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Hydrolysis of P_2-Purinoceptor Agonists by a Purified Ectonucleotidase from the Bovine Aorta, the ATP-Diphosphohydrolase

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ABSTRACT. Pharmacologists are becoming more and more aware of the possibility that certain ATP analogues currently used to classify the P_2-purinoceptors are dephosphorylated by ectonucleotidases. In this study, we provide evidence that in the vascular system, these purine analogues are hydrolysed by an ATP-diphosphohydrolase (ATPDase). This enzyme is known as the major plasma membrane nucleotidase of endothelial and smooth muscle cells, and is believed to dephosphorylate extracellular triphospho- and diphosphonucleosides. Assays were conducted with a purified ATPDase from smooth muscle cells of bovine aorta. At a concentration of 250 μM, adenosine 5′-(α,β-methylene) triphosphonate (α,β-metATP), adenosine 5′-(β,γ-methylene) triphosphonate (β,γ-metATP), adenosine 5′-(α,β-methylene) diphosphonate (α,β-metADP), adenylyl 5′-(β,γ-imido) diphosphonate (β,γ-imidoATP) and adenosine 5′-O-(2-thiodiphosphate) (ADPβS) all resisted dephosphorylation, whereas 2-chloroadenosine triphosphate (2-chloroATP), 2-methylthioadenosine triphosphate (2-MeSATP) and 8-bromoadenosine triphosphate (8-bromoATP) were hydrolysed at 99, 63, and 20% of the rate of ATP hydrolysis, respectively. All the non-hydrolysable analogues tested, except α,β-metADP, competed with ATP and ADP for the ATPDase catalytic site, reducing their hydrolysis by 35–50%. Apparent K_m values for ATP and ADP were estimated at 14.1 and 12.0 μM, respectively, whereas apparent K_m and K_i values for the purine analogues ranged from 12 to 28 μM. These results strongly support the view that (1) the ATPDase is expected to reduce substantially the P_2-response induced by ATP, ADP, and some hydrolysable agonists; and (2) by competing with the hydrolysis of endogenously released ATP and ADP, non-hydrolysable analogues could alter the amplitude or direction of the cellular response induced by these natural substrates. BIOCHEM PHARMACOL 51;11:1453–1460, 1996.

KEY WORDS. ATP-diphosphohydrolase; Apyrase; ectonucleotidase; ATP analogues; nucleotide analogues; P_2-purinoceptors; vasoconstriction

Adenine nucleotides are released in the extracellular space from a variety of cells in response to several stimuli and as a result of tissue damage or cell death [1–3]. Extracellular adenine nucleotides initiate a large number of cellular responses by binding to purinergic receptors [4, 5]. The design, synthesis, and pharmacology of nucleotide analogues have been extremely useful in the classification of these receptors, and have provided evidence for at least six subtypes of P_2-purinoceptors designated as P_{2D}, P_{2T}, P_{2U}, P_{2X}, P_{2Y}, and P_{2Z} [6].

Analysis of the potency and physiological effects of P_2-purinoceptor ligands may be complicated by the presence of ectonucleotidases. Indeed, a number of studies conducted on vascular cells [7, 8], non-vascular smooth muscles [9–14], and striated muscles [15] have indicated that in many cases, the relative potency of ATP and ADP analogues was related to their resistance to hydrolysis. In the absence of ectonucleotidase activity, the order of potency of purine analogues of P_{2X}-purinoceptors: α,β-metATP§>>2-MeSATP > ATP [16] becomes ATP = 2-MeSATP > α,β-metATP [17, 18].

