



Regulation of epidermal homeostasis through P2Y₂ receptors

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1 Previous studies have indicated a role for extracellular ATP in the regulation of epidermal homeostasis. Here we have investigated the expression of P2Y₂ receptors by human keratinocytes, the cells which comprise the epidermis.

2 Reverse transcriptase-polymerase chain reaction (RT-PCR) revealed expression of mRNA for the G-protein-coupled, P2Y₂ receptor in primary cultured human keratinocytes.

3 *In situ* hybridization studies of skin sections revealed that P2Y₂ receptor transcripts were expressed in the native tissue. These studies demonstrated a striking pattern of localization of P2Y₂ receptor transcripts to the basal layer of the epidermis, the site of cell proliferation.

4 Increases in intracellular free Ca²⁺ concentration ([Ca²⁺]_i) in keratinocytes stimulated with ATP or UTP demonstrated the presence of functional P2Y receptors.

5 In proliferation studies based on the incorporation of bromodeoxyuridine (BrdU), ATP, UTP and ATPγS were found to stimulate the proliferation of keratinocytes.

6 Using a real-time firefly luciferase and luciferin assay we have shown that under static conditions cultured human keratinocytes release ATP.

7 These findings indicate that P2Y₂ receptors play a major role in epidermal homeostasis, and may provide novel targets for therapy of proliferative disorders of the epidermis, including psoriasis.

Keywords: Epidermis; keratinocytes; skin; proliferation; extracellular nucleotides; ATP; P2Y₂ receptors; *in situ* hybridization; intracellular calcium

Abbreviations: BrdU, bromodeoxyuridine; [Ca²⁺]_i, intracellular free calcium concentration; cps, counts per second; DEPC, diethyl pyrocarbonate; GAPDH, glyceraldehyde phosphate dehydrogenase; Ins(1,4,5)P₃, inositol(1,4,5)trisphosphate; MAPK, mitogen-activated protein kinase

Introduction

Human epidermis is a stratified squamous epithelium comprised of keratinocytes. Cell proliferation is largely confined to the basal layer of the epidermis where cells divide to generate either replacement progenitor cells, or cells committed to terminal differentiation. Differentiating cells migrate to the skin surface. The morphological and biochemical changes which occur during differentiation characterize the distinct layers of the epidermis (see Fuchs, 1990). At the skin surface, the stratum corneum is composed of dead, terminally-differentiated keratinocytes which form the main cutaneous barrier. The balance between proliferation and differentiation of basal cells, which maintains the epidermis at constant thickness, is regulated by many extracellular factors; cell proliferation and differentiation usually follow an inverse relationship. Positive growth factors include epidermal growth factor (Rheinwald, 1980), transforming growth factor α (TGF α ; Barrandon & Green, 1984; Gottlieb *et al.*, 1988) and keratinocyte growth factor (Finch *et al.*, 1989). The best characterized negative growth regulators, and hence stimulators of differentiation, are the TGF β s (Shipley *et al.*, 1986), extracellular Ca²⁺ (Hennings *et al.*, 1980) and vitamin D (Jones & Sharpe, 1994).

Changes in keratinocyte intracellular free Ca²⁺ concentration ([Ca²⁺]_i) have been recorded in response to both differentiation- and proliferation-stimulating agents,

although the pattern of change is dissimilar. Differentiation is associated with a sustained increase in [Ca²⁺]_i which requires influx of extracellular Ca²⁺ to be maintained (Pillai *et al.*, 1988, 1990; Bikle *et al.*, 1996; Sharpe *et al.*, 1989). Recent evidence suggests that expression of the plasma membrane Ca²⁺-ATPase is decreased upon differentiation, and that this contributes to the increased [Ca²⁺]_i (Cho & Bikle, 1997). In contrast to this requirement for a sustained increase in [Ca²⁺]_i, growth stimulation is associated with a temporary rise in [Ca²⁺]_i lasting only a few minutes, consistent with release of intracellular stores, but not Ca²⁺ influx (Pillai & Bikle, 1992; McGovern *et al.*, 1995).

