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Nucleotide hydrolytic activity of isolated intact rat mesenteric small arteries

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Segments of isolated intact rat mesenteric small arteries were incubated in physiological bicarbonate buffer in the presence of nano- to millimolar concentrations of ATP. ATP was hydrolysed, and when the vessel was transferred from one incubation to another, the enzyme activity was transferred with the vessel, consistent with the presence of an ecto-ATPase. The substrate, ATP, was shown to induce a modification of the hydrolytic activity which occurred the more rapidly the higher the concentration of ATP. The modified system hydrolysed ATP with a decreased substrate affinity. As the substrate induced a modification of the hydrolytic activity, steady-state velocity measurements for determination of kinetic parameters could not be obtained. Nevertheless, it was possible to compare the modification caused by ATP and UTP, and to compare the hydrolysis rates measured with [^{32}P]ATP, [^{32}P]UTP and [^{32}P]GTP. It was concluded that the hydrolytic activity of the vessels did not distinguish between the nucleoside triphosphates (NTPs). In a histidine buffer, the activity was shown to be activated by micromolar concentrations of either Ca^{2+} or Mg^{2+}, and not to be influenced by inhibitors of P-type, F-type and V-type ATPases. Functional removal of the endothelium before assay did not reduce the measured NTP hydrolysis. At millimolar concentrations of trinucleotide the hydrolysis rate was 10–15 μmol per min per gram of tissue or 0.11–0.17 μmol per min per 10^6 vascular smooth muscle cells. This value is equivalent to the maximal velocity obtained for the Ca^{2+} or Mg^{2+}-dependent NTPase released to the medium upon 2 s of sonication of the vessels (Plesner, L., Juul, B., Skriver, E. and Aalkjær, C. (1991) Biochim. Biophys. Acta 1067, 191–200). Comparing the characteristics of the released NTPase to the characteristics of the activity of the intact vessel, they showed a strong resemblance, but the substrate-induced modification of the enzyme was seen only in the intact preparation.

Introduction

Rat small arteries have been characterized through a number of studies [2]. In response to sympathetic nerve stimulation a dual response occurs which in part may be due to release of ATP [3,4]. In perfusion experiments P_2-receptors in the rat mesenteric bed were demonstrated [5] and we (Juul, B., Plesner, L. and Aalkjær, C. (1991), submitted) have characterized the effects of extracellular ATP in rat mesenteric small arteries and found evidence for the presence of P_{2x}-purinoceptors, which mediate contraction, depolarisation and potentiation of the noradrenaline-induced contraction.

Termination of purinoceptor-stimulation is ascribed to the rapid degradation of extracellular ATP [6,7]: in blood vessels ATP is hydrolysed by circulating ATPase(s) [8–11], by ecto-ATPases of blood cells [8,12–14] and endothelium [15–17], but also the smooth muscle cells of the media [15,18] and the fibroblasts of the adventitia [17] contain highly active ecto-ATPases.

In a number of other tissues ecto-ATPase activities have been described [19–26], and lately evidence was presented that the ecto-ATPase is identical to the (Ca^{2+}-Mg^{2+})-ATPase [22,23], which is also referred to as the (Ca^{2+}-Mg^{2+})-nucleotidase [27]. This enzyme is

Abbreviations: NTPase, nucleoside triphosphatase (EC 3.6.1.15); NTP, nucleoside triphosphate; CDTA, *trans*-1,2-diaminocyclohexanetetraacetic acid; EDTA, ethylenedinitrilotetraacetic acid; NEM, *N*-ethylmaleimide; PSS, physiological salt solution (see Methods); *p*NPP, *para*-nitrophenyl phosphate; VSMC, vascular smooth muscle cells.