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§ *Abbreviations:* α,β-metATP, adenosine 5′-(α,β-methylene) triphosphonate; ATPDase, ATP-diphosphohydrolase; ATPase, adenosine triphosphatase; ADPase, adenosine diphosphatase; AMPase, adenosine monophosphatase; ADPβS, adenosine 5′-O-(2-thiodiphosphate); α,β-metADP, adenosine 5′-(α,β-methylene) diphosphonate; β,γ-metATP, adenosine 5′-(β,γ-methylene) triphosphonate; β,γ-imidoATP, adenylyl 5′-(β,γ-imido) diphosphonate; 8-bromoATP, 8-bromoadenosine triphosphate; 2-chloroATP, 2-chloroadenosine triphosphate; 2-MeSATP, 2-methylthioadenosine triphosphate; DEAE-agarose, diethylaminoethyl-agarose; and EDRF, endothelial-derived relaxing factor.

Up until recently, it was believed that two distinct enzymes were involved in the hydrolysis of extracellular ATP and ADP in blood vessels [8]. This concept is now questioned by the finding of ATPDases that dephosphorylate extracellular triphospho- and diphosphonucleosides in many organs and tissues including blood vessels [19, 20]. We have demonstrated recently the presence of an ATPDase in the plasma membrane of bovine aorta endothelial and smooth muscle cells [21–23], an enzyme that was purified to homogeneity using two totally different approaches [24, 25].

Several direct and indirect pieces of evidence demonstrate that the aorta ATPDase is an ectonucleotidase. First, this ATPDase has been identified at the surface of bovine aorta endothelial and smooth muscle cells in culture using several inhibitors including a polyclonal antibody raised against purified bovine aorta ATPDase [26]. Second, we have recently confirmed these findings by comparing substrate specificity and enzyme inhibition obtained with purified bovine aorta ATPDase fractions and intact endothelial cells in suspension.[‖] Third, an intracellular orientation of the catalytic site for the ATPDase, which has a K_m in the micromolar range, is incompatible with the observation that ATP intracellular concentrations are in the millimolar range. Since little pyrophosphatase and non-specific phosphatase activities have been found in these cell types [7, 21, 27], this ATPDase is probably responsible for most of the hydrolysis of extracellular nucleotides in this tissue. We have also established that this ATPDase inhibits ADP-induced platelet aggregation in both platelet-rich plasma and whole blood [28]. Along with eicosanoids and the EDRF, this ATPDase constitutes one of the major protective mechanisms for limiting the size of hemostatic plugs [29].

In this paper, we wanted to establish whether the ATPDase could be responsible for the degradation of ATP and ADP analogues in blood vessels. Our first goal was to evaluate the hydrolysis of various ATP and ADP analogues with a highly purified bovine aorta ATPDase preparation. We also conducted a series of experiments to verify if analogues and nucleotides compete for the same catalytic site. Finally, kinetic parameters were estimated to compare the affinity of ATP, ADP, and nucleotide analogues for the ATPDase. We demonstrated that most nucleotide analogues interact with the bovine aorta ATPDase, some of them being rapidly hydrolysed, whereas the others act as competitive inhibitors. These results strongly suggest that the ATPDase is responsible for the hydrolysis of the purine analogues reported during pharmacological investigations on the vascular system. This information should prove to be useful for the design and synthesis of specific inhibitors of the hydrolysis of extracellular nucleotides, which has become a prerequisite for the characterization of P_2-purinoceptors.

[‖] Levesque FP, Sévigny J and Beaudoin AR, manuscript in preparation.

MATERIALS AND METHODS

Chemicals

Tris base (tris[hydroxymethyl]aminomethane), imidazole, ammonium molybdate, ATP, α,β-metATP, α,β-metADP, β,γ-metATP, ADPβS, β,γ-imidoATP, 8-bromoATP, sodium thiophosphate, and malachite were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.); 2-chloroATP and 2-MeSATP were bought from Research Biochemicals Inc. (Natick, MA, U.S.A.). KH_2PO_4 was obtained from Anachemia Science (Montréal, Québec, Canada); Triton X-100 and ADP were obtained from Boehringer–Mannheim (Laval, Québec, Canada). $CaCl_2$ and Tween 20 (polyoxyethylene [20] sorbitan monolaureate) were purchased from the Fisher Scientific Co. (Fair Lawn NJ, U.S.A.), and the protein assay kit was obtained from Bio-Rad Laboratories Ltd. (Mississauga, Ontario, Canada).