In the vicinity of a wound, keratinocytes will be exposed to ADP, ATP and UTP released from dead or damaged cells as well as from platelets (Boarder & Hourani, 1998). There is accumulating evidence that keratinocytes possess specific receptors for these nucleotides, which may have important effects on keratinocyte proliferation and differentiation. Extracellular nucleotides such as ATP act at P2 receptors, a family of cell-surface receptors which have been divided into two classes based on modes of signal transduction and subsequently molecular cloning: the ligand-gated ion channels, or P2X receptors; and G-protein-coupled, P2Y receptors. There are a number of subdivisions of both classes that display differential responsiveness to nucleotide agonists (see Boarder & Hourani, 1998); in the absence of specific antagonists this has formed the basis of pharmacological characterization of

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receptor subtypes. All mammalian P2Y receptors cloned to date are coupled to hydrolysis of phosphatidylinositol 4,5-bisphosphate and hence to inositol 1,4,5-trisphosphate-(Ins(1,4,5)P₃-mediated release of intracellular Ca²⁺ stores (Boarder & Hourani, 1998). In addition P2Y₂ receptor occupation leads to activation of the mitogen-activated protein kinase (MAPK) cascade (see Boarder & Hourani, 1998); MAPK activation is closely associated with proliferation.

The presence of P2Y receptors on primary human (Pillai & Bikle, 1992) and canine keratinocytes (Suter *et al.*, 1991) has been indicated through recordings of increased [Ca²⁺]_i in response to a range of extracellular nucleotides. The results of desensitization experiments in both studies suggested that ATP and UTP were competing for a single receptor, indicating that keratinocytes express P2Y₂ receptors. Consistent with the reported transient nature of the ATP-evoked [Ca²⁺]_i rise, this nucleotide was also found to induce proliferation of human keratinocytes and to inhibit differentiation (Pillai & Bikle, 1992). However, in a separate study by Cook *et al.* (1995) ATP was shown to have an inhibitory effect on human keratinocyte growth. This anti-proliferative effect was not mediated by P2Y₂ receptors, as UTP, an equipotent agonist at this receptor, was ineffective.

To further understand the action of extracellular nucleotides on keratinocytes we have investigated the presence and function of P2Y₂ receptors in human skin and cultured keratinocytes. These findings have important implications for the actions of ATP and UTP in epidermal wound repair and for the pathogenesis of hyperproliferative skin diseases. We have additionally demonstrated that under static conditions cultured human keratinocytes release ATP in significant quantities. This indicates that keratinocytes *in vivo* may be exposed to ATP, not only following injury, but also in the normal skin micro-environment, raising the possibility that ATP has a role in epidermal homeostasis.

Methods

Cell culture

Normal human keratinocytes were isolated from foreskins or neonatal rectoauricular skin removed during plastic surgery and cultured in MCDB153 with supplements, as described previously (Jones & Sharpe, 1994). Cells were passaged with the addition of 100 µg ml⁻¹ bovine hypothalamic extract to aid cell attachment (Tsao *et al.*, 1982) and used from passage 1–4.

Isolation of RNA and first strand cDNA synthesis

Total RNA was extracted from cultured keratinocytes or tissue samples as described previously (Bowler *et al.*, 1995), treated with RNAase-free DNase 1 (35 U µl⁻¹) for 30 mins and stored as an ethanolic precipitate at –20°C. An aliquot of 5 µg of total RNA was used as template for first strand cDNA synthesis as described earlier (Bowler *et al.*, 1995).

PCR analysis

PCR reactions were carried out using a 50 µl reaction volume as described earlier (Bowler *et al.*, 1995). Following an initial denaturation step (94°C for 2 mins), the conditions for denaturation, annealing and extension were: 25, 30 or 35 cycles of 94°C for 10 s; 58°C for 30 s (60°C for glyceraldehyde

phosphate dehydrogenase (GAPDH); 72°C for 30 s.

Primer sequences:

P2Y₂: Sense 5'-CCAGGCCCCCGTGTCTACTTTG

Anti-sense 5'-CATGTTGATGGCGTTGAGGGTGTG

Sense 5'-GGTGAAGGTCCGAGTCAACGG

GAPDH Anti-sense 5'-GGTCATGAGTCCTTCCACGAT

In situ hybridization

Sections of skin were prepared and stored according to the protocol described earlier (Bowler *et al.*, 1998). *In situ* hybridization was performed as previously (Littlewood-Evans *et al.*, 1997; Bowler *et al.*, 1998). Briefly, sections were rehydrated, treated with citric acid buffer, and proteinase K to allow access of the RNA probes and to decrease non-specific binding. Sections were post-fixed in paraformaldehyde to retain cellular localization of the mRNA. Remaining proteins were acetylated with acetic anhydride. Prehybridization was carried out in a humidified box at 42°C for 3 h, before addition of 300,000 c.p.m. of 500 bp, sense or anti-sense ³²P-dUTP-labelled P2Y₂ riboprobe per section. Hybridization was carried out overnight at 42°C. Washing of the sections and development of slides were conducted as described previously (Bowler *et al.*, 1998). Finally, tissues were counter-stained in methylene blue, mounted and photographed.

