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## Short- and long-term (trophic) purinergic signalling

Geoffrey Burnstock

*Professor Geoffrey Burnstock, Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London, NW3 2PF. Tel: +44 20 7830 2948; Fax: +44 20 7830 2949; E-mail: [g.burnstock@ucl.ac.uk](mailto:g.burnstock@ucl.ac.uk) and Department of Pharmacology and Therapeutics, The University of Melbourne, Australia*

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### Main Text

#### Summary

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 addition to fast purinergic signalling in neurotransmission, neuromodulation and secretion. [It is not always easy to distinguish between short- and long-term signalling. For example, ATP can sometimes act as a short-term trigger for long-term trophic events that become evident days or even weeks after the original challenge.](#) Examples of short-term purinergic signalling during sympathetic, parasympathetic and enteric neuromuscular transmission and in synaptic transmission in ganglia and in the central nervous system are described, as well as in neuromodulation and secretion. Long-term trophic signalling is described in the immune/defence system, stratified epithelia in visceral organs and skin, embryological development, bone formation and resorption and in cancer. It is likely that the increase in intracellular  $\text{Ca}^{2+}$  in response to [both P2X and P2Y](#) purinoceptor activation participates in many short- and long-term physiological effects.

#### Introduction

The proposal that purine nucleotides are extracellular signalling molecules, as well as an intracellular energy source was first reported by [1]. Then in 1970, adenosine 5'-triphosphate (ATP) was shown to be a transmitter in autonomic neuromuscular transmission [2] and in a later review the term 'purinergic' signalling introduced [3]. This concept was not accepted by many for the next 20 years. Separate purinergic receptor families, P1 (adenosine) and P2 (ATP/adenosine 5'-diphosphate (ADP)) were described in 1978 [4], but the turning point in acceptance of purinergic signalling came after the receptors for purines and pyrimidines were cloned and characterised in the early 1990's (see [5]). Four P1 receptor subtypes ( $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ ,  $A_3$ ), seven P2X ion channel receptors (P2X1-7) and eight G protein-coupled receptors (P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub>, P2Y<sub>13</sub>, P2Y<sub>14</sub>) are currently recognised [6]. Activation of P2 receptors leads to increase in intracellular  $\text{Ca}^{2+}$ ; from extracellular

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Author for correspondence ([g.burnstock@ucl.ac.uk](mailto:g.burnstock@ucl.ac.uk)).

Present address: Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London, NW3 2PF

sources for P2X receptors and from intracellular sites for P2Y receptors. Perhaps because of their ancient origin, the array of purinoceptor subtypes has a unique property of being extraordinarily widely distributed throughout living cells and tissues [7]. 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 it is almost impossible to find a cell without sensitivity to ATP and its analogues. There has been a rapid expansion of the field since 1995 (see [8,9]).

### Short-Term Purinergic Signalling

ATP was shown to be a transmitter released from non-adrenergic, non-cholinergic (NANC) nerves to produce short-term purinergic signalling from inhibitory enteric nerves in the guinea pig taenia coli [2] and from excitatory parasympathetic nerves in the urinary bladder [10]. Short-term purinergic signalling was demonstrated when ATP was identified as a cotransmitter with noradrenaline in sympathetic nerves in the taenia coli [11], cat nictitating membrane [12], vas deferens [13,14] and in blood vessels (see [15,16]). ATP is also a cotransmitter with acetylcholine in motor nerves supplying developing skeletal muscle (see [17]), bladder [18] and carotid body [19] and in sensory motor nerves with substance P and calcitonin gene-related peptide [20]. Later ATP was shown to be a cotransmitter, mediating short-term purinergic signalling, in neurons in the central nervous system (CNS) [8,21,22]. The involvement of short-term purinergic signalling in the control of vascular tone is illustrated in Figure 1. Purinergic synaptic transmission between nerves was shown in the coeliac ganglion [23,24] and the medial habenula in the brain [25]. ATP released during synaptic transmission can activate astrocyte receptors, which in turn initiate  $Ca^{2+}$  signals and propagate  $Ca^{2+}$  waves in the astroglial networks via the activation of P2Y receptors and the diffusion of inositol trisphosphate ( $IP_3$ ) through the gap junctions [26]. Ionotropic P2X receptors are responsible for rapid astrocytic signalling, while metabotropic P2Y receptors mediate long-term effects (see [27]).

