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Minireview

Cross-talk between the ATP and ADP nucleotide receptor signalling pathways in glioma C6 cells★✪

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In this review we summarize the present status of our knowledge on the enzymes involved in the extracellular metabolism of nucleotides and the receptors involved in nucleotide signalling. We focus on the mechanism of the ATP and ADP signalling pathways in glioma C6, representative of the type of nonexcitable cells. In these cells, ATP acts on the $P2Y_2$ receptor coupled to phospholipase C, whereas ADP on two distinct P2Y receptors: $P2Y_1$ and $P2Y_{12}$. The former is linked to phospholipase C and the latter is negatively coupled to adenylyl cyclase. The possible cross-talk between the ATP-, ADP- and adenosine-induced pathways, leading to simultaneous regulation of inositol 1,4,5-trisphosphate and cAMP mediated signalling, is discussed.

Extracellular nucleotides interact with receptors present on the surface of the cellular membrane. The large family of these receptors was in the seventies termed "purinergic" (Burnstock, 1972; 1978). Since it was later shown that not only adenine but also uridine nucleotides act on the same class of receptors (King *et al.*, 1998), they are presently termed

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Abbreviations: DAG, diacylglycerol; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; PCR, polymerase chain reaction; PLC, phospholipase C; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid; PTX, pertussis toxin.

"nucleotide receptors", although one can still find in the literature their previous designation.

There are three routes by which nucleotides can reach the extracellular compartment (Bodin & Burnstock, 2001). The first is lysis of cells, the second, exocytosis. There are numerous arguments that prove that ATP stored in synaptic vesicles is released as a co-transmitter from nerve terminals by exocytosis (Zimmermann, 1994). The third route, suggested in some non-neuronal cells, could be carrier-mediated ATP release that would involve ATP-binding cassete proteins (ABC), a family of transport ATPases. However, the precise mechanism of this process remains obscure (Bodin & Burnstock, 2001).

Once released, the nucleotides interact with various nucleotide receptors and finally are degraded by several ecto-enzymes (Grobben *et al.*, 1999). Nucleotide receptors are divided into two major classes, P1, responding to adenosine, and P2, responding to ATP, ADP, UTP and UDP (Burnstock, 1978). Since their first cloning in 1993 and 1994, P2 receptors can be further divided into two families: P2X, ligand gated, intrinsic ion channel, ionotropic receptors, and P2Y, G protein-coupled, seven transmembrane domain, metabotropic receptors (Abbracchio & Burnstock, 1994; Boarder & Hourani, 1998; Boeynaems *et al.*, 2000; Dubyak, 1991; Fredholm *et al.*, 1997).

The present review is focused on the mechanism of action of the nucleotide P2Y metabotropic receptors that respond to ATP and ADP in glioma C6 cells. Attention is also focused on the interaction (cross-talk) that may occur between these receptors.

ECTO-ENZYMES

The released nucleotides are degraded by ecto-enzymes. The term "ecto-enzymes" used in this review concerns the enzymes present on the extracellular side of the cell plasma membrane that exclusively metabolize nucleotides. Several ecto-nucleotidases have been cloned from various tissues, including brain (Chen *et al.*, 1996; Khakh & Kennedy, 1998; Zimmermann, 1994). Due to their action, ADP, AMP and adenosine are formed from ATP. Extracellular degradation and inactivation of ATP proceeds by a cascade of enzymes: ecto-ATPase (EC 3.6.1.3), ecto-apyrase (EC 3.6.1.5), ecto-nucleotide pyrophosphatase (EC 3.6.1.9) and ecto-5′-nucleotidase (EC 3.1.3.5). Ecto-ATPase splits off the terminal phosphate and cleaves preferentially ATP into ADP and P_i, whereas ecto-apyrase equipotently cleaves ATP, ADP and other nucleoside tri- and diphosphates. Ecto-nucleotide pyrophosphatase converts ATP into AMP and PP_i and ecto-5′-nucleotidase, AMP into adenosine and P_i. Ecto-nucleotide pyrophosphatase uses as substrate not only ATP but also UTP, GTP, ADP and UDP. AMP and pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) are inhibitors of this enzyme (Grobben *et al.*, 1999). PPADS is also well known inhibitor of ecto-ATPase (Chen *et al.*, 1996). Ecto-5′-nucleotidase resides largely on glia (astrocytes, oligodendrocytes and microglia). This enzyme is strongly inhibited by ATP and ADP. In neural tissues, the activity of ecto-5′-nucleotidase is lower than that of ecto-ATPase and ecto-ADPase. This results in a delayed formation of adenosine after release of ATP (Zimmermann, 1994). In the synaptic cleft the concentration of ATP is in the micromolar range (Grobben *et al.*, 1999).

