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REVIEW

Interaction of purinergic receptors with GPCRs, ion channels, tyrosine kinase and steroid hormone receptors orchestrates cell function

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Abstract Extracellular purines and pyrimidines have emerged as key regulators of a wide range of physiological and pathophysiological cellular processes acting through P1 and P2 cell surface receptors. Increasing evidence suggests that purinergic receptors can interact with and/or modulate the activity of other classes of receptors and ion channels. This review will focus on the interactions of purinergic receptors with other GPCRs, ion channels, receptor tyrosine kinases, and steroid hormone receptors. Also, the signal transduction pathways regulated by these complexes and their new functional properties are discussed.

Keywords Purinergic receptors · GPCRs · Receptor tyrosine kinases · Steroid hormone receptors · Ion channels

Introduction

Extracellular purines and pyrimidines have widespread and specific signalling actions in the regulation of a variety of functions in many tissues. They have emerged as physiological regulators of cell growth, differentiation, and death [1]. Moreover, they have been implicated in neoplastic transformation, embryogenesis, platelet aggregation, cardiovascular function, bone and muscle regeneration, insulin release, inflammation and immunomodulation, neuroprotection, and initiation of pain [2–5]. Taking these facts into account, there is increasing interest in the therapeutic potential of purinergic and pyrimidinergic compounds [1, 4].

Purinergic and pyrimidinergic nucleotides cannot be transported across the plasma membrane by simple diffusion, so they are released to the extracellular environment via lytic (diffusion through the damaged plasma membrane during trauma, injury, apoptosis, and necrosis) [6–10] and non-lytic mechanisms (mechanical distension, ATP release channels, ATP-binding cassette proteins, facilitated diffusion by nucleotide-specific transporters, and vesicular exocytosis) [11–19] either under physiological and pathophysiological conditions. However, some of the transport mechanisms involved in ATP release are controversial, for instance, it has been reported that cystic fibrosis transmembrane conductance regulator cannot carry this nucleotide [20].

Nucleotides have a short half-life due to the presence of ectonucleotidases that rapidly degrade them, so they can activate plasma membrane receptors, called purinergic receptors, in an autocrine and paracrine manner [5, 21]. Many receptor subtypes for purines and pyrimidines have been identified on the basis of cloning, signal transduction and pharmacology. They are divided into P1 adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3 subtypes), P2Y metabotropic receptors ($P2Y_{1,\ 2,\ 4,\ 6,\ 11–14}$), and P2X ionotropic receptors ($P2X_{1–7}$ subtypes forming both homomultimers and heteromultimers) [22].

P1 receptors are all members of the rhodopsin-like family of G protein-coupled receptors (GPCRs). They have a short extracellular N-terminal domain, seven transmembrane domains, and a short intracellular C-terminal loop. They couple principally to adenylate cyclase, either negatively (A_1 and A_3) or positively (A_{2A} and A_{2B}). The human A_{2B} receptor has also been observed to couple through $G_{q/11}$ to regulate phospholipase C (PLC) activity [23, 24].

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P2Y receptors are also members of the GPCR family; their structure consists of an extracellular N-terminal domain, seven transmembrane spanning regions that form the ligand binding pocket, and a C-terminal domain containing several binding/phosphorylation sites for protein kinases and G proteins. Particularly, the second and third intracellular loops (IL-2 and IL-3) of GPCRs are important for G protein coupling. Studies showed that when IL-2 and IL-3 are deleted, GPCRs are no longer able to couple to G proteins [25, 26].

Each P2Y receptor subtype is directly coupled to multiple G proteins triggering the activation of various intracellular signalling cascades. $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors couple to $G_{\alpha q}$ protein to induce the activation of PLC which catalyses the hydrolysis of phosphatidylinositol 4,5-biphosphate to generate inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) [27]. DAG induces the activation of protein kinase C (PKC) leading to the stimulation of diverse downstream effectors; IP_3 stimulates intracellular calcium (Ca^{2+}) mobilization. In addition, some of these P2Y receptors also couple to adenylyl cyclase inducing changes in intracellular cyclic adenosine monophosphate (cAMP) levels. $P2Y_{13}$ receptors can simultaneously couple to G_i and G_s inducing opposite effects on intracellular cAMP levels [28].

P2X receptors are trimers or hexamers formed by protein subunits, each consisting of intracellular N and C termini possessing consensus binding motifs for protein kinases, two transmembrane spanning regions involved in channel gating and ion pore lining, respectively, and a large extracellular loop containing the ATP-binding site [23]. Six homomultimers ($P2X_{1,\ 2,\ 3,\ 4,\ 5,\ 7}$) and three heteromultimers ($P2X_2/P2X_3$, $P2X_4/P2X_6$, and $P2X_1/P2X_5$) have been functionally characterized. These receptors trigger the activation of many intracellular signalling pathways by increasing the $[Ca^{2+}]$i concentration [21].

Therefore, P1 and P2 receptors can lead to the activation of several signalling pathways such as the mitogen-activated protein kinase (MAPK) cascade [23, 27, 29–34], and the phosphatidylinositol-3 kinase (PI3K)/Akt signalling pathway [27, 34–39] to regulate cell survival, cell differentiation, programmed cell death, cell cycle progression, and cellular growth.

It is well known that interactions between GPCRs can modulate their activity either potentiating or inhibiting it. Such interactions can take place through the formation of a physical complex (receptor dimerization), or through receptor cross-talk, when second messengers integrate coincident signals from multiple receptors, which are not physically associated [4, 23]. Thus, P2Y receptors can interact and/or regulate the activity of other P2Y receptors and, also, of other GPCRs. In addition, P2Y receptors can also modulate the activity of P2X ion channels and receptor tyrosine kinases (RTKs), recently recognized as important in the regulation of signalling and cellular responses [40–44]. Related to this, it is known that purinergic receptors, particularly A_1, A_{2A}, $P2X_{1,3,4,7}$, and $P2Y_{1,2,4,6,12}$ subtypes as well as ectonucleotidases and nucleotide transporters are assembled in specialized sub-membrane compartments (lipid rafts, raft-like structures, and caveolae). Altogether, these reciprocal influences control the duration, magnitude, and/or direction of the signals triggered by purines and pyrimidines, and the impact that each single ligand has on a variety of short- and long-term functions [45]. However, the interaction of receptors for purines and pyrimidines with other receptor types is one issue that remains unresolved [22]. Thus, in this review, we focus on the interplay occurring between P2 receptors and other receptor families. Particularly, we discuss the relationship between purinergic receptors and other GPCRs and tyrosine kinase and steroid hormone receptors.

Interactions between purinergic receptors and other GPCRs

As previously mentioned in the introduction, GPCRs exist as dimers or higher-order oligomers that may modify their functions. P2Y metabotropic receptors tend to form homo- or heterodimers with GPCRs not only of different families but also of the same purinergic receptor families, leading to alterations in functional properties. Such dimerization occurs constitutively in the endoplasmic reticulum where it could have an important role in the quality control of newly synthesized receptors and specific subcellular localization [46]. Only limited information is available on the physiological relevance of the various GPCR dimers identified to date in cell culture systems and native tissues, because of difficulties associated with demonstrating any physiological significance of dimerization in the native systems [47].

P2Y homodimerization and homomultimerization

It has been reported that $P2Y_1$ receptors exist as dimers, in HEK293 cell membranes, in the resting state. Agonist exposure induces a rise in receptor dimerization. This effect follows desensitization and is fully reversible upon withdrawal of agonist. Both monomer and constitutive dimers are fully active [48]. In addition, this receptor may form oligomers in other types of cells [49]. Moreover, it was demonstrated that $P2Y_2$ receptors form homo-oligomeric assemblies and that the formation of $P2Y_2$ receptor oligomers does not depend on the presence of UTP as an agonist [50]. The $P2Y_4$ receptor subunit can also form higher-order complexes. These multimers appear stable,

being to some extent resistant to denaturing and reducing conditions, thus indicating that they derive, at least in part, from covalent disulphide bonds occurring between the subunits. Moreover, both rat and human endogenous $P2Y_4$ receptors appear as stable dimers in cell lines or primary neurons from the peripheral and central nervous system. This also occurs for the heterologous $P2Y_4$ receptor transiently transfected in the neuroblastoma SH-SY5Y cell line [43]. Endogenously expressed $P2Y_4$ and $P2Y_6$ receptors form high-order complexes in neurons. The protomeric unit at the basis of the $P2Y_6$ receptor complex appeared to be the monomer while the dimer seems to be the unit for $P2Y_4$ subtypes. Moreover, dimeric $P2Y_4$ and monomeric $P2Y_6$ proteins display selective microdomain partitioning in lipid rafts from specialized subcellular compartments such as synaptosomes. Receptor activation by UTP induced the oligomerization of the $P2Y_6$ but not of the $P2Y_4$ receptor. Transfected $P2Y_4$ and $P2Y_6$ proteins homo-interact and possess the appropriate domains to associate with $P2Y_{1,\ 2,\ 4,\ 6,\ 11}$ receptor subtypes as judged by the results obtained using a direct method of double co-transfection (i.e. co-transfection with Myc-$P2Y_4$ plus, respectively, FLAG-$P2Y_{1,\ 2,\ 6,\ 11}$ or with FLAG-$P2Y_6$ plus, respectively, Myc-$P2Y_{1,\ 2,\ 4,\ 11}$); however, endogenous $P2Y_4$ form hetero-oligomers only with $P2Y_6$ receptors [51].