Short-term signalling involved in prejunctional neuromodulation via both P1 and P2 receptors was also recognised in both peripheral (see [20,28,29]) and central nervous systems [30,31].

Purinoceptors are extensively present in the CNS, where they mediate neuronal excitability and they are important for signalling in neuronal-glia circuitry, being an important gliotransmitter [8,32,33].

Purinoceptors are present in all peripheral tissues, being involved in short-term as well as long-term regulation of different functions, including neuromuscular and synaptic transmission and secretion in gut [34], and secretion in kidneys [35], liver [36] and reproductive systems [37]. In vascular [16] and respiratory systems it mediates reflex activities via activation of sensory nerves [38]. Activation of purinoceptors can mediate rapid responses in the immunological system [39], in

blood cells [40], skin [41], bones and muscles [42], urinary tract [43] and heart [44]. Short-term purinergic signalling also takes place in secretion from endocrine (see [45]) and non-endocrine cells [34]. P2X3 and P2X2/3 receptors are involved in nociception (see [46]). Purinergic signalling via P2Y<sub>12</sub> receptors is well established for control of platelet aggregation.

### Long-Term (Trophic) Purinergic Signalling

ATP and its analogues are involved in tissue remodelling in response to injury and play a key role in the regulation of subsequent repair and regeneration (see [47]). Stimulation of purinoceptors triggers astrogliosis, the generalised response of astrocytes to brain damage, involving cell proliferation and remodelling of the neural circuitry [48,49]. Reactive astrogliosis is instrumental for both formation of scar and limitation of brain damaged area (through anisomorphic astrogliosis), as well as for the post-insult remodelling and recovery of neural function (by isomorphic astrogliosis). The initial events in the responses of astroglia to purinergic signalling are instrumental for glial Ca<sup>2+</sup> excitability or can initiate long-term effects [50]. [For reactive astrogliosis, not only was increase in intracellular calcium absolutely necessary, but ATP was also shown to be one of the key factors involved in its initiation via the activation of P2Y G protein-coupled receptors linked to phospholipase C and IP<sub>3</sub> \[51\].](#) These trophic/astrogliotic proliferative effects of P2 agonists were found both *in vitro*, in glial cultures and *in vivo*, in nucleus accumbens of rats [524-554]. P2X receptors mediate long-term potentiation in the hippocampus [565]. ~~Long-term purinergic signalling also occurs in chronic inflammation and neuropathic pain [56].~~ Activation of P2X receptors can have multiple effects on synaptic plasticity, either inhibiting or facilitating the long-term changes of synaptic strength depending on the physiological context [577]. [Long-term purinergic signalling also occurs in chronic inflammation and neuropathic pain \[58\].](#)

### Embryological development

P2 receptor subtypes appear transiently during both embryological and postnatal development suggesting that ATP is involved in the sequential proliferation, differentiation, motility and death of cells during the complex events involved [8,58,59,60]. For example, in *Xenopus* embryos a novel P2Y<sub>8</sub> receptor was cloned and 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 [610]. Transient expression of P2Y<sub>1</sub> receptors in the limb buds of chick embryos mediates rapid cell proliferation [624]. During postnatal development of cerebellum [632] and skeletal muscle [643] changes in expression of P2X receptor subtypes have been described. [During early development of the myotube P2X5 receptors were present, followed by P2X6 receptor expression and then P2X2](#)

~~receptors were expressed during the development of the neuromuscular junction. ATP-evoked  $\text{Ca}^{2+}$  transients in the chicken retina were strongest as early as E3, but were drastically reduced at E11-13.5 [64]. Purinergic signalling in development is likely to involve cross-talk between several other signalling pathways, including growth factors, cytokines and extracellular matrix components [5960]. During early development of the myotube P2X5 receptors were present, followed by P2X6 receptor expression and then P2X2 receptors were expressed during the development of the neuromuscular junction. ATP-evoked  $\text{Ca}^{2+}$  transients in the chicken retina were strongest as early as E3, but were drastically reduced at E11-13.5 [65]. Similar mechanisms are involved in adult neurogenesis [66].~~

### Bone formation and resorption

Osteoclast activity and bone resorption are activated by ADP via P2Y<sub>1</sub> receptors, whereas ATP and uridine 5'-triphosphate (UTP) signalling via P2Y<sub>2</sub> receptors in osteoblasts inhibit bone growth and mineralisation (Figure 2) [42,675,686]. P2X7 receptors have trophic regulatory roles in bone formation and resorption [697,7068]. Osteoblasts activated by P2X7 receptors show enhanced differentiation and bone formation [6971], whereas P2X7 receptor activation of osteoclasts evokes apoptosis and bone resorption [720-742].