In glioma C6 cells, which are transformed glial cells with a phenotype similar to astrocytes, Grobben *et al.* (1999) have demonstrated that the main ecto-enzyme that hydrolyzes ATP is ecto-nucleotide pyrophosphatase producing AMP and PP_i . The authors found that at 1–10 μM ATP concentration, the hydrolysis of ATP by this enzyme amounted to 75%, whereas at a higher, 100 μM ATP concentration, the degradation of ATP into AMP and PP_i decreased to 50%. At such concentration, ATP was also hydrolyzed by ecto-ATPase that produces ADP and P_i.

AMP, formed by ecto-nucleotide pyrophosphatase, was easily converted into adenosine by the ecto-5′-nucleotidase present in glioma C6 cells. Due to the action of ecto-nucleotide pyrophosphatase and ecto-5′-nucleotidase, adenosine was found to be the main final product of ATP degradation in this line of cells (Grobben *et al.*, 1999).

The authors have also described the presence of a nucleoside diphosphate kinase in the conditioned medium of glioma C6 cell cultures that is able to convert ATP + UDP into ADP and UTP (Grobben *et al.*, 1999). Due to the action of the ecto-enzymes, the P2Y receptor pathways activated by ATP are terminated and the hydrolysis of ATP into AMP and adenosine shifts the signalling pathway from the P2 into P1 nucleotide receptors.

NUCLEOTIDE RECEPTORS

Nucleotide receptors have been classified as P1 and P2 (Fig. 1) and such classification was accepted by the International Union of Pharmacology (IUPHAR) Subcommittee for the Nomenclature and Classification of Purinoceptors (Abbracchio & Burnstock, 1998; Fredholm *et al.*, 1994; 1997).

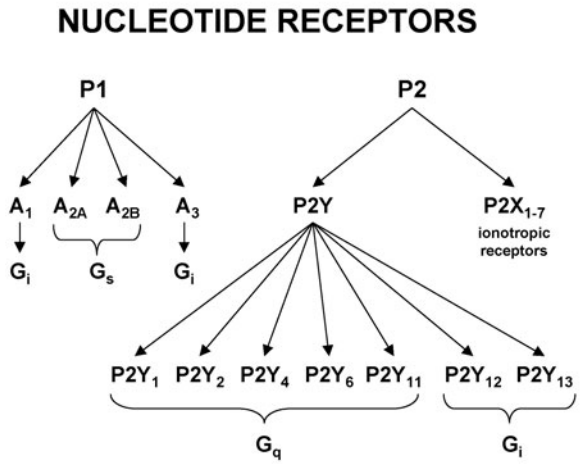

Figure 1. Nucleotide receptors and their effectors.

P1 receptors are activated by adenosine and P2 receptors by ATP, ADP, UTP and UDP. Ionotropic receptors (P2X) form ligand gated channels. Metabotropic receptors (P1, P2Y) are coupled to protein G that stimulates phospholipase C (G_q) and stimulates (G_s) or inhibits (G_i) adenylyl cyclase.

P1 receptors

P1 receptors respond to adenosine. Adenosine is known to act *via* four major receptors, A_1, A_{2A}, A_{2B} and A_3 (Fig. 1), which have been characterized according to their primary sequences and their biochemical and pharmacological properties (Fredholm *et al.*, 1994; Stiles, 1992). Extracellular ATP and ADP are rapidly broken down by ecto-enzymes to adenosine. However, under stress condition, such as hypoxia when the cellular energetics is depressed, intracellular adenosine production is greatly enhanced and adenosine can be released into the extracellular space by specific transporters (Khakh & Kennedy, 1998; Stiles, 1992). The released adenosine, similarly as that formed by the action of ecto-enzymes can act on adenosine receptors. It is worth adding that extracellular adenosine can be also transported back into the cell by a specific nucleotide transporter or cross the cell membrane by simple diffusion (Stiles, 1992). In contrast, extracellular ATP and ADP are unable to cross the cell membrane. Adenosine can be also deaminated by a cytosolic, as well as an extracellular, adenosine deaminase. All of these events cause that adenosine in circulation has a short half-life (Stiles, 1992).

P1 receptors are seven transmembrane domain, G protein-coupled receptors (Ciruela *et al.*, 1997; Stiles, 1992; Windscheif, 1996). In contrast to P2, P1 receptors are competitively antagonized by methylxanthines (Stiles, 1992). Whereas A_1 and A_3 receptors are coupled to protein G_i and inhibit adenylyl cyclase, both types of A_2 receptors are coupled to protein G_s and stimulate adenylyl cyclase. Activation of A_1 receptor increases also K^+ conductance by opening diverse K^+ channels (Belardinelli, 1993; Zimmermann, 1994), whereas activation of A_2 receptors opens ATP-sensitive K^+ channels and produces K^+

efflux in the process that does not involve the pertussis toxin (PTX) sensitive G protein (Furukawa *et al.*, 1993).

In glioma C6 cells, Pianet *et al.* (1989) showed that adenosine strongly stimulates basal and isoproterenol-elicited cAMP synthesis. These data indicate the presence of the A_2 adenosine receptor positively coupled to adenylyl cyclase in these cells.