Enzyme Purification and Assays

Bovine aorta ATPDase activity was first partially purified in a plasma membrane enriched fraction according to Côté *et al.* [21], then solubilized with Triton X-100, and further purified by DEAE-agarose and Affi-Gel blue chromatography columns [25]. Enzyme activity was routinely measured by the liberation of inorganic phosphate using a very sensitive malachite green colorimetric assay [30]. Unless stated otherwise, incubations were carried out in the following medium: 50 mM Tris base, 50 mM imidazole, 8 mM $CaCl_2$, and 250 μM substrate in a final volume of 1.0 mL (pH 7.5). Reactions were started by adding 50 ng of protein at 37° and stopped after 10 min with 250 μL of the malachite reagent. These incubation conditions maintained substrate hydrolysis to less than 5%, which was necessary to measure linear rates and to avoid any contribution of nucleotide diphosphate dephosphorylation when nucleotide triphosphates were used as the substrate. This substrate concentration allowed hydrolysis rates to reach at least 80% of V_{max} values. Rates of phosphorothioate analogue dephosphorylation were derived from a standard curve constructed with sodium thiophosphate instead of KH_2PO_4. As for the analogues modified on the phosphate chain, the reaction product methylene phosphate is considered highly unstable, yielding rapidly inorganic phosphate, and β,γ-imidoATP degradation yields imidoADP and inorganic phosphate [31]. Therefore, degradation rates for this category of analogues could be safely estimated from standard curves of inorganic phosphate. Rates of phosphate release were expressed in micromoles per minute per milligram of protein (units/mg). Protein concentration was estimated by the technique of Bradford [32] using bovine serum albumin as a standard.

The high sensitivity of the malachite green method allowed us to evaluate kinetic parameters without the use of radioactive substrates. Apparent K_m and V_{max} values for ATP, ADP, and each hydrolysable purine analogue were

derived from Woolf–Augustinson–Hoftsee plots, with substrate concentrations ranging between 6 and 100 μM. Apparent K_i values for non-hydrolysable purine analogues were derived from Dixon replots of Lineweaver–Burk curves, constructed with substrate concentrations ranging between 6 and 100 μM, and inhibitor concentrations of 0, 20, 40 and 80 μM. In all cases, less than 5% of the substrate was dephosphorylated.

RESULTS

Characterization of the Purified ATPDase

The bovine aorta ATPDase was partially purified in a plasma membrane enriched fraction [21] and further purified on DEAE-agarose and Affi-Gel blue chromatography columns. The presence of a single band having ATPase and ADPase, but not AMPase activities, after migration on polyacrylamide gel electrophoresis under non-denaturing conditions, confirmed that other ATPases and non-specific phosphatase had been eliminated [25]. This enzyme preparation represents a 1700-fold purification from the homogenate. Taking into account enzyme inhibition by Triton X-100, the purified fraction dephosphorylated 250 μM ATP and ADP at rates of 14.8 ± 0.1 and 14.7 ± 0.1 units/mg, respectively, compared to 1.4 ± 0.1 and 1.0 ± 0.1 units/mg for the plasma membrane enriched fraction (Fig. 1, A and B). A reduction in the ratio of ATP/ADP rates of hydrolysis is consistent with the loss of other ATPases or phosphatases along the purification steps.

