**Review**

**P2X7 receptor: Death or life?**

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**Abstract**

The P2X7 plasma membrane receptor is an intriguing molecule that is endowed with the ability to kill cells, as well as to activate many responses and even stimulate proliferation. Here, the authors give an overview on the multiplicity and complexity of P2X7-mediated responses, discussing recent information on this receptor. Particular attention has been paid to early and late signs of apoptosis and necrosis linked to activation of the receptor and to the emerging field of P2X7 function in carcinogenesis.

**P2X7: From an elusive receptor to an ATP-gated channel/pore**

The P2X7 receptor (P2X7R) for extracellular ATP is expressed by a variety of cell types as different as neurons, macrophages, dendritic and microglial cells, fibroblasts, lymphocytes, and endothelial cells. At least a subpopulation of human osteoblasts also express P2X7 [1]. Expression of the receptor has been demonstrated in human fibroblasts [2, 3] and epithelia from the human bladder [4, 5], human and rat uterus [6, 7], male genital organs of the rats [8], human fetal keratinocytes [9] and mouse parotid acinar and duct cells [10].

Although interest in this molecule has increased exponentially since the late 1990s, initial observations on its peculiar biochemical properties go back to the 1970s, when Cockcroft and Gomperts reported that extracellular ATP caused degranulation and striking morphological changes in rat mast cells [11]. These authors also hypothesized that cell responses triggered by ATP, such as histamine secretion and phosphatidyl inositol formation, were due to activation of an as yet unknown receptor (the `ATP receptor`) activated by the fully dissociated ATP form (ATP4+) [12]. Based on its intriguing ability to cause a reversible permeabilization of the plasma membrane, this receptor was also referred to as the `permeabilizing ATP receptor` later named P2Z [13]. P2Z was described not only in rat mast cells but also in mouse macrophages [14, 15]. Following these early observations, the peculiar pharmacological and biochemical properties of the P2Z receptor were recognized in many other cells such as human macrophages, human B lymphocytes, mouse microglial cells, mouse and human monocyte-derived dendritic cells.

The permeabilizing ATP receptor was cloned in 1996 from a rat brain library and named P2X7 for its homology with the other P2X receptors [16]. P2X7 is a 595 AA protein with a predicted structure comprising two transmembrane domains and a bulky extracellular cysteine rich region, with conserved lysine and glycine residues and several potential N-linked glycosylation sites, followed by a long stretch (from Phe188 to Val321) forming six putative antiparallel β sheets. The amino and carboxyl-terminal domains are both cytoplasmic. Similarities have been found between the β sheets region and the catalytic domains of class II aminoacyl-tRNA synthetases [17]. These β sheets are likely to include residues comprising the ATP-binding site, since substitution of two aminoacids comprised in this region, Lys193 and Lys311, completely abrogates ethidium and barium uptake [18].

P2X7 is an ionotropic, ligand-gated, cation channel [19, 20]. Stimulation of the receptor with low ATP doses, reversibly opens a membrane channel permeable to small cations (Na+, Ca2+, K+), while sustained stimulation with higher ATP doses or repeated stimulation with sequential ATP pulses, induces the formation of a pore permeable to large molecular weight molecules such as choline (100 Da),

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methylglucamine (190 Da), ethidium (314 Da), YO-PRO-1 (376 Da), propidium (414 Da), lucifer yellow (457 Da) [21–24]. ATP generates in B and T lymphocytes a pore with a smaller molecular cut-off of slightly over 300 Da (ethidium is admitted, propidium is excluded) [25–27].

Alkalization of the medium or removal of Mg$^{2+}$ and Ca$^{2+}$ increases the apparent affinity of ATP and BzATP for the native and recombinant P2X$\gamma$ receptor [11, 28, 29]. Metal ions such as Cu$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, Co$^{2+}$ inhibit P2X$\gamma$ evoked currents [30, 31]. The widely used calmodulin antagonist calmidazolium, inhibits BzATP-induced currents in HEK293 cells stably expressing the rat P2X$\gamma$ receptor while, it has no effect on YO-PRO-1 uptake. This evidence was interpreted as proof that it is possible to dissociate the channel from pore function, and therefore these might be two separate molecular entities [24].