P2 receptors

Nucleotides, such as ATP and ADP interact with cell surface P2 receptors (Fig. 1). P2 fall into two groups: the intrinsic ion channel P2X receptors and the seven transmembrane domain, G protein-coupled P2Y receptors.

The P2X subtype

At least seven different P2X ionotropic receptors ($P2X_{1-7}$) have been cloned so far (Fig. 1). P2X receptors are characterized by only two membrane-spanning regions, a large cysteine-rich extracellular loop, and both C- and N-termini located intracellularly (Abbracchio & Burnstock, 1998; Khakh & Kennedy, 1998). As is the case for other ionotropic receptors (nicotinic, $GABA_A$, etc.), functional P2X receptors may be composed of several subunits and may form homo- or hetero-multimers (Windscheif, 1996). The P2X channel formed by these subunits is permeable to cations, mainly to Ca^{2+}. $P2X_7$ receptor opens non-selective large pores up to 5 nm in diameter. All of these P2X receptors respond to ATP and are even called ATP-gated ion channels. They also respond to ADP and some of them ($P2X_1$–$P2X_3$) to UTP and are sensitive to suramin and PPADS as antagonists (Boarder & Hourani, 1998). α,β-Methylene-ATP interacts with the P2X receptors but is ineffective as an agonist for all P2Y receptors (Boarder & Hourani, 1998). In glioma C6 cells, addition of α,β-methylene-ATP to the extracellular medium does not produce entry of extracellular Ca^{2+} into the cells, which indicates a lack of this type of ionotropic receptors (Sabała *et al.*, 2001).

The P2Y subtype

Metabotropic, G-protein-coupled P2Y receptors fall into the seven transmembrane domain superfamily of receptors with extracellular N- and intracellular C-termini and a large intracellular III loop (Abbracchio & Burnstock, 1998). Cloning and pharmacological studies revealed seven distinct P2Y receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ (Fig. 1). Cloned $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors activate phospholipase C (PLC) and cause an increase in inositol 1,4,5-trisphosphate (IP_3) concentration (Boarder & Hourani, 1998). The $P2Y_{12}$ receptor and its close relative, $P2Y_{13}$ receptor, have been cloned only recently (Communi *et al.*, 2001; Hollopeter *et al.*, 2001; Savi *et al.*, 2001; Zhang *et al.*, 2002). Both are coupled to the inhibition of adenylyl cyclase. Detailed characteristics of the $P2Y_{12}$ receptor will be described in one of the following chapters. It was found that the $P2Y_1$ receptor responds selectively to ADP compared to ATP while ATP may act as a partial antagonist. 2-Methylthio-ATP and 2-methylthio-ADP are also selective agonists of high potency for $P2Y_1$ receptor, whereas UTP is not effective. In contrast, the $P2Y_2$ receptor responds both to ATP and UTP, while 2-methylthio-ATP has no effect on its activity. These two receptors are sensitive to suramin but differ in their antagonist selectivity to PPADS (Boarder & Hourani, 1998). $P2Y_1$ is blocked by PPADS while $P2Y_2$ is resistant to this compound. The $P2Y_4$ and $P2Y_6$ receptors are selective for UTP and UDP, respectively. The $P2Y_4$ receptor is activated most potently by UTP, less so by ATP and not at all by nucleoside diphosphates. The $P2Y_6$ receptor is activated most potently by UDP, weakly by UTP and does not respond to ATP (Boarder & Hourani, 1998; Communi *et al.*, 1996; Lazarowski & Harden, 1994; Nicholas *et al.*, 1996). The $P2Y_6$ receptor has been shown

to be expressed in glioma C6–2B cells and is linked to PLC (Nicholas *et al.*, 1996). The $P2Y_{11}$ receptor is selective to ATP but not to UTP and UDP (Boarder & Hourani, 1998).

In glioma C6 cells, ATP has been reported to mediate phosphoinositide turnover (Kitanaka *et al.*, 1992; Lin & Chuang, 1993). We have also demonstrated that in these cells ATP treatment generates IP_3 and simultaneously results in an increase in intracellular Ca^{2+} concentration due to the PLC-mediated release from intracellular stores (Sabała *et al.*, 1997; 2001).