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distinct from the Ca^{2+}-pump of the plasma membrane [27], and characterized by (1) lack of substrate specificity, (2) dependence on micromolar concentrations of Ca^{2+} or Mg^{2+} for activity and (3) insensitivity to inhibitors of P-type, V-type and F-type ATPases (for references, see Ref. 1). Recently, the amino acid sequence of the cloned liver enzyme was published [28]. Based on this work, it was concluded that the protein is identical to biliary glycoprotein I [28,29] and to the cell adhesion molecule Cell-CAM 105 [30]; furthermore, the protein was found to be phosphorylated by the tyrosine kinase of the insulin receptor [31]. Thus, the system seems to be physiologically controlled, and ATP may play a role not only through interaction with purinoceptors, but also through influence on cell adhesion.

In the preceding paper [1] we describe the release of an ATPase following brief sonication of small arteries. Evidence is presented that the enzyme originates from the plasma membranes of the smooth muscle cells and exhibits the characteristic properties of the (Ca^{2+}-Mg^{2+})-ATPase or (Ca^{2+}-Mg^{2+})-nucleotidase mentioned above. When the concentration of divalent cations was in the micromolar range a strong influence of monovalent cation salts was revealed and taken into account to establish optimal conditions for the assay of potential inhibitors and optimal conditions for a sensitive assay of the specific activity of the enzyme. It was suggested that the name nucleoside triphosphatase (EC 3.6.1.15), or Ca^{2+} or Mg^{2+}-dependent NTPase should be preferred, as the enzyme does not distinguish between the trinucleotides and possibly the dinucleotides, whereas the activity measured with mononucleotides as substrates was repeatedly found to be low or absent.

The aim of this paper was to characterize the nucleotide hydrolytic activity of intact isolated rat mesenteric small arteries and compare it to the released system, the Ca^{2+} or Mg^{2+}-dependent NTPase described in Ref. 1.

Experimental procedures

Buffers. Physiological bicarbonate buffer (physiological salt solution (PSS)) had the following composition (in mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5. pH was 7.4 at 37 °C through equilibration with 95% O_2/5% CO_2, the aeration was continued during preincubations and assay of hydrolytic activity. Phosphate-free PSS was PSS where KCl was exchanged for KH_2PO_4 on an equimolar basis.

Preparation of vessels. Male Wistar rats (200–400 g) of our own colony were killed by a blow on the head, and the mesenterium immediately removed and placed in PSS, where the dissection took place. Mesenteric arteries were dissected as previously described [32]. Segments of 5 to 20 mm were taken out from first- to fourth-order branches of the superior mesenteric artery. The length of each segment was measured and the artery then mounted on a 40 μm stainless steel wire. The ends of the wire were taken through a short segment of plastic tubing which was slipped onto a stick. This mounting ensured easy transfer of the vessels with minimum damage. After mounting the vessels were allowed to equilibrate for at least 15 min in PSS at 37 °C.

Measurements of nucleotide hydrolytic activity in intact small arteries. The assay mixture was PSS except in the experiments reported in Sections 3, 4 and 5 (see these Sections and legend to Fig. 6). Assay temperature was 37 °C. Assay volume and time are given in the legends to the figures and so are the nucleotide concentrations. The assay was started with transfer of the vessel to the assay tube. Nucleotide hydrolysis in the absence of vessel was measured as a blank under all assay conditions and subtracted. The specific activity of the ^{32}P-labeled nucleotides was $(0.5–50) \cdot 10^3$ cpm/nmol, when the trinucleotides were present in millimolar concentrations, and $(0.5–5) \cdot 10^7$ cpm/nmol, when the ATP concentration was in the nanomolar range.

(γ-^{32}P)-labeled nucleotide hydrolysis was determined on samples of 500 μl. Each sample was mixed with 50 μl ice-cold 50% trichloroacetate and placed in an ice-bath for 10 min before measuring $^{32}P_i$ release according to Ref. 33. Hydrolysis of non-labeled dinucleotides was measured in phosphate-free PSS according to Ref. 34.

Functional removal of endothelium. The inside of the vessel was rubbed gently with a 40 μm stainless steel wire [35]. It was shown (Juul, B., Plesner, L. and Aalkjær, C. (1991), submitted) that this procedure removes the relaxation response to 1 μM acetylcholine, which is dependent on an intact endothelium [36].

