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Analysis of the influence of nucleotidases on the apparent activity of exogenous ATP and ADP at $P2Y_1$ receptors

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1 ADP is a potent agonist of rat and human $P2Y_1$ purinoceptors. ATP is a weak competitive antagonist. This study analyses the situation in which $P2Y_1$ receptors are exposed to ATP in the presence of exogenous ecto-nucleotidases (apyrases) that have high or low ATPase/ADPase activity ratio.

2 Rat brain capillary endothelial cells of the B10 clone express $P2Y_1$ receptors that couple to intracellular Ca^{2+} mobilization. They have low endogenous ecto-ATPase and ecto-ADPase activities.

3 ATP did not raise intracellular Ca^{2+} in B10 cells. Addition of apyrases III or VII (1 u ml^{-1}) to ATP treated cells induced large intracellular Ca^{2+} transients. Apyrases had no action in the absence of ATP.

4 A 1 u ml^{-1} apyrase III solution generated 20 μM ADP from 0.1 mM ATP within 15 s. This concentration of ADP was sufficient to produce maximal activation of $P2Y_1$ receptors.

5 ATP was a full agonist of $P2Y_1$ receptors in the presence of 1 u ml^{-1} apyrase III. Dose response curves for the apparent actions of ATP were bell shaped in the presence of 0.1 u ml^{-1} apyrase III. Apyrase III did not alter ADP dose response curves when coincubated with ADP for 15 s.

6 Apyrase VII (1 u ml^{-1}) shifted dose response curves for the actions of ADP to larger concentrations. It induced a bell shaped ATP dose response curve.

7 Results suggest that ATPDases prevent $P2Y_1$ receptor activation by degrading ADP but may contribute to $P2Y_1$ receptor activation by generating ADP from ATP.

Keywords: $P2Y_1$ receptors; apyrase; endothelial cells; ecto-nucleotidase

Introduction

Purinoceptors of the P_2 type constitute a new class of receptors that recognize nucleotides and that mediate the actions of extracellular nucleotides in many cell types (Dubyak & El-Moatassim, 1993; Fredholm *et al*., 1994). They comprise two large families: ionotropic P2X receptors and metabotropic P2Y receptors (Abbracchio & Burnstock, 1994; North & Barnard, 1997). Receptors of the P2Y family are seven transmembrane domain receptors that couple to different intracellular signalling pathways via heterotrimeric G proteins.

$P2Y_1$ receptors are expressed in platelets and vascular endothelial cells (Webb *et al*., 1996; Léon *et al*., 1997; Daniel *et al*., 1998). Brain capillary endothelial cells of the B10 clone only express $P2Y_1$ receptor mRNA sequences (Webb *et al*., 1996). In these cells, adenine nucleotides induce a mobilization of intracellular Ca^{2+} stores that is not associated with a measurable production of inositol phosphates (Feolde *et al*., 1995) and that can be antagonized by pyridoxal phosphate-6-azophenyl-2′,4′ disulphonic acid (PPADS) (Vigne *et al*., 1998), adenosine-2′-phosphate-5′-phosphate (A2P5P) and adenosine-3′-phosphate-5′-phosphate (A3P5P) (Hechler *et al*., 1998a). We also showed that while ADP and its derivatives are agonists of the receptor, ATP and its derivatives are weak antagonists (Hechler *et al*., 1988b). Identical properties have been observed for the human $P2Y_1$ receptor expressed in Jurkat cells (Hechler *et al*., 1988b). These findings suggested that the $P2Y_1$ receptor in B10 cells couples to intracellular Ca^{2+} mobilization. Another action of ADP in B10 cells is to inhibit adenylyl cyclase (Webb *et al*., 1996). This action is insensitive to PPADS and A3P5P (Webb *et al*., 1996; Hechler *et al*., 1998a). These studies together with results on the platelet ADP receptor (Schachter *et al*., 1996, 1997; Jin *et al*., 1998; Daniel *et al*., 1998) suggest that adenylyl cyclase inhibition may be mediated by a receptor distinct from the $P2Y_1$ receptor.