It has been established that $P2Y_{12}$ receptors exist predominantly as homo-oligomers, essential for their functionality, which are situated in lipid rafts of mammalian cells and in freshly isolated platelets. Upon *in vitro* treatment with the active metabolite of clopidogrel or *in vivo* oral clopidogrel administration to rats, the homo-oligomers are disrupted into non-functional dimers and monomers that are sequestered outside the lipid rafts [52].

P2Y heterodimerization

$P2Y_1$ and $P2Y_{11}$ receptors were found to associate together when co-expressed in HEK293 cells. The hetero-oligomer formation promotes agonist-induced internalization of the $P2Y_{11}$ receptor, which by itself is unable to undergo endocytosis. This interaction and subsequent internalization has an important impact on $P2Y_{11}$ receptor desensitization. Co-internalization of these receptors was also seen in 1321N1 astrocytoma cells upon stimulation with ATP or with the $P2Y_1$ receptor-specific agonist 2-MeS-ADP. In addition, the association of $P2Y_1$ with the $P2Y_{11}$ receptor influences the ligand selectivity of the $P2Y_{11}$ receptor. In this way, the specific $P2Y_1$ receptor antagonist MRS2179 inhibited both the rise in $[Ca^{2+}]i$ induced by the potent $P2Y_{11}$ receptor agonist 2′,3′-*O*-(4-benzoyl-benzoyl)-ATP (BzATP) and the internalization of the $P2Y_{11}$ receptor in response to ATP, whereas the highly potent $P2Y_{11}$ receptor antagonist NF157 was not able to inhibit any of these effects. Thus, the hetero-oligomerization of these receptors allows novel functions of the $P2Y_{11}$ receptor in response to extracellular nucleotides [53]. Heterodimerization also takes place between purinergic receptors and other types of GPCRs. For instance, it has been reported that adenosine A_1 and $P2Y_1$ receptors can form constitutive hetero-oligomers in co-transfected cells. This process is promoted by the simultaneous activation of both receptors [54–56]. Oligomeric association of A_1 and $P2Y_1$ receptors generates $P2Y_1$-like agonistic pharmacology and provides a molecular mechanism for an increased diversity of purine signalling [55, 57]. Co-localization of A_1–$P2Y_1$ receptors at glutamatergic synapses and surrounding astrocytes has also been demonstrated in rat hippocampus. $P2Y_1$ receptor stimulation impaired the potency of A_1 receptor coupling to G protein, whereas the stimulation of A_1 receptors increased the functional responsiveness of $P2Y_1$ receptors. This may be particularly important during pathological conditions, when large amounts of these mediators are released. The same complex was also demonstrated in human astroglial cells [58, 59]. A_1 and $P2Y_2$ receptors can also associate in co-transfected HEK293T cells and intact rat brain. This heterodimerization affects the receptor binding site attenuating A_1 agonist binding by $P2Y_2$ receptor agonists in membranes of co-transfected cells. Moreover, A_1 receptor activity is suppressed and $P2Y_2$ receptor activity synergistically enhanced, upon simultaneous addition of A_1 and $P2Y_2$ receptor agonists [60].

Cross-talk between purinergic receptors and other GPCRs

Hypertonic stress-induced cell shrinkage releases ATP from polymorphonuclear neutrophils (PMNs), released ATP augments PMNs functions through P2 receptors and p38 MAPK activation, or ATP is converted to adenosine, which suppresses PMNs functions via A_2 receptors that activate cAMP/PKA signalling. This bidirectional control by released ATP allows PMNs to register and differentially respond to osmotic changes in their extracellular environment [61]. Cross-talk between A_1 and $P2Y_2$ receptors has additionally been reported to function in local regulation of water transport and homeostasis by the kidney [62]. There is evidence supporting cross-talk between $P2Y_{12}$ and $P2Y_1$ receptors in platelets. There, $P2Y_{12}$ receptor activation by ADP positively modulates the $P2Y_1$-dependent calcium response, whereas $P2Y_1$ receptor activation negatively modulates $P2Y_{12}$ receptor function through Src kinase activation. Moreover, modulation of both receptors is mediated by PI3K and inhibition of adenylate cyclase. In turn, a negative feedback pathway from $P2Y_1$ receptors, mediated by Src tyrosine kinase, inhibits the PI3K-dependent signalling component. Ca^{2+} signalling, therefore, represents a point of cross-talk between these receptors and

a key regulator of platelet response to ADP [63]. On the other hand, the calcium response evoked by $P2Y_1$ receptors is potentiated by the activity of $P2Y_{12}$ receptor-dependent signalling pathways in glioma C6 cells. There, Ca^{2+} influx, enhanced by the cooperation of $P2Y_1$ and $P2Y_{12}$ receptor activities, directly depends on the capacitative calcium entrance mechanism [64]. Simultaneous activation of P2Y and adenosine A1 receptors synergistically increases Ca^{2+} transients and translocation of PKC to the plasma membrane in DDT1 MF-2 cells [65].

$P2Y_2$ receptor activation by ATP decreases angiotensin type 1 receptor density through nitric oxide (NO)-mediated *S*-nitrosylation of nuclear factor κB in rat cardiac fibroblasts [66]. In transfected CHO cells, the G_i/G_o protein-coupled adenosine A_1 receptor activates MAPK via a pathway which is independent of PKC but involves tyrosine kinase, PI3K and MEK1 activation. Moreover, co-activation of adenosine A_1 and $P2Y_2$ receptors induces synergistic increases in MAPK activity [67]. This effect may be related to the enhancement of $G_{q/11}/[Ca^{2+}]$ signalling observed upon the simultaneous activation of these receptors [60]. In this way, the PKC/Raf-1 upstream mediators of the MAPK cascade may synergistically increase MAPK signalling. In addition, simultaneous activation of endogenous A_1 and $P2Y_2$ receptors in DDT1 MF-2 cells synergistically increases translocation of PKC to the plasma membrane [65]. However, identifying the mechanism(s) underlying the synergistic increases in MAP kinase activity will require further research.

Channel regulation by P2 receptors

Growing evidence implicates a key role for extracellular nucleotides in the regulation of ion channels, but the mechanism for such action is poorly defined. ATP and other nucleotides, including UTP, decrease epithelial Na^+ channel (ENaC) activity via apical $P2Y_2$ receptors. $P2Y_2$ receptors couple to ENaC via PLC. In this way, locally released ATP acts in an autocrine/paracrine manner to tonically regulate ENaC in mammalian collecting duct. Loss of this intrinsic regulation leads to ENaC hyperactivity and contributes to hypertension that occurs in $P2Y_2$ receptor−/− mice. $P2Y_2$ receptor activation by nucleotides thus provides physiologically important regulation of ENaC and electrolyte handling in mammalian kidney [68]. A paracrine regulation of ENaC by UTP has also been reported in lung epithelia of mice infected with the respiratory syncytial virus (RSV). RSV infection resulted in higher levels of pyrimidines and purines in the alveolar space which mediated, at least in part, the harmful effects of RSV on lung epithelia [69].

In layer V pyramidal neurons of the prefrontal cortex post-synaptically localized P2Y receptors interact with NMDA receptor channels [70].

Activation of neuronal $P2Y_1$ receptors may gate calcium-dependent K^+ channels (K(Ca)2 channels) via PLC-dependent increases in intracellular Ca^{2+}, thereby defining an additional class of neuronal ion channels as novel effectors for P2Y receptors. This mechanism may form the basis for the control of synaptic plasticity via $P2Y_1$ receptors [71]. $P2Y_1$ receptors can transduce information from central sensory neurons through regulation of hyperpolarization-activated cation channel activities [72].

P2 receptors and RTKs

As previously mentioned, in addition to ion channel activity and GPCRs, P2Y receptors can modulate the activity of RTKs [73]. This latter family comprises high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones, which regulate normal cellular processes and also have a critical role in the development and progression of many types of cancer. At least 20 classes of RTKs have been identified, including the epidermal growth factor receptor (EGFR) family, the insulin receptor family, the platelet-derived growth factor receptor (PDGFR) family, the fibroblast growth factor receptor (FGFR) family, the nerve growth factor receptor (NGFR) family, and the vascular endothelial growth factor receptor (VEGFR) family [74].