While the presence of $P2Y_2$ receptor in glioma C6 cells seems to be generally accepted, the presence of the $P2Y_1$ receptor that stimulates PLC activity had previously been a subject of serious debate. Studies on glioma C6-2B and C6 cells have shown the presence of a $P2Y_1$-like receptor effecting inhibition of adenylyl cyclase but not accumulation of IP_3 and resistant to PPADS (Boarder & Hourani, 1998; Schater *et al.*, 1996; 1997). It has been proposed therefore that two different $P2Y_1$ receptors exist: one coupled to PLC and blocked by PPADS and another one, native to glioma C6, negatively coupled to adenylyl cyclase. However, this interpretation was questioned by Webb *et al.* (1996), who found that cloned rat $P2Y_1$ receptor cDNA and that derived from glioma C6 are 100% identical in the entire coding region. Thus, the authors suggested that the same $P2Y_1$ receptor was coupled in different cell types to either PLC activation or to adenylyl cyclase inhibition, as it had been found in glioma C6 cells. However, results from our laboratory revealed that both the $P2Y_1$ and $P2Y_2$ receptors co-exist in these cells and both are linked to PLC (Sabała *et al.*, 1997; 2001).

GLIOMA C6 AS A MODEL OF NONEXCITABLE CELLS

Concerning calcium signalling, only metabotropic receptors are expressed in the plasma membrane of nonexcitable cells (the term used to indicate the absence of voltage-dependent Ca^{2+} channels). These receptors are coupled to intracellular signal transduction processes. In such type of cells, Ca^{2+} signalling is mediated by the inositol phosphate system. We have previously shown that glioma C6 belongs to such type of cells (Barańska *et al.*, 1995; 1999).

When hormones or neurotransmitters interact with plasma membrane receptors that contain seven transmembrane domains and are coupled to protein G_q, such interaction results in stimulation of PLCβ activity (Fig. 2). PLC converts phosphatidylinositol (4,5)-bisphosphate (PIP_2) into second messengers: IP_3 and diacylglycerol (DAG). IP_3 diffuses through the cytosol to the endoplasmic reticulum (ER) and binds to the IP_3 receptor present at this membrane (Berridge, 1993). After binding, the channel in this receptor opens and Ca^{2+} stored in the ER is released into the cytosol. The initial rise of Ca^{2+} in the cytosol results from the direct action of IP_3 on the ER stores and can be observed even in the absence of extracellular Ca^{2+}. This increase has a transient nature and constitutes the first phase in the process of the cytosolic Ca^{2+} mobilization that occurs after activation of metabotropic receptors. When extracellular Ca^{2+} is present, the depletion of the ER stores causes opening of specific voltage-independent Ca^{2+} channels in the plasma membrane and Ca^{2+} can enter from the extracellular space (Fig. 2). This is the second, sustained phase with a more prolonged rise in the cytosolic Ca^{2+}. This biphasic process was termed "capacitative Ca^{2+} entry" (Berridge, 1995; Putney & Bird, 1993) or "store mediated Ca^{2+} entry" (Rosado *et al.*, 2000) and is typical for the nonexcitable type of cells. Ca^{2+} entry coupled to the state of filling of the ER Ca^{2+} stores is independent on the mechanism of its depletion and can be also caused by thapsigargin, an irreversible inhibitor of the SERCA ATPase, that promotes the leak of $Ca^{2+}$from the ER without IP_3 formation (Fig. 2). The nature of the signal linking

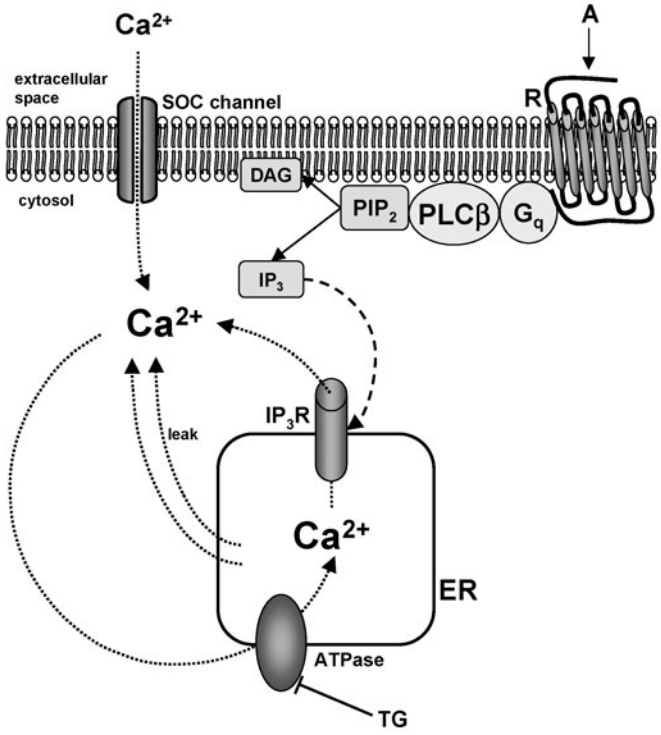

Figure 2. Inositol phosphate-Ca^{2+} signalling system illustrating capacitative (store-mediated) Ca^{2+} entry in nonexcitable cells.