This paper analyses the functional consequences of ATP antagonism of $P2Y_1$ receptors. *In vivo*, $P2Y_1$ receptors encounter both ATP and ADP. Concentrations of the two nucleotides at the vicinity of the receptors are not usually known. They are dependent on the source of nucleotides. Platelets release both ATP and ADP. Neuronal cells and vascular endothelial cells mainly release ATP (Gordon, 1986). Concentrations of ATP and ADP may be altered by ATP diphosphohydrolase (ATPDase, EC 3.6.1.5), a family of ecto-enzymes also known as apyrase, ecto-ATPase, ecto-ADPase, nucleotide phosphohydrolase or ATP pyrophosphohydrolase (Plesner, 1995). ATPDases have now been characterized from a diversity of tissues and species. They usually have both ATPase and ADPase activities but their substrate specificity varies broadly. CD39, the major ATPDase of vascular endothelial cells, has similar ADPase and ATPase activities (Kaczmarek *et al*., 1996; Wang & Guidotti, 1996; Marcus *et al*., 1997). The T-tubule enzyme is mainly an ATPase (Delgado *et al*., 1997). ATPDases are usually considered to be membrane bound enzymes and indeed structural data indicate that CD39 is a transmembrane protein (Kaczmarek *et al*., 1996). However recent evidence has suggested the existence of soluble nucleotidases (Todorov *et al*., 1997).

The situation in which $P2Y_1$ receptors are exposed to their natural antagonist (ATP) in the presence of ATPDases that generate the agonist of the receptor (ADP) and degrade it into an inactive product (AMP), is unusual and has not yet been considered. It cannot be modelled since the kinetics of ATPDases are complex (Plesner, 1995) and has to be assessed experimentally. In this study, we use rat brain capillary

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endothelial cells of the B10 clone. This paper first shows that B10 cells have very low endogenous ecto-ATPase and ecto-ADPase activities. Exogenous apyrases were then used to analyse the influence of ATPDase activity on P2Y$_1$ receptor function. Results suggest that ATPDases can function as more than scavenging enzymes. They may contribute to P2Y$_1$ receptor activation by transforming antagonistic ATP into agonistic ADP.

Methods

Rat brain capillary endothelial cells of the B10 clone were cultured as described previously (Feolde *et al*., 1995). The culture medium was Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, 100 u ml^{-1} penicillin and 100 μg ml^{-1} streptomycin.

For intracellular Ca^{2+} measurements, suspended B10 cells were incubated for 30 min in the presence of 5 μM indo-1/AM, centrifuged at 1000 $\times$ *g* and resuspended into an Earle's salt solution (composition; mM) NaCl 140, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.8, glucose 5, HEPES 25, pH 7.4, at a density of 10^6 cells ml^{-1}. Adenine nucleotides and apyrases (10 μl of the appropriate dilution) were added to test tubes while carefully avoiding mixing of the two drops. Reaction was then initiated by adding 90 μl of the cell suspension. After mild vortexing, tubes were inserted into a FacStar Plus cytometer (Becton Dickinson) (Vigne *et al*., 1994). Mean fluorescence ratio's were determined for 1000 cells sampled at different times after the addition of agonists. The acquisition time was 2 s. Dose response curves for the actions of agonists were determined at the peak of the intracellular Ca^{2+} transient (15 s). All experiments were performed at 20°C. Fluorescence ratio were calculated in arbitrary units set to a value of 100 for unstimulated cells. All experiments shown in a given figure were performed on the same batch of cells.

Definition of the pharmacological properties of P2Y$_1$ receptors requires that solutions of ATP used are not contaminated by trace amounts of ADP (Léon *et al*., 1997). ATP solutions were cleaned of contaminant ADP by using an ATP regenerating system prior to the experiments (Hechler *et al*., 1998b). ATP (1 mM in Earle's salt solution) was treated with 20 u ml^{-1} creatine phosphokinase and 10 mM creatine phosphate for at least 10 min at 20°C. Creatine phosphokinase was then inactivated with 10 mM iodoacetamide. Serial dilutions were made and used to stimulate cells. Under the conditions used, iodoacetamide had no action on apyrase activities.