Materials. All reagents were of analytical grade. [γ-^{32}P]ATP, [γ-^{32}P]GTP and [γ-^{32}P]UTP were from Amersham and nonlabeled tri-, di- and mono-nucleotides were from Boehringer. Stock solutions of nucleotides were prepared in bidistilled water, titrated to pH 7.4 with NaOH and stored in aliquots at −20 °C. Oligomycin was from Boehringer, dissolved in 96% ethanol and kept at 4 °C. Monosodium vanadate (Merck) was dissolved in water and each day boiled for 10 min to avoid polymerisation. NEM (Sigma) and ouabain (Serva) were dissolved in water and freshly prepared daily and weekly, respectively.

Results and Discussion

1. Modification of the hydrolytic activity by the substrate

The ATP-hydrolysing activity of isolated intact rat mesenteric small arteries was measured in physiological buffer (PSS) to minimize time-dependent changes

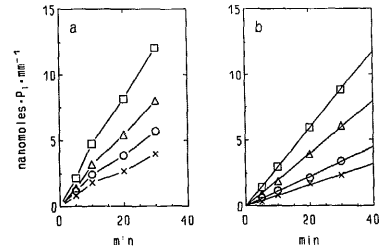

Fig. 1. Time courses: [^{32}P]ATP hydrolysed per millimeter of vessel vs. time. (a) At time zero a vessel was added to each of four tubes containing 3.5 ml PSS and [ATP] = 2.5 mM (□), 1.43 mM (△), 0.9 mM (○), and 0.67 mM (x). At the indicated intervals samples (500 μl) were drawn for measurement of released [^{32}P]P_i. After 30 min, the vessels were removed, but the tubes were left at 37 °C, and samples were drawn at 40 and 50 min for measurement of leak of enzyme (result not shown, see text). (b) The experiment of Fig. 1a was repeated: The four vessels used in Fig. 1a had been allowed to recover (for 10 min in PSS without ATP at 37 °C) and at time zero, each vessel was added to the assay tube which contained the same ATP concentration as the vessel had been hydrolysing in the incubation in (a).

in cell function that might influence the ATPase activity during assay. Still, non-linear time courses were obtained, the deviation from linearity being more pronounced the lower the ATP concentration. Representative examples of time courses are shown in Fig. 1a, where four vessels were incubated each with a different ATP concentration, and the ATP hydrolysis was measured as a function of incubation time.

In all cases less than 10% of the substrate present in the incubation-medium was used during the time course experiment. Therefore, when the hydrolysis rate decreased with incubation time, it was not due to a decrease of substrate concentration; nor was it caused by product inhibition, as it was verified that none of the possible products of the ATP hydrolysis would cause measurable inhibition in the concentrations that could be present (results not shown).

High concentrations of ATP have been reported to permeabilize certain cells [37,38]. If the cells of our preparation were permeabilized by extracellular ATP, there would be a leak of intracellular ATPases. These would contribute to the hydrolysis rate, but also they would be removed with each sample drawn, which could result in non-linear time courses. Therefore, in several experiments like the one shown in Fig. 1a, the ATP hydrolysis rate was measured in the medium after removal of the vessel. In all cases this rate was less than 10% of the rate measured in the preceding period with the vessel present, and the percentage was decreasing with increasing ATP concentrations. The lack of linearity in the time courses, therefore, could not be explained by extracellular ATP permeabilizing the cells of the tissue.

In the time courses of Fig. 1a, the deviation from linearity was more pronounced the lower the ATP concentration of the incubation. This could be interpreted to show that the effect of ATP might be to protect the ATPase activity from a time-dependent inactivation. From control experiments, however, it was evident, that in the absence of ATP there was no measurable inactivation: initial rates identical to those of Fig. 1a were measured with vessels that had been incubated for up to 70 min in the absence of ATP, but otherwise under the same conditions as those of Fig. 1a (results not shown). Therefore, the modification of the ATPase activity that caused the deviation from linearity in the time course of Fig. 1a was induced by ATP.

