B}, and A_3 receptors, with A_{2B} receptors having the strongest expression. Stimulation of A_{2B} receptors resulted in enhanced growth (Braun *et al.*, 2006).

Other studies have reported the antiproliferative effects of adenosine nucleotides for both human and porcine kerati-

Adenosine, ATP, and ADP were also shown to be antiproliferative for normal human epidermal keratinocytes cultured in the absence or presence of exogenous epidermal growth factor (Cook *et al.*, 1995), probably via A_{2B} receptors (Brown *et al.*, 2000). In this study, ATP had a more potent effect than adenosine. The antiproliferative effect of adenosine was not blocked by the A_1/A_2 receptor antagonist, 8-(*p*-sulfophenyl)theophylline, confirming the findings in a previous study that used theophylline (Brown *et al.*, 2000). The adenosine transport blocking agent, dipyridamole, had an inhibitory effect of its own and did not potentiate the effect of adenosine. This suggested that extracellular adenosine receptors were not being activated in this system. The effects of adenosine might be mediated via an intracellular site of action (Brown *et al.*, 2000). 8-Chloro-adenosine, presumably acting via P1 receptors, induced ''growth arrest without differentiation of primary mouse epidermal keratinocytes'' (Dransfield *et al.*, 2001).

In a study of the effect of various purinoceptor agonists on primary human keratinocyte cultures, the response to ATP was not blocked by 8-(*p*-sulfophenyl)theophylline, which meant that the effect of ATP was not due to breakdown of ATP to adenosine and activation of extracellular adenosine receptors (Greig *et al.*, 2003a). Dipyridamole did not potentiate the ATP response, and hence blocking adenosine transport into the cell, and thereby increasing local extracellular adenosine concentrations, did not have any effect. This suggests that the main trophic agent in human keratinocyte cultures is ATP, working via P2 receptors, whereas adenosine has a minor effect, which is largely obscured by the ATP response.

P2 receptors

The presence of P2 purinergic receptors was suggested by a study on canine keratinocytes (Suter *et al.*, 1991). Extracellular ATP stimulates P2 receptors to transiently increase intracellular calcium levels. An increase in intracellular calcium in cultured human keratinocytes is an early event in terminal differentiation (Sharpe *et al.*, 1989), which is of primary importance during this process.

Proliferation. The first clue to a role for ATP in proliferation came from the finding that ATP stimulates phosphoinositide, which mobilizes $[Ca^{2+}]_i$, stimulates thymidine incorporation, thus inhibiting differentiation in cultures of human epidermal keratinocytes (Pillai and Bikle, 1992). ''In whole-cell recordings from HaCaT cells,'' an immortalized human keratinocyte cell line, ATP ''caused a bipolar change in membrane potential, transient depolarisation followed by long-lasting hyperpolarisation'' (Koegel and Alzheimer, 2001). ''Extracellular ATP stimulates HaCaT cell proliferation via purinoceptor-mediated $[Ca^{2+}]_i$ mobilization'' (Lee *et al.*, 2001).

Reverse transcription-PCR and *in situ* hybridization studies identified $P2Y_2$ receptors on the basal layer of the epidermis, which is the site of cell proliferation, and also identified $P2Y_2$ receptors in primary cultured human keratinocytes. ATP and consistent with the pharmacological profile of $P2Y_2$ receptors (Dixon *et al.*, 1999). This study also showed that cultured human keratinocytes released ATP under static conditions.

Double labeling of human skin with proliferation markers Ki-67, proliferating cell nuclear antigen, and $P2Y_1$ and $P2Y_2$ receptors, identified a proliferating subpopulation of basal and parabasal keratinocytes. Cells positive for these two markers were also positive for $P2Y_1$ and $P2Y_2$ receptors (Greig *et al.*, 2003a) (Figure 2). Low concentrations of ATP, UTP, and 2-methylthio ADP caused an increase in keratinocyte cell number in primary human keratinocyte cultures, also suggesting a role for $P2Y_1$ and $P2Y_2$ receptors in keratinocyte proliferation (Greig *et al.*, 2003a). Further evidence has demonstrated functional $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors in human keratinocytes, which are involved in the regulation of cell proliferation (Burrell *et al.*, 2003).