Endogenous ATPase activity of B10 cells was assessed using [γ-^{32}P]-ATP as described previously (Wang & Guidotti, 1996). Briefly, cells grown in 60 mm Petri dishes were washed of their culture medium and further incubated at 37°C into an Earle's salt solution supplemented with 0.1 mM ATP and 2–4 μCi ml^{-1} [γ-^{32}P]-ATP. After different periods of time ranging from 15 s to 30 min, 500 μl samples of the incubation solutions were harvested and added to 0.5 ml of ice cold 0.5 M HCl supplemented with 0.1 g ml^{-1} activated charcoal. The suspension was kept on ice for 10 min and centrifuged at 4200 r.p.m. for 15 min and free $^{32}PO_4$ was determined by liquid scintillation counting 200 μl of the supernatant. Cells were harvested into 0.1 N NaOH and the protein content was determined according to Bradford (Bradford, 1976). The Ca^{2+} and Mg^{2+} dependent ATPase activity was determined by subtracting values obtained in the presence of 4 mM EDTA.

Endogenous ADPase activity of B10 cells was assessed using a bioassay. Suspended B10 cells (2×10^7 cells ml^{-1}) were incubated with 10 μM ADP at 37°C. After selected times (0 to 20 min), 10 μl aliquots of the cell suspension were diluted into 90 μl of a suspension of indo-1 loaded B10 cells and indo-1 fluorescence ratio was measured after 15 s. The concentration of ADP was calculated from a reference curve defined using the same pool of indo-1 loaded cells and freshly prepared ADP solutions.

The degradation of ATP by apyrase III was analysed using [α-^{32}P]-ATP. The incubation solution was an Earle's salt solution supplemented with 90 μM ATP and 0.14 μCi ml^{-1} [α-^{32}P]-ATP. The reaction was initiated by addition of 1 u ml^{-1} apyrase III and allowed to proceed at 20°C. After different times of incubation, 200 μl aliquots were diluted into 3 ml of 1 M NH_4OH/ethanol (3 vol/2 vol) and immediately applied to 2 ml washed Dowex AG1X8 columns (Biorad). ATP metabolites were eluted using a procedure adapted from published methodologies (Lin, 1974; Boyer & Stempel, 1979). Columns were washed with 2 ml water followed by 5 ml of 0.2 M Tris-HCl pH 8.0. AMP, ADP and ATP were then sequentially eluted with 2×3 ml HCl 30 mM, 3×3 ml HCl 60 mM and 3×3 ml HCl 1 M. Recovery of the nucleotides was determined by measuring the absorbance at 260 nm in parallel experiments using unlabelled nucleotides. Fractions recovered from the column (1 ml) were counted and the rates of conversion of ATP into ADP and AMP were calculated.

ATPase and ADPase activities of apyrases were determined as follows. Apyrases (1 u ml^{-1}) were incubated at 37°C into an Earle's salt solution in the presence of different concentrations of ATP or ADP. Reactions were stopped after 10 s and inorganic phosphate was assessed colorimetrically as described previously (Kates, 1986). Lineweaver-Burk reciprocal plots of the data indicated Km(ATP) values of 50 μM and 20 μM for apyrase III and VII respectively. Km(ADP) values were 60 μM and 11 μM for apyrases III and VII respectively. These values are close to the values reported previously (Molnar & Lorand, 1961). Maximum ATPase to ADPase activity ratio were 6.2 for apyrase III and 0.7 for apyrase VII.

ATP, ADP, PPADS, creatine phosphate, creatine phosphokinase (Type III from bovine heart), indo-1/AM, apyrases III and VII were purchased from the Sigma Chemical Co. All reagents were dissolved into an Earle's salt solution. [α-^{32}P]-ATP (111 TBq mmol^{-1}) and [γ-^{32}P]-ATP (167 TBq mmol^{-1}) were purchased from ICN Pharmaceuticals Inc.

