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Review

Long-term (trophic) purinergic signalling: purinoceptors control cell proliferation, differentiation and death

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The purinergic signalling system, which uses purines and pyrimidines as chemical transmitters, and purinoceptors as effectors, is deeply rooted in evolution and development and is a pivotal factor in cell communication. The ATP and its derivatives function as a 'danger signal' in the most primitive forms of life. Purinoceptors are extraordinarily widely distributed in all cell types and tissues and they are involved in the regulation of an even more extraordinary number of biological processes. In addition to fast purinergic signalling in neurotransmission, neuromodulation and secretion, there is long-term (trophic) purinergic signalling involving cell proliferation, differentiation, motility and death in the development and regeneration of most systems of the body. In this article, we focus on the latter in the immune/defence system, in stratified epithelia in visceral organs and skin, embryological development, bone formation and resorption, as well as in cancer.
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ATP – The Universal Intercellular Signalling Molecule

The molecule of adenosine 5′-triphosphate or ATP was discovered 80 years ago simultaneously in Heidelberg and Boston by Lohman,[1] Fiske and SubbaRow.[2] Very soon afterwards, the central role of ATP in cell energetics was fully appreciated.[3] In fact, the role of ATP in living matter is unique and without ATP we, in all probability, would not witness life in its present forms.

Indeed, the life forms, which we know on earth, are built around the genetic code that is stored in the relatively simple molecules of DNA and RNA, composed from the purine adenine and the pyrimidines, guanine, uracil and thymine. The purines and pyrimidines, as well as ATP and GTP, most likely appeared in the prebiotic period, with adenine derivatives being preferentially synthesised as a result of purely thermal reactions.[4,5] Very early in evolution, ATP was chosen as an energy substrate, thus shaping the metabolism of all forms of life.[6] The preponderance of ATP stimulated the evolution of enzymes with preferential binding properties, and adenine nucleotides began to be used in various intracellular signalling cascades, such as, for example, the cAMP cascade.[7] At the very same time, ATP probably became the first extracellular signalling molecule, because of its sheer availability. Indeed, as every cell contained high concentrations of ATP, cell damage inevitably results in the appearance of ATP gradients in the surrounding milieu, which thus became a universal 'danger' signal. As a result, virtually every known cell or single-cell organism has a form of ATP sensitivity, and purinergic signalling represents the primordial form of chemical intercellular signalling.[8]

Although the intracellular signalling and metabolic roles for ATP were established quite early, its importance as an extracellular signalling molecule was acknowledged much later. The possible signalling role for AMP was postulated in 1929[9] and purinergic signalling (i.e., signalling mediated by purines and pyrimidines) was initially suggested in 1970, when ATP was identified as a transmitter in the autonomic nervous system.[10] In 1972, the concept of purinergic nerves and purinergic transmission was formulated,[11] and after initial

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Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine 5′-triphosphate; ATPγS, adenosine 5′-O-(3-thiotriphosphate); BzATP, 2′-&3′-O-(4-benzoyl-benzoyl)-ATP; DNA, deoxyribose nucleic acid; cAMP, cyclic adenosine monophosphate; GTP, guanosine triphosphate; IL, interleukin; InsP3, inosine trisphosphate; LTP, long-term potentiation; 2-MeSADP, 2-methylthio ADP; NGF, nerve growth factor; NO, nitrous oxide; TNF-α, tumour necrosis factor-α; UTP, uridine 5'-triphosphate
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resistance is now widely accepted and has a major role in both the nervous system[12–14] and non-neuronal cells.[15] Initially, the focus was on short-term purinergic signalling in neurotransmission, neuromodulation and secretion, but more recent studies have also established roles in long-term (trophic) signalling in cell proliferation, differentiation, motility and death in development and regeneration.[16,17]

The Omnipresent Purinoceptors

The action of purines and pyrimidines is mediated through an extended family of purinoceptors,[18,19] generally divided into P1 adenosine receptors[20] and P2 receptors for ATP and related nucleotides.[21,22] In the early 1990s, receptors for purines and pyrimidines were cloned and characterised[12,23–25] and it is currently recognised that there are four subtypes of P1 receptors (A_1, A_{2A}, A_{2B} and A_3), seven subtypes of P2X ligand-gated ion channel receptors ($P2X_{1-7}$) and eight subtypes of P2Y G-protein-coupled receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$[26]). P2 receptors appeared very early in evolution, for example, P2X receptors have been found in early prokaryotes;[27–29] despite little sequence homology with later evolutionary forms of the receptors, the functional properties are very much conserved. Similarly, functional metabotropic (P2Y-like) receptors are present in *Protozoa*, and in the most primitive plants in which they regulate numerous vital functions.[8,30]

The P1 and P2Y receptors are classical 7-transmembrane domain receptors, the action of which is mediated through G-proteins and numerous intracellular second messengers, including the cAMP and $InsP_3$ cascades. In addition, some of these receptors are linked to membrane ion channels, thus mediating plasmalemmal ion fluxes and electrophysiological effects. P2X receptors are archetypal ligand-operated cationic channels,[31–33] many of which have an appreciable Ca^{2+} permeability.[34] The P2X channels are assembled (in a homo- or heteromeric manner) from seven subunits, designated as $P2X_1$–$P2X_7$, which determines the variability of their biophysical and pharmacological properties.