Costimulation of HaCaT cells by ATP acting on $P2Y_2$ receptors and parathyroid hormone–related protein increased proliferation (Burrell *et al.*, 2008). Confocal optical sectioning through mulilayered HaCaT cultures showed that the responsiveness to ATP differs dramatically between proliferating cells and cells undergoing partial differentiation (Burgstahler *et al.*, 2003). It was claimed that UTP caused IL-6 production in HaCaT keratinocytes via $P2Y_2$ and/or $P2Y_4$ receptors (Kobayashi *et al.*, 2006; Yoshida *et al.*, 2006). This finding might be important as studies have shown that IL-6 has a physiological role in the repair processes of wounds (Gallucci *et al.*, 2000). Calcium-permeable transient receptor potential canonical 7 is a purinoceptor-operated 1,2-diacylgycerol-activated channel in HaCaT cells (Beck *et al.*, 2006), thought to be an additional mediator of calcium influx into keratinocytes, which in turn can lead to differentiation. ''Calcium-independent phospholipase A is required for Ca^{2+} entry into HaCaT keratinocytes following ATP or UTP stimulation'' (Ross *et al.*, 2007, 2008), and Ca^{2+} movement is involved in the control of numerous cellular process.

''Retinoids, vitamin A derivatives, are important regulators of growth and differentiation of skin cells'' and have been used therapeutically for aging skin. An agonist for the retinoic receptor was shown to enhance expression of mRNA for $P2Y_2$ receptors in basal keratinocytes involved in proliferation of the epidermis (Fujishita *et al.*, 2006).

Differentiation. Changes in intracellular Ca^{2+} were found to regulate differentiation of keratinocytes in the granular layer of the epidermis (Menon *et al.*, 1985), which is an interesting observation in view of the later discovery of high expression of $P2X_5$ receptors in this layer of the epidermis, the purinoceptor subtype that is known to mediate cell differentiation (Gröschel-Stewart *et al.*, 1999; Greig *et al.*, 2003a). Double labeling of $P2X_5$ receptors with cytokeratin K10 or involucrin showed that $P2X_5$ receptors were expressed in differentiating keratinocytes within the epidermis (Greig *et al.*, 2003a). The increase in $[Ca^{2+}]_i$ in response to ATP varied in each layer of the epidermis and was greater in the basal than in the outer layers (Tsutsumi *et al.*, 2009a). Both extracellular ATP and UTP ''induce transient rises in

(Sharpe *et al.*, 1989; Suter *et al.*, 1991), suggesting in retrospect that $P2Y_2$ and/or $P2Y_4$ receptors were involved.

Multiple P2X receptor subtypes were identified later in cultured human epidermal keratinocytes using whole-cell patch clamp techniques, reverse transcription-PCR, and intracellular Ca^{2+} measurements (Inoue *et al.*, 2005). $P2X_2$, $P2X_3$, $P2X_5$, and $P2X_7$ receptor mRNA was increased in differentiated cells, whereas $P2Y_2$ receptor mRNA was downregulated in differentiated cells.

Protein kinase C-α has been shown to decrease cell proliferation and augment cell differentiation of human keratinocytes (Hegemann *et al.*, 1994). The expression of $P2X_7$ receptors was significantly increased in keratinocytes overexpressing protein kinase C-α (Gönczi *et al.*, 2008).

Terminal differentiation/apoptosis. An immunohistochemical study of rat stratified squamous epithelium showed that $P2X_7$ receptors were clearly associated with the keratinization process in the outer layer (Gröschel-Stewart *et al.*, 1999). This was confirmed in human epidermis with colocalization with markers for keratinocyte apoptosis: $P2X_7$ receptors control death of keratinocytes in the outer stratum corneum (Greig *et al.*, 2003a). The $P2X_7$ receptor is unlike other P2X receptors because it is a bifunctional molecule that can be triggered to act as a channel permeable to small cations, or on prolonged stimulation can form a cytolytic pore permeable to large hydrophilic molecules up to 900 Da (Surprenant *et al.*, 1996). The opening of this pore results in the increase in intracellular cytosolic free calcium ions and the induction of cell death (Zheng *et al.*, 1991; Ferrari *et al.*, 1996). There is evidence that this process is dependent on the caspase signaling cascade (Coutinho-Silva *et al.*, 1999; Ferrari *et al.*, 1999). Double labeling of $P2X_7$ receptors with anti-caspase-3 showed colocalization within the stratum corneum (Greig *et al.*, 2003a) (Figure 2f). High concentrations of ATP and 2′,3′-O(benzoyl-4-benzoyl-ATP) (BzATP, a $P2X_7$ receptor agonist) caused a significant decrease in keratinocyte cell number in primary human keratinocyte cultures, providing evidence for a functional role for $P2X_7$ receptors (Greig *et al.*, 2003a).

Fetal keratinocytes

The expression of $P2X_5$, $P2X_7$, $P2Y_1$, and $P2Y_2$ receptors in 8–11-week-old human fetal epidermis was investigated using immunohistochemistry (Greig *et al.*, 2003b). P2 purinergic receptors are likely to be involved in fetal keratinocyte proliferation via $P2Y_1$ receptors found on basal cells and fetal keratinocyte differentiation via activation of $P2X_5$ receptors.