All experiments were carried out in triplicate and were repeated at least three times. Data are expressed as overall means $\pm$ s.e.mean, whereas Figures show representative plots. When no error bars are presented on the Figures, it is smaller than the size of the points.

Results

Endogenous ATPDase activity in intact B10 cells was first determined. The Ca^{2+} and Mg^{2+} dependent ecto-ATPase activity was estimated as 0.46 ± 0.06 nmoles min^{-1} mg of protein^{-1} ($n = 4$). The endogenous ADPase activity was 15 times lower (< 0.03 nmoles min^{-1} mg of protein^{-1}). These activities were too low to produce significant degradations of ATP or ADP during short term experiments such as intracellular Ca^{2+} assays. In these experiments, indo-1 fluorescence ratio were measured 15 s after addition of ATP to the cells.

Exogenous apyrases were then used to analyse the influence of ATPDases on P2Y$_1$ receptor function. Different forms of apyrases are commercially available. Among these, apyrase III and apyrase VII were selected for they have properties similar

to known vertebrate ATPDases. Apyrase III has an ATPase to ADPase ratio of 6.2, similar to that of the T-tubule enzyme (Plesner, 1995). Apyrase VII has an ATPase to ADPase ratio of 0.7, similar to that of CD39 (Kaczmarek *et al*., 1996; Wang & Guidotti, 1996; Marcus *et al*., 1997). Figure 1 presents the results of a typical experiment in which cells were sequentially treated with ATP and apyrases. It shows that, as previously described (Hechler *et al*., 1998b), ATP, that had been cleaned of contaminating ADP, did not raise intracellular Ca^{2+}. It further shows that the addition of 1 unit ml^{-1} grade III or grade VII apyrases to ATP treated cells induced large and transient increases in intracellular Ca^{2+}. Transients induced by apyrase III were as large as those induced by 0.1 mM ADP in the same cells. Apyrase VII induced smaller transients. Control experiments showed that apyrases had no action by themselves and that they potentiated actions of subsequent additions of ATP.

An obvious hypothesis for these results was that apyrases generated enough ADP from ATP to activate P2Y$_1$ receptors. This hypothesis was tested by measuring the degradation of [α-^{32}P]-ATP under the same conditions as those used to measure intracellular Ca^{2+}. Figure 2 shows that apyrase III rapidly degraded ATP into ADP and AMP. Within 15 s, the concentration of ATP decreased by 55%. The concentration of ADP increased to 22 μM. This value was 20 times larger than the concentration of ADP necessary to induce half maximum activation of P2Y$_1$ receptors (1 μM). The ADP concentration remained stable for at least 2 min. AMP accumulated slowly. Taken together these results indicated (i) that apyrase III sequentially produced ADP and AMP from ATP and (ii) that a 1 u ml^{-1} apyrase III activity generated enough ADP within a few seconds to maximally activate P2Y$_1$ receptors.

We next analysed actions of ATP on P2Y$_1$ receptor function in the presence of different activities of apyrase III. Figure 3 shows that ATP which was inactive by itself became a full agonist in the presence of 1 u ml^{-1} apyrase. Its potency was however three times less than that of ADP, the real agonist of the receptors. At a lower apyrase activity (0.1 u ml^{-1}), the dose response curve for ATP action was bell shaped. Apyrase III (1 u ml^{-1}) had no action on ADP dose response curves in experiments in which it was added to the cells at the same time as ADP and in which intracellular Ca^{2+} was measured after 15 s. A 2 min treatment of ADP solutions with 1 u ml^{-1} apyrase III shifted however the ADP dose response curve to larger concentrations (data not shown). The low efficiency of apyrase III on ADP responses was expected from the large ATPase to ADPase activity ratio of the enzyme (6.2).

Figure 4 shows dose response curves for apyrase III actions. Activities of 0.03 to 3 u ml^{-1} were necessary to potentiate actions of 0.1 mM ATP. Larger activities were necessary to potentiate actions of 1 mM ATP. Thus, apyrase III was more potent at low concentrations of ATP.