Human carcinomas frequently express high levels of receptors of the EGFR family, and overexpression of at least two of these receptors has been associated with a more aggressive clinical behaviour [75, 76]. This could be explained by the fact that the EGFR function is trans-regulated by a variety of stimuli, including agonists of certain GPCRs [77]. Different P2Y receptor subtypes have been involved in the transactivation of the EGFR in normal and cancer cells. For example, in the PC12 cell line, derived from a pheochromocytoma of the rat adrenal medulla, $P2Y_2$ receptors mediate EGFR transactivation to finally induce MAPK activation. This occurs downstream of related adhesion focal tyrosine kinase (RAFTK, a member of the focal adhesion PTK family). As a consequence, although $P2Y_2$ and EGFRs may both activate a similar multiprotein signalling cascade immediately upstream of MAPK, the $P2Y_2$ receptor appears to uniquely utilize $[Ca^{2+}]i$, PKC, and, subsequently, RAFTK [73]. Also in the human colonic cancer cell line, Caco-2, ATP-mediated stimulation of MAPKs involves cross-communication between $P2Y_{2/4}$ receptor subtypes and EGFR signalling systems [32]. Furthermore, in tumoral HeLa cells and normal female reproductive tract epithelial cells, cell-released nucleotides

stimulate $P2Y_1$ receptors to trigger mitogenic signals by transactivating the EGFR. The pathway involves PKC, Src, and cell surface metalloproteases. Strikingly, the canine kidney epithelial cell line which ectopically expresses $P2Y_1$ receptors displays a highly proliferative phenotype that depends on EGFR activity associated with an increased level of EGFR. This discloses a novel aspect of GPCR-mediated regulation of EGFR function [77]. Similarly, an *in vitro* wound healing assay performed in human corneal and BEAS 2B (human bronchial) epithelial cells suggested that ATP released as a consequence of the wound triggers EGFR transactivation resulting in the stimulation of the PI3K and ERK signalling pathways to lead wound closure [78]. Moreover, ATP, acting through P2Y receptors, transactivates both PDGFR and EGFR leading to the activation of ERK1/2 and PI3K and to an increase in the proliferation rate of Müller glial cells. PDGF-induced proliferation may depend on transactivation of the EGFR kinase while metalloproteinase 9 was implicated in the signal transfer from P2Y to EGFRs [79]. As can be inferred, many reports do not determine the P2Y receptor subtype/s involved in EGFR transactivation. On the other hand, metalloproteinase-dependent transactivation of the EGFR is stimulated by ATP-induced ERK1/2 phosphorylation through $P2Y_2$/$P2Y_4$ receptors in bovine adrenal chromaffin cells [80]. In astrocytes, $P2Y_2$ receptors are also involved in the phosphorylation of the EGFR. This occurs due to cell stress-released nucleotides which induce the activation of $P2Y_2$ receptors leading to pro-inflammatory responses that can protect neurons from injury, including the stimulation and recruitment of glial cells. $P2Y_2$ receptor activation induces the phosphorylation of the EGFR, a response dependent upon the presence of SH_3 binding domains in the intracellular C terminus of the $P2Y_2$ receptor that promote Src binding and transactivation of EGFR, a pathway that regulates the proliferation of cortical astrocytes [81].

P2X receptors have also been recently implicated in the transactivation of the EGFR. In HEK 293 human embryonic kidney cells, transactivation of the EGFR by BzATP is essential for $P2X_7$ receptor-induced expression of Egr-1 [82].

P2 receptors can potentiate or synergize with growth factors to regulate a cellular response. In the human breast cancer cell line MCF-7, ATP-γ-S, or EGF lead to ERK activation and phosphorylation of the transcription factors CREB and Elk-1. Co-stimulation synergistically activated c-Fos expression and notably increased the phosphorylation of ERK, CREB, and EGFR. Nevertheless, the ERK pathway does not fully account for this synergy since Fos induction was differentially sensitive to the MEK inhibitor U0126, indicating that ATP and EGF signal differently to c-Fos. Thus, extracellular nucleotides cooperate with growth factors to activate genes linked to the proliferative response in MCF-7 cells [83]. ATP, ADP, and UTP acting through $P2Y_1$ and $P2Y_2$ receptors, and low concentrations of adenosine, augmented adult multipotent neural stem cell proliferation in the presence of growth factors. This result infers nucleotide receptor-mediated synergism that augments growth factor-mediated cell proliferation, supporting the notion that extracellular nucleotides contribute to the control of adult neurogenesis [84]. In addition, Grimm and collaborators established that nucleotides and EGF, acting in a paracrine or autocrine manner, both induce converging intracellular signalling pathways (Akt and focal adhesion kinase) that carry potential for synergism in the control of neural stem cell proliferation and cell survival [85]. ATP and insulin act synergistically to stimulate the activation of ERK1/2, and also induce an additive activation of Raf and Ras in coronary artery smooth muscle cells (CASMCs), leading to synergistic stimulation of CASMCs proliferation [86]. Opposite, UTP or UDP significantly reduced the proliferative response to PDGF in vascular smooth muscle cells [87]. The mechanism underlying these opposite effects of P2Y receptor activation is not known. More than one P2Y receptor subtype may contribute and also P2Y receptors can respond differently depending on the expression of effector proteins and on the cross-talk occurring between different signalling pathways and receptors in a particular cell type. Therefore, interactions between P2Y receptors and RTKs can be complex [1].

Recently, it has been shown that plasma membrane distribution of $P2Y_2$ receptors is transregulated by the EGFR in smooth muscle cells isolated from human chorionic arteries. There, the use of AG1478, a selective and potent inhibitor of the EGFR tyrosine kinase activity, not only blocked the UTP-induced vasomotor activity but also abrogated both RhoA and Rac1 activation, the $P2Y_2$ receptor association with membrane rafts, and its internalization. These results reveal an unsuspected functional interplay that controls both the membrane distribution and the vasomotor activity of the $P2Y_2$ receptor in intact human blood vessels [88].

Extracellular purines can stimulate the synthesis and release of nerve growth factor (NGF) [89], which is essential for neuronal growth and differentiation, and they can also act in combination with this factor to regulate differentiation and growth of various cell lines [90].

It has been reported that the use of P2 receptor antagonists reversibly prevents diverse NGF-dependent responses in PC12 cells. Furthermore, NGF modulates extracellular release of ATP and also the expression levels of $P2X_2$ receptor protein [91]. These authors established that P2 receptor agonists can behave as neurotrophic factors for neuronal cells. They reported that ATP and 2-Cl-ATP promote neurite regeneration after priming of PC12 cells with NGF, whereas various P2 receptor antagonists were

inhibitory. Moreover, NGF and ATP induced the expression of $P2X_2$, $P2X_3$, $P2X_4$ and $P2Y_2$ receptor proteins under neurite-regenerating conditions in PC12 cells [92]. On the other hand, the induction of PC12 cell differentiation by NGF altered mRNA expression of several P2Y and P2X receptors, but only increased $P2X_{1–4}$ protein expression. NGF enhanced the ability of the non-hydrolyzable ATP analog ATPγS to stimulate catecholamine (norepinephrine) release. These responses characterize sympathetic neuronal differentiation and appear to be physiologically important [93]. Additionally, both ATP and NGF enhanced the expression of the stress-induced heat shock proteins 70 and 90 [94], as well as the phosphorylation of ERK1/2 in PC12 cells [92]. In parallel with NGF, ATP prevented the cleavage and activation of caspase-2 and inhibits the release of cytochrome *c* from mitochondria into the cytoplasm. Finally, neither NGF nor ATP modulated the expression of P2 receptors suggesting a potential interaction between ATP and NGF signalling in the neuritic outgrowth and survival of PC12 cells [94]. Therefore, extracellular ATP potentiates the neurite outgrowth induced by NGF. On the other hand, it was shown that ATP and BzATP acting through $P2X_7$ receptors can induce biochemical and/or morphological changes characteristic of apoptotic cell death in some cell types [95–97]. These opposite effects exerted by ATP on cell apoptosis may be due to the interaction between purinergic and growth factor signalling. However, different expression of P2 receptors should also be considered.

The neurotrophic effect of ATP and other nucleotides was determined in the NGFR-negative mouse neuroblastoma neuro2a cell line. There, ATP stimulated neurite outgrowth, apparently, via $P2Y_{11}$ receptors as determined by the potency order of the P2 agonists ATP=ATPγS>ADP>>2Me-S-ATP on the neuritogenic effect, the insensibility to UTP and to the antagonist PPADS. This neurotrophic effect was mediated by Src kinase, PLC and ERK1/2 MAPK, suggesting that ATP can stimulate neurite outgrowth independent of other neurotrophic factors and can be an effective trophic agent [98].

ATPγS in the presence of NGF leads to phosphorylation of tyrosine receptor kinase A (TrkA, high-affinity nerve growth factor receptor) and to the co-localization (determined by immunocytochemistry) and association (determined by immunoprecipitation) of TrkA with $P2Y_2$ receptors; these events are required to enhance neuronal differentiation [99]. The use of Src family kinase inhibitor blocked ATPγS/$P2Y_2$ receptor-promoted enhancement of NGF/TrkA signalling and neuronal differentiation in PC12 cells, abrogated the enhancement by ATPγS of neurite outgrowth in primary cultures of dorsal root ganglion neurons, and also blocked co-immunoprecipitation of TrkA, $P2Y_2$ receptors, and Src family kinases. Thus, Src family kinases regulate $P2Y_2$ receptor-TrkA molecular cross-talk suggesting that they are key convergence points between RTKs and GPCRs [42]. Furthermore, ATPγS promotes phosphorylation of ERK1/2 and p38, thereby enhancing sensitivity to NGF and accelerating neurite formation in both PC12 cells and dorsal root ganglion neurons. In conclusion, the interactions of tyrosine kinase- and $P2Y_2$ receptor-signalling pathways provide a paradigm for the regulation of neuronal differentiation and suggest a role for $P2Y_2$ as a morphogen receptor that potentiates neurotrophin signalling in neuronal development and regeneration [99].