Probably because of their ancient origin, the extensive array of purinoceptors has a unique property of being extraordinarily widely distributed throughout living cells and tissues (Table 1). In contrast to all other chemical transmitters, which are, as a rule, segregated to certain cell types and certain functions, the receptors for purines and pyrimidines are found everywhere and as a matter of fact it is almost impossible to find a cell without sensitivity to ATP and its analogues. Indeed, purinoceptors are extensively present in the central nervous system, where they mediate fast synaptic transmission, provide for presynaptic inhibition and regulation of neuronal excitability and are particularly important for signalling in neuronal–glial circuitry, being one of the most important gliotransmitters.[12,13,35,36] In the peripheral nervous system, purinoceptors are involved in sensory[37,38] and autonomic functions.[39] Purinoceptors are present in all peripheral tissues, being involved in the regulation of very different functions in the gut, kidneys, in the cardiovascular and respiratory systems, the immunological system, in blood cells, skin, bones and muscles.[8,15] Furthermore, the purinergic signalling system possesses another unique property – the release of the principal mediator, ATP, initiates the appearance of a trail of derivatives, ADP, AMP and adenosine, to which extracellular ATP rapidly degrades due to the activity of ectonucleotidases that represent an important component of purinergic signalling.[13] As a result of the single event of ATP release from different cell types (which occurs through different concomitant mechanisms, including Ca^{2+}-regulated exocytosis, membrane transporters and diffusion through large-permeability plasmalemmal channels[40,41]), several classes of receptors (sometimes having opposite actions) are activated at effector cells. Finally, purinoceptors are linked to an extensive array of intracellular signalling cascades that underlie their long-term trophic effects (Figure 1).

Purinergic Signalling Controls Biological Defence Systems

The ancient 'damage signaller' characteristic of ATP is evolutionarily conserved in several systems of biological defence. First, ATP functions as one of the main mediators of pain, both in acute and chronic contexts, as indeed $P2X_{2/3}$ receptors are involved in fast pain perception,[38,42] whereas $P2X_4$ and $P2Y_{12}$ receptors assume a leading role in the pathogenesis of neuropathic pain.[43–45]

Second, purinergic agonists regulate the immune response in various tissues. In particular, in the brain and spinal cord, activation of several types of purinoceptors (most notably $P2X_4$, $P2X_7$, $P2Y_6$ and $P2Y_{12}$), which occurs in a highly coordinated temporal sequence, controls motility and activation of microglia, thus being central to the brain immune response.[46,47] In particular, the $P2X_7$ receptor seems to be critical for microglial activation by β-amyloid, being therefore pathologically relevant for Alzheimer's disease.[48] Similarly, purinergic signalling is intimately involved in the activation of the peripheral immune response being not only a stimulator but also a precise regulator of the differentiation and function of immunocompetent cells,[49,50] as well as in driving chemotaxis of neutrophiles, eosinophiles, macrophages and mast cells.[51–53]

Third, ATP and its analogues are directly involved in tissue remodelling in response to injury and have a key role in the regulation of subsequent repair and regeneration. In the nervous system, stimulation of purinoceptors triggers astrogliosis, the generalised response of astrocytes to brain damage, which is characterised by cell proliferation and remodelling of the neural circuitry.[54,55] Reactive astrogliosis is instrumental for both formation of scar and limitation of the brain damaged area (through anisomorphic astrogliosis), as well as for post-insult remodelling and recovery of neural function (by isomorphic astrogliosis). The initial events in astroglial responses to purinergic signallers are often associated with $P2Y_{1/2}$ receptor-mediated Ca^{2+} astroglial signalling in astrocytes,[56,57] which, depending on the context, is instrumental for glial Ca^{2+} excitability or can initiate long-term effects.[58] These trophic/astrogliotic effects of P2 agonists (manifested by proliferative and morphological responses) were found both *in vitro*, in glial cultures, and