[Ca²⁺]_i measurements

Keratinocytes were grown to approximately 70% confluency on 22 mm diameter coverslips. The equipment and experimental protocol followed for the determination of [Ca²⁺]_i have been described previously (Dixon *et al.*, 1997). Measurements were made from groups of approximately 8–10 fura-2-loaded cells and addition of agonists was performed manually by pasteur pipette. Autofluorescence values were obtained *in situ* using ionomycin and 50 µM manganese, as described by Thomas & Delaville (1991). The ratio of fluorescence intensities at 340 nm and 380 nm after subtraction of autofluorescence was plotted as an indicator of [Ca²⁺]_i.

Cell proliferation assay

The effect of extracellular nucleotides on proliferation of keratinocytes was assessed using a Biotrak cell proliferation ELISA system (version 2). Briefly, keratinocytes were seeded at subconfluent levels into 96 well plates and incubated in MCDB153 at 37°C in a humidified atmosphere of 5% CO₂, 95% air for 48 h. Medium was removed and replaced with medium containing bromodeoxyuridine- (BrdU)-labelling reagent (1 : 1000) and the nucleotide of interest in the presence of 10% FCS. Cells were incubated for a further 24 h before fixing. Incorporation of BrdU was assessed by ELISA, according to the protocol of the manufacturer. Data were analysed by randomized block analysis of variance (ANOVA) followed by Newman Keuls procedure.

Real-time measurement of ATP release

ATP release was measured using a firefly luciferase and luciferin assay in a custom-built photon-counting luminometer described in detail in Cobbold & Lee (1991). A circular flat-bottomed stainless steel chamber at 37°C, with a capacity of approximately 300 µl was filled with HEPES buffer ((mM) HEPES 10, NaCl 121, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2, NaHCO₃ 5, glucose 10, BSA 0.2%; pH 7.2)

containing 10 $\mu\text{g ml}^{-1}$ firefly luciferase and 10 μM luciferin. A 13 mm diameter coverslip of cells at confluency was floated gently into the full chamber to rest on its base. The chamber was then sealed with a glass coverslip and positioned immediately below the photocathode of the photomultiplier (EMI type 9789) housed in a constant-temperature incubator, held at 0–4°C to reduce electrical noise. The pulses of current generated when light hit the photomultiplier were fed into a computer fitted with an EMI photon-counting board. Data, collected in 50 ms bins, were integrated over 1 s intervals to give counts s^{-1} (c.p.s.).

The signals were calibrated by measuring the luciferase luminescence over a range of ATP concentrations (1 nM–1 μM). The calibration curve, plotted as log of luminescence intensity (c.p.s.) against log of [ATP], was linear over this range of concentrations.

Materials

FCS (Myoclonal grade), Taq DNA polymerase and Superscript II reverse transcriptase were purchased from GIBCO BRL, Paisley, U.K.; Fura-2 acetoxymethyl ester (fura-2 AM) was from Molecular Probes, Eugene, Oregon, U.S.A.; dNTPs, oligo(dT), RNAase inhibitor and restriction enzymes were obtained from Boehringer Mannheim, Lewes, U.K.; LM1 emulsion and the Biotrak proliferation kit were from Amersham, Little Chalfont, U.K.; MCDB153 culture medium, firefly luciferase and luciferin, and all other chemicals were purchased from Sigma-Aldrich, Poole, U.K.

Results

Expression of P2Y₂ receptor transcripts

cDNA derived from primary cultured human keratinocytes was subjected to 25, 30 and 35 rounds of PCR amplification using oligonucleotide primers specific for the human P2Y₂ receptor. The 362 bp product was clearly visible following 30 cycles of PCR amplification demonstrating expression of P2Y₂ receptor transcripts by this cell type (Figure 1A). We have previously demonstrated that human osteoclasts of giant cell tumour abundantly express P2Y₂ receptor mRNA (Bowler *et al.*, 1995). cDNA derived from a purified population of human osteoclasts was therefore included as a positive control; the 362 bp product was visible following 25 cycles of amplification (Figure 1A). The specificity of the amplified product was confirmed by sequence analysis, and amplification of GAPDH at 25 and 35 cycles confirmed the integrity of cDNAs used (Figure 1B).