We verified that the catalytic properties of the ATPDase were not modified after solubilization and purification by comparing the rates of hydrolysis of different purine analogues (250 μM) by the plasma membrane enriched fraction and the purified ATPDase. Panels A and B of Fig. 1 illustrate typical results obtained with α,β-metATP. This purine analogue was not dephosphorylated by either enzyme preparation. Moreover, the presence of α,β-metATP reduced to about 50% the rate of ATP or ADP hydrolysis, measured with the plasma membrane or the purified ATPDase fraction. These results demonstrate that the purification procedures did not modify the properties of the ATPDase. We therefore used the purified ATPDase in all our assays in order to eliminate any interference by other contaminating nucleotidases.

Degradation of Nucleotides and Nucleotide Analogues

Table 1 compares the rates of hydrolysis of purine analogues (250 μM) modified either on the purine ring or in the phosphate chain. All three ATP analogues modified on the purine ring were dephosphorylated by the bovine aorta ATPDase. However, the rates of hydrolysis depended both on the position and the nature of the substitution. For instance, 2-chloroATP was dephosphorylated at approximately the same rate as ATP, whereas 2-MeSATP was dephosphorylated at 63% the rate of ATP hydrolysis. And

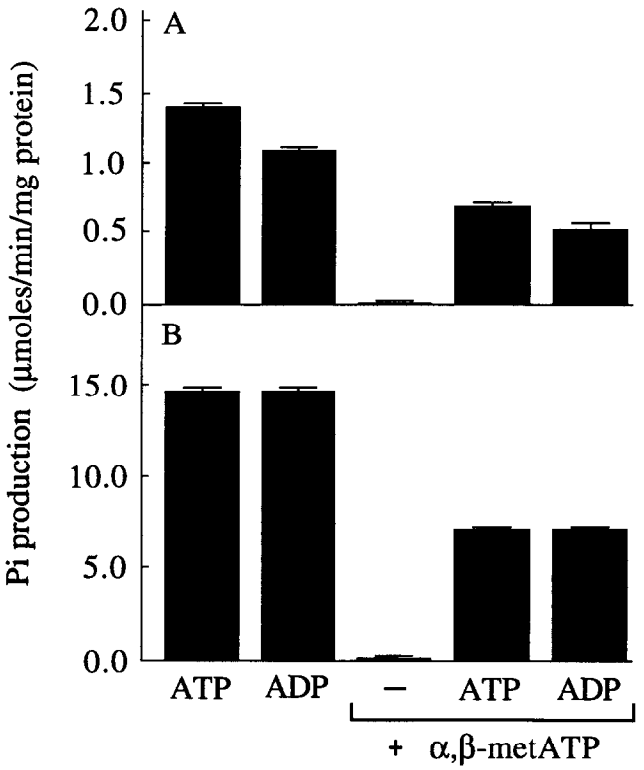

FIG. 1. Catalytic properties of (A) the plasma membrane enriched fraction and (B) the purified bovine aorta ATPDase. Assays were carried out with 250 μM ATP, ADP, and α,β-metATP, as well as combinations of α,β-metATP (250 μM) and each nucleotide (250 μM). Values (means ± SEM) represent the rates of inorganic phosphate release in μmol/min/mg protein, and were calculated from three sets of experiments, each run in triplicate.

8-bromoATP was much more resistant to hydrolysis, with a mean rate equal to one-fifth that of ATP. In contrast, the purine analogues modified in the phosphate chain, ADPβS, α,β-metATP, β,γ-metATP, α,β-metADP, or β,γ-imidoATP, were not hydrolysed by the ATPDase, regardless of the nature of the substitution.

Competition Studies

We performed a series of competition studies to assess whether ATP, ADP, and the purine analogues are hydrolysed by the same catalytic site. Table 1 compares the rates of phosphate release measured in the presence of an analogue (250 μM) plus ATP or ADP (250 μM), expressed as a percentage of the rate of hydrolysis of the natural nucleotide alone (250 μM). Simultaneous addition of an analogue modified on the purine ring (2-MeSATP, 2-chloroATP, or 8-bromoATP) with either ATP or ADP resulted in similar or lower rates of phosphate release than with the natural substrate alone. Since those analogues were shown to be hydrolysed by the ATPDase, and since their presence did not generate rates superior to 100%, they were probably