### Vascular remodelling in atherosclerosis and post-angioplasty restenosis

ATP and UTP acting via P2Y<sub>2</sub> receptors cause proliferation of vascular smooth muscle cells. Proliferation of endothelial cells is produced by ADP acting via P2Y<sub>1</sub> receptors. Adenosine via A<sub>2</sub> receptors mediates inhibition of smooth muscle proliferation but stimulation of endothelial cell proliferation (Figure 3) [753]. This suggests that 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 [764-786] and in post-angioplasty restenosis [797]. P2Y<sub>4</sub> receptors appear to be regulators of angiogenesis [780]. DNA synthesis and migration of vascular endothelial cells in vasa vasorum is increased by ATP in diseased pulmonary vessels [7981]. Microvascular disease is characterised by an increased wall-lumen ratio in diabetic patients. This is probably because of an increase in vascular smooth muscle cells leading to higher rates of restenosis after angioplasty. Release of ATP, induced by high glucose, stimulates vascular smooth muscle cell growth via P2Y receptors [820]. An unusual type of long-term purinergic signalling is the evidence that at a critical concentration ATP, acting on both erythrocytes [834] and endothelial cells [842], leads to increase in ATP release into the circulating blood for several hours.

### Skin

Stratified squamous epithelia in rat skin as well as cornea, oesophagus, soft palate, vagina and tongue showed heavy immunostaining of the P2X5 receptor associated with cell differentiation in the spinous and granular cell layers, but not in basal cuboidal outer layers. There was heavy immunostaining of P2X7 receptors in the outer layer, associated with apoptotic cell death [853]. There is rapid turnover of the epithelium of the small intestine. P2X5 receptors are expressed on the narrow 'stem' of villus goblet cells, while P2X7 receptor immunoreactivity is seen only on the membranes of the enterocytes and goblet cells at the tip of the villus, where cells are undergoing apoptosis [864].

P2X5, P2X7, P2Y<sub>1</sub> and P2Y<sub>2</sub> receptor subtype expression was studied in healthy human epidermal keratinocytes in relation to markers for proliferation (PCNA and Ki-67), differentiation (cytokeratin K10 and involucrin) and apoptosis (TUNEL and anticaspase-3) [875]. P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors were immunoreactive in basal and parabasal keratinocytes. Expression of P2X5 receptors within the stratum spinosum and P2X7 receptors in the stratum corneum, was associated with cell differentiation (and subsequent anti-proliferation) and apoptotic cell death, respectively (Figure 4). Functional experiments on cultured keratinocytes showed an increase in cell numbers in response to the P2Y<sub>1</sub> receptor agonist 2-methylthio ADP and the P2Y<sub>2</sub> receptor agonist UTP. In contrast there was a significant decrease in cell numbers with the P2X5 receptor agonist ATP $\gamma$ S and the P2X7 receptor agonist 2'(3')-O-(4-benzoylbenzoyl) BzATP. It was also shown that P2Y<sub>1</sub> receptors in the basal layer of the developing human foetal epidermis were associated with proliferation [886]. P2X5 receptors, predominantly in the basal and intermediate layers, were associated with differentiation, while P2X7 receptors in the periderm were associated with apoptotic cell death.

Purinergic signalling is involved in wound healing. In regenerating epidermis of denervated wounds, P2Y<sub>1</sub> receptor expression was increased in keratinocytes, while P2Y<sub>2</sub> receptor expression was decreased [897]. Nerve growth factor (NGF) treatment of denervated wounds reduced expression of P2Y<sub>1</sub> receptors and increased expression of P2Y<sub>2</sub> receptors. NGF treatment enhanced both P2X5 and P2Y<sub>1</sub> receptors in keratinocytes in innervated wounds. In all experimental wound healing processes, P2X7 receptors were absent.