P2Y₂ receptor transcripts are localized to the stratum basale

In situ hybridization revealed a striking pattern of localization of P2Y₂ receptor transcripts in the basal cell layer of skin sections (Figure 2B, D and E). In control experiments with a sense RNA probe no specific granular localization was detected (Figure 2A and C). Interestingly, high levels of P2Y₂ receptor mRNA were detected in both the sweat glands (Figure 2F) and the hair follicles (data not shown).

ATP and UTP increase $[\text{Ca}^{2+}]_i$ in keratinocytes

Application of 10 μM ATP ($n=5$) or 10 μM UTP ($n=5$) to groups of 8–10 fura-2 loaded keratinocytes induced a rise in

$[\text{Ca}^{2+}]_i$. Figure 3 depicts the response of a group of keratinocytes to increasing concentrations of ATP and UTP. The threshold concentrations for ATP and UTP were in the low micromolar range; 1 μM UTP evoked a response in 2/4 preparations, and 2 μM ATP was effective in 3/4 preparations (1 μM ATP did not elicit a rise in $[\text{Ca}^{2+}]_i$ in 4/4 preparations).

P2Y₂ agonists stimulate proliferation of human keratinocytes

Keratinocytes cultured in MCDB153, supplemented as described above, are undergoing near maximal proliferation. In order then to study the effect of nucleotide stimulation the rate of proliferation needs to be reduced. This was achieved by addition of 10% FCS, which slows keratinocyte growth as noted previously (Sharpe & Fisher, 1990). In the presence of 10% FCS, ATP (0.1–100 μM) significantly increased proliferation (Figure 4A). The poorly-hydrolyzable analogue of ATP, ATP γS , and UTP similarly stimulated keratinocyte proliferation in the presence of serum (Figure 4B).

Keratinocytes constitutively release ATP

As depicted in Figure 5, introducing a coverslip of confluent keratinocytes into the chamber of the photon-counting detection system led to an immediate increase in the intensity of the luminescence. The signal rose from the background level of approximately 9 c.p.s. to 604 ± 166 c.p.s. (mean \pm s.e. mean,

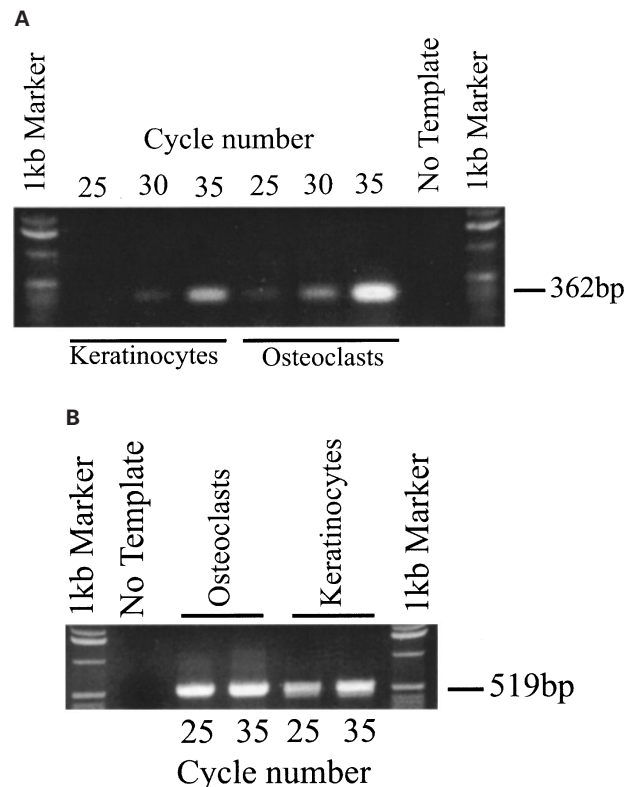


Figure 1 Expression of P2Y₂ receptor transcripts in primary cultured human keratinocytes and osteoclasts. (A) PCR amplification of a specific 362 bp fragment of the human P2Y₂ receptor using cDNA from primary cultured human keratinocytes. cDNA from a sorted human osteoclast population was included as a positive control. (B) Amplification of a specific 519 bp GAPDH product confirmed the integrity of the cDNA used. This result is typical of three analyses using keratinocytes derived from skin samples from three individual patients.