The carboxyl-terminal cytoplasmic domain of P2X$\gamma$ (AA 352–595) is longer than in other members of the P2X subtype. This domain is crucial for P2X$\gamma$ pore formation, transduction and signalling [16, 29, 32]. Allelic mutations, leading to loss of function, have been identified both in the human and mouse receptor. A mutation (P451L) in the P2X$\gamma$ cytoplasmic tail occurring in some mice strains reduces the capacity of ATP to induce pore formation [33, 34]. It has been suggested that pore formation requires over 95% of the C-terminal tail of the receptor. Experiments performed with truncated receptors expressed in HEK-293 cells and Xenopus oocytes show that truncation of the protein at residue 581 allows only negligible influx of ethidium while, surprisingly, cells expressing a receptor truncated at position 582 have unchanged uptake [35]. In contrast, formation of the ionic channel occurs even in cells expressing receptors truncated at position 380, suggesting that only a limited portion of the cytosolic region is needed for channel activity [35].

The glutamic acid 496 seems to be important for the pore-forming activity of P2X$\gamma$, and substitution of Glu$^{496}$ with Ala (E496A), occurring in the ankyrin repeat motif of the carboxyl-terminal domain of the receptor, leads to loss of function of the receptor in homozygous individuals and substitution of Ile$^{568}$ with Asn (I568N) and is located in a tracking motif in the carboxyl terminus; this polymorphism blocks normal trafficking and membrane expression of the receptor [35, 39].

The Hill coefficients obtained from the ATP dose-dependency curves are consistent with multiple ATP-binding sites [28, 40]. Studies performed with P2X$\gamma$ receptors fused with enhanced green fluorescent protein (EGFP), reveal that there are no large scale changes in P2X$\gamma$ receptor density when pore formation occurs [41].

Li and colleagues postulated a differential assembling of the P2X$\gamma$ receptor complex in diverse cell types. Experiments performed in rat parotid duct and acinar cells would indicate that while in the first cell type, P2X$\gamma$ gating is fast and independent on cytoskeleton, in acinar cells, activation of the receptor is slower and requires actin polymerisation [10]. It has been suggested that the ATP-induced P2X$\gamma$ pores increase or decrease by very small units [42]. It is not known why in some cell types P2X$\gamma$ expression gives rise to the ATP-dependent pore, while in other cells (human lymphoblastoid cells, human and rat fibroblasts) the channel–pore transition does not occur [2, 25, 43, 44].

A subject that has interested numerous authors in the last few years is the membrane blebbing/vesiculation associated with P2X$\gamma$ activation. Among other features, the vesicles have been shown to contain active IL-1$\beta$, an important proinflammatory cytokine. P2X$\gamma$ activation leads to loss of plasma membrane and to phospholipid flip. Upon a brief stimulation with P2X$\gamma$ agonists, phosphatidylycerine exposure reverses within hours, without concomitant cell death [45]. The ROCKI kinase pathway plays a key role in the cytoskeletal rearrangement following P2X$\gamma$ activation [46, 47].

Western blot analysis of native proteins shows that P2X$\gamma$ forms multimeric complexes in rat bone marrow cells and peritoneal macrophages, while it is present as a monomer in brain glia or astrocyte lysates [48]. This could be due to expression of regulatory proteins modulating the channel–pore transition process. Different P2X$\gamma$-interacting proteins have been identified. Mass spectrometry on immunoprecipitates showed that a complex comprising 11 proteins interacts with the receptor: laminin α3, integrin β2, β-actin, α-actinin, supervillin, matrix activated MAGuK (membrane-associated guanylate kinase P55), heat shock proteins 70 and 90 (Hsp70, Hsp90), heat shock cognate protein 71 (Hsc71), phosphatidylinositol 4-kinase (PI4K) and receptor protein tyrosine phosphatase-beta (RPTPβ). Among those RPTPβ and Hsp90 have been proposed to functionally modulate P2X$\gamma$ [49, 50]. To isolate other potentially P2X$\gamma$ related proteins the yeast two hybrid approach has also been used, resulting in isolation of various epithelial membrane protein family members (EMP). EMPs have been linked to cell blebbing and in general to receptor-mediated apoptosis [51].