Agonist (A) acts on receptor (R) coupled with protein G_q and stimulates phospholipase Cβ (PLCβ) activity. Breakdown of phosphatidylinositol (4,5)-bisphosphate (PIP_2) produces diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 binds to IP_3 receptor (IP_3R) present in the endoplasmic reticulum (ER) and releases Ca^{2+} into the cytosol. The depletion of the ER Ca^{2+} store causes opening of voltage-independent, store operated Ca^{2+} channels (SOC) in the plasma membrane and extracellular Ca^{2+} influx. Thapsigargin (TG) inhibits Ca^{2+}-ATPase that pumps Ca^{2+} into the ER lumen and due to that causes a leak of Ca^{2+} from this structure (for details see text).

the ER Ca^{2+} store with Ca^{2+} channels in the plasma membrane is being still debated. Recently, a role of the actin cytoskeleton in this process has been proposed (Rosado *et al.*, 2000; Sabała *et al.*, 2002).

We have previously shown that glioma C6 cells are characterized by the typical biphasic, capacitative Ca^{2+} entry pathway (Barańska *et al.*, 1995; 1999; Czajkowski & Barańska, 1999; Czajkowski *et al.*, 2002; Sabała *et al.*, 1997; 2001; 2002) (Fig. 3). In such type of cells, the existence of such a pathway points to PLC activation and IP_3-mediated signal transduction, responsible for the increase in the intracellular Ca^{2+} concentration.

$P2Y_1$ AND $P2Y_2$ RECEPTORS IN GLIOMA C6 CELLS

In glioma C6 cells, we have characterized $P2Y_1$ and $P2Y_2$ receptors using the measurement of intracellular Ca^{2+} concentration and testing the effect of specific pharmacological agents (agonists and antagonists of $P2Y_1$ and $P2Y_2$ receptors) on Ca^{2+} mobilization (Sabała *et al.*, 2001). We have shown that stimulation of nucleotide receptors by ADP, ATP and UTP initiates a typical, biphasic Ca^{2+} response compatible with the capacitative model of Ca^{2+} influx (Figs. 3A, 3C, 3E). The presence of external Ca^{2+} is not necessary to elevate Ca^{2+}(Figs. 3B, 3D, 3F). Figure 3A shows that the kinetics of ADP-evoked Ca^{2+} changes differs from those evoked by ATP and UTP (Figs. 3C, 3E). The ATP and UTP induced Ca^{2+} elevation starts with an initial peak response and is followed by a long sustained plateau phase, whereas the response to ADP is more transient (Fig. 3A).

We have also shown that cells treated with ATP fail to respond to UTP, indicating that the action of ATP and UTP is non-additive, whereas ADP and UTP effects are additive. Similarly, cross-desensitization between ATP and UTP, but not between ADP and UTP, occurred. The pharmacological properties of nucleotide receptors generating Ca^{2+} mobilization in response to various nucleotide analogues indicated the following order of potencies: 2-methyltio-ADP > ADP > 2-methylthio-ATP = 2-chloro-ATP > ATP > UTP. Adenosine, AMP and $\alpha\beta$-methylene-ATP, were ineffective (Sabała *et al.*, 2001). Furthermore, the effect