To elucidate whether the modification induced by ATP would be reversed in its absence, experiments like the one shown in Fig. 1a was repeated with the same vessels, after the vessels had been allowed to recover for 10 min in PSS without ATP. It is seen from the results that the modification was not reversed: The hydrolysis rates derived from the time courses that were now linear (Fig. 1b) were lower still than the hydrolysis rates at the end of the previous incubation (Fig. 1a).

Compared to the initial rates of the first incubation (Fig. 1a), the percentage reduction in hydrolysis rate seen in the second incubation (Fig. 1b) was twice as large with 0.67 mM ATP as with 4 mM. A suggestion as to the nature of the modification induced by ATP was obtained (Fig. 2) when the initial hydrolysis rates of the first incubation (Fig. 1a) and the hydrolysis rates of the second incubation (Fig. 1b) were plotted against the ATP concentration (Fig. 2, triangles and diamonds, respectively): the ATP induced modification seemed to imply a decreased affinity for the substrate or an apparent disappearance of saturation.

To test this hypothesis a series of experiments was performed where the same vessel was incubated with ATP for 10 min in each of five consecutive tubes containing increasing concentrations of ATP. Whereas the measured ATP hydrolysis rates (Fig. 2, circles), at [ATP] = 0.67, 0.9 and 1.43 mM, were equal to the initial rates of the first incubation (Fig. 1a), then at [ATP] = 2.5 and 4 mM, the rates became comparable to the rates of the second incubation (Fig. 1b). When, on the other hand, a vessel was incubated first at the high ATP concentration (Fig. 2, squares), all of the hydrolysis rates measured were close to the rates of Fig. 1b.

The results thus support the suggestion that ATP induces a modification of the activity. The modified enzyme hydrolyzes ATP but with a lower affinity. The data also indicate that the modification occurs more

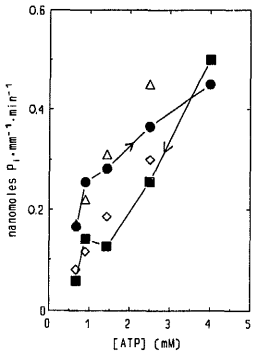

Fig. 2. ATP hydrolysis rate vs. ATP concentration, influence of incubation time and ATP concentration. ATP hydrolysis rates were measured in an experiment where one vessel was taken through *increasing* concentrations of ATP (●) and allowed to hydrolyse the nucleotide for 10 min in each incubation, starting in [ATP] = 0.67 mM. Another vessel was taken through *decreasing* concentrations of ATP (■), starting in [ATP] = 4 mM, and again the ATP hydrolysis rates were measured over 10 min in each incubation. Assay volume was 500 μl and assay buffer was PSS. For comparison, the ATP hydrolysis rates measured over the first 10 min of the time course of Fig. 1a were included (△) as well as the hydrolysis rates measured in Fig. 1b (◇). The lines were drawn as an aid to the eye.

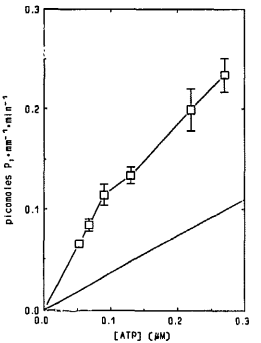

Fig. 3. ATP hydrolysis rate vs. ATP concentrations in the submicromolar range. Note that the ATP hydrolysis rate is given in pmol/min per mm. Each point corresponds to the mean of three assays of the activity of freshly prepared vessels incubated for 10 min in a volume of 500 μl. The length of the bars indicates 2 S.E. The assay buffer was PSS. The line has the equation $v = 1.14/(1 + 2.8/[ATP])$ (see text).