Putative LPS-binding domains have been identified in the C-terminal domain. This seems to fit with the property of P2X$\gamma$ to modulate secretion of different immunomodulatory molecules (IL-1β, TNF-α, NO) in LPS-stimulated macrophages. Protein–protein and protein–lipid interaction motifs within the receptor tail were also identified [52]. P2X$\gamma$-derived peptides are able to bind LPS and block two LPS-modulated intracellular events, i.e. the capacity of activating extracellular signal-regulated kinases 1 and 2 (ERK1, ERK2) and stimulation of IkappaB-alpha degradation. In human leukaeinic lymphocytes, P2X$\gamma$ ion channel activation by ATP stimulates phospholipase D (PLD) through the influx of bivalent cations [53].
Although most of the studies on P2X7 have been performed in the immune system, recent reports showed expression and putative functions of the receptor also in other cell types such as the human neuroblastoma cell line SH-SY5Y [54], glial Müller cells from the human retina [55], mouse Schwann cells [56], and rat pituitary cells [57]. The presence of transcripts for the P2X7 subtype has been detected in preparations of medulla oblongata, spinal cord, and nodose ganglion. P2X7 protein has been found in the presynaptic terminals in the central nervous system [58]. The receptor is localized to the excitatory terminals in the hippocampus and its stimulation induces the release of glutamate and GABA [59]; primary astrocytes cultures from rat hippocampus are also immunopositive for P2X7. Contrasting observations have been recently published on involvement of P2X7 in mossy fiber-CA3 synaptic responses. Activation of this receptor has been found to depress mossy fiber-CA3 synaptic transmission through activation of p38 MAP kinase [60], but BzATP-mediated decrease of mossy fiber-C3 potential was shown not to be mediated by P2X7 [61]. The authors hypothesize that BzATP is extracellularly catabolized to Bz-adenosine and subsequently hetero-exchanged for intracellular adenosine that would be responsible for depressing mossy fibers potential through presynaptic A1 receptors [61]. Accordingly, Sim and colleagues failed to detect P2X7 receptor in hippocampal neurons [62].

P2X7 receptor stimulation provides a new route for excitatory amino acid release (L-glutamate and D-aspartate) from murine cortical astrocytes [63]. Activation of the receptor is also coupled to the phosphoinositide 3-kinase (PI3K)/Akt pathway in rat cortical astrocytes and in P2X7-expressing 1321N1 cells [64].

Death or life?

It was soon evident that extracellular ATP was a potent permeabilizing and cytotoxic factor for macrophages. Steinberg and Silverstein demonstrated that, upon exposure of macrophages to high ATP concentrations, most of the cells died and the surviving ones were insensitive to ATP-mediated permeabilization and refractory to ATP cytotoxic effects [14]. These findings suggested that ATP-mediated responses were due to expression of cytotoxic receptor/s and not to the mere perturbation of the plasma membrane integrity by ATP [14, 21]. These observations were later confirmed by experiments performed with pharmacological inhibitors of the receptor having different properties and showing diverse species/specificity, i.e. periodate oxidized-ATP (oATP), 1-[(N,O-Bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl)-4-phenylpiperazine (KN-62), and brilliant blue G. Oxidized ATP [65], is active not only at P2X7 but also at other P2X subtypes. KN-62 only inhibits human P2X7 [66], while brilliant blue G is active at the murine subtype [67]. A further proof of the existence of the receptor was given by the demonstration that blockade of P2X7 by a specific monoclonal antibody fully abrogated ATP-mediated cytotoxicity. Furthermore, cells lacking reactivity to anti-P2X7 antibodies were also resistant to the ATP permeabilizing and cytotoxic effects [68].

P2X7 would thus be a cytotoxic receptor, capable of killing the cell by forming membrane pores, and ATP not just an intermediate of intracellular energy transactions but a potent cytotoxic molecule. Since the cytotoxic effect occurs only at high extracellular ATP concentrations, massive release of the nucleotide due to cell damage or lysis, or decreased activity of the ATP hydrolysing enzymes (ecto-ATPases and ecto-nucleotidases) have to be postulated. For example, hematopoietic precursor cells isolated from mouse bone marrow, which express P2X7 receptors, are very sensitive to ATP and are killed by stimulation with the nucleotide. This high sensitivity has permitted the establishment of an efficient procedure to isolate highly purified marrow stromal cells, by deleting hematopoietic cell precursors [69]. Elimination of P2X7-expressing cells can also be significantly increased by incubating cells in the presence of a toxic agent such as potassium thiocyanate that enters the cell membrane upon P2X7 receptor opening [70]. This same procedure might be helpful for the treatment of tumors of hematopoietic or other origins.