Table 1 Functional consequences of genetic deletion of purinoceptors

Receptor subtype	Phenotype[reference]
P1 Adenosine receptors	
A_1	(i) Behavioural phenotype: increased aggression and anxiety; decreased motor activity (ii) Neural phenotype: neuroprotection in newborns; hyperalgesia; no inhibition of synaptic transmission; decreased long-term potentiation; reduced hypoxia-associated decrease in neural activity and recovery after hypoxia (iii) Kidney phenotype: absent tubuloglomerular feedback (iv) Metabolic phenotype: increased insulin and glucagon secretion[103–106]
A_{2A}	(i) Behavioural phenotype: increased aggression and anxiety; decreased exploratory activity; attenuated psychostimulant responses; decreased alcohol sensitivity and withdrawal; decreased amphetamine- and cocaine-induced locomotor response (ii) Neural phenotype: neuroprotection in adults; hypoalgesia (iii) Cardiovascular phenotype: increased blood pressure, heart rate and rennin activity (iv) Haemostatic phenotype: increased platelet aggregation; increased brain damage after focal ischaemia (v) Immunological phenotype: increased inflammatory response (vi) Sensory phenotype: decreased pain threshold[107–111]
A_{2B}	(i) Immunological phenotype: increased histamine release but decreased IL-13 release from mast cells[112,113]
A_3	(i) Behavioural phenotype: increased despair and motor activity (ii) Neural phenotype: reduced neuroprotection; hyperalgesia (iii) Immunological phenotype: attenuated lipopolysaccharide-induced TNFα production and adenosine-induced histamine release from mast cells; decreased neutrophil infiltration of damaged myocardium; decreased local inflammatory response (iii) Cardiovascular phenotype: decreased infarct size following ischaemic–reperfusion injury; loss of adenosine-induced cutaneous vasopermeability; i.v. adenosine produces and greater drop in blood pressure; increased tolerance to ischaemia; lower intraocular pressure[114–119]
P2X receptors	
$P2X_1$	(i) Kidney phenotype: absent tubuloglomerular feedback (ii) Reproductory phenotype: male infertility due to the reduction of sperm in the ejaculate and severely impaired contractility of vas deference (iii) haemostatic phenotype: reduced thrombosis associated with injury of the walls of small arterioles[120–123]
$P2X_2$	(i) Neural phenotype: impaired synaptic facilitation in hippocampal interneurones (ii) Sensory phenotype: impaired taste (iii) Chemosensory phenotype: affected excitation of afferent nerves in carotid body by hypoxia (iv) Gut phenotype: reduced peristalsis of the small intestine[124–126]
$P2X_3$	(i) Sensory phenotype: affected nociception, impaired temperature sensitivity, impaired taste (ii) Urinary phenotype: affected voiding reflex[39,125,127–129]
$P2X_2$&$P2X_3$	(i) Sensory phenotype: affected nociception, impaired temperature sensitivity, severely impaired taste (ii) Chemosensory phenotype: reduced ventilatory responses to a decrease in the level of inspired O_2[39,125]
$P2X_4$	(i) Neural phenotype: reduced hippocampal LTP (ii) Sensory phenotype: reduced chronic pain (both inflammatory and neuropathic) (ii) Vascular phenotype: impaired flow-sensitivity of blood vessels; decrease in NO production by endothelial cells, decreased vasodilatation, higher blood pressure[130,131]
$P2X_7$	(i) Immunological phenotype: impaired immune response (ii) Sensory phenotype: reduced inflammatory and neuropathic chronic pain (iii) Exocrine phenotype: impaired saliva production (iv) Bone phenotype: abnormal bone formation and resorption[25,132–134]
P2Y receptors	
$P2Y_1$	(i) Haemostatic phenotype: mildly prolonged bleeding times (ii) Metabolic phenotype: increases systemic glucose levels[21,135]
$P2Y_2$	(i) Epithelial phenotype: abnormal secretion (ii) Bone phenotype: inhibited bone formation[88,136]
$P2Y_4$	(i) Epithelial phenotype: abnormal secretion[21,137]
$P2Y_6$	(i) Immunological phenotype: UDP-induced IL-6 and macrophage-inflammatory protein-2 release to lipopolysaccharide and macrophage UDP-induced inositol phosphate production are lost (ii) Cardiovascular phenotype: loss of endothelium-dependent UDP vasodilation[138]
$P2Y_{12}$	(i) Haemostatic phenotype: prolonged bleeding time, inhibition of platelet aggregation to ADP, and resistance to arterial thrombosis[139]