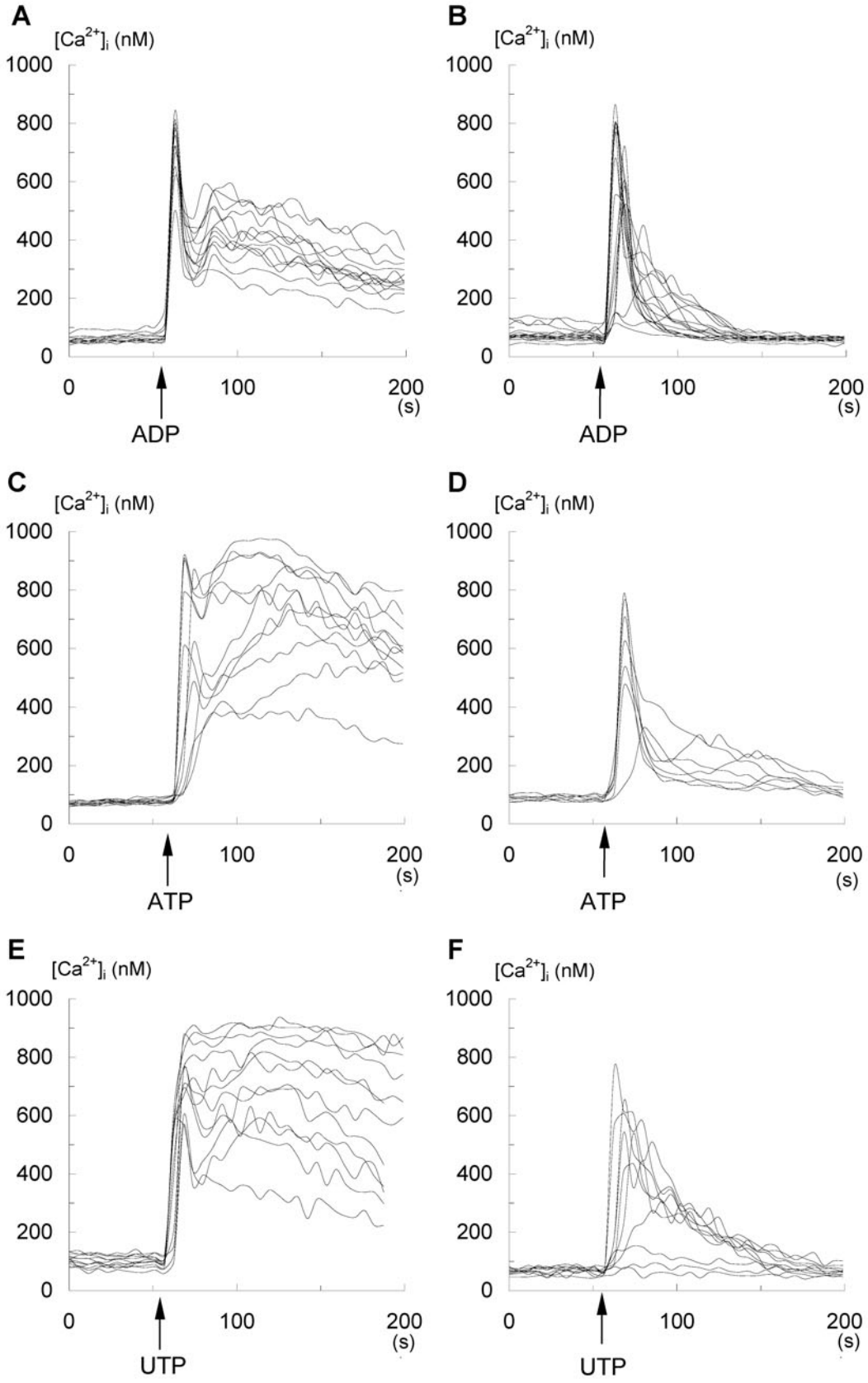

Figure 3. Effect of ADP (A, B), ATP (C, D) and UTP (E, F) on changes in intracellular Ca^{2+} signalling in single glioma C6 cells measured in the presence of extracellular Ca^{2+} (A, C, E) or in its absence (B, D, F).

Each trace represents the response of an individual cell. (According to Sabała *et al.*, 2001).

of antagonists indicated that suramin that distinctly blocked both $P2Y_1$ and $P2Y_2$ receptors, blocked the effect of ATP, UTP and ADP on the intracellular Ca^{2+} rise, whereas PPADS, a selective $P2Y_1$ but not $P2Y_2$ antagonist, decreased only the Ca^{2+} response to ADP (by 94%) (Sabała *et al.*, 2001). A similar effect was observed with MRS2179, a specific and selective antagonist of $P2Y_1$ receptor (Czajkowski *et al.*, 2002).

Those data suggested the presence of $P2Y_1$ and $P2Y_2$ receptors in glioma C6 cells. To confirm their identity, polymerase chain reaction on reverse transcribed total RNA was employed. The RT-PCR analysis revealed the molecular identity of the P2Y receptors expressed by glioma C6 as both $P2Y_1$ and $P2Y_2$ (Fig. 4A). ADP acts as an agonist on the first and ATP and UTP on the second one. The molecular identity, together with the pharmacological analysis and the typical, biphasic Ca^{2+} response, led us to the conclusion that both of these receptors are linked to PLC (Sabała *et al.*, 2001).

The conclusion that in glioma C6, the $P2Y_1$ receptor stimulates PLC and is effective in the production of IP_3 and release of Ca^{2+} from the ER, was supported by direct visualization of a PIP_2-bound PLCδ-PH domain coupled to GFP expressed in these cells (Czajkowski *et al.*, 2002). After stimulation of the cells with ADP, the PIP_2-bound GFP fluorescence at the cell surface was distinctly reduced, indicating the breakdown of PIP_2 by activated PLC (Czajkowski *et al.*, 2002). The absence of accumulation of IP_3 in these cells after ADP treatment was the reason for the former belief that in glioma C6 ADP-responding nucleotide receptor stimulation does not lead to PLC activation. This phenomenon was probably caused by the ADP-evoked transient increase in IP_3 level, too short to be examined. It is worth adding that such increase in IP_3 level in glioma C6 cells was observed only at high ADP