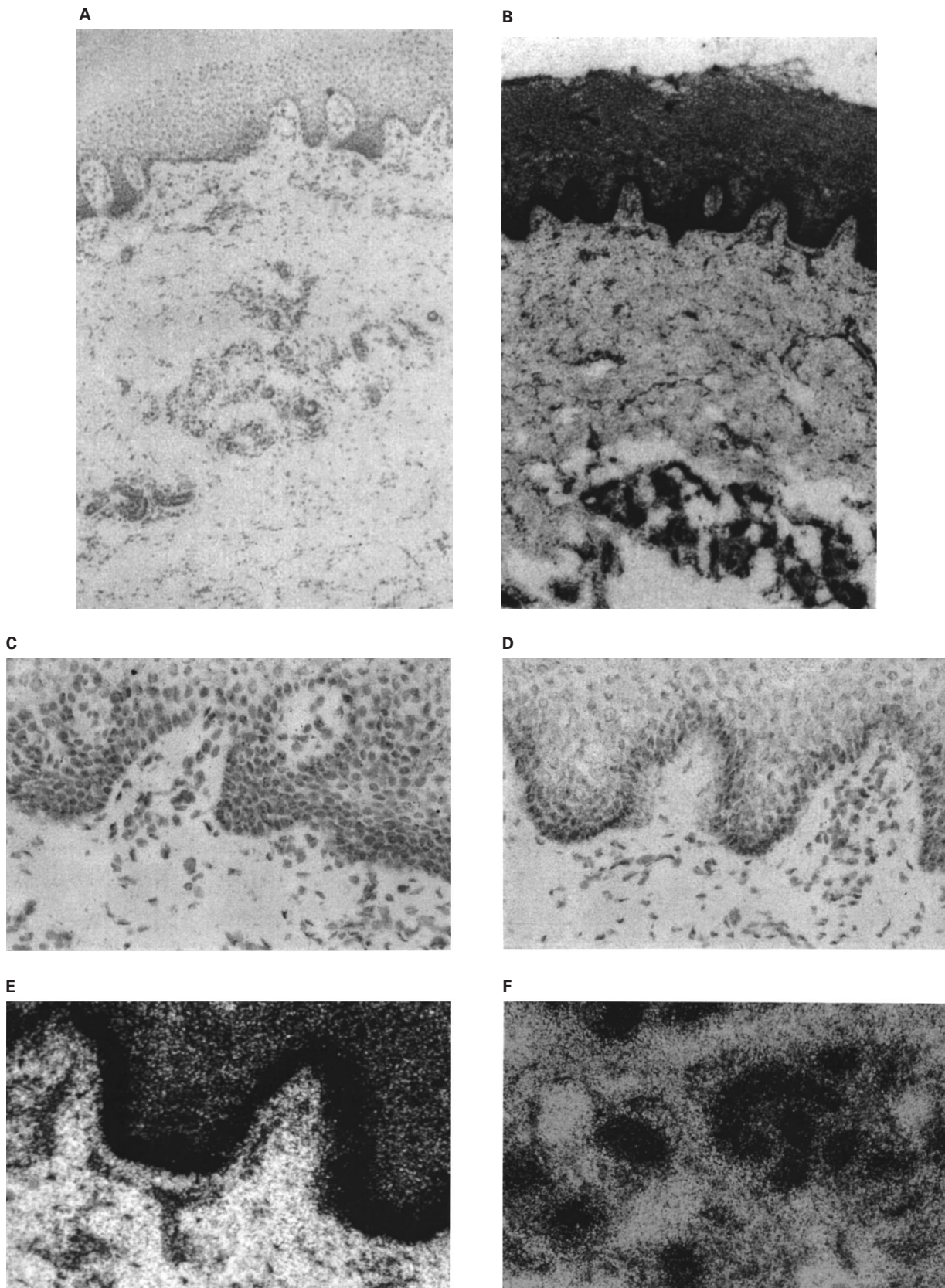


Figure 2 *In situ* localization of P2Y₂ receptor transcripts in sections of human skin using ³²P-dUTP-labelled probes. (A) Low power photomicrograph of hybridization with a sense probe (negative control) showing no specific granular localization. (B) Similar section hybridized with an anti-sense probe and exposed for 15 days. Note the localization over the basal (proliferative) layer of the epidermis and in the glandular structures deep in the dermis. (C) High magnification view of a skin section hybridized with a sense probe (negative control) and exposed for 5 days. Note the absence of signal. (D and E) High magnification detail of skin sections hybridized with anti-sense probe and exposed for 5 days and 15 days respectively. Note the intense specific localization of signal over the basal (proliferative) layer of the epidermis. (F) High magnification view showing specific hybridization of the anti-sense probe over sweat glands.