We further observed that ATP (0.1 mM) responses in the presence of 1 u ml^{-1} apyrase III were completely inhibited by PPADS. Half maximum inhibition was observed at 200 μM

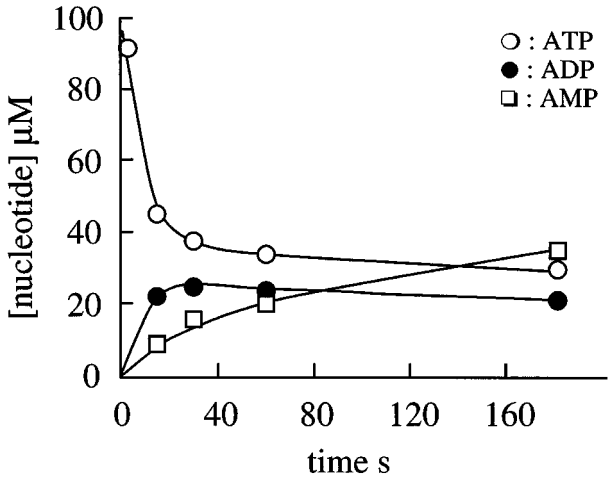

Figure 2 Degradation of ATP by apyrase III. [α-^{32}P]-ATP, ATP (90 μM) and apyrase III (1 u ml^{-1}) were mixed and the concentrations of AMP, ADP and ATP were determined after different times as described under Methods.

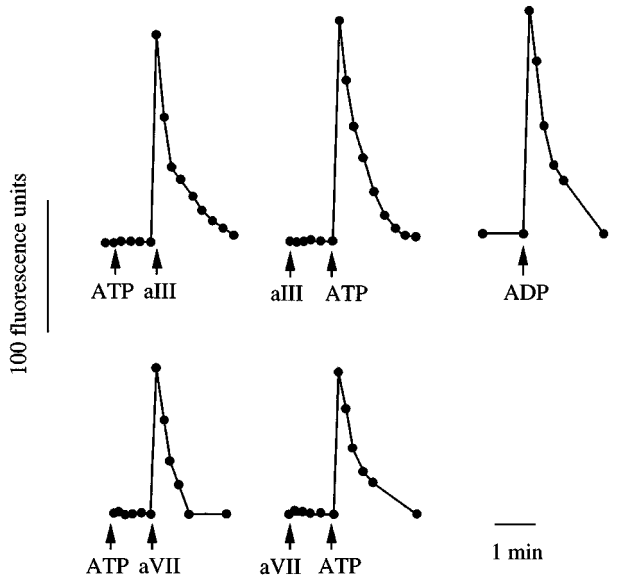

Figure 1 Apyrases potentiated actions of ATP. Indo-1 loaded cells were stimulated with 0.1 mM ATP and 1 u ml^{-1} apyrase III (aIII) or VII (aVII) as indicated and the mean indo-1 fluorescence ratio was measured. The action of 0.1 mM ADP in cells of the same preparation is shown for comparison. Each point is the mean of 1000 individual cell measurements.

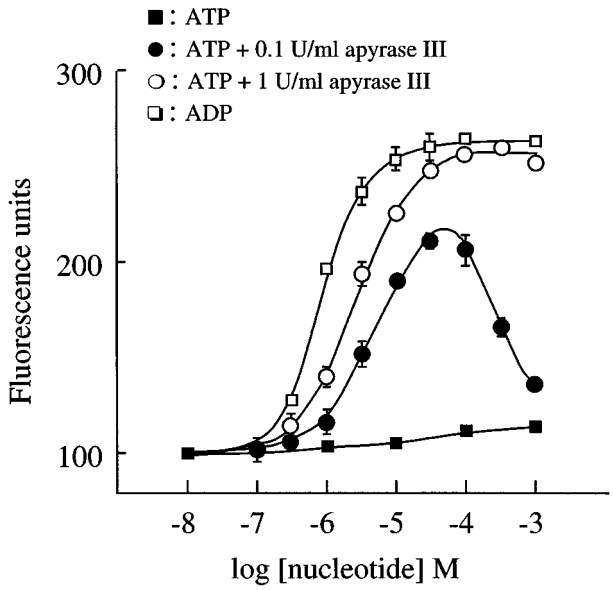

Figure 3 Apparent agonist properties of ATP in the presence of apyrase III. Indo-1 loaded cells were treated with the indicated concentrations of ATP in the absence or the presence of apyrase III. Indo-1 fluorescence ratio were measured at 15 s. The dose response curve for the action of ADP is shown for comparison.