The GPR17 is a new P2Y-like receptor, responsive to uracil nucleotides and cysteinyl-leukotrienes (cysLTs), which may have a potential role in the regulation of both cell viability and differentiation state of central nervous system cells [100]. To distinguish GPR17 functions from other P2Y receptor activities, Daniele et al. [101] have demonstrated that the expression of GPR17 mRNA is selectively induced during PC12 cell differentiation to neuronal cells, whereas $P2Y_{2, 4, 6, 12, 13, 14}$ receptors are constitutively expressed in PC12 cells and do not undergo modulation following NGF treatment. In addition, the specificity of GPR17 ligands (UDP glucose and LTD_4) was evaluated by the use of the GPR17 selective antagonists cangrelor and montelukast. Furthermore, to unequivocally prove a role for GPR17 some experiments were performed in PC12-differentiated cells following silencing of the receptor upon incubation of cells with small interfering RNAs. Thus, in NGF-differentiated PC12 cells, GPR17 ligands induced a significant pro-survival effect. They activated the intracellular phosphorylation of both ERK1/2 and p38 MAPKs, which have been identified as important signalling pathways for neurotrophins in PC12 cells. Additionally, GPR17 agonists promoted, both alone and synergistically with NGF, neurite outgrowth in PC12 cells, suggesting a possible interplay between endogenous uracil derivatives, cysLTs and NGF in the signalling pathways involved in neuronal survival and differentiation. GPR17 ligands were also able to confer a NGF-like activity to the EGF which also promoted cell differentiation and neurite elongation. Thus, GPR17, like other P2Y receptors, can act as a neurotrophic regulator for neuronal-like cells [101].

$P2Y_2$ receptors have been shown to transactivate VEGFR in human coronary endothelial cells. In these cells, $P2Y_2$ receptor activation by UTP induces rapid tyrosine phosphorylation of the VEGFR-2, and co-localization of both receptors. Consequently, the expression of the pro-inflammatory vascular cell adhesion molecule-1 (VCAM-1) augments through RhoA activation. Deletion or mutation of two Src homology-3-binding sites in the C-terminal tail of $P2Y_2$ receptors, or inhibition of Src kinase activity abolishes $P2Y_2$ receptor-mediated transactivation of VEGFR-2 and subsequently inhibits UTP-induced VCAM-1 expression. These data indicate a novel mechanism whereby a nucleotide receptor transactivates a

receptor tyrosine kinase to generate an inflammatory response associated with atherosclerosis [102].

$P2Y_1$ receptors have also been found to transactivate the VEGFR in vascular endothelial cells. It was found that $P2Y_1$ receptor stimulation of VEGFR phosphorylation by 2-*methyl*-thio-ATP (2Me-S-ATP) was suppressed by the VEGFR-2 tyrosine kinase inhibitor, SU1498. In addition, phosphorylation of VEGFR-2 by VEGF was comparable with 2Me-S-ATP stimulation of the $P2Y_1$ receptor, and both 2Me-S-ATP and VEGF stimulation increased tyrosine phosphorylation of VEGFR-2 at Tyr 1175 [103, 104].

As previously mentioned, extracellular nucleotides can also stimulate the release of growth factors. In platelets, for example, activation of $P2Y_1$ and $P2Y_{12}$ receptors by ADP results in an increase in soluble VEGF concentrations. This suggests that ADP release in the tumour microenvironment may be, on balance, pro-angiogenic. P2Y receptor antagonism abrogates ADP-mediated pro-angiogenic protein release and thus may represent a potential pharmacologic strategy for regulating platelet-mediated angiogenesis [105]. It was reported that VEGF is released from primary human monocytes through $P2X_7$ receptor stimulation by ATP. This effect is calcium-dependent and is associated with reactive oxygen species production. Thus, $P2X_7$ receptors are also likely to be important in the control of angiogenesis and wound repair [106]. $P2Y_2$ receptor activation in human salivary gland cells promotes the formation of EGFR/ErbB3 heterodimers and metalloprotease-dependent neuregulin 1 release, resulting in the activation of both EGFR and ErbB3 [107]. $P2X_7$ receptors were also implicated in VEGF release in rat C6 glioma cells. Cell exposure to BzATP augmented $P2X_7$ receptor expression, increased intracellular calcium $[Ca^{2+}]i$ mobilization, induced the formation of large pores, and enhanced the expression of pro-inflammatory factors including MCP-1, IL-8, and VEGF [108].

Interactions between P2Y, P2X, and polypeptide growth factor signalling pathways may have important implications for CNS development as well as injury and repair. Besides, reports suggest that fibroblast growth factor 2 (FGF-2) is increased after injury and can stimulate astrocyte proliferation. It has been shown that extracellular nucleotides can potentiate FGF-2-mediated signalling. In primary cultures of rat cortical astrocytes, for example, extracellular ATP enhances FGF-2-induced proliferation in a process mediated by P2Y receptors, phosphorylation of ERK1/2 MAPK and increased cyclin expression. However, when $P2X_7$ receptors are activated, FGF-2-dependent proliferation is inhibited shifting cells to a state of reversible growth arrest that may involve phosphorylation of p38 and JNK MAPKs. Thus, P2Y and $P2X_7$ receptors mediate opposing effects on FGF-2-induced mitogenesis [109–111]. Furthermore, in adult mouse olfactory epithelium ATP also induces cell proliferation by promoting FGF-2 and TNF-α synthesis and activation of their receptors (FGFR and EGFR, respectively) [112].

P2 receptors and steroid hormone receptors

Purinoceptors are widely expressed in endocrine glands. For instance, in testicular Sertoli and in Leydig cells, they are involved in estradiol and testosterone secretion and are also expressed in the ovary where they mediate the antagonism of estradiol and progesterone secretion from granulosa cells [3, 4]. Recently, it has been found that 17β-estradiol acting via estrogen receptor alpha promotes proliferation of MCF-7 breast cancer cells by down-regulating $P2Y_2$ receptor expression and attenuating $P2Y_2$ receptor-induced increase of $[Ca^{2+}]i$ [113]. A similar P2 receptor down-regulation mechanism by this female gonadal hormone was determined in dorsal root ganglion (DRG) primary sensory neurons. In these cells, $P2X_3$ receptor subunit mRNA was significantly decreased by the application of 17β-estradiol in a concentration-dependent manner. The use of the estrogen receptor antagonist, ICI 182,780, blocked the reduction in the receptor subunit protein level. Thus, 17β-estradiol participates in the control of peripheral pain signal transduction by modulating the expression of the $P2X_3$ subunit and, consequently, $P2X_3$ receptor-mediated events [114]. On the other hand, $P2X_3$ receptor mRNA was significantly decreased in DRG neurons of ovariectomized rats. However, estrogen replacement could reverse this effect [115]. 17β-Estradiol may then participate in the regulation of P2 receptors, either decreasing or increasing their expression, to control cell signalling pathways.

Besides, it has been shown that estrogens can modulate cell events in a non-genomic manner by affecting signalling mediated by P2 receptors. For instance, purinergic agonists, acting mainly through $P2Y_2$ receptors, potently stimulate HCO_3^- secretion in highly differentiated cultures of monkey oviductal epithelium. When phenol red (an estrogen) is removed from the culture medium, ATP-dependent HCO_3^- secretion is markedly reduced but could be restored by treatment with estradiol. Therefore, estradiol induces changes in HCO_3^- concentration by mediating purinergic signalling pathways or ATP secretion [116]. In normal human cervical epithelial cells, apoptosis is mediated predominantly through $P2X_7$ receptors. In this case, estradiol inhibited the apoptotic effect induced by ATP or BzATP independent of its mitogenic function, implying a novel anti-apoptotic mechanism exerted by estradiol which antagonizes $P2X_7$ receptor-induced apoptosis [117]. Another example of the antagonistic effect of estradiol on $P2X_7$ receptors was established in CV-1 monkey kidney cells

transformed by SV40 (COS cells) expressing the human $P2X_7$ receptor (hP2X7). ATP or BzATP induced a cation current through $hP2X_7$ receptor which was rapidly and reversibly inhibited by 17β-estradiol, in a concentration-dependent and non-genomic manner [118].

Although no reports suggest a role for progesterone in the regulation of the expression of P2 receptors, some authors showed that the hormone can act in a non-genomic manner to antagonize or potentiate ATP-mediated signalling. For instance, progesterone can selectively potentiate homomeric $P2X_2$ receptor cation influx [119]. On the other hand, in T47D-Y cells, a breast cancer cell line lacking expression of the classical nuclear progesterone receptors, progesterone can act in a rapid non-nuclear manner to inhibit extracellular ATP effects on intracellular calcium mobilization and ERK activation [120]. In addition, in human granulosa-luteal cells, human corionic gonadotrophin (hCG)-induced progesterone production was reduced by ATP treatment. Additionally, PD98059, an ERK1/2 MAPK inhibitor, reversed the inhibitory effect of ATP on hCG-induced progesterone production, suggesting that extracellular ATP inhibits progesterone production by hCG through ERK1/2 MAPK [121].

Androgens have important physiological effects, not only are they the precursors for steroid hormone biosynthesis in gonadal and extragonadal tissues, but also act directly via androgen receptors throughout the body [122]. Little is known about the regulation of P2 receptors by androgens or vice versa. In Leydig cells, ATP induces an increase in $[Ca^{2+}]i$ and testosterone secretion, supporting the hypothesis that Ca^{2+} signalling through purinergic receptors contributes to the process of testosterone secretion in these cells [123]. The receptors involved in this response were investigated. The presence of $P2X_2$, $P2X_4$, $P2X_6$, and $P2X_7$ receptor subunits was demonstrated, but functional results suggested that a heteromeric channel, possibly $P2X_{2/4/6}$, is responsible for testosterone secretion in Leydig cells [124]. In addition, sustaining the regulation of P2 receptors by androgens, it was determined that testosterone administration to adult hypogonadal mice restored purinergic excitatory transmission and $P2X_1$ receptor immunofluorescence of vasa deferentia [125].