ATP can induce cell death by either necrosis or apoptosis, depending upon the incubation time, ATP dose and cell type [71]. The ability of extracellular ATP to cause apoptosis of mouse cell lines was initially documented in P-815 mastocytoma and YAC lymphoid cells, and then subsequently extended to mouse thymocytes [22, 72, 73]. P2X7-expressing J774 mouse macrophages mostly die by colloid-osmotic lysis as they quickly swell, change refrangence, detach from the substrate and release cytoplasmic components. Recently, P2X7 has also been shown to cause apoptotic death of BAC1 macrophages [74]. Mouse microglial cell lines N9 and N13 also show condensation of nuclei and DNA fragmentation, which are both hallmarks of apoptosis. Furthermore, stimulation with ATP of N9 and N13 cells induces activation of different caspases. Caspase-1, 3 and 8 are activated and caspase substrates such as PARP and lamin B are processed [75, 76]. Activation of casp-3 and casp-9 has been shown in human cervical epithelial cells [77]. Triggering of P2X7 induces phosphatidylserine externalization and membrane blebbing [45]. These effects are mediated by P2X7 as they are blocked by P2X7 inhibitors and are absent in P2X7-less cells. Increased P2X7 expression has been found in the peri-infarct region in the rat brain cortex after cerebral artery occlusion; augmented P2X7 protein levels were detected in microglia, neurons and apoptotic cells [78]. Stimulation of P2X7 receptor induces release of tumor necrosis factor alpha (TNF-α) from microglia [79].

Sugiyama and colleagues reported that in pericyte-containing retinal microvessels, activation of P2Y1 receptors by UTP prevented P2X7 pores from forming. These data would point to a cross-talk between the P2X and P2Y subtypes in inducing/preventing cell death. They also showed that maximal activation of P2X7 resulted in voltage-dependent calcium channels (VDCCs) opening, exacerbating the death process [80].
The mammalian ectoenzyme ART-2 catalyzes protein ADP-ribosylation. It has been shown that exposure of T lymphocytes to NAD, the substrate of ART-2, causes P2X7 receptor ADP-ribosylation and P2X7-dependent apoptosis characterized by exposure of phosphatidylserine [81], shedding of CD62L, propidium iodide uptake and cell shrinkage [82, 83].

Human fibroblasts from diabetic patients show enhanced P2X7-mediated responses e.g. increased shape change, microvesiculation, increased IL-6 secretion and accelerated apoptosis [84]. Lymphocytes from type 1 diabetic (NOD) mice show an increased apoptosis upon stimulation of the P2X7 receptor, that also results in an augmented shedding of the lymphocyte homing receptor CD62L [85].

Purinoceptors can also be activated by spontaneous or stimulated ATP release through an autocrine or paracrine loop. A basal ATP release occurs in vitro, thus providing a chronic stimulation of P2 receptors. Murine macrophages expressing high levels of P2X7 show an unusually high rate of spontaneous cell death that can be significantly reduced by inhibiting P2X7, or by incubation of the cells in the presence of the ATP-hydrolyzing enzyme apyrase, suggesting that the P2X7 receptor is activated by constitutively released ATP [86].

Extracellular ATP induces apoptosis of human peripheral monocytes infected with Mycobacterium bovis (bacillus Calmette Guerin, BCG) as demonstrated by DNA fragmentation and nuclear condensation. Interestingly and more importantly, ATP also induces swelling of the BCG-containing vacuoles and reduces BCG viability [87]. Pore formation upon P2X7 receptor stimulation is responsible for induction of cell death of BCG-infected human macrophages. Triggering of P2X7 receptor also induces death of the intracellular bacteria, at variance with CD95 or complement activated cell death that only kills macrophages [88]. The crucial step in the killing of the intracellular parasite is fusion of the parasite-containing phagosomes with intracellular lysosomes [89].

Li and colleagues showed that heterogeneity in cell donors with respect to ATP responsiveness could depend on p2x7 polymorphisms and suggested an association between a single-nucleotide polymorphism in the P2X7 promoter (position −762) and infection by Mycobacterium tuberculosis [90]. Another P2X7 polymorphism, this time situated in the coding region (A/C 1531), has been associated with the inability of macrophages to kill mycobacteria [91]. A putative role for P2X7 during infection has also been hypothesized since two inflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) upregulate P2X7 expression [92–94].