PPADS (data not shown). In comparison, the concentration of PPADS required to inhibit half of ADP (3 μM) responses in the same cells was 20 μM. The difference can be accounted for by the competitive nature of PPADS antagonism (see Discussion).

Finally Figure 5 analyses the influence of apyrase VII on the apparent activities of ATP and ADP. Apyrase VII induced a biphasic action of ATP and was less potent than apyrase III. Thus, a 10 μM ATP solution was inactive in the presence of 1 u ml^{-1} apyrase VII (Figure 5) but produced large intracellular Ca^{2+} transients in the presence of 0.1 or 1 u ml^{-1} apyrase III (Figure 3). Figure 5 also shows that apyrase VII (1 u ml^{-1}) shifted the dose response curve for ADP action to larger concentrations. Apyrase III was inactive under the same conditions (data not shown).

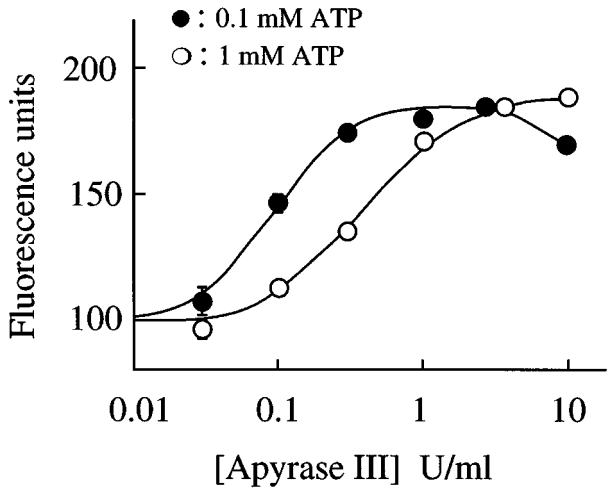

Figure 4 Large concentrations of ATP reduced the efficacy of apyrase III. Indo-1 loaded cells were exposed at the same time to ATP (0.1 or 1 mM) and the indicated activities of apyrase III. Indo-1 fluorescence ratio were measured at 15 s.

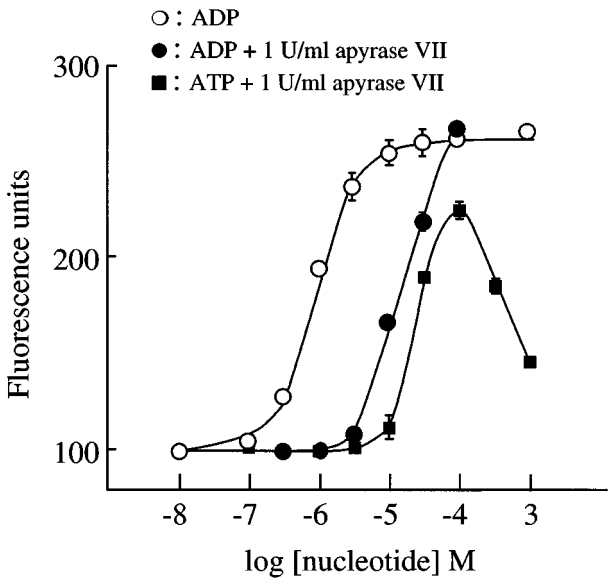

Figure 5 Apparent agonist properties of ATP and ADP in the presence of apyrase VII. Indo-1 loaded cells were exposed at the same time to the indicated concentrations of ATP or of ADP and to 1 u ml^{-1} apyrase VII. Indo-1 fluorescence ratio were measured at 15 s.