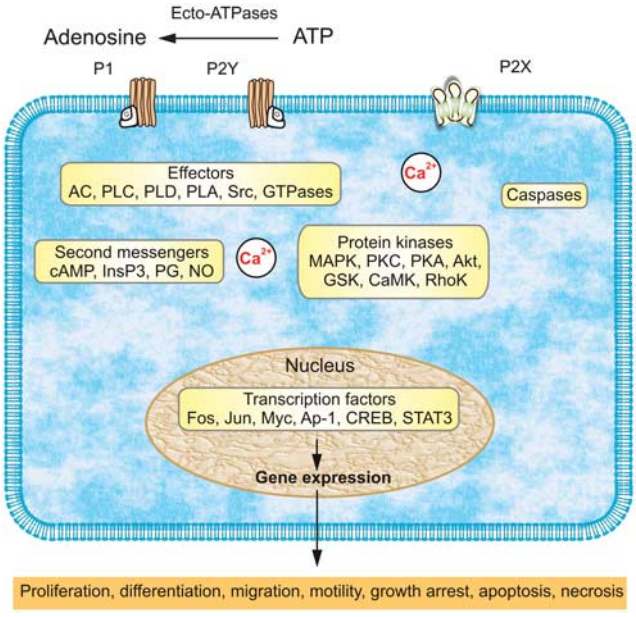

Figure 1 Overview of purinergic signalling mechanisms that regulate long-term, trophic effects. Extracellular nucleotides and nucleosides bind to purinoceptors coupled to signal-transducing effector molecules. Activation of effectors leads to generation of second messengers and/or stimulation of protein kinases that regulate expression of genes needed for long-term, trophic actions. Trophic action of P2X receptors can be mediated by increases in cytosolic Ca^{2+} concentration; activation of $P2X_7$ receptors can also be coupled to protein kinase cascades and caspases that can mediate proliferation and apoptosis. Cell-specific and/or receptor subtype-specific differences are likely to account for variations in signalling pathways and functional outcomes. It should be noted that the list of elements is not meant to be all-inclusive. Other protein kinases, for example, MEK and PI3K, are upstream of the listed kinases involved in purinergic signalling, whereas others are downstream, for example, p70S6K. In addition, dashed arrows indicate that not all listed elements are activated by the upstream component, for example, not all P1 receptors are coupled to all listed effectors. AC, adenylyl cyclase; AP-1, activator protein-1; CaMK, calcium/calmodulin protein kinase; CREB, cAMP response element binding protein; DG, diacylglycerol; GSK, glycogen synthase kinase; $InsP_3$, inositol trisphosphate; MAPKs, mitogen-activated protein kinases (including extracellular signal-regulated protein kinase (ERK), p38 MAPK, and stress-activated protein kinase (SAPK)/c-Jun NH2-terminal kinase (JNK)); MEK, MAPK/ERK kinase; NO, nitric oxide; PG, prostaglandin; PI3K, phosphoinositide 3-kinase; PLC, phosphatidylinositol-specific phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PLD, phospholipase D; PLA, phospholipase A; STAT3, signal transducer and activator of transcription-3 (based on Figure 11 from Burnstock[12] with permission from the American Physiological Society)

in vivo, in nucleus accumbens of rats.[17,59–61] Similarly, purinergic signalling has a fundamental role in remodelling and healing of lesions in other tissues, including skin and bone. In the next part of this review, we will focus on the long-term trophic roles of purines and pyrimidines in cell proliferation, differentiation and death in the turnover of epithelial cells in skin and in cells lining visceral organs, in restenosis, embryological development, bone formation and resorption and cancer.

Stratified Squamous Epithelia

Stratified squamous epithelia in several sites, including two non-keratinised cell types, namely, rat cornea and oesophagus, and four keratinised cell types, soft palate, foot pad skin, vagina and tongue, showed heavy immunostaining of the $P2X_5$ receptor associated with cell differentiation in spinous and granular cell layers, but not in basal cuboidal or keratinised outer layers. In contrast, there was heavy immunostaining of $P2X_7$ receptors in the outer keratinised layer, perhaps associated with apoptotic cell death.[62]

Rapid turnover rates are found in the epithelium of the small intestine. The crypts contain undifferentiated progenitor cells from which almost all other epithelial cell types, including goblet cells and enterocytes, arise. The differentiated cells glide towards the villus tips where they are finally ejected into the lumen. In the rat, this whole process takes 3–4 days.[63] $P2X_5$ receptors are expressed on the narrow 'stem' of villus goblet cells, whereas $P2X_7$ receptor immunoreactivity is seen only on the membranes of enterocytes and goblet cells at the tip of the villus, where cells undergo apoptosis, before shedding into the lumen.[64]

Skin

The expression of $P2X_5$, $P2X_7$ and $P2Y_2$ receptor subtypes was studied in healthy human epidermal keratinocyes in relation to markers for proliferation (PCNA and Ki-67), differentiation (cytokeratin KIO and involucrin) and apoptosis (TUNEL and anti-caspase-3).[65] It was shown that $P2Y_1$ and $P2Y_2$ receptors were immunoreactive in basal and parabasal keratinocytes, $P2X_5$ receptor immunostaining within the stratum spinosum and $P2X_7$ receptor immunostaining in the stratum corneum, associated with cell proliferation, differentiation and apoptotic cell death, respectively (Figure 2). Functional experiments on cultured keratinocytes were also carried out in this study, which showed the following: an increase in cell numbers in response to the $P2Y_1$ receptor agonist 2-methylthio ADP and the $P2Y_2$ receptor agonist UTP; and a significant decrease in cell numbers with the $P2X_5$ receptor agonist ATPγS and the $P2X_7$ receptor agonist BzATP (Figure 3). Later studies from this group examined the purinergic signalling profile in human fetal epidermis.[66] They showed $P2Y_1$ receptors in the basal layer of the developing epidermis associated with proliferation; $P2X_5$ receptors predominantly in the basal and intermediate layers associated with differentiation; and $P2X_7$ receptors in the periderm associated with apoptotic cell death. $P2Y_2$ receptors were also found in the periderm, where they may have a role in chloride and fluid secretion into the amniotic fluid.