TABLE 1. Effects of nucleotide analogues on ATPase and ADPase activities

	Rate of phosphate release (%)		
	Analogue (250 μM)	**Analogue + ATP (500 μM total)**	**Analogue + ADP (500 μM total)**
2-MeSATP	63.0 ± 1.0	104.1 ± 3.9	104.5 ± 2.6
2-chloroATP	98.8 ± 2.8	103.5 ± 1.3	100.3 ± 3.3
8-bromoATP	20.0 ± 0.7	57.6 ± 3.8	56.1 ± 2.5
ADPβS	0.4 ± 0.5	53.1 ± 0.7	53.0 ± 0.4
α,β-metATP	0	48.9 ± 0.5	49.2 ± 0.7
α,β-metADP	0	100.1 ± 2.6	98.0 ± 1.7
β,γ-metATP	0	59.5 ± 0.6	57.4 ± 0.3
β,γ-imidoATP	2.2 ± 2.0	67.7 ± 3.2	61.2 ± 2.2

Rates of phosphate release were expressed as the percent relative to the rate of ATP hydrolysis, estimated at 14.8 units/mg. Values are means ± SEM of three series of experiments, each run in triplicate.

competing for the same catalytic site. Furthermore, the fact that similar rates of phosphate release were obtained with ATP and ADP is in agreement with the properties of an ATPDase.

The weakly- or non-hydrolysable nucleotide analogues, with the exception of α,β-metADP, all competed with ATP and ADP for the same catalytic site, reducing their rates of hydrolysis by more than 35% (Table 1). And comparable inhibitions were obtained with ATP or ADP. For instance, the presence of ADPβS reduced the rates of ATP and ADP hydrolysis to about 53%. The methylene phosphonate analogues, α,β-metATP and β,γ-metATP, reduced the rate of ATP and ADP hydrolysis to 49 and 58%, respectively. Finally, β,γ-imidoATP reduced nucleotide hydrolysis by more than 35%. Consequently, all purine analogues, except α,β-metADP, interact with the catalytic site of the purified bovine aorta ATPDase.

Kinetic Parameters

To further characterize the interaction between the nucleotides and the catalytic site of the ATPDase, we analysed the kinetic properties of the enzyme in the presence of various concentrations of substrate. Figure 2 shows that the rate of ATP hydrolysis as a function of ATP concentration (6–500 μM) followed a Michaelis representation. From a Woolf–Augustinson–Hoftsee replot of the data (Fig. 2; inset), apparent K_m and V_{max} values were estimated at 14.1 ± 1.3 μM and 16.3 ± 0.1 μmol/min/mg protein, respectively. Since this K_m is in the same range as the one we previously measured with [γ-^{32}P]ATP for the partially purified bovine aorta ATPDase [23], this malachite green technique was used to estimate apparent K_m and V_{max} values for ADP and each hydrolysable analogue. Table 2 indicates that the purified ATPDase hydrolysed ATP, ADP, and the purine substituted analogues (2-chloroATP, 2-MeSATP, and 8-bromoATP) with similar affinities, K_m values ranging between 12 and 28 μM.

The type of inhibition of ATP hydrolysis by the non-hydrolysable analogues was also investigated. Lineweaver–Burk curves of ATP hydrolysis (12.5 to 100 μM) in the presence of various concentrations of β,γ-imidoATP (0, 20, 40, and 80 μM) indicate a competitive type of inhibition (Fig. 3). The apparent K_i values estimated by Dixon replots of the Lineweaver–Burk curves for β,γ-imidoATP, α,β-metATP, β,γ-metATP, or ADPβS all ranged between 12 and 18 μM, which is in the same order of magnitude as the apparent K_m values calculated for the hydrolysable nucleotides (Table 2). These results demonstrate that the bovine aorta ATPDase has a comparable affinity for all hydrolysable and non-hydrolysable nucleotides that we have tested, besides α,β-metADP. Finally, comparable apparent K_i values were obtained when β,γ-imidoATP was incubated with either ATP or ADP (12.5 ± 1.3 and 13.5 ± 1.8 μM, respectively; data not shown).