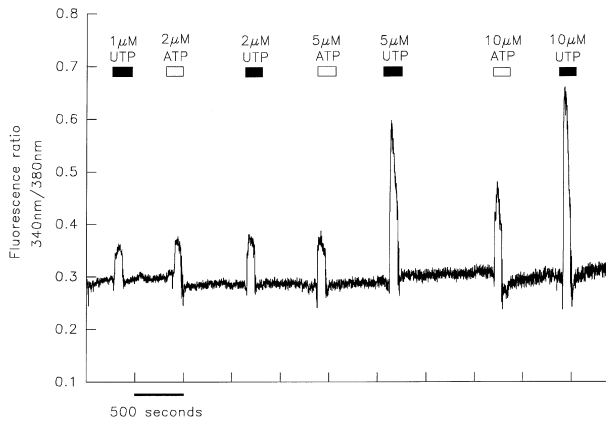


Figure 3 Increases in $[Ca^{2+}]_i$ induced by ATP and UTP in cultured human keratinocytes. Cultured keratinocytes were loaded with fura-2 by incubation with 5 μM fura-2 AM. Groups of 8–10 cells were excited with light at 340 and 380 nm and the emission at 510 nm recorded. The application of ATP or UTP led to increases in $[Ca^{2+}]_i$ as indicated by the change in the ratio of fluorescence at 340 and 380 nm.

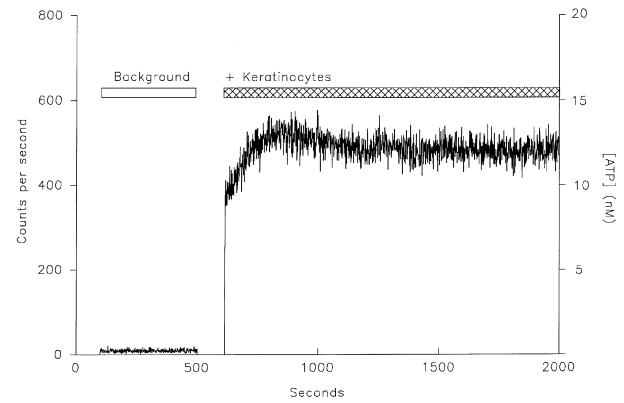


Figure 5 ATP release from human keratinocytes. The chamber of the photon-counting luminometer was filled with firefly luciferase (10 μg ml⁻¹) and luciferin (10 μM) in HEPES buffer and the background signal recorded. Introduction of a coverslip of keratinocytes at confluency resulted in an increase in signal to approximately 500 c.p.s. The signal was calibrated by measuring the luciferase luminescence over a range of ATP concentrations as shown on the right hand axis. This result is typical of five coverslips although considerable variability was seen in the signals recorded from individual coverslips.

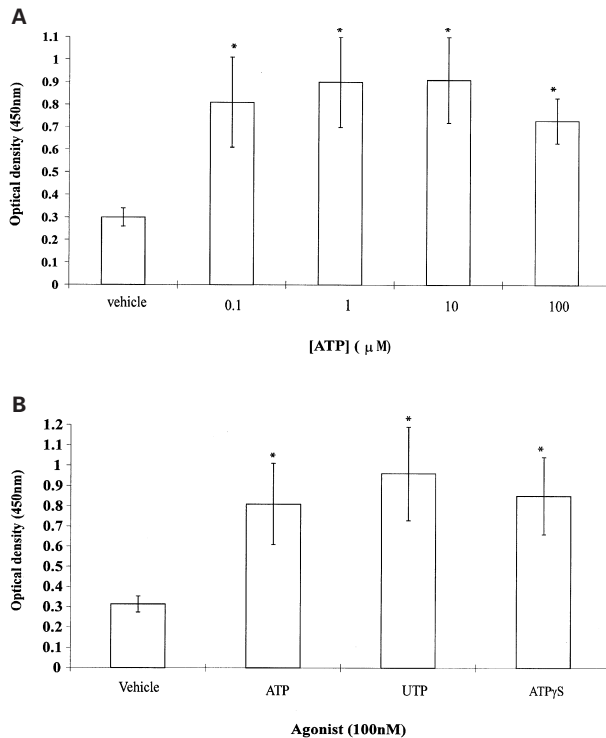


Figure 4 Proliferation of keratinocytes in response to (A) increasing concentrations of ATP and (B) 100 nM ATP, UTP and ATP_γS was assessed using a Biotrak cell proliferation ELISA system. All data are represented as mean ± s.e.mean ($n=16$ wells). * denotes significant difference at $P<0.01$. This result is typical of four separate experiments.