Extracellular ATP is a powerful stimulus for IL-1β secretion [95]. Endotoxin (LPS) induced IL-1β release is in fact a very inefficient process, since it is slow and leads to secretion of a modest amount of the cytokine. Addition of extracellular ATP to endotoxin-primed macrophages or microglial cells causes a fast release of a large quantity of processed IL-1β. The process is dependent on P2X7 stimulation and ATP is an efficient stimulus for IL-1β secretion only if cells have been previously primed with LPS, pointing to a role for the nucleotide in accelerating the proteolytic maturation of the cytokine [76, 96–98]. This hypothesis was then validated by studies performed in p2x7−/− mice. In peritoneal macrophages obtained from these animals pro-IL-1β is not processed and externalised in response to ATP. In contrast to what is observed in cells from wild type mice. Injection of ATP in endotoxin treated p2x7−/− animals also failed to increase IL-1β production [99].

p2x7−/− mice are also less prone to develop cartilage lesions, loss of proteoglycan content, and presence of collagen degradation products [100].

**P2X7 receptor in cell growth and tumor models**

In apparent contrast with the role of P2X7 receptor in necrosis and apoptosis, an increasing number of reports has also correlated this protein with increased cell proliferation and tumor transformation. The first cellular model in which a role for P2X7 in cell growth was suggested were T lymphocytes [101].

In human peripheral blood lymphocytes (PBL) and T CD4+ and CD8+ subpopulations, ATP increases mitogenic activity via the P2X7 receptor, when co-applied with anti-CD3 antibodies, phytohaemagglutinin (PHA) or heterologous leucocytes, all stimuli that mimic TCR/MHC activation [101]. Extracellular nucleotides were known to stimulate proliferation through activation of P2Y receptors, the novelty of this study was to attribute a proliferative activity to a pro-apoptotic receptor. A further step in the analysis of the possible involvement of the receptor in promoting proliferation, was made by analysing the behaviour of human B lymphoid cells stably transfected with a P2X7 receptor cDNA [102]. In contrast to wild type cells, the P2X7 transfectants acquired the ability to survive and proliferate in the absence of serum, a hallmark of cancerous cells. Proliferation is likely supported by an autocrine/paracrine stimulation of released ATP. Indeed, P2X7-expressing cells release an amount of ATP that is four-fold larger than in control cells. Moreover incubation of the P2X7 transfectants with the ATP-hydrolyzing enzyme apyrase or pre-treatment with oxidized ATP (oATP) abrogates proliferation [101]. Recently, Budagian and coworkers dissected the signalling pathways responsible for P2X7 effects in lymphocytes, demonstrating that in Jurkat cells, a human T lymphocyte cell line, P2X7 activation results in phosphorylation and activation of p56lck [103]. p56lck is a lymphoid specific tyrosine kinase mediating the initial events of TCR/CD3 signalling leading to mitogenic activation of T lymphocytes. Active p56lck phosphorylates the TCR allowing the docking of other kinases such as Zap-70 and Syk to its complex; p56lck also stimulates MAP kinases. The P2X7-dependent activation of p56lck may offer an explanation for the proliferation-promoting effects of ATP on CD3 activated T lymphocytes [101]. Activation of P2X7 has been shown to be able to trigger the signalling pathway of mitogen-activated MAPK/ERK kinases, that has been extensively investigated in
different cell types such as mouse macrophages and microglia, human astrocytoma and rat parotid acinar salivary cells [74, 104–106]. MAP kinases are known to promote cell growth and proliferation by inducing de novo synthesis of pyrimidine nucleotides in the nucleus [107]. They also activate transcription factors, triggering expression of early response genes coding for growth-promoting proteins [108].

Chronic lymphocytic leukaemia B lymphocytes were one of the first cellular models in which P2X7 like activity was investigated [109, 110] and are currently a hot field of investigation in view of the possible application in the prognosis and therapy of this disease [111–113]. B chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in the western world, and despite a known familial incidence and higher percentage of male individuals among patients, it has not been associated with a specific genetic ‘hallmark’ [114]. Recently it has been shown that P2X7R expression and function is higher in CLL patients with the aggressive variant of the disease, compared to those affected by the indolent form [111]. Patients affected by the aggressive form showed accordingly higher resting calcium levels and ATP evoked calcium influx as well as higher sensitivity to ATP-mediated cytotoxicity. The proposed model predicts that a tonic, low activation of P2X7 receptor will lead to an increased proliferation, while an acute stimulation with high concentrations of nucleotide causes death of tumor lymphocytes (Figure 1) [111].