In a study on purinergic signalling in wound healing[67] in regenerating epidermis of denervated wounds, $P2Y_1$ receptor protein expression was significantly increased in keratinocytes, whereas $P2Y_2$ receptor protein expression was significantly decreased. However, NGF treatment of denervated wounds reduced the expression of $P2Y_1$ receptors and enhanced the expression of $P2Y_2$ receptors. In innervated wounds, NGF treatment enhanced both $P2X_5$ and $P2Y_1$ receptor proteins in keratinocytes. $P2X_7$ receptors were absent in all experimental wound healing processes.

$P2X_5$ and $P2X_7$ receptors were shown to be present in human warts and CIN612 organotypic raft cultures of human papillomavirus-infected keratinocytes and may provide a novel approach for the treatment of warts.[68] $P2Y_1$, $P2Y_2$ and $P2X_5$ receptors are expressed on human anagen hair

follicles, with $P2Y_1$ receptors present in proliferating cells in the outer root sheath and bulb, whereas $P2X_5$ receptors were present on the inner and outer root sheaths and medulla, and were associated with differentiation.[65] $P2Y_2$ receptors were found in living cells at the edge of the cortex/medulla. $P2X_7$ receptors were not present.

Cancer

There were early reports of the beneficial effect of ATP in the treatment of cancer,[69] and analysis of the purinergic receptor subtypes involved in the development of tumours in the prostate,[70] bladder,[71] melanoma,[72,73] breast[74–76] and other organs has been described (see review by White and Burnstock[77]). Again, it was shown that $P2Y_1$ and $P2Y_2$ receptors were expressed and involved in cell proliferation, $P2X_5$ receptors were involved in differentiation (and were therefore antiproliferative) and $P2X_7$ receptors were involved in cell death (Figure 4). Human melanomas express functional $P2X_7$ receptors that mediate the apoptotic functions of ATP,[72] whereas $P2Y_1$ and $P2Y_2$ receptor agonists caused a decrease and increase in cell numbers, respectively.[73] In human squamous cell carcinoma, $P2Y_2$, $P2X_5$ and $P2X_7$ receptors seem to be associated with proliferation, differentiation and cell death, respectively.[78]

In high-grade bladder cancer, using the HT-1376 cell line, $P2X_5$ and $P2Y_{11}$ receptors were shown to mediate the antineoplastic effects of ATP, whereas $P2X_7$ receptors mediated apoptotic cell death.[71] Similar results are described for cell lines of hormone-refractory prostate cancer[79] and ATP was shown to reduce the *in vivo* growth of advanced hormone-refractory prostate cancer implanted into mice.[80]

Finally, several clinical trials have demonstrated that systemic administration of ATP may have beneficial effects (prolongation of survival and reduced cachexia) in inoperable lung cancer patients (for details see White and Burnstock[77]).

Long-Term Purinergic Signalling in Embryological Development

The transient appearance of P2 receptors during both embryological and postnatal development suggests that ATP is involved in the sequential proliferation, differentiation, motility and death of cells during the complex events involved in development[12,81,82]. For example, a novel $P2Y_8$ receptor was cloned in *Xenopus* embryos and was shown to be transiently expressed in the neural plate and tube from stages 13–18 and again at stage 28, when secondary neurulation occurs in the tail bud, suggesting an involvement of this receptor in the development of the nervous system.[83] $P2Y_1$ receptors were transiently expressed in the limb buds of chick