$n=5$). This equates to a concentration of ATP in the chamber of 17 ± 4 nM, although considerable variability in the level of ATP release from individual coverslips was seen (range: 7–30 nM). The plateau in signal was achieved when the rate of release of ATP was equal to the rate of its removal through hydrolysis and the action of extracellular enzymes. The signal remained steady at this level for the length of the recording; no

change was seen even over a period of 50 min. If extreme care was not taken when introducing keratinocytes into the chamber, a large increase in signal was recorded, which could peak at 2000–3000 c.p.s., and which decayed gradually over a period of 2–3 h (data not shown). This signal may reflect lysis of some cells with concomitant release of intracellular ATP. Alternatively, it may represent the physiological response of cells to the mechanical stresses generated when a coverslip is introduced into the chamber.

Discussion

In these studies we have described the expression of P2Y₂ receptor mRNA transcripts in cultured human keratinocytes. P2Y₂ receptor expression has been shown to be up-regulated in response to culture conditions (Turner *et al.*, 1997). However, *in situ* hybridization studies presented here clearly demonstrate that transcripts for these receptors are present in the native tissue. These studies illustrate a striking pattern of localization of transcripts to the actively-proliferating basal cells of the epidermis. P2Y₂ receptor transcripts were also detected in the outer, more differentiated layers of the epidermis, although at much lower levels. This suggests that P2Y₂ mRNA receptor expression is down-regulated in a differentiation-dependent manner, and is therefore reminiscent of the P2Y₂ receptor mRNA down-regulation observed during phorbol ester-induced monocytic differentiation of HL60 human promyelocytic leukocytes (Dubyak *et al.*, 1996; Martin *et al.*, 1997).

The most common primary effectors of G-protein-coupled P2Y receptors are the phospholipase C β enzymes; activation of receptors leads to Ins(1,4,5)P₃-mediated release of intracellular Ca²⁺ stores. In keeping with an earlier study on human keratinocytes (Pillai & Bikle, 1992) stimulation with ATP and UTP, equipotent agonists for the P2Y₂ receptor, resulted in increased $[Ca^{2+}]_i$. This suggests that the detected transcripts are translated into functional receptors, although a contribution from other receptors (e.g. P2Y₄ and P2Y₁₁) to the responses to ATP and UTP cannot be excluded.

The intense localization of P2Y₂ mRNA transcripts to the actively-dividing basal layer suggests a role for nucleotides as

regulators of epidermal proliferation. Keratinocytes cultured in MCDB153 medium, supplemented as described above, proliferate at near maximal rate and further stimulation of the growth rate will not readily be observed. In order then to study the effect of nucleotide stimulation on keratinocyte growth, the rate of proliferation must first be slowed. This was achieved by the addition of 10% FCS, which slows proliferation and promotes differentiation of cells. The inhibitory effect of serum has been noted previously and has been attributed, at least in part, to the high concentrations of TGF β it contains (Sharpe & Fisher, 1990). Under these conditions then, stimulation with ATP and UTP led to a significant increase in proliferation, thus over-riding the inhibitory effects of factors present in serum. However, this response to ATP appears to be independent of [Ca²⁺]_i, as proliferation was seen at concentrations lower than the threshold for [Ca²⁺]_i mobilization. This may reflect involvement of alternative signalling pathways in mediating the ATP-stimulated proliferation. Indeed P2Y₂ occupation has recently been shown to lead to activation of the p42/p44 MAPK cascade which is associated with proliferation (Boarder & Hourani, 1998). Mitogenic effects of ATP have been reported previously in a number of cell types including aortic smooth muscle cells (Wang *et al.*, 1992; Harper *et al.*, 1998), mesangial cells (Schulze-Lohoff *et al.*, 1992), Swiss 3T3 and 3T6 fibroblasts (Huang *et al.*, 1989) and significantly in primary human keratinocytes (Pillai & Bikle, 1992).