Glucocorticoids are essential for stress responses. Also ATP released from stressed cells is implicated in inflammation. However, little is known about the effects of glucocorticoids on ATP-induced inflammation. In a human microvascular endothelial cell line, dexamethasone enhanced ATP-induced interleukin 6 (IL-6) secretion through PLC and p38 MAPK. In addition, dexamethasone induced $P2Y_2$ receptor mRNA expression, and when the $P2Y_2$ receptor was silenced by its small interfering RNA, ATP-induced IL-6 production decreased [126]. Dexamethasone also enhanced the ATP-induced $[Ca^{2+}]i$ increase and nitric oxide (NO) production in type I spiral ganglion neurons of the guinea pig cochlea. These effects were dependent on the presence of extracellular Ca^{2+} thereby suggesting that dexamethasone may rapidly enhance the Ca^{2+} influx through the activation of ionotropic P2X receptors which may interact with glucocorticoid receptors [127]. Different results were obtained in HT4 mouse neuroblastoma cells, where ATP-induced elevation of $[Ca^{2+}]i$ was inhibited by corticosterone, cortisol and dexamethasone. Both extracellular Ca^{2+} influx through P2X receptors, and internal Ca^{2+} release were attenuated. Therefore, glucocorticoids modulate P2X receptor-medicated Ca^{2+} influx through a membrane-initiated, non-genomic pathway in HT4 cells [128]. Besides, corticosterone inhibited ATP-induced cation currents through $P2X_3$ receptors in rat DRG neurons. These effects diminished after adding protein kinase A inhibitor H89. Thus, glucocorticoid hormones might participate in the modulation of $P2X_3$ receptor-associated events in sensory neurons, and the effect is mediated by glucocorticoid receptors and the downstream activation of protein kinase A [129].

It has been reported that $P2Y_2$ receptors contribute to NaCl homeostasis and blood pressure regulation in aldosterone-sensitive distal nephron [130]. In addition, the same role for $P2Y_2$ receptors was established in knockout mice lacking $P2Y_2$ receptors, which showed salt-resistant arterial hypertension linked to an inhibitory influence on renal Na^+ and water reabsorption [131]. However, there are no reports suggesting a relation between mineralocorticoid receptors and P2 receptors.

Retinoids, vitamin A derivatives, are important regulators of the growth and differentiation of skin cells. It was established that, in normal human epidermal keratinocytes (NHEKs), all-trans-retinoic acid (ATRA) and 9-cis-retinoic acid, agonists to retinoic acid receptor, enhanced the expression of the $P2Y_2$ receptor mRNA and receptor function. So, retinoids, at least in part, exert their proliferative effects by up-regulating $P2Y_2$ receptors in NHEKs [132]. Besides, ATRA and 9-cis-retinoic acid significantly increased the mRNA and protein levels of $P2X_2$ receptors in rat pheochromocytoma PC12 cells [133]. On the other hand, retinoic acid (RA) induces neuronal differentiation and down-regulates $P2X_7$ receptor expression in human SH-SY5Y neuroblastoma cells, thus protecting them from extracellular nucleotide-$P2X_7$ receptor-induced neuronal death [134]. Similar results were obtained in the case of Neuro-2a cells, where RA-induced neuronal differentiation associated with decreased expression and function of $P2X_7$ receptors [135]. Together, these evidences suggest that retinoids can transcriptionally regulate the expression and function of P2 receptors, at least, in the skin and nervous system. However, in contrast with these results, RA-induced human neuroblastoma SK-N-BE(2)C

cell differentiation did not alter the expression level of $P2Y_6$ receptors [136].

The existence of an interaction between P2 receptors and the vitamin D receptor (VDR) has not been studied yet. However, it was found that 1α, $25(OH)_2$ vitamin D_3 induces ATP exocytosis in static ROS 17/2.8 and SAOS-2 cells and primary calvarial osteoblasts expressing VDR; this effect was abolished by inhibitors of vesicular exocytosis. Furthermore, silencing of VDR by siRNA prevented 1α, $25(OH)_2$ vitamin D_3 stimulation of ATP exocytosis in ROS 17/2.8 and SAOS-2 cells. Similarly, 1α, $25(OH)_2$ vitamin D_3 failed to activate ATP secretion in primary osteoblasts from a VDR knockout mouse. Thus, 1α, $25(OH)_2$ vitamin D_3 stimulation of ATP exocytosis involves non-transcriptional VDR functions in osteoblasts [137].

Concluding remarks

Extracellular nucleotides can regulate many cellular effects through activation of P2 receptors. Nevertheless, it seems that these receptors can form membrane complexes with other P2 receptors or other classes of receptors. P2 receptors can homodimerize, heterodimerize, and even modulate the expression and/or activity of other GPCRs, receptor tyrosine kinases and steroid hormone receptors. This clearly affects intracellular signalling pathways either in physiological or pathophysiological conditions. Thus, P2 receptors should be viewed as components of homo/heteroreceptor complexes rather than self-dependent entities, although it remains unclear to what extent they can associate with each other to form signalling units. In addition, several metabolites and agonists can play a potential role in purinergic signalling. Therefore, P2 receptors can be considered as attractive targets for novel drug development.

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References

1. Abbracchio MP, Burnstock G (1998) Purinergic signalling: pathophysiological roles. Jpn J Pharmacol 78:113–145
2. Burnstock G (2006) Purinergic signalling—an overview. Novartis Found Symp 276:26–48
3. Burnstock G (2006) Purinergic signalling. Br J Pharmacol 147: S172–S181
4. Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol Rev 58:281–341
5. Nandigama R, Padmasekar M, Wartenberg M, Sauer H (2006) Feed forward cycle of hypotonic stress-induced ATP release, purinergic receptor activation, and growth stimulation of prostate cancer cells. J Biol Chem 281:5686–5693
6. Boucher I, Rich C, Lee A, Marcincin M, Trinkaus-Randall V (2010) The $P2Y_2$ receptor mediates the epithelial injury response and cell migration. Am J Physiol Cell Physiol 299:C411–C421
7. Yin J, Xu K, Zhang J, Kumar A, Yu FS (2007) Wound-induced ATP release and EGF receptor activation in epithelial cells. J Cell Sci 120:815–825
8. Gourine AV, Dale N, Llaudet E, Poputnikov DM, Spyer KM, Gourine VN (2007) Release of ATP in the central nervous system during systemic inflammation: real-time measurement in the hypothalamus of conscious rabbits. J Physiol 585:305–316
9. Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P, Lysiak JJ, Harden TK, Leitinger N, Ravichandran KS (2009) Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 461:282–286
10. Martins I, Tesniere A, Kepp O, Michaud M, Schlemmer F, Senovilla L, Séror C, Métivier D, Perfettini JL, Zitvogel L, Kroemer G (2009) Chemotherapy induces ATP release from tumor cells. Cell Cycle 8:3723–3728
11. Eltzschig HK, Eckle T, Mager A, Küper N, Karcher C, Weissmüller T, Boengler K, Schulz R, Robson SC, Colgan SP (2006) ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. Circ Res 99:1100–1108
12. Knight GE, Bodin P, De Groat WC, Burnstock G (2002) ATP is released from guinea pig ureter epithelium on distension. Am J Physiol Renal Physiol 282:F281–F288
13. Samuels SE, Lipitz JB, Dahl G, Muller KJ (2010) Neuroglial ATP release through innexin channels controls microglial cell movement to a nerve injury. J Gen Physiol 136:425–442
14. Ballerini P, Di Iorio P, Ciccarelli R, Nargi E, D'Alimonte I, Traversa U, Rathbone MP, Caciagli F (2002) Glial cells express multiple ATP binding cassette proteins which are involved in ATP release. Neuroreport 13:1789–1792
15. Reigada D, Lu W, Zhang M, Mitchell CH (2008) Elevated pressure triggers a physiological release of ATP from the retina: possible role for pannexin hemichannels. Neuroscience 157:396–404
16. Zhang Z, Chen G, Zhou W, Song A, Xu T, Luo Q, Wang W, Gu XS, Duan S (2007) Regulated ATP release from astrocytes through lysosome exocytosis. Nat Cell Biol 9:945–953
17. Li A, Leung CT, Peterson-Yantorno K, Stamer WD, Mitchell CH, Civan MM (2011) Mechanisms of ATP release by human trabecular meshwork cells, the enabling step in purinergic regulation of aqueous humor outflow. J Cell Physiol. doi:10.1002/jcp.22715
18. Praetorius HA, Leipziger J (2009) ATP release from non-excitable cells. Purinergic Signal 5:433–446
19. Tu J, Le G, Ballard HJ (2010) Involvement of the cystic fibrosis transmembrane conductance regulator in the acidosis-induced efflux of ATP from rat skeletal muscle. J Physiol 588:4563–4578
20. Li C, Ramjeesingh M, Bear CE (1996) Purified cystic fibrosis transmembrane conductance regulator (CFTR) does not function as an ATP channel. J Biol Chem 271:11623–11626
21. Erb L, Liao Z, Seye CI, Weisman GA (2006) P2 receptors: intracellular signalling. Pflugers Arch 452:552–562
22. Burnstock G (2008) Unresolved issues and controversies in purinergic signalling. J Physiol 586:3307–3312
23. Burnstock G (2007) Purine and pyrimidine receptors. Cell Mol Life Sci 64:1471–1483