Thus, apyrases which potentiated agonist actions of ATP, inhibited actions of ADP. Apyrase III which has a high ATPase to ADPase activity ratio was more potent for potentiating actions of ATP than for inhibiting actions of ADP. Apyrase VII was more potent for inhibiting actions of ADP than for potentiating actions of ATP.

Discussion

The first finding of this study is that cultured rat brain capillary endothelial cells express low ecto-ATPase and ADPase activities. The Ca^{2+} and Mg^{2+} dependent ecto-ATPase activity was estimated to 0.5 u/10^9 cells (i.e. about 0.25 u/10^9 cells by considering a protein content of 0.5 mg of protein/10^6 cells and that 1 unit of activity represents 1 μmole of substrate hydrolyzed per min). The estimated ecto-ADPase activity was 15 times lower. In agreement with these observations, we observed (Frelin & Vigne, unpublished observations) that B10 cells do not express mRNA sequences coding for CD39, the major ATPDase in endothelial cells (Kaczmarek *et al*., 1996; Wang & Guidotti, 1996; Marcus *et al*., 1997). Ecto-ATPase activity is much lower than that previously reported for macrovascular endothelial cells (20–70 u/10^9 cells) (Plesner, 1995). Whether the low activity in B10 cells is a genuine property of microvascular endothelial cells or is a consequence of the culturing conditions is not known. A low endogenous nucleotidase activity is a major advantage for analysing purinergic responses for exogenous ATP is not degraded to a significant extent during short term experiments.

The second finding of this study is that although ATP is an antagonist of $P2Y_1$ receptors (Hechler *et al*., 1998b), it has agonist actions in the presence of apyrases (Figures 3 to 5). The agonist action of ATP is apparent and is due to ADP formed by apyrases during the course of the experiments (Figure 2). Apyrases which potentiate actions of ATP reduce those of ADP. These responses are observed both with grade III and grade VII apyrases. The two enzymes differ in that while apyrase III is more potent than apyrase VII for promoting apparent agonist actions of ATP, it is less potent than apyrase VII for inhibiting actions of ADP. These differences can be accounted for by the 10 times larger ATPase to ADPase activity ratio of apyrase III. It may appear surprising that ADP formed by apyrases activated receptors in spite of the large ADPase activities of apyrases. This is expected however if newly formed ADP molecules dissociate from apyrases and preferentially bind to $P2Y_1$ receptors. Two observations are in favor of such a mechanism: (i) apyrases have separate ATPase and ADPase activities (Plesner, 1995); (ii) the apparent Kd value of the complexes formed by ADP and $P2Y_1$ receptors (1 μM) (Feolde *et al*., 1995) is much lower than the Km value of apyrases for ADP (11–60 μM).

ATP responses in the presence of apyrase III were inhibited by PPADS. This observation rules out a possible involvement of adenylyl cyclase coupled P2Y receptors that are insensitive to PPADS (Webb *et al*., 1996). The concentration of PPADS required to produce half maximum inhibition of ATP (0.1 mM in the presence of 1 u ml^{-1} apyrase III) responses was 200 μM. In comparison, the concentration of PPADS required to inhibit half of ADP (3 μM) responses in the same cells was 20 μM. The difference can be accounted for by the competitive nature of PPADS antagonism. A 1 u ml^{-1} apyrase III activity generates 20 μM ADP from 0.1 mM ATP (Figure 2), a concentration 20 times larger than the apparent Kd value of ADP for $P2Y_1$ receptors.

Pharmacological actions of ATP in the presence of apyrases are unusual. First ATP dose response curves are bell shaped in the presence of low apyrase III activities and monophasic at larger activities. Second high concentrations of ATP decrease the potency of apyrase III (Figure 4). These two findings are related consequences of the weak antagonistic action of ATP at $P2Y_1$ receptors (Hechler *et al*., 1998b). At low concentrations of ATP, apyrase produces enough ADP to activate receptors. ADP being generated at the expense of ATP, ATP concentrations decrease (Figure 2), but not enough ATP is left to antagonize receptors. At larger concentrations of ATP, ADP is also generated but more ATP is left to antagonize ADP responses, hence a lower potency of apyrase (Figure 4) and a lower apparent efficacy of ATP (Figures 3 and 5).