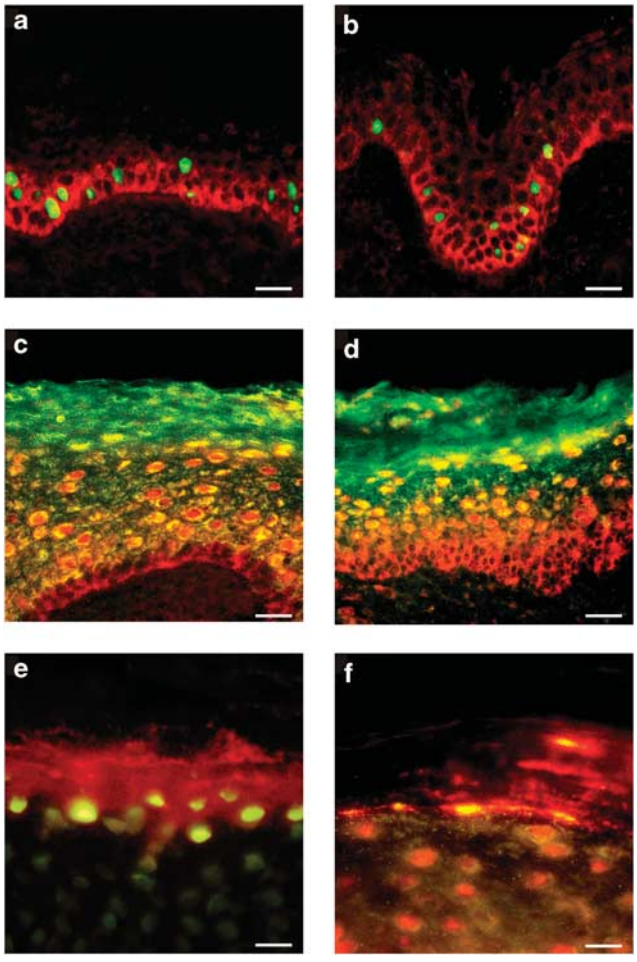

Figure 2 Double labelling of $P2Y_1$ and $P2Y_2$ receptors with markers of proliferation shows colocalisation within a sub-population of basal and parabasal keratinocytes. Double labelling of $P2X_5$ receptors with markers of differentiated keratinocytes shows colocalisation within the stratum spinosum, and double labelling of $P2X_7$ receptors with markers of apoptosis in human leg skin shows colocalisation within the stratum corneum. (**a**) Ki-67 immunolabelling (a marker for proliferation) stained the nuclei (green) of a sub-population 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 μm. (**b**) PCNA immunolabelling (a marker for proliferation) stained the nuclei (green) of a sub-population 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 μm. (**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 colocalisation 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 labelled differentiated keratinocytes, even in dying cells. Scale bar 30 μm. (**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 mid-granular 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 labelled with involucrin, which is a late marker of keratinocyte differentiation. Scale bar 30 μm. (**e**) TUNEL (green) labelled 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 μm. (**f**) Anti-caspase-3 (green) colocalised with areas of $P2X_7$ receptor immunostaining (red) both at the junction of the stratum granulosum and within the stratum corneum. Areas of colocalisation were yellow. Note that the differentiating keratinocytes in the upper stratum granulosum were also positive for anti-caspase-3. Scale bar 15 μm (reproduced with permission from Greig *et al.*[140])