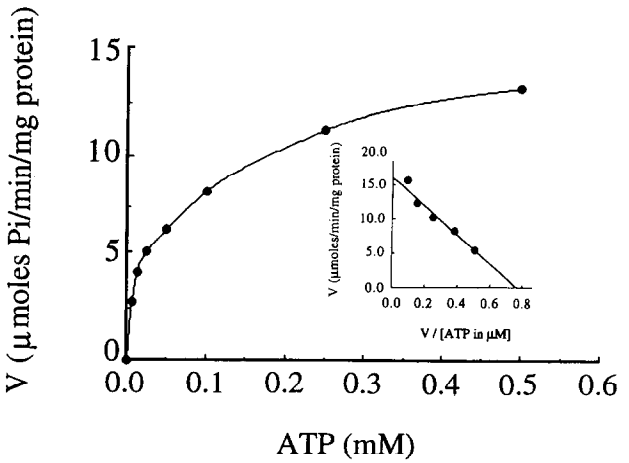

FIG. 2. Kinetic analysis of the hydrolysis of ATP by the purified bovine aorta ATPDase: Michaelis–Menten relationship of enzyme activity (50 ng protein) determined with Ca^{2+}-ATP in a concentration range of 6–100 μM. Inset: Woolf–Augustinson–Hoftsee plot used to estimate apparent K_m and V_{max} values at 14.1 μM and 16.3 μmol phosphate/min/mg protein, respectively. Results are the means of three series of experiments, each run in triplicate. Best-fit analysis indicated a linear relationship (Statview for Macintosh, USA).

TABLE 2. Kinetic parameters of the bovine aorta ATPDase for ATP, ADP, and purine analogues

Substrates	K_m (μM)	V_{max} (μmol/min/mg protein)	Inhibitors	K_i (μM)
ATP	14.1 ± 1.3	16.3 ± 0.1	α,β-metATP	18 ± 4.2
ADP	12.0 ± 1.9	16.2 ± 0.3	β,γ-metATP	17 ± 2.1
2-chloroATP	12.9 ± 0.4	18.7 ± 0.1	ADPβS	15 ± 3.0
2-MeSATP	27.9 ± 0.4	19.8 ± 0.1	β,γ-imidoATP	12 ± 3.0
8-BromoATP	27.7 ± 1.2	19.6 ± 0.2		

Values are the means ± SEM of three experiments, each run in triplicate. Apparent K_m and K_i values are estimated using substrate concentrations ranging from 6 to 100 μM, and inhibitor concentrations ranging from 20 to 80 μM.

DISCUSSION

Because ATP and ADP are dephosphorylated rapidly, stable analogues have been synthesized to study and classify P_2-purinoceptors [33–36]. However, several purine analogues were shown to be hydrolysed by ectonucleotidases [9, 10, 12–14]. In this work, we have demonstrated that these analogues are dephosphorylated by the bovine aorta ATPDase. The bovine aorta ATPDase was characterized [21, 23, 28] and recently purified to homogeneity [25] in our laboratory. In the present study, we further confirmed the ATPDase character of the purified enzyme. First, we demonstrated that this enzyme hydrolyses ATP and ADP at comparable rates and with similar apparent affinities (K_m). Second, ADPβS, an analogue of ADP, was found to inhibit ATPase activity competitively, which supports the view of a single catalytic site for ATP and ADP. And finally, ATP and ADP hydrolysis were both inhibited competitively by β,γ-imidoATP, with similar K_i values. Such characteristics have been reported for other ATPDases [37–40]. Consequently, we have clearly established that the purified enzyme is an ATPDase, capable of hydrolysing triphosphonucleosides and diphosphonucleosides.