Ecto-nucleotidases are usually considered as scavenging enzymes that prevent purinergic receptor activation (Gordon, 1986; Plesner, 1995; Todorov *et al*., 1997). Scavenging actions of apyrases were observed in experiments using ADP (Figure 5). Our *in vitro* experiments thus indicate that ATPDases may also contribute to $P2Y_1$ receptor activation by generating the agonist of the receptor (ADP) from its antagonist (ATP). Whether ATPDases have the same function *in vivo* is not currently known. We observed however that an apyrase III activity of 1 u ml^{-1} generated apparent full agonist actions of ATP (Figure 3). The same activity would be obtained by diluting 10^7 cells, that have an ATPDase activity of 10 u/10^9 cells, into 1 ml. Considering (i) that endogenous ATPDase activities as large as 20–70 u/10^9 cells have been reported for macrovascular endothelial cells (Plesner, 1995) and (ii) that cells in tissues face very small volumes of extracellular space, it seems likely that ATPDases also favour apparent agonist actions of ATP *in vivo*. It is of further interest to note that a variety of situations can be encountered *in vivo* depending on the type of receptor and ATPDase expressed and on the ligand present. For instance a low ATPase activity would favour $P2Y_2$ receptor activation by ATP in a tissue that expresses both $P2Y_1$ and $P2Y_2$ receptors. Conversely, a large ATPase activity would favour $P2Y_1$ receptor activation by ADP under the same conditions.

ATP has long been considered as an agonist of $P2Y_1$ receptors. The recent identification of the antagonist properties of ATP (Hechler *et al*., 1988b) was possible (i) as the cells used had very low levels of ecto-ATPase activity and (ii) as responses to nucleotides were assessed after only 15 s of exposure of the cells to nucleotides. This study suggests that identification of antagonist properties of ATP is probably more difficult in preparations in which activity of ecto-ATPases cannot be controlled or in preparations that have been treated with apyrases to prevent receptor desensitization by endogenously produced ATP. Under these conditions, ATP appears as a partial agonist rather than as an antagonist. One way of distinguishing true from false agonist actions of ATP in such preparations is to establish complete ATP dose response curves and to look for possible bell shaped ATP dose response curves. Such curves cannot be accounted for by simple partial agonism.

Identification of P2Y receptor responses is severely limited by the lack of potent and specific receptor antagonists, by the presence of ecto-nucleotidases in most cell preparations and by the fact that commercially available ATP derivatives are contaminated by trace amounts of ADP derivatives. For instance 2-methyl thio ATP and 2-chloro ATP have long been considered as potent agonists of $P2Y_1$ receptors. These actions are now known to be due to the presence of low amounts of 2-methyl thio ADP and 2-chloro ADP in the solutions used (Léon *et al*., 1997; Hechler *et al*., 1998b). One indirect way of identifying P2Y receptor responses in complex multicellular preparations is to use exogenous enzymes. Creatine phosphokinase (in the presence of creatine phosphate) has previously been used to identify false agonist actions of ATP and its 2-substituted derivatives at $P2Y_1$ receptors (Hechler *et al*., 1998b). Similarly, hexokinase (in the presence of glucose) has been used to identify false agonist actions of ADP and UDP at $P2Y_2$ receptors and of UTP at $P2Y_6$ receptors (Nicholas *et al*., 1996). A potentiation of ATP actions by apyrase III (Figure 3) and an inhibition of ADP actions by apyrase VII (Figure 5) are characteristic features that may be used to identify $P2Y_1$ receptor responses in situations in which activity of endogenous ATPDases cannot be controlled.

In conclusion, this paper suggests that ATPDases have a dual function. They prevent $P2Y_1$ receptor activation by degrading ADP. They may also contribute to receptor activation in a space limited environment by generating ADP from ATP. These stress the unique autocrine/paracrine properties of ATP and their dependence on local conditions.

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