24. Panjehpour M, Castro M, Klotz KN (2005) Human breast cancer cell line MDA-MB-231 expresses endogenous A_{2B} adenosine receptors mediating a Ca^{2+} signal. Br J Pharmacol 145:211–218
25. Chicchi GG, Graziano MP, Koch G, Hey P, Sullivan K, Vicario PP, Cascieri MA (1997) Alterations in receptor activation and divalent cation activation of agonist binding by deletion of intracellular domains of the glucagon receptor. J Biol Chem 272:7765–7769
26. Umanah GK, Huang LY, Maccarone JM, Naider FR, Becker JM (2011) Changes in Conformation at the cytoplasmic ends of the fifth and sixth transmembrane helices of a yeast G protein-coupled receptor in response to ligand binding. Biochemistry. doi:10.1021/bi200254h
27. Van Kolen K, Slegers H (2006) Integration of P2Y receptor-activated signal transduction pathways in G protein-dependent signalling networks. Purinergic Signal 2:451–469
28. White N, Burnstock G (2006) P2 receptors and cancer. Trends Pharmacol Sci 27:211–217
29. Katz S, Boland R, Santillán G (2006) Modulation of ERK 1/2 and p38 MAPK signalling pathways by ATP in osteoblasts: involvement of mechanical stress-activated calcium influx, PKC and Src activation. Int J Biochem Cell Biol 38:2082–2091
30. Katz S, Boland R, Santillán G (2008) Purinergic (ATP) signalling stimulates JNK1 but not JNK2 MAPK in osteoblast-like cells: contribution of intracellular Ca^{2+} release, stress activated and L-voltage-dependent calcium influx, PKC and Src kinases. Arch Biochem Biophys 477:244–252
31. Scodelaro Bilbao P, Boland R, Russo de Boland A, Santillán G (2007) ATP modulation of mitogen activated protein kinases and intracellular Ca^{2+} in breast cancer (MCF-7) cells. Arch Biochem Biophys 466:15–23
32. Buzzi N, Bilbao PS, Boland R, de Boland AR (2009) Extracellular ATP activates MAP kinase cascades through a P2Y purinergic receptor in the human intestinal Caco-2 cell line. Biochim Biophys Acta 1790:1651–1659
33. Neary JT, McCarthya M, Kanga Y, Zuniga S (1998) Mitogenic signalling from P1 and P2 purinergic receptors to mitogen-activated protein kinase in human fetal astrocyte cultures. Neurosci Lett 242:159–162
34. Milton SL, Dirk LJ, Kara LF, Prentice HM (2008) Adenosine modulates ERK1/2, PI3K/Akt, and p38MAPK activation in the brain of the anoxia-tolerant turtle Trachemys scripta. J Cereb Blood Flow Metab 28:1469–1477
35. Huwiler A, Rölz W, Dorsch S, Ren S, Pfeilschifter J (2002) Extracellular ATP and UTP activate the protein kinase B/Akt cascade via the P2Y(2) purinoceptor in renal mesangial cells. Br J Pharmacol 136:520–529
36. Heo JS, Han HJ (2006) ATP stimulates mouse embryonic stem cell proliferation via protein kinase C, phosphatidylinositol 3-kinase/Akt, and mitogen-activated protein kinase signalling pathways. Stem Cells 24:2637–2648
37. Montiel M, de la Blanca EP, Jiménez E (2006) P2Y receptors activate MAPK/ERK through a pathway involving PI3K/PDK1/PKC-zeta in human vein endothelial cells. Cell Physiol Biochem 18:123–134
38. Burgos M, Neary JT, González FA (2007) P2Y2 nucleotide receptors inhibit trauma-induced death of astrocytic cells. J Neurochem 103:1785–1800
39. Scodelaro Bilbao P, Santillán G, Boland R (2010) ATP stimulates the proliferation of MCF-7 cells through the PI3K/Akt signalling pathway. Arch Biochem Biophys 499:40–48
40. Erb L, Liu J, Ockerhausen J, Kong Q, Garrad RC, Griffin K, Neal C, Krugh B, Santiago-Pérez LI, González FA, Gresham HD, Turner JT, Weisman GA (2001) An RGD sequence in the P2Y(2) receptor interacts with alpha(V)beta(3) integrins and is required for G(o)-mediated signal transduction. J Cell Biol 153:491–501
41. Vial C, Tobin AB, Evans RJ (2004) G protein-coupled receptor regulation of $P2X_1$ receptors does not involve direct channel phosphorylation. Biochem J 382:101–110
42. Arthur DB, Georgi S, Akassoglou K, Insel PA (2006) Inhibition of apoptosis by $P2Y_2$ receptor activation: novel pathways for neuronal survival. J Neurosci 26:3798–3804
43. D'Ambrosi N, Iafrate M, Vacca F, Amadio S, Tozzi A, Mercuri NB, Volonté C (2006) The P2Y(4) receptor forms homo-oligomeric complexes in several CNS and PNS neuronal cells. Purinergic Signal 2:575–582
44. Köles L, Gerevich Z, Oliveira JF, Zadori ZS, Wirkner K, Illes P (2008) Interaction of P2 purinergic receptors with cellular macromolecules. Naunyn Schmiedebergs Arch Pharmacol 377:1–33
45. Amadio S, Apolloni S, D'Ambrosi N, Volonté C (2011) Purinergic signalling at the plasma membrane: a multipurpose and multidirectional mode to deal with amyotrophic lateral sclerosis and multiple sclerosis. J Neurochem 116:796–805
46. Bulenger S, Marullo S, Bouvier M (2005) Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. Trends Pharmacol Sci 26:131–137
47. Nakata H, Suzuki T, Namba K, Oyanagi K (2010) Dimerization of G protein-coupled purinergic receptors: increasing the diversity of purinergic receptor signal responses and receptor functions. J Recept Signal Transduct Res 30:337–346
48. Choi RC, Simon J, Tsim KW, Barnard EA (2008) Constitutive and agonist-induced dimerizations of the $P2Y_1$ receptor: relationship to internalization and scaffolding. J Biol Chem 283:11050–11063
49. Wang L, Karlsson L, Moses S, Hultgårdh-Nilsson A, Andersson M, Borna C, Gudbjartsson T, Jern S, Erlinge D (2002) P2 receptor expression profiles in human vascular smooth muscle and endothelial cells. J Cardiovasc Pharmacol 40:841–853
50. Kotevic I, Kirschner KM, Porzig H, Baltensperger K (2005) Constitutive interaction of the $P2Y_2$ receptor with the hematopoietic cell-specific G protein G(alpha16) and evidence for receptor oligomers. Cell Signal 17:869–880
51. D'Ambrosi N, Iafrate M, Saba E, Rosa P, Volonté C (2007) Comparative analysis of $P2Y_4$ and $P2Y_6$ receptor architecture in native and transfected neuronal systems. Biochim Biophys Acta 1768:1592–1599
52. Savi P, Zachayus JL, Delesque-Touchard N, Labouret C, Hervé C, Uzabiaga MF, Pereillo JM, Culouscou JM, Bono F, Ferrara P, Herbert JM (2006) The active metabolite of clopidogrel disrupts $P2Y_{12}$ receptor oligomers and partitions them out of lipid rafts. Proc Natl Acad Sci USA 103:11069–11074
53. Ecke D, Hanck T, Tulapurkar ME, Schäfer R, Kassack M, Stricker R, Reiser G (2008) Hetero-oligomerization of the $P2Y_{11}$ receptor with the $P2Y_1$ receptor controls the internalization and ligand selectivity of the P2Y11 receptor. Biochem J 409:107–116
54. Yoshioka K, Saitoh O, Nakata H (2002) Agonist-promoted heteromeric oligomerization between adenosine A_1 and $P2Y_1$ receptors in living cells. FEBS Lett 523:147–151
55. Yoshioka K, Hosoda R, Kuroda Y, Nakata H (2002) Hetero-oligomerization of adenosine A_1 receptors with $P2Y_1$ receptors in rat brains. FEBS Lett 531:299–303
56. Sichardt K, Nieber K (2007) Adenosine A(1) receptor: functional receptor-receptor interactions in the brain. Purinergic Signal 3:285–298
57. Yoshioka K, Saitoh O, Nakata H (2001) Heteromeric association creates a P2Y-like adenosine receptor. Proc Natl Acad Sci USA 98:7617–7622
58. Tonazzini I, Trincavelli ML, Storm-Mathisen J, Martini C, Bergersen LH (2007) Co-localization and functional cross-talk between A_1 and $P2Y_1$ purine receptors in rat hippocampus. Eur J Neurosci 26:890–902