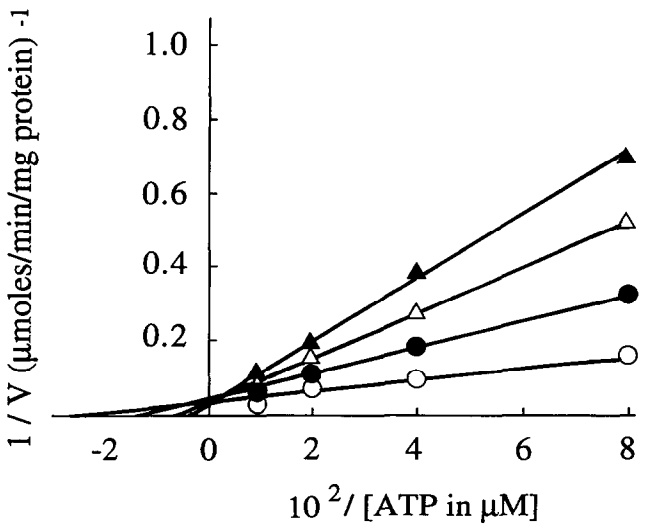

FIG. 3. Competitive inhibition of the bovine aorta ATPDase activity by β,γ-imidoATP. Lineweaver–Burk plots of ATP (12.5 to 100.0 μM) hydrolysis were constructed with 0 μM ○), 20 μM (●), 40 μM (△), or 80 μM (▲) β,γ-imidoATP. Lines of best fit were derived from the original data. Best-fit analysis indicated linear relationships (Statview for Macintosh, USA).

In this study, two categories of P_2-purinoceptor agonists have been considered: ATP analogues modified on the purine base (2-chloroATP, 2-MeSATP, and 8-bromo ATP), and analogues modified on the phosphate chain, by substitution of (1) an ionized oxygen by an ionized sulfur group (ADPβS), (2) a bridging oxygen by a methylene group (α,β-metATP, α,β-metADP, and β,γ-metATP), and (3) a bridging oxygen by an imido group (β,γ-imido-ATP). In the first category, the three analogues modified on the purine ring were hydrolysed by the purified bovine aorta ATPDase. Hydrolysis rates depended on both the position and nature of the substitute, 2-chloroATP, 2-MeSATP, and 8-bromoATP being hydrolysed at 99, 63, and 20% of the rate of ATP hydrolysis, respectively. Apparent K_m values were 13, 28, and 28 μM, respectively. Since they are in the same range as those estimated for ATP and ADP, with 14 and 12 μM, these analogues would be expected to compete with ATP and ADP for the catalytic site of the ATPDase. Very few studies have dealt with the metabolism of analogues modified on the purine moiety. Frog satorius muscles also degrade ATP (10 μM) faster than 2-MeSATP (10 μM), with half-lives of 8.3 and 18.1 min, respectively [15]. However, one cannot generalize, since dephosphorylation rates obtained for ATP, ADP, AMP, 2-chloroATP, 2-MeSATP, and 8-bromoATP (100 μM) with sections of guinea pig urinary bladder are comparable, with half-lives of about 20 min [14].

The analogues modified on the phosphate chain were not hydrolyzed by the purified bovine aorta ATPDase (ADPβS, α,β-metATP, α,β-metADP, β,γ-metATP, and β,γ-imidoATP). These results are in agreement with other studies conducted on partially purified ATPDases, such as pig pancreas [41], bovine spleen [42], and mouse sarcoma Li-7m [38], and with ectonucleotidases [7, 8, 13, 14, 43]. On pig aorta endothelial cells, a dinucleotide pyrophosphatase slowly hydrolyses 100 μM β,γ-imidoATP and β,γ-metATP [7], whereas an ectoATPase would be responsible

for the slow degradation of α,β-metATP (100 μM) into α,β-metADP, 85% of the substrate remaining after 180 min of incubation [8].