rapidly the higher the concentration of ATP. If so, it implies that ATP hydrolysis at very low ATP concentrations (Fig. 3) should be catalysed by a less modified enzyme, i.e., by an enzyme with higher substrate affinity, and therefore the ATP hydrolysis rates at low ATP-concentrations should exceed the value predicted when extrapolating to small ATP concentrations from the hydrolysis rates measured in the millimolar ATP range. Such values (ATP hydrolysis rates at millimolar ATP-concentrations), obtained with freshly prepared vessels, are given in Fig. 2 (open triangles), and in Fig. 4 (open triangles). These data may be fitted to a hyperbola of the form $v = 1.14/(1 + 2.8/[ATP])$ (see Section 2). This function is plotted in Fig. 3 and compared to the measured ATP hydrolysis rates. The latter clearly exceed those predicted from the above equation and the results thus support the suggestion that the ATP induced modification occurs less rapidly the lower the concentration of ATP.

The experiments described above where one vessel was incubated consecutively with increasing of decreasing concentrations of ATP were repeated with UTP, and the results obtained were indistinguishable from the results presented for the hydrolysis of ATP (Figs. 2 and 3) indicating that UTP caused the same modification of the enzyme as did ATP.

For rat pancreas cells non-linear time courses were reported [22] and in cultured VSMC [18] the rate of adenosine appearance was less when the initial concen-

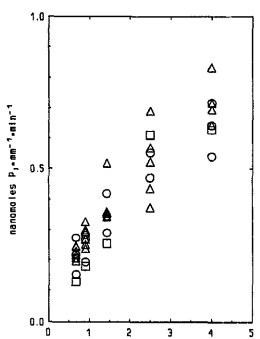

Fig. 4. Trinucleotide hydrolysis rate vs. concentration of trinucleotides. [γ-^{32}P]ATP hydrolysis rate (△), [γ-^{32}P]UTP hydrolysis (○) and [γ-^{32}P]GTP hydrolysis rate (□). Each point corresponds to an assay of the activity of a freshly prepared vessel for 10 min in a volume of 500 μl. The assay buffer was PSS.

tration of ATP was higher; but this could be due to feed forward inhibition of 5'-nucleotidase, and is not necessarily caused by substrate inhibition. To our knowledge a substrate induced modification (inhibition) of ecto-ATPases has not been described before.

2. Substrates

In Fig. 4 are shown the hydrolysis rates that were measured with trinucleotide concentrations in the millimolar range and with freshly prepared vessels incubated with the trinucleotide for 10 min. The data could be fitted to a hyperbola of the form

$$v = 1.14/(1+2.8/[\mathrm{NTP}]) \quad (1)$$

v, the hydrolysis rate, is expressed in nmol/min per mm; [NTP] is expressed in mM. But, since the substrate induces a time-dependent modification of the enzyme, this equation must be considered an empirical fit of the data, for extrapolation purposes only, and its parameters are not V_{max} and K_m for the enzyme. In double-reciprocal form which is the form used in Fig. 5, Eqn. 1 becomes