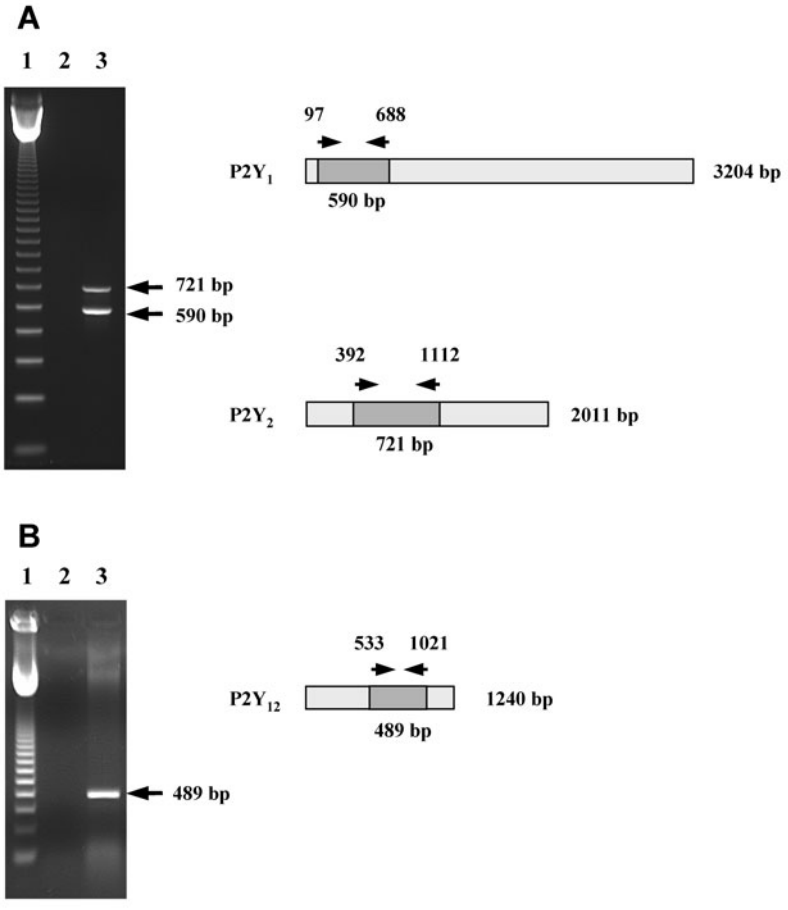

Figure 4. Detection of $P2Y_1$, $P2Y_2$ (A) and $P2Y_{12}$ (B) mRNA by RT-PCR.

Amplification products of 590 and 721 bp (A) and 489 bp (B) corresponding to $P2Y_1$, $P2Y_2$ and $P2Y_{12}$ mRNA, respectively, were analyzed. Lane 1, 123 kb DNA ladder; Lane 2, control reaction in the absence of cDNA template; Lane 3, PCR products. (Adapted from Sabała *et al.*, 2001 (A) and from Czajkowski *et al.*, 2002 (B)).

concentration and for 30 min stimulation of the cells (Grobben *et al.*, 2001).

In contrast to the PLC activation, ADP-evoked adenylyl cyclase inhibition has long been documented (Boyer *et al.*, 1993). In glioma C6 cells, ADP, in addition to its effect on the Ca^{2+} response, inhibits by 80% the isoproterenol-elicited cAMP accumulation (Sabała *et al.*, 2001). This effect was reversed by treatment of the cells with PTX and was insensitive to PPADS. PTX is a specific inhibitor of G_i protein coupled to the inhibition of adenylyl cyclase. Those data suggested two possibilities: that in glioma C6 the $P2Y_1$ receptor is dually coupled to both G_q (PLC) and G_i (adenylyl cyclase) or that two ADP receptors exist, one, $P2Y_1$, coupled to PLC, and the second, $P2Y_1$-like, coupled to adenylyl cyclase (Sabała *et al.*, 2001).

Most recently, a P2Y receptor that couples to the inhibition of adenylyl cyclase in rat blood platelets, previously termed $P2T_{AC}$, has been cloned and designated as $P2Y_{12}$ (Hollopeter *et al.*, 2001; Savi *et al.*, 2001). The presence of this receptor has also been reported in glioma C6-2B cells (Jin *et al.*, 2001). Thus, to differentiate whether ADP interacts with one or with two receptors, we used the antisense oligonucleotide technique and showed that knock-down of the $P2Y_1$ receptor causes a decrease in the $P2Y_1$ mRNA level and loss of the receptor-evoked PLC activity (Czajkowski *et al.*, 2002). Adenylyl cyclase inhibition by ADP remains unchanged under this condition. To confirm the identity of the $P2Y_{12}$ receptor responsible for the inhibition of adenylyl cyclase, PCR on reverse-transcribed total mRNA was employed (Czajkowski *et al.*, 2002). Primers were selected to cover the unique sequence of rat platelet $P2Y_{12}$ receptor. Figure 4B shows that mRNA of this receptor is expressed and localized in glioma C6 cells. Thus, in these cells, ADP acts on two receptors: $P2Y_1$, linked to the stimulation of PLC and Ca^{2+} release, and $P2Y_{12}$, negatively coupled to adenylyl cyclase through G_i protein.