Human anagen hair follicles express P2Y<sub>1</sub>, P2Y<sub>2</sub> and P2X<sub>5</sub> receptors [9088]. P2Y<sub>1</sub> receptors were present in proliferating cells in the outer root sheath and bulb, while P2X<sub>5</sub> receptors were associated with differentiation of the inner and outer root sheaths and medulla. P2Y<sub>2</sub> receptors were found in cells at the edge of the cortex/medulla, while P2X<sub>7</sub> receptors were not present.

### Cancer

Analysis of the purinergic receptor subtypes involved in the development of tumours in the prostate [891], bladder [920], melanoma [934,942], breast [953-975] and other organs have been described

[986,997]. P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors were expressed and involved in cell proliferation, P2X<sub>5</sub> receptors were involved in differentiation (and were therefore antiproliferative) while P2X<sub>7</sub> receptors were involved in cell death [in many tumours](#) (Figure 5). [However, P2X7 receptors have been shown to mediate both proliferation of cancer cells and apoptotic cell death \(see \[100\]\). It may be that low concentrations of released ATP promote proliferation, while high concentrations lead to cell death.](#) In human melanomas functional P2X<sub>7</sub> receptors are expressed that mediate apoptosis [934], while P2Y<sub>1</sub> and P2Y<sub>2</sub> receptor agonists caused a decrease and increase in cell numbers, respectively [942]. In human squamous cell carcinoma, P2Y<sub>2</sub>, P2X<sub>5</sub> and P2X<sub>7</sub> receptors appear to be associated with proliferation, differentiation and cell death, respectively [10198].

Using the HT-1376 high grade bladder cancer cell line, P2X<sub>5</sub> and P2Y<sub>11</sub> receptors mediated the anti-neoplastic effects of ATP, while P2X<sub>7</sub> receptors mediated apoptotic cell death [920]. Cell lines of hormone-refractory prostate cancer showed similar results [10299]. ATP reduced the *in vivo* growth of advanced hormone-refractory prostate cancer implanted into mice [1030]. Clinical trials have demonstrated that systemic administration of ATP may have beneficial effects (prolongation of survival and reduced cachexia) in lung cancer patients (see [997]).

## [Second Messenger Mechanisms And](#) Transcription Factors Involved In Short- And Long-Term Purinergic Signalling

The second messenger mechanisms involved in short-term purinergic signalling have been analysed in a number of studies for P2X ion channel receptors (see [1044-1085]). [Occupation of both P2X and P2Y receptors leads to increase in intracellular Ca<sup>2+</sup>, P2X receptors from extracellular sources and P2Y receptors from intracellular sources](#) [Calcium ions contribute to P2X channel responses](#) [see 5, 1096]. It was shown that extracellular ATP activates the P2X channel trimeric structure by binding the three intersubunit-binding sites, which leads to conformational rearrangements that are transferred to transmembrane helices linked to ATP-binding domains by  $\beta$  strands [1107]. Coupling of the P2Y receptor subtypes to specific G proteins was initially inferred from indirect evidence from movement of intracellular levels of inositol phosphate, calcium, cyclic AMP (cAMP) and determination of pertussis toxin sensitivity. Direct evidence followed by measuring the effect of ADP and GTP hydrolysis in vesicles reconstituted with P2Y<sub>1</sub> and either G $\alpha_q\beta_1\gamma_2$  or G $\alpha_{11}\beta_1\gamma_2$  [11108]. G protein-coupled P2Y receptors also modulate the activity of voltage-gated ion channels in the cell membrane through the activity of activated G proteins (see [11209] for a detailed analysis).

The transcription factors involved in long-term trophic signalling are more complex, as indicated in Figure 6. A role for calcium influx in cell proliferation has been proposed [1130]. External calcium concentration is important for calcium channel function and it also regulates

calcium sensing receptor activity. Activation of the P2Y<sub>11</sub> receptor by ATP, for example, leads to a rise in cAMP and in IP<sub>3</sub> and cytosolic calcium, whereas activation by UTP was shown to produce calcium mobilization without IP<sub>3</sub> or cAMP increase [1144].

## Conclusions

Trimeric P2X ion channel receptors largely mediate short-term purinergic signalling, although there are examples of P2X receptor-mediated long-term signalling. P1 and P2Y G protein-coupled receptors are predominantly involved in long-term (trophic) purinergic signalling, but there are also examples of mediation of short-term events. Examples of both types of purinergic signalling are explored and the intracellular translational mechanisms involved discussed. Knowledge of the underlying mechanisms involved in both short-term and long-term purinoceptor-mediated signalling will help in the development of purinergic drugs for therapeutic purposes.