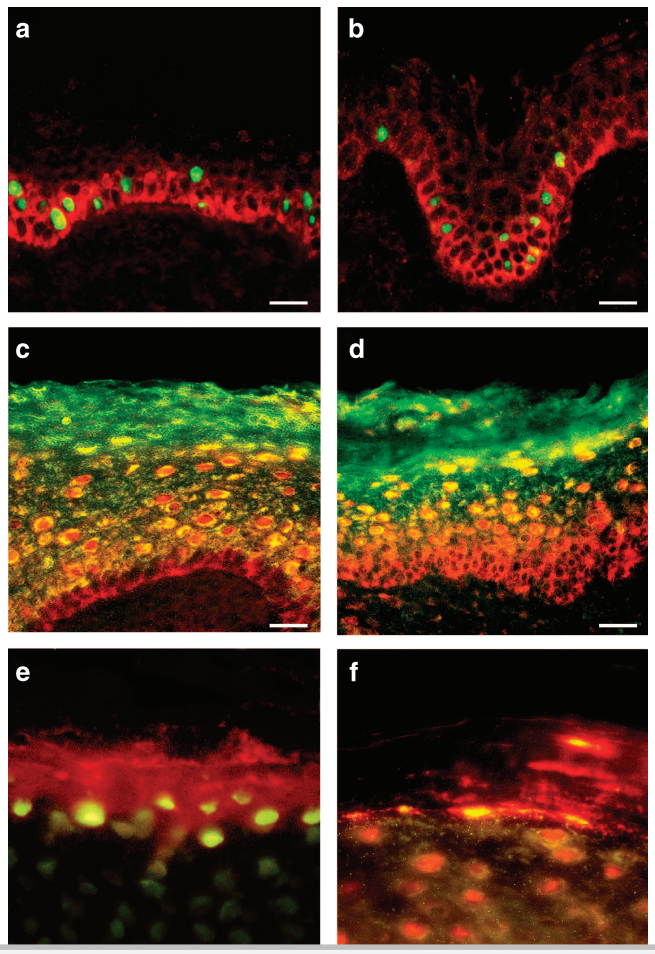

Figure 2. Double labeling of $P2Y_1$ and $P2Y_2$ receptors with markers of proliferation show colocalization within a subpopulation of basal and parabasal keratinocytes. Double labeling of $P2X_5$ receptors with markers of differentiated keratinocytes show colocalization within the stratum spinosum, and double labeling of $P2X_7$ receptors with markers of apoptosis in human leg skin show colocalization within the stratum corneum. (**a**) Ki-67 immunolabeling (a marker for proliferation) stained the nuclei (green) of a subpopulation of keratinocytes in the basal and parabasal layers of the epidermis. $P2Y_1$ receptor immunostaining (red) was found in the basal layer on cells also staining for Ki67. Scale bar = 30 mm. (**b**) Proliferating cell nuclear antigen (PCNA) immunolabeling (a marker for proliferation) stained the nuclei (green) of a subpopulation of keratinocytes. These nuclei were often distributed in clusters and found in the basal and parabasal layers of the epidermis. $P2Y_2$ receptor immunostaining (red) was also expressed in basal and parabasal epidermal cells. Scale bar = 30 mm. (**c**) $P2X_5$ receptor immunostaining (red) showed overlap (yellow) with cytokeratin K10 (green), an early marker of keratinocyte differentiation. $P2X_5$ receptors were present in the basal layer of the epidermis up to the mid-granular layer. Cytokeratin K10 was distributed in most suprabasal keratinocytes. The stratum basale stained only for $P2X_5$ receptors, indicating that no differentiation was taking place in these cells. The colocalization of $P2X_5$ receptors and cytokeratin K10 appeared mainly in the cytoplasm of differentiating cells within the stratum spinosum and partly in the stratum granulosum. Note that the stratum corneum also stained for cytokeratin K10, which labeled differentiated keratinocytes, even in dying cells. Scale bar = 30 mm. (**d**) $P2X_5$ receptor immunostaining (red) showed overlap (yellow) with involucrin (green). $P2X_5$ receptors were present in the basal layer of the epidermis up to the midgranular layer. Note that the pattern of staining with involucrin was similar to that seen with cytokeratin K10, except that cells from the stratum basale up to the midstratum spinosum were not labeled with involucrin, which is a late marker of keratinocyte differentiation. Scale bar = 30 mm. (**e**) TUNEL (green) labeled the nuclei of cells at the uppermost level of the stratum granulosum and $P2X_7$ antibody (red) mainly stained cell fragments within the stratum corneum. Scale bar = 15 mm. (**f**) Anti-caspase-3 (green) colocalized with areas of $P2X_7$ receptor immunostaining (red) both at the junction of the stratum granulosum and within the stratum corneum. Areas of colocalization are yellow. Note that the differentiating keratinocytes in the upper stratum granulosum were also positive for anti-caspase-3. Bar = 15 mm (reproduced from Greig *et al.*, 2003a

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