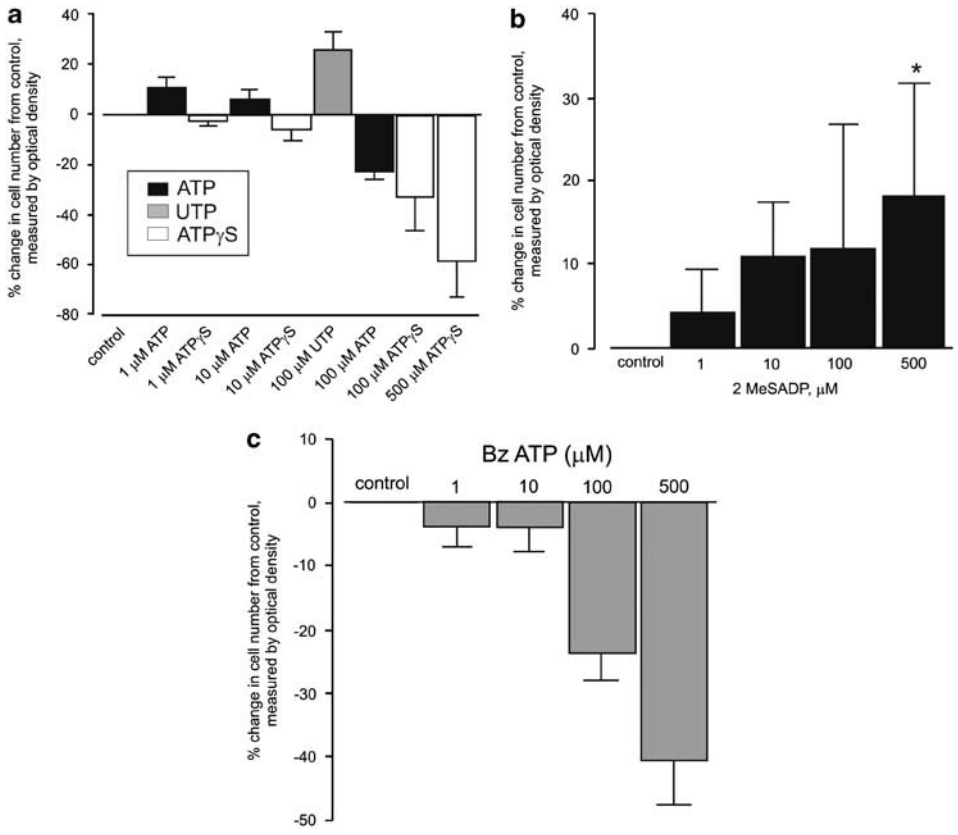

Figure 3 At 48 h after application of drugs to primary human keratinocyte cultures. (**a**) ATP (1–10 μM) and UTP (100 μM) cause an increase in cell number, whereas ATPγS (100–500 μM) and ATP (100 μM) cause a significant decrease. Results represent the mean of eight experiments. *$P<0.001$ compared with that of control. (**b**) 2MeSADP (500 μM) causes a significant increase in cell number. Results represent the mean of eight experiments. *$P<0.05$ compared with that of control. (**c**) BzATP (100–500 μM) causes a significant decrease in cell number. Results represent the mean of nine experiments. *$P<0.001$ compared with that of control. Error bars represent mean ± S.E.M (reproduced with permission from Greig *et al.*[140])

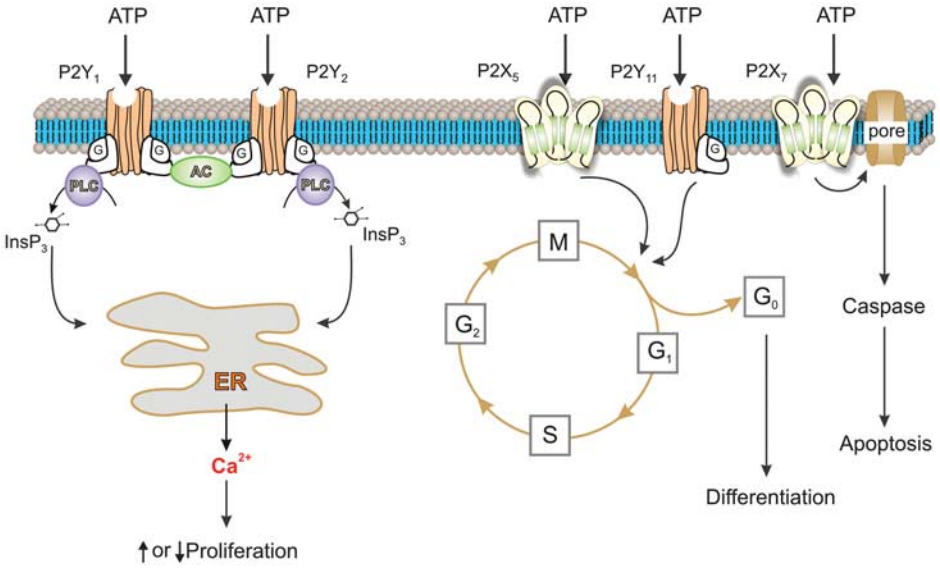

Figure 4 Schematic diagram illustrating the different mechanisms by which P2 receptor subtypes might alter cancer cell function. $P2Y_1$ and $P2Y_2$ receptors could affect the rate of cell proliferation through altering the intracellular levels of cAMP by modulating adenylyl cyclase (AC) or by increasing intracellular calcium levels through the phospholipase C (PLC) pathway. $P2X_5$ and $P2Y_{11}$ receptor activation might switch the cell cycle from proliferation into a state of differentiation. The $P2X_7$ receptor activates the apoptotic caspase enzyme system (redrawn from White and Burnstock[77] with permission from Elsevier)