59. Tonazzini I, Trincavelli ML, Montali M, Martini C (2008) Regulation of A_1 adenosine receptor functioning induced by P2Y1 purinergic receptor activation in human astroglial cells. J Neurosci Res 86:2857–2866
60. Suzuki T, Namba K, Tsuga H, Nakata H (2006) Regulation of pharmacology by hetero-oligomerization between A_1 adenosine receptor and $P2Y_2$ receptor. Biochem Biophys Res Commun 351:559–565
61. Chen Y, Shukla A, Namiki S, Insel PA, Junger WG (2004) A putative osmoreceptor system that controls neutrophil function through the release of ATP, its conversion to adenosine, and activation of A_2 adenosine and P2 receptors. J Leukoc Biol 76:245–253
62. Rieg T, Vallon V (2009) ATP and adenosine in the local regulation of water transport and homeostasis by the kidney. Am J Physiol Regul Integr Comp Physiol 296:R419–R427
63. Hardy AR, Jones ML, Mundell SJ, Poole AW (2004) Reciprocal cross-talk between $P2Y_1$ and $P2Y_{12}$ receptors at the level of calcium signalling in human platelets. Blood 104:1745–1752
64. Suplat D, Krzeminski P, Pomorski P, Baranska (2007) $P2Y_1$ and $P2Y_{12}$ receptor cross-talk in calcium signalling: evidence from nonstarved and long-term serum-deprived glioma C6 cells. Purinergic Signal 3:221–230
65. Fredholm BB, Assender JW, Irenius E, Kodama N, Saito N (2003) Synergistic effects of adenosine A_1 and P2Y receptor stimulation on calcium mobilization and PKC translocation in DDT1 MF-2 cells. Cell Mol Neurobiol 23:379–400
66. Nishida M, Ogushi M, Suda R, Toyotaka M, Saiki S, Kitajima N, Nakaya M, Kim KM, Ide T, Sato Y, Inoue K, Kurose H (2011) Heterologous down-regulation of angiotensin type 1 receptors by purinergic $P2Y_2$ receptor stimulation through *S*-nitrosylation of NF-{kappa}B. Proc Natl Acad Sci USA 108:6662–6667
67. Dickenson JM, Blank JL, Hill SJ (1998) Human adenosine A_1 receptor and $P2Y_2$-purinoceptor-mediated activation of the mitogen-activated protein kinase cascade in transfected CHO cells. Br J Pharmacol 124:1491–1499
68. Pochynyuk O, Bugaj V, Rieg T, Insel PA, Mironova E, Vallon V, Stockand JD (2008) Paracrine regulation of the epithelial Na^+ channel in the mammalian collecting duct by purinergic $P2Y_2$ receptor tone. J Biol Chem 283:36599–36607
69. Song W, Wei S, Matalon S (2010) Inhibition of epithelial sodium channels by respiratory syncytial virus *in vitro* and *in vivo*. Ann N Y Acad Sci 1203:79–84
70. Wirkner K, Köles L, Thümmler S, Luthardt J, Poelchen W, Franke H, Fürst S, Illes P (2002) Interaction between P2Y and NMDA receptors in layer V pyramidal neurons of the rat prefrontal cortex. Neuropharmacology 42:476–488
71. Schicker KW, Chandaka GK, Geier P, Kubista H, Boehm S (2010) $P2Y_1$ receptors mediate an activation of neuronal calcium-dependent K^+ channels. J Physiol 588:3713–3725
72. Huang W, Xiu Y, Yan JA, He WJ, Zhao YD, Hu ZA, Ruan HZ (2010) Facilitation of Ih channels by $P2Y_1$ receptors activation in mesencephalic trigeminal neurons. Neurosci Lett 482:156–159
73. Soltoff SP (1998) Related adhesion focal tyrosine kinase and the epidermal growth factor receptor mediate the stimulation of mitogen-activated protein kinase by the G-protein-coupled $P2Y_2$ receptor. Phorbol ester or $[Ca^{2+}]i$ elevation can substitute for receptor activation. J Biol Chem 273:23110–23117
74. Robinson DR, Wu YM, Lin SF (2000) The protein tyrosine kinase family of the human genome. Oncogene 19:5548–5557
75. Gullick WJ, Srinivasan R (1998) The type 1 growth factor receptor family: new ligands and receptors and their role in breast cancer. Breast Cancer Res Treat 52:43–53
76. Mendelsohn J, Baselga J (2000) The EGF receptor family as targets for cancer therapy. Oncogene 19:6550–6565
77. Buvinic S, Bravo-Zehnder M, Boyer JL, Huidobro-Toro JP, González A (2007) Nucleotide $P2Y_1$ receptor regulates EGF receptor mitogenic signalling and expression in epithelial cells. J Cell Sci 120:4289–4301
78. Yin J, Xu K, Zhang J, Kumar A, Yu F-SX (2007) Wound-induced ATP release and EGF receptor activation in epithelial cells. J Cell Sci 120:815–825
79. Milenkovic I, Weick M, Wiedemann P, Reichenbach A, Bringmann A (2003) P2Y receptor-mediated stimulation of Müller glial cell DNA synthesis: dependence on EGF and PDGF receptor transactivation. Invest Ophthalmol Vis Sci 44:1211–1220
80. Luke TM, Hexum TD (2008) UTP and ATP increase extracellular signal-regulated kinase 1/2 phosphorylation in bovine chromaffin cells through epidermal growth factor receptor transactivation. Purinergic Signal 4:323–330
81. Liu J, Liao Z, Camden J, Griffin KD, Garrad RC, Santiago-Pérez LI, González FA, Seye CI, Weisman GA, Erb L (2004) Src homology 3 binding sites in the $P2Y_2$ nucleotide receptor interact with Src and regulate activities of Src, proline-rich tyrosine kinase 2, and growth factor receptors. J Biol Chem 279:8212–8218
82. Stefano L, Rössler OG, Griesemer D, Hoth M, Thiel G (2007) P2X(7) receptor stimulation upregulates Egr-1 biosynthesis involving a cytosolic Ca(2+) rise, transactivation of the EGF receptor and phosphorylation of ERK and Elk-1. J Cell Physiol 213:36–44
83. Wagstaff SC, Bowler WB, Gallagher JA, Hipskind RA (2000) Extracellular ATP activates multiple signalling pathways and potentiates growth factor-induced c-fos gene expression in MCF-7 breast cancer cells. Carcinogenesis 21:2175–2181
84. Mishra SK, Braun N, Shukla V, Fullgrabe M, Scomerus C, Korf H-W, Gachet C, Ikehara Y, Sévigny J, Robson SC, Zimmermann H (2005) Extracellular nucleotide signalling in adult neural stem cells: synergism with growth factor-mediated cellular proliferation. Development 133:675–684
85. Grimm I, Ullsperger SN, Zimmermann H (2010) Nucleotides and epidermal growth factor induce parallel cytoskeletal rearrangements and migration in cultured adult murine neural stem cells. Acta Physiol 199:181–189
86. Agazie YM, Bagot JC, Trickey E, Halenda SP, Wilden PA (2001) Molecular mechanisms of ATP and insulin synergistic stimulation of coronary artery smooth muscle growth. Am J Physiol Heart Circ Physiol 280:H795–H801
87. White PJ, Kumari R, Porter KE, London NJ, Ng LL, Boarder MR (2000) Antiproliferative effect of UTP on human arterial and venous smooth muscle cells. Am J Physiol Heart Circ Physiol 279:2735–2742
88. Norambuena A, Palma F, Poblete MI, Donoso MV, Pardo E, González A, Huidobro-Toro JP (2010) UTP controls cell surface distribution and vasomotor activity of the human $P2Y_2$ receptor through an epidermal growth factor receptor-transregulated mechanism. J Biol Chem 285:2940–2950
89. Neary JT, Kang Y, Shi YF (2004) Signalling from nucleotide receptors to protein kinase cascades in astrocytes. Neurochem Res 29:2037–2042
90. Franke H, Illes P (2006) Involvement of P2 receptors in the growth and survival of neurons in the CNS. Pharmacol Ther 109:297–324
91. D'Ambrosi N, Cavaliere F, Merlo D, Milazzo L, Mercanti D, Volonté C (2000) Antagonists of P2 receptor prevent NGF-dependent neuritogenesis in PC12 cells. Neuropharmacology 39:1083–1094
92. D'Ambrosi N, Murra B, Cavaliere F, Amadio S, Bernardi G, Burnstock G, Volonté C (2001) Interaction between ATP and nerve growth factor signalling in the survival and neuritic outgrowth from PC12 cells. Neuroscience 108:527–534