CROSS-TALK BETWEEN THE ATP AND ADP NUCLEOTIDE RECEPTORS

The biological response to extracellular ATP could be due to ATP or to one of its metabolic products, ADP or adenosine. In glioma C6 cells (Fig. 5), ATP can interact directly with the $P2Y_2$ receptor linked to PLC, whereas its metabolic product, ADP, interacts with the $P2Y_1$ receptor coupled to PLC and with the $P2Y_{12}$ receptor, negatively coupled to adenylyl cyclase (Czajkowski *et al.*, 2002; Sabała *et al.*, 2001). Adenosine, interacting with the A_2 type of receptor, stimulates the activity of this enzyme (Pianet *et al.*, 1989) (Fig. 5).

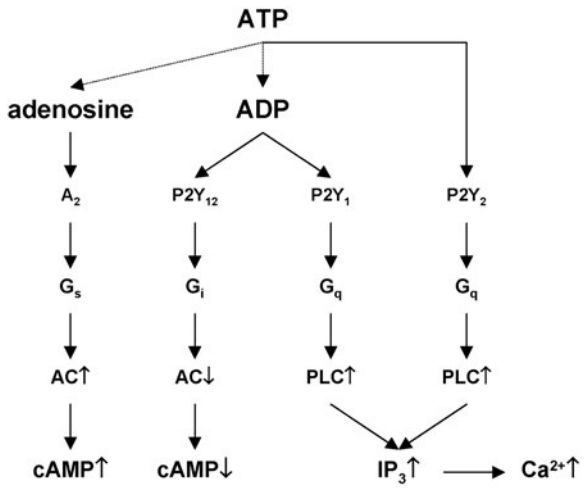

Figure 5. Schematic diagram of ATP, ADP and adenosine signalling pathways in glioma C6 cells.

For details see text. $P2Y_1$, $P2Y_2$, $P2Y_{12}$, A_2, nucleotide receptors; G_s, G_i, G_q, proteins G; AC, adenylyl cyclase, PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate.

In the brain, astrocytes, neurons, and endothelial cells are all ATP-releasing cells. The presence of nucleotide receptors in their plasma membranes points to the function of ATP in the communication between these cells (Zimmermann, 1994). In neurons, the effect of ATP and adenosine are often opposite. Adenosine, *via* A_1 receptor inhibits the release of most transmitters, probably by inhibi-

tion of presynaptic Ca^{2+} channels (Zimmerman, 1994). In cholinergic nerve terminals isolated from striatum, breakdown of ATP to adenosine by ecto-nucleotidase is a major feedback in the autoinhibitory mechanism depressing further release of ATP. Adenosine, by increasing K^+ conductance, acts to hyperpolarize neurons. In contrast, ATP depolarizes the neuronal membrane (Illes & Norenberg, 1993). This depolarization may occur by opening of nonselective cationic channels, or by the action of G protein subunits, activated by $P2Y_2$ receptor. These events inhibit the resting K^+ conductance, close K^+ channels and lead to a long-lasting depolarization (Stiles, 1992; Illes & Norenberg, 1993; Zimmerman, 1994). Moreover, acting *via* P2X receptor and producing an influx of Ca^{2+}, ATP might play a neurotoxic role, similar to that of glutamate. Adenosine blocks this process. The neuroprotective role of adenosine in cerebral ischemia has long been recognized (Zimmerman, 1994). However, all of these events concern the effect of adenosine on A_1 receptor that *via* G_i protein inhibits adenylyl cyclase. The A_{2A} receptor that stimulates adenylyl cyclase, has an opposite function. This type of receptor, present on cholinergic nerve terminals, stimulates acetylcholine release from stratial synaptosomes (Kurokawa *et al.*, 1996).

In glioma C6 cells, adenosine stimulates adenylyl cyclase activity (Fig. 5). Chiono *et al.* (1995) described that in C6-2B glioma cells the increase in the intracellular Ca^{2+} level observed during capacitative Ca^{2+} entry inhibits type VI adenylyl cyclase that predominates in such cells. Thus, the increase in cAMP accumulation induced by adenosine may be negatively regulated by the increase in the Ca^{2+} level produced by ATP (Fig. 5).

Perhaps the most significant result described in this review is the evidence that in glioma C6 cells, ADP interacts with two distinct receptors, $P2Y_1$ and $P2Y_{12}$ (Czajkowski *et al.*, 2002). The first of them increases the intracellular Ca^{2+} level, the second inhibits cAMP synthesis (Fig. 5). Since in these cells adenosine, by G_s protein, stimulates the activity of adenylyl cyclase, ADP is the only agonist acting on the nucleotide receptor that activates G_i protein. This protein not only inhibits adenylyl cyclase by its α subunit, but also by the $\beta\gamma$ subunits regulates functions of many plasma membrane channels (Hur & Kim, 2002; Selbie & Hill, 1998). Thus, the cross-talk between the two distinct pathways generated by ADP, the pathways induced by ATP and adenosine, and among all of them, leads to a multiple network of simultaneous regulation of the IP_3 and cAMP mediated signalling.

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