More controversial at the moment is the association of a loss of function P2X7 polymorphism with B-CLL incidence and progression. This polymorphism (1513A→C) codes for glutamic acid to alanine at amino acid position 496 in the C-terminal tail of the receptor [36]. It was first identified in normal subjects in which it caused a reduced ATP-mediated barium and ethidium uptake. Subsequently, this mutation was proposed as a prognostic marker in B cells, but no consensus has been reached as yet on this matter [115–118].

Besides B chronic lymphocytic leukaemia, several other tumors show an altered expression of the P2X7 receptor. For example, non-melanoma skin cancers express this receptor and die upon massive application of ATP or BzATP [119]. In this case the receptor seems to be associated with cells undergoing apoptosis as it colocalises with TUNEL and active caspase-3 staining [119]. For other widely diffused neoplasia, such as prostate and breast cancer, the expression of P2X7-R has been immunologically detected specifically in transformed cells [6, 120]. One hundred fourteen out of 116 prostate cancer biopsies stained positively for the P2X7 receptor and also cells well distinct from the tumor show expression of the receptor along with tumor progression [120]. Likewise, all cases of in situ lobular and ductal carcinoma showed intense P2X7 labelling in the nuclei and cytoplasm, while more aggressive forms tended to present the receptor at the cell surface [6]. The authors of these studies infer that the expression of the P2X7 receptor could be an attempt of cancer cells to undergo apoptosis, that fails because the receptor might be nonfunctional. However no functional studies were performed.

This increasing number of reports suggests that application of nucleotide-derived drugs, able to modulate P2X7 receptor functions, might be useful in tumour therapy [121]. To this aim, development of allosteric modulators of the P2X7 receptor that potentiate the effect of ATP might be an alternative approach to decrease ATP dose and therefore reduce the side effects of ATP break-down products or of ATP itself.

The anti-inflammatory drug tenidap synergises with extracellular ATP for activation of the P2X7 receptor, by increasing the affinity of the P2X7 for ATP [122]. The natural antibiotic polymyxin B (PMB) is a well-known agent binding and neutralizing bacterial endotoxin. It has recently been shown that PMB amplifies ATP-induced responses by acting at both recombinant or natively expressed P2X7-R in several cell models [123]. Another possible therapeutical approach to neoplasias could be the use of P2X7 antagonists to counteract the proliferative advantage conferred by the receptor to the transformed cells. Several P2X7 receptors antagonist blockers have been identified but none of them is selective [20]. Brilliant blue G [67] and KN-62 display a good potency, the latest compound is also a CaM kinase II (calcium calmodulin dependent protein kinase type 2) inhibitor. There have been different attempts to obtain KN-62 analogues [124], more active and specific for P2X7 [125–127]. The benzophenanthridine alkaloid chelerythrine, a potent PKC inhibitor, has

![Figure 1. Low tonic or massive stimulation with ATP of P2X7-expressing cells causes a transient or a prolonged opening of the receptor-pore, respectively; this in-turn induces a modest or high intracellular Ca^{2+} concentration increase. Although changes in calcium are unlikely to be solely responsible for the duality in P2X7-mediated effects, they represent physiological triggering points in diverse upstream and downstream signalling processes, activating different cell functions and responses.](image_url)
a noncompetitive inhibitory action on the P2X7 receptor expressed by human B lymphocytes [128]. Quite recently new adamantane amide antagonists, which show a high potency at P2X7 receptor, have been isolated by two groups at Astra Zeneca [129, 130]. Further studies on this direction could provide useful tools to better understand the physiological functions of P2X7 not only in tumour models but also in other cell types such as immune and epithelial cells.

Conclusions

In conclusion the P2X7 receptor seems to play a role in both cell death and survival. These two opposite functions are only apparently contradictory as they may depend on the specific intracellular pathway activated by P2X7 at different points or in diverse phases of the cell cycle. Low tonic stimulation by ATP is postulated to produce a low-level activation of P2X7 that supports growth. On the contrary, massive P2X7 stimulation would lead to cell death (Figure 1). In this view, it would be of great interest to measure ATP concentration at cell membrane.

Pro-apoptotic, pro-inflammatory receptors acting in some cases as growth-promoting tumorigenic proteins are not novel: TNF-α receptor (p55) for example, is known for both its cytotoxic activity and for promoting cancer cell growth [131].

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