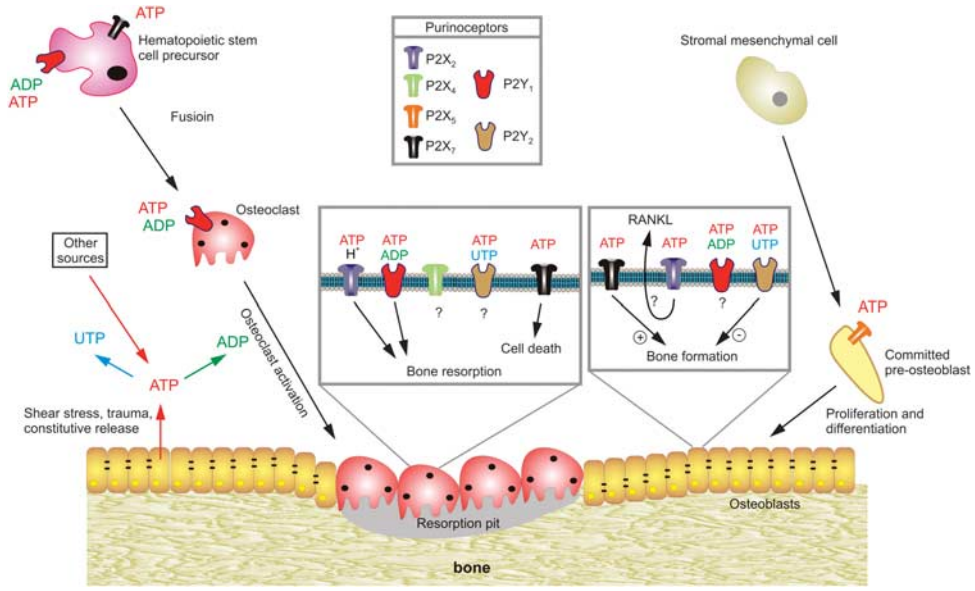

Figure 5 Schematic diagram illustrating the potential functions of extracellular nucleotides and P2 receptors in modulating bone cell function. ATP released from osteoclasts (e.g., through shear stress or constitutively) or from other sources, can be degraded to adenosine 5′-diphosphate (ADP) or converted into uridine 5′-triphosphate (UTP) through ecto-nucleotidases. All three nucleotides can function separately on specific P2 receptor subtypes, as indicated by the colour coding. ATP is a universal agonist, whereas UTP is only active at the $P2Y_2$ receptor and ADP is only active at the $P2Y_1$ receptor. ADP acting on $P2Y_1$ receptors seems to stimulate both the formation (i.e., fusion) of osteoclasts from haematopoietic precursors and the resorptive activity of mature osteoclasts. For the latter, a synergistic action of ATP and protons has been proposed by the $P2X_2$ receptor. ADP could also stimulate resorption indirectly through actions on osteoclasts, which in turn release pro-resorptive factors (e.g., receptor activator of nuclear factor κB ligand, RANKL) ATP at high concentrations might facilitate fusion of osteoclast progenitors through $P2X_7$ receptor pore formation or induce cell death of mature osteoclasts through $P2X_7$ receptors. In osteoblasts, ATP, through $P2X_5$ receptors, might enhance proliferation and/or differentiation. By contrast, UTP, through $P2Y_2$ receptors, is a strong inhibitor of bone formation by osteoblasts. For some receptors (e.g., $P2X_4$ and $P2Y_2$ receptors on osteoclasts or $P2X_2$ receptors on osteoblasts), evidence for expression has been found but their role is still unclear (based on schemes from Hoebertz *et al.*[88])

embryos and were shown to mediate rapid cell proliferation.[84] Changes in expression in P2X receptor subtypes during postnatal development of cerebellum have been described.[85] Transient changes in P2X receptor subtype expression during the development of skeletal muscle have been described.[86] $P2X_5$ receptors were present during the early development of the myotube, followed by $P2X_6$ receptor expression and then, during the development of the neuromuscular junction, $P2X_2$ receptors were expressed. In the chicken retina, ATP-evoked Ca^{2+} transients were strongest as early as E3 and were drastically reduced at E11–13.5.[87] Nucleotide signalling in development probably involves a cross-talk between several other signalling pathways, including growth factors, cytokines and extracellular matrix components.[82]

Trophic Purinergic Signalling in Bone Formation and Resorption

Activation of $P2Y_1$ receptors by ADP stimulates osteoclast activity and bone resorption (Figure 5), whereas ATP and UTP signalling through $P2Y_2$ receptors in osteoblasts inhibits bone growth and mineralisation.[88] More recently, $P2X_7$ receptors have been shown to have trophic regulatory roles in bone formation and resorption.[89,90] $P2X_7$ receptor activation of osteoblasts enhances differentiation and bone formation,[91] whereas $P2X_7$ receptor activation of osteoclasts results in apoptosis and bone resorption.[92–94]

Long-Term Trophic Actions of Purines and Pyrimidines in the Pathogenesis of Atherosclerosis and Post-Angioplasty Restenosis

ATP and UTP, acting through $P2Y_2$ receptors, cause proliferation of vascular smooth muscle cells and proliferation of endothelial cells through $P2Y_1$ receptors. Adenosine acting through A_2 receptors inhibits smooth muscle proliferation but stimulates endothelial cell proliferation.[95] The increase in vascular smooth muscle and endothelial cells in both atherosclerosis and hypertension may be mediated by the trophic actions of purines and pyrimidines released from nerves and endothelial cells[96–98] and in post-angioplasty restenosis.[99] $P2Y_4$ receptors seem to be regulators of angiogenesis.[100] ATP increases DNA synthesis and migration of vascular endothelial cells in vasa vasorum in diseased pulmonary vessels.[101] Diabetic patients express microvascular disease characterised by an increased wall–lumen ratio, mainly because of an increase in vascular smooth muscle cells, and have higher rates of restenosis after angioplasty. High glucose-induced release of ATP exerts an effect on P2Y receptors to stimulate vascular smooth muscle cell growth.[102]

Conclusions

Purinergic signalling, mediated by ATP, related nucleotides and adenosine, operates in all types of tissues and cells. Purinergic agonists mediate fast cell signalling, and exert

numerous long-term trophic effects, involved in regulation of cell replication, proliferation, differentiation and death. It is hoped that there will be further exploration of the roles of this primitive and widespread signalling system in cell biology.

Conflict of interest

The authors declare no conflict of interest.

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