## Additional Information

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### Competing Interests

I have no competing interests

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# Figures

## Figure 1.

Short-term (acute) purinergic signalling controlling vascular tone. Schematic illustrating the main receptor subtypes for purine and pyrimidines present in most blood vessels. Perivascular nerves in the adventitia release ATP as a cotransmitter: ATP is released with noradrenaline (NA) and neuropeptide Y (NPY) from sympathetic nerves to act on smooth muscle P2X<sub>1</sub> and in some vessels P2X<sub>2</sub>, P2X<sub>4</sub> and P2Y<sub>2</sub> purinoceptors, resulting in vasoconstriction. ATP is also released together with calcitonin gene-related peptide (CGRP) and substance P (SP) from sensory nerves during 'axon reflex' activity and broken down to adenosine diphosphate (ADP) to act on smooth muscle P2Y<sub>1</sub>-purinoceptors in some regions of some vessels resulting in vasodilatation. P1(A<sub>1</sub>)-purinoceptors on nerve terminals of sympathetic and sensory nerves mediate adenosine (AD) (arising from enzymatic breakdown of ATP) modulation of transmitter release. P2X<sub>2/3</sub> purinoceptors are present on a subpopulation of sensory nerve terminals. P1(A<sub>2</sub>)-purinoceptors on vascular smooth muscle mediate vasodilatation. Endothelial cells release ATP and uridine 5'-triphosphate (UTP) during shear stress and hypoxia to act on P2Y<sub>1</sub>, P2Y<sub>2</sub> and sometimes P2Y<sub>4</sub> purinoceptors leading to the production of nitric oxide (NO) and subsequent vasodilatation. ATP, following its release from aggregating platelets, also acts on these endothelial receptors. Blood-borne platelets possess P2Y<sub>1</sub> and P2Y<sub>12</sub> ADP-selective purinoceptors as well as P2X<sub>1</sub> receptors, while immune cells of various kinds possess P2X<sub>7</sub>, as well as P2X<sub>1</sub>, P2Y<sub>1</sub> and P2X<sub>2</sub> purinoceptors. P2X<sub>2</sub>, P2X<sub>3</sub> and P2X<sub>4</sub> receptors have also been identified on endothelial cell membranes. (Modified from [753], with permission from Lippincott Williams & Wilkins.)

**Figure 2.** 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<sub>2</sub> receptor and ADP is only active at the P2Y<sub>1</sub> receptor. ADP acting on P2Y<sub>1</sub> 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<sub>2</sub> 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<sub>7</sub> receptor pore formation or induce cell death of mature osteoclasts through P2X<sub>7</sub> receptors. In osteoblasts, ATP, through



P2X5 receptors, might enhance proliferation and/or differentiation. By contrast, UTP, through P2Y<sub>2</sub> receptors, is a strong inhibitor of bone formation by osteoblasts. For some receptors (e.g., P2X<sub>4</sub> and P2Y<sub>2</sub> receptors on osteoclasts or P2X<sub>2</sub> receptors on osteoblasts), evidence for expression has been found but their role is still unclear. Please see online article for colour figure. (Reproduced from [1152], with permission.)

### Figure 3.

Schematic diagram of long-term (trophic) actions of purines released from nerves, platelets, and endothelial cells (which also release UTP) acting on P2 receptors to stimulate or inhibit cell proliferation. ATP released as a cotransmitter from sympathetic nerves and sensory-motor nerves (during axon reflex activity) stimulates smooth muscle cell proliferation via P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors via a mitogen activated protein kinase (MAPK) cascade, whereas adenosine resulting from enzymatic breakdown of ATP acts on P1 (A<sub>2</sub>) receptors to inhibit cell proliferation (via elevation of cAMP). ATP and UTP released from endothelial cells stimulate endothelial and smooth muscle cell proliferation via P2Y<sub>1</sub>, P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors. Adenosine resulting from ATP breakdown acts on P1 (A<sub>2</sub>) receptors to stimulate endothelial cell proliferation and regulate the release of platelet-derived growth factor (PDGF) from platelets. NA, noradrenaline; CGRP, calcitonin gene-related peptide; SP, substance P. (Reproduced from [753], with permission from Lippincott, Williams and Wilkins.)