We also provide evidence that several nucleotide analogues interfere with the dephosphorylation of ATP and ADP. Simultaneous addition of 250 μM 8-bromoATP, α,β-metATP, β,γ-metATP, β,γ-imidoATP, or ADPβS with either 250 μM ATP or ADP reduced the rate of inorganic phosphate release by at least 35%. Beukers *et al.* [44] reported that intact human blood cell ectoATPase activity is inhibited by these analogues. However, the importance of ATP hydrolysis by blood cells, relative to vascular endothelial and smooth muscle cells, would be negligible. The half-life of exogenous ATP in whole blood was estimated at 5 min, compared to a few seconds when perfused into the microcirculation [45]. Enzyme kinetics showed that all inhibitions were competitive, with K_i values ranging between 12 and 18 μM. These values are in the same range as the K_m values estimated for ATP and ADP (14 and 12 μM, respectively). Competitive inhibitions by α,β-metATP, β,γ-metATP, or β,γ-imidoATP have been reported for other mammalian nucleotidases, the bovine spleen ATPDase [42] and the natural killer cell ectoATPase [46]. The analogue α,β-metADP is an exception, since it was neither dephosphorylated nor did it interfere with ATP and ADP hydrolysis. It is noteworthy that this analogue displays almost no affinity for P_{2x}-purinoceptors [47, 48], and that it is used as a specific inhibitor of the 5′-nucleotidase [27, 49].

In this work, we have shown that P_2-purinoceptor agonists are recognized by the purified bovine aorta ATPDase. They act either as substrates or as inhibitors of the hydrolysis of natural substrates of the enzyme, ATP and ADP. Most purine receptors are activated by nucleotide concentrations close to the micromolar range. For instance, the threshold of platelet activation by ADP is reached between 2 and 5 μM [50], whereas 20 μM ADP causes vasodilation and vasoconstriction of most vessels [4, 36]. In this work, we have demonstrated that the K_m and K_i values for ATP, ADP, and purine analogue hydrolysis by the bovine aorta ATPDase fall in the same range as the nucleotide concentrations needed to activate P_2-purinoceptors. Hence, the non-hydrolysable ATP analogues are expected to influence the concentrations of endogenously released purine nucleotides by competing for the catalytic site of the ATPDase. On the other hand, the ATPDase may reduce significantly the applied concentration of hydrolysable P_2-purinoceptor agonists, and thus restrain the magnitude and/or duration of the cell responses. Bo *et al.* [48] have reported that divalent cations facilitate the dephosphorylation of ATP and its derivatives, and influence interactions between purine nucleotides and P_{2x}-purinoceptors. Such dependence on Ca^{2+} or Mg^{2+} is a characteristic of most ATPDases [19].

Fedan and co-workers [51, 52] proposed that the tonic phase of the contraction induced by P_{2x}-purinoceptor activation depends on the hydrolysis of ATP into metabolites that bind to a different receptor, probably P_1 purinoceptors. From the recent literature, different types of ectoATPDases would be distributed on a variety of cells [19]. We believe that in many cases, this enzyme has been confused with ectoATPases which, by definition, have a different substrate specificity. By acting in tandem with the ecto 5′-nucleotidase, the ATPDase would convert ATP to adenosine, thereby shutting off first a P_2-effect and then eliciting a P_1-effect [3, 19]. Considerable efforts have been deployed to synthesize ectoATPase and ectoADPase inhibitors in order to isolate these two types of cellular responses [53–55]. Yet some of these compounds, such as suramin, tend to lack specificity and to interact with P_2-purinoceptors. The design of more specific inhibitors requires more information on the identity and properties of these ectonucleotidases. The identification of ATPDase as a potential target should prove to be useful in the synthesis of specific inhibitors of ectonucleotidases.

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