In vivo, keratinocytes may be exposed to high levels of ATP and UTP following injury to the skin when nucleotides are released from dead or damaged tissue, and from platelets. The resulting proliferation of cells in the basal layer would promote healing of an epidermal wound by replacing damaged epidermis. Growth factors, including TGF α which is known to stimulate keratinocyte proliferation, are released along with nucleotides upon degranulation of platelets at the site of an injury. ATP has been shown to interact synergistically with growth factors to promote cell proliferation in a range of cell types including a mouse keratinocyte-derived cell line, BALB/MK (Wang *et al.*, 1990).

In partial epidermal loss, proliferation of the basal keratinocytes re-forms the full epidermis. In deeper injury, new epidermal growth occurs from the hair follicles where preserved, or from the wound edges. The dense localization of P2Y₂ mRNA transcripts to the hair follicles reported here may explain the ability of the skin to regenerate from these regions; extracellular nucleotides acting at the P2Y₂ receptors would stimulate proliferation of basal cells within the inner root sheath of the follicle. High levels of P2Y₂ mRNA were also detected in the sweat glands, consistent with an established role for these receptors in regulating ion conductance into sweat (Ko *et al.*, 1997; Wilson *et al.*, 1996).

Luciferase/luciferin experiments demonstrate that under static conditions keratinocytes are releasing ATP. The amount of ATP released into the 300 μ l volume of the chamber gave rise to an ATP concentration of approximately 17 nM. Released into a restricted volume at the cell surface, ATP may reach sufficient concentrations to activate P2Y₂ receptors

and hence stimulate proliferation of keratinocytes. The stimulation of ATP release by extracellular ATP has been demonstrated in endothelial cells (Bodin & Burnstock, 1996), and thus the basal release recorded here may reflect ATP-induced ATP release. Interestingly, serum contains high concentrations of enzymes which hydrolyze ATP (Suter *et al.*, 1991). If released ATP contributes to the proliferation of keratinocytes in culture, the inhibitory effects of serum on proliferation may be partly explained by the removal of ATP, and hence the proliferative stimulus.

Release of ATP has been demonstrated from a number of cell types in response to mechanical stimulation (Milner *et al.*, 1990; Schlosser *et al.*, 1996; Grygorczyk & Hanrahan, 1997). Indeed, the large signal observed when introducing a coverslip into the chamber without exercising extreme care may reflect the response of keratinocytes to mechanical stress. In skin, mechanical forces of pressure and shearing are transmitted to the basal layer and basement membrane (Kennedy, 1998). Repeated shearing forces acting on an area of skin results in thickening of the area. This physiological response may be mediated by ATP, released in response to shearing forces. Recently, release of UTP has been demonstrated from 1321N1 astrocytoma cells (Lazarowski *et al.*, 1997). If UTP is similarly released from keratinocytes in response to mechanical stimulation, this nucleotide may act in concert with ATP to regulate proliferation.

It is likely that keratinocytes express other P2Y subtypes in addition to the P2Y₂ receptor, possibly also in a differentiation-dependent manner. Indeed, Pillai & Bikle (1992) reported that cultured keratinocytes respond to ADP with increased [Ca²⁺]_i; this nucleotide is not an agonist for the P2Y₂ receptor, but acts at both the P2Y₁ and P2Y₆ subtypes (see Boarder & Hourani, 1998). Further expression and functional studies are required to determine the full complement of P2 receptors expressed by keratinocytes.

In conclusion, we have described the expression and involvement of P2Y₂ receptors in the control of keratinocyte proliferation. ATP and UTP, released in the vicinity of a wound from damaged cells or as a result of the inflammatory response, may act to promote keratinocyte proliferation and inhibit differentiation through activation of P2Y₂ receptors. This mechanism may be responsible for epidermal thickening due to repeated minor trauma or shearing forces if, as seems likely, keratinocytes release ATP (and possibly also UTP) in response to mechanical stimulation. Agonists for the P2Y₂ receptor thus represent potential therapeutic agents for the enhancement of wound healing, whilst antagonists may be useful in the treatment of pathologies associated with hyperproliferation of keratinocytes, including psoriasis. In addition, the significant concentrations of ATP released by keratinocytes under static conditions, indicate that ATP may act as an important positive growth regulator.

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