### Figure 4.

Double-labeling of P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors with markers of proliferation show colocalization within a subpopulation of basal and parabasal keratinocytes. Double-labeling of P2X<sub>5</sub> receptors with markers of differentiated keratinocytes show colocalization within the stratum spinosum, and double-labeling of P2X<sub>7</sub> receptors with markers of apoptosis in human leg skin show colocalization within the stratum corneum. (a) Ki-67 immunolabelling (a marker for proliferation) stained the nuclei (green) of a subpopulation of keratinocytes in the basal and parabasal layers of the epidermis. P2Y<sub>1</sub> 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 subpopulation of keratinocytes. These nuclei were often distributed in clusters and found in the basal and parabasal layers of the epidermis. P2Y<sub>2</sub> receptor immunostaining (red) was also expressed in basal and parabasal epidermal cells. Scale bar 30 µm. (c) P2X<sub>5</sub> receptor immunostaining (red) showed overlap (yellow) with cytokeratin K10 (green), an early marker of keratinocyte differentiation. P2X<sub>5</sub> 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<sub>5</sub> receptors, indicating that no differentiation was taking place in these cells. The

colocalization of P2X5 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  $\mu\text{m}$ . (d) P2X5 receptor immunostaining (red) showed overlap (yellow) with involucrin (green). P2X5 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 labelled with involucrin, which is a late marker of keratinocyte differentiation. Scale bar 30  $\mu\text{m}$ . (e) TUNEL (green) labelled the nuclei of cells at the uppermost level of the stratum granulosum and P2X7 antibody (red) mainly stained cell fragments within the stratum corneum. Scale bar 15  $\mu\text{m}$ . (f) Anti-Caspase-3 (green) colocalized with areas of P2X7 receptor immunostaining (red) both at the junction of the stratum granulosum and within the stratum corneum. Areas of colocalization were yellow. Note that the differentiating keratinocytes in the upper stratum granulosum were also positive for anti-Caspase-3. Scale bar 15  $\mu\text{m}$ . Please see online article for colour figure. (Reproduced from [875], with permission.)

**Figure 5.** Schematic diagram illustrating the different mechanisms by which P2 receptor subtypes might alter cancer cell function. P2Y<sub>1</sub> and P2Y<sub>2</sub> 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<sub>5</sub> and P2Y<sub>11</sub> receptor activation might switch the cell cycle from proliferation into a state of differentiation. The P2X<sub>7</sub> receptor activates the apoptotic caspase enzyme system. InsP<sub>3</sub>, inositol trisphosphate . (Redrawn from [986], and reproduced from [1152] with permission.)

**Figure 6.**

Schematic overview of purinergic signalling mechanisms that regulate long-term, trophic effects. Extracellular nucleotides and nucleosides bind to purinergic receptors coupled to signal transducing effector molecules. Activation of the effectors leads to generation of second messengers and/or stimulation of protein kinases that regulate expression of genes needed for long-term, trophic actions. In some cases, P2X receptors such as P2X<sub>7</sub> are also coupled to protein kinase cascades and can mediate proliferation and apoptosis. Cell-specific and/or receptor subtype-specific differences are likely to account for variations in signaling pathways and functional outcomes. It should be noted that the list of elements is not meant to be all-inclusive. Other protein kinases, e.g., MEK, PI3K, are upstream of the listed kinases involved in purinergic signaling while others are downstream, e.g., p70S6K. In addition, dashed arrows indicate that not all listed elements are activated by the upstream component, e.g., not all P1 receptors are coupled to all listed effectors. Abbreviations: AC, adenylyl

cyclase; AP-1, activator protein-1; CaMK, calcium-calmodulin protein kinase; CREB, cyclic AMP response element binding protein; DG, diacylglycerol; GSK, glycogen synthase kinase; IP<sub>3</sub>, inositol trisphosphate; MAPKs, mitogen-activated protein kinases [including extracellular signal regulated protein kinase (ERK), p38 MAPK, and stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK)]; MEK, MAPK/ERK kinase; NO, nitrous oxide; PG, prostaglandin; PI3K, phosphoinositide 3-kinase; PI-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. (Reproduced from [8], with permission.)