93. Arthur DB, Taupenot L, Insel PA (2007) Nerve growth factor-stimulated neuronal differentiation induces changes in P2 receptor expression and nucleotide-stimulated catecholamine release. J Neurochem 100:1257–1264
94. D'Ambrosi N, Murra B, Vacca F, Volonté C (2004) Pathways of survival induced by NGF and extracellular ATP after growth factor deprivation. Prog Brain Res 146:93–100
95. Kong Q, Wang M, Liao Z, Camden JM, Yu S, Simonyi A, Sun GY, Gonzalez FA, Erb L, Seye CI, Weisman GA (2005) $P2X_7$ nucleotide receptors mediate caspase-8/9/3-dependent apoptosis in rat primary cortical neurons. Purinergic Signal 1:337–347
96. Wang Q, Wang L, Feng Y-H, Li X, Zeng R, Gorodeski GI (2004) $P2X_7$ receptor-mediated apoptosis of human cervical epithelial cells. Am J Physiol Cell Physiol 287:C1349–C1358
97. Schulze-Lohoff E, Hugo C, Rost S, Arnold S, Gruber A, Brüne B, Sterzel RB (1998) Extracellular ATP causes apoptosis and necrosis of cultured mesangial cells via $P2Z/P2X_7$ receptors. Am J Physiol Renal Physiol 275:F962–F971
98. Lakshmi S, Joshi PG (2006) (2006) Activation of Src/kinase/phospholipase C/mitogen activated protein kinase and induction of neurite expression by ATP, independent of nerve growth factor. Neuroscience 141:179–189
99. Arthur DB, Akassoglou K, Insel PA (2005) $P2Y_2$ receptor activates nerve growth factor/TrkA signalling to enhance neuronal differentiation. Proc Natl Acad Sci U S A 102:19138–19143
100. Ceruti S, Viganò F, Boda E, Ferrario S, Magni G, Boccazzi M, Rosa P, Buffo A, Abbracchio MP (2011) Expression of the new P2Y-like receptor GPR17 during oligodendrocyte precursor cell maturation regulates sensitivity to ATP-induced death. Glia 59:363–378
101. Daniele S, Lecca D, Trincavelli ML, Ciampi O, Abbracchio MP, Martini C (2010) Regulation of PC12 cell survival and differentiation by the new P2Y-like receptor GPR17. Cell Signal 22:697–706
102. Seye CI, Yu N, González FA, Erb L, Weisman GA (2004) The $P2Y_2$ nucleotide receptor mediates vascular cell adhesion molecule-1 expression through interaction with VEGF receptor-2 (KDR/Flk-1). J Biol Chem 279:35679–35686
103. Rumjahn SM, Baldwin KA, Buxton IL (2007) P2Y receptor-mediated angiogenesis via vascular endothelial growth factor receptor 2 signalling. Proc West Pharmacol Soc 50:58–60
104. Rumjahn SM, Yokdang N, Baldwin KA, Thai J, Buxton IL (2009) Purinergic regulation of vascular endothelial growth factor signalling in angiogenesis. Br J Cancer 100:1465–1470
105. Bambace NM, Levis JE, Holmes CE (2010) The effect of P2Y-mediated platelet activation on the release of VEGF and endostatin from platelets. Platelets 21:85–93
106. Hill LM, Gavala ML, Lenertz LY, Bertics PJ (2010) Extracellular ATP may contribute to tissue repair by rapidly stimulating purinergic receptor X7-dependent vascular endothelial growth factor release from primary human monocytes. J Immunol 185:3028–3034
107. Ratchford AM, Baker OJ, Camden JM, Rikka S, Petris MJ, Seye CI, Erb L, Weisman GA (2010) $P2Y_2$ nucleotide receptors mediate metalloprotease-dependent phosphorylation of epidermal growth factor receptor and ErbB3 in human salivary gland cells. J Biol Chem 285:7545–7555
108. Wei W, Ryu JK, Choi HB, McLarnon JG (2008) Expression and function of the P2X(7) receptor in rat C6 glioma cells. Cancer Lett 260:79–87
109. Neary JT, Kang Y, Shi Y-F (2005) Cell cycle regulation of astrocytes by extracellular nucleotides and fibroblast growth factor-2. Purinergic Signal 1:329–336
110. Neary JT, Kang Y, Shi YF, Tran MD, Wanner IB (2006) P2 receptor signalling, proliferation of astrocytes, and expression of molecules involved in cell–cell interactions. Novartis Found Symp 276:131–143
111. Neary JT, Shi YF, Kang Y, Tran MD (2008) Opposing effects of P2X(7) and P2Y purine/pyrimidine-preferring receptors on proliferation of astrocytes induced by fibroblast growth factor-2: implications for CNS development, injury, and repair. J Neurosci Res 86:3096–3105
112. Jia C, Cussen AR, Hegg CC (2011) ATP differentially upregulates fibroblast growth factor 2 and transforming growth factor alpha in neonatal and adult mice: effect on neuroproliferation. Neuroscience 177:335–346
113. Li HJ, Wang LY, Qu HN, Yu LH, Burnstock G, Ni X, Xu M, Ma B (2011) P2Y(2) receptor-mediated modulation of estrogen-induced proliferation of breast cancer cells. Mol Cell Endocrinol 338:28–37
114. Ma B, Yu L, Fan J, Cong B, He P, Ni X, Burnstock G (2011) Estrogen modulation of peripheral pain signal transduction: involvement of $P2X_3$ receptors. Purinergic Signal 7:73–83
115. Fan J, Yu LH, Zhang Y, Ni X, Ma B, Burnstock G (2009) Estrogen altered visceromotor reflex and P2X(3) mRNA expression in a rat model of colitis. Steroids 74:956–962
116. Rajagopal M, Fischer H, Widdicombe JH (2008) Hormonal and purinergic stimulation of bicarbonate secretion in oviducts of rhesus monkey. Am J Physiol Endocrinol Metab 295:55–62
117. Wang Q, Li X, Wang L, Feng Y-H, Zeng R, Gorodeski G (2004) Antiapoptotic effects of estrogen in normal and cancer human cervical epithelial cells. Endocrinology 145:5568–5579
118. Cario-Toumaniantz C, Loirand G, Ferrier L, Pacaud P (1998) Non-genomic inhibition of human $P2X_7$ purinoceptor by 17beta-estradiol. J Physiol 508:659–666
119. De Roo M, Boué-Grabot E, Schlichter R (2010) Selective potentiation of homomeric $P2X_2$ ionotropic ATP receptors by a fast non-genomic action of progesterone. Neuropharmacology 58:569–577
120. Lee KL, Dai Q, Hansen EL, Saner CN, Price TM (2010) Modulation of ATP-induced calcium signalling by progesterone in T47D-Y breast cancer cells. Mol Cell Endocr 319:109–115
121. Tai C-J, Kang SK, Tzeng C-R, Leung PCK (2001) Adenosine triphosphate activates mitogen-activated protein kinase in human granulosa-luteal cells. Endocrinology 142:1554–1560
122. Lin H-Y, Sun M, Lin C, Tang H-Y, London D, Shih A, Davis FB, Davis PJ (2009) Androgen-induced human breast cancer cell proliferation is mediated by discrete mechanisms in estrogen receptor-α-positive and -negative breast cancer cells. J Steroid Biochem Mol Biol 113:182–188
123. Foresta C, Rossato M, Nogara A, Gottardello F, Bordon P, Di Virgilio F (1996) Role of P2-purinergic receptors in rat Leydig cell steroidogenesis. Biochem J 320:499–504
124. Antonio LS, Costa RR, Gomes MD, Varanda WA (2009) Mouse Leydig cells express multiple P2X receptor subunits. Purinergic Signal 5:277–287
125. Brock JA, Handelsman DJ, Keast JR (2007) Postnatal androgen deprivation dissociates the development of smooth muscle innervation from functional neurotransmission in mouse vas deferens. J Physiol 581:665–678
126. Ding Y, Gao ZG, Jacobson KA, Suffredini AF (2010) Dexamethasone enhances ATP-induced inflammatory responses in endothelial cells. J Pharmacol Exp Ther 335:693–702
127. Yukawa H, Shen J, Harada N, Cho-Tamaoka H, Yamashita T (2005) Acute effects of glucocorticoids on ATP-induced Ca^{2+} mobilization and nitric oxide production in cochlear spiral ganglion neurons. Neuroscience 130:485–496
128. Han JZ, Lin W, Chen YZ (2005) Inhibition of ATP-induced calcium influx in HT4 cells by glucocorticoids: involvement of protein kinase A. Acta Pharmacol Sin 26:199–204
129. Liu XH, Zeng JW, Zhao YD, Chen PH, Xiao Z, Ruan HZ (2008) Rapid inhibition of ATP-induced currents by corticosterone in rat dorsal root ganglion neurons. Pharmacology 82:164–170

130. Pochynyuk O, Rieg T, Bugaj V, Schroth J, Fridman A, Boss GR, Insel PA, Stockand JD, Vallon V (2010) Dietary Na^+ inhibits the open probability of the epithelial sodium channel in the kidney by enhancing apical $P2Y_2$-receptor tone. FASEB J 24:2056–2065
131. Rieg T, Bundey RA, Chen Y, Deschenes G, Junger W, Insel PA, Vallon V (2007) Mice lacking $P2Y_2$ receptors have salt-resistant hypertension and facilitated renal Na^+ and water reabsorption. FASEB J 21:3717–3726
132. Fujishita K, Koizumi S, Inoue K (2006) Upregulation of $P2Y_2$ receptors by retinoids in normal human epidermal keratinocytes. Purinergic Signal 2:491–498
133. Tozaki-Saitoh H, Koizumi S, Sato Y, Tsuda M, Nagao T, Inoue K (2006) Retinoic acids increase $P2X_2$ receptor expression through the 5′-flanking region of P2rx2 gene in rat phaeochromocytoma PC-12 cells. Mol Pharmacol 70:319–328
134. Orellano EA, Rivera OJ, Chevres M, Chorna NE, González FA (2009) Inhibition of neuronal cell death after retinoic acid-induced down-regulation of $P2X_7$ nucleotide receptor expression. Mol Cell Biochem 337:83–99
135. Wu PY, Lin YC, Chang CL, Lu HT, Chin CH, Hsu TT, Chu D, Sun SH (2009) Functional decreases in $P2X_7$ receptors are associated with retinoic acid-induced neuronal differentiation of Neuro-2a neuroblastoma cells. Cell Signal 21:881–891
136. Lee H, Choi BH, Suh BC, Lee SK, Kim KT (2003) Attenuation of signal flow from $P2Y_6$ receptor by protein kinase C-alpha in SK-N-BE(2)C human neuroblastoma cells. J Neurochem 85:1043–1053
137. Biswas P, Zanello LP (2009) 1α, $25(OH)_2$ vitamin D_3 induction of ATP secretion in osteoblasts. J Bone Miner Res 24:1450–1460