$$v^{-1} = 0.88 + 2.46[\mathrm{NTP}]^{-1}$$

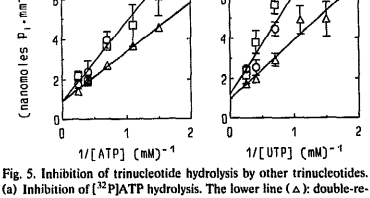

Fig. 5. Inhibition of trinucleotide hydrolysis by other trinucleotides. (a) Inhibition of [^{32}P]ATP hydrolysis. The lower line (△): double-reciprocal presentation of the data given in Fig. 4. for the ATP hydrolysis rates. The least-squares regression line has the form: $v^{-1} = 0.88 + 2.47[\mathrm{ATP}]^{-1}$ ($r = 0.944$). Upper line: reciprocal of ATP hydrolysis rates vs. reciprocal of ATP concentrations in the presence of 2.5 mM of UTP (□) or 2.5 mM of GTP (○). The least-squares regression line has the form: $v^{-1} = 0.89 + 3.94[\mathrm{ATP}]^{-1}$ ($r = 0.933$). (b) Inhibition of [^{32}P]UTP hydrolysis. Lower line (△): double-reciprocal presentation of the data given in Fig 4 for the UTP hydrolysis rates. The least-squares regression line has the form: $v^{-1} = 1.05 + 2.86[\mathrm{UTP}]^{-1}$ ($r = 0.88$). Upper line: reciprocal of UTP hydrolysis rates vs. reciprocal UTP concentrations in the presence of 2.5 mM ATP (□) or 2.5 mM GTP (○). The least squares regression line has the form: $v^{-1} = 1.21 + 4.97[\mathrm{UTP}]^{-1}$ ($r = 0.95$). The values given in the presence of competing substrates are means of two or three determinations. The length of the bars indicates 2 S.E.

The data of Fig. 4 indicate that the NTP-hydrolysing activity of the intact vessel did not distinguish between the substrates ATP, UTP and probably GTP. Furthermore, when the ATP substrate curve was performed in the presence of 2.5 mM of UTP (square) or GTP (circle) (Fig. 5a), and when the UTP substrate curve was performed in the presence of 2.5 mM ATP (square) or GTP (circle) (Fig. 5b), only the nucleotide concentration needed to obtain half maximal activity was changed. The inhibition pattern is the pattern of competing substrates, thus indicating that the trinucleotides are probably hydrolysed by the same enzyme.

Dinucleotide, mononucleotide and *p*NPP hydrolysis, catalysed by intact vessels, were measured by colorimetric determination of released inorganic phosphate in phosphate-free PSS, where the ATP hydrolysis rate was found to be the same as in PSS. At 1 mM of either ADP, UDP or GDP, the dinucleotide hydrolysis rate in nmol/min per mm was 0.219 ± 0.049 (S.E.) ($n = 5$) for ADP, whereas for UDP and GDP is was 0.178 and 0.158, respectively. The latter two figures were the mean of two determinations. The hydrolysis rate for AMP at 1 mM was 0.036 ± 0.006 (S.E.) ($n = 3$) nmol/min per mm but with 1 mM *p*NPP there was no measurable hydrolysis.

Lack of distinction between trinucleotides has often been reported for ecto-ATPases [9,13,15,19–23,38] as well as for the Ca^{2+}- or Mg^{2+}-dependent NTPase (Ref. 1 (and references in Ref. 1) and Refs. 24 and 27). Whether di- and trinucleotides are hydrolysed by the same enzyme is a matter of controversy [12,13,18,19,25]. Our experiments did not address this question.

3. Effect of divalent cations

To test the effect of divalent cations, Ca^{2+} and Mg^{2+}, on the nucleotide hydrolytic activity of the intact vessel, two vessels were incubated in a histidine buffer, 30 mM (pH 7.4 at 37 °C), with [NaCl] = 20 mM, [sucrose] = 193 mM, and [[^{32}P]ATP] = 1 mM. Under these conditions and in the presence of 2 μM Mg^{2+} and 50 μM Ca^{2+} the ATP hydrolysis rate was 0.260 ± 0.031 (S.E.) nmol/min per mm. This is not significantly different from the hydrolysis rate measured at 1 mM ATP in PSS (Fig. 4). The ATP hydrolysis rate measured in the histidine buffer with NaCl and sucrose and with only endogenous divalent cations present was 0.209 ± 0.024 (S.E.) ($n = 4$); it was sufficient to add only one of the divalent cations, either Mg^{2+} (2 μM) or Ca^{2+} (50 μM) to obtain full activity (0.248 and 0.265 nmol/min per mm, respectively), however, when the endogenous divalent cations were chelated by the addition of 1 mM CDTA to the assay, the ATP hydrolysis rate decreased to 0.020 ± 0.002 (S.E.) ($n = 4$) nmol/min per mm. The ecto-ATPase [19,21–24,26,38], as well as the Ca^{2+} or Mg^{2+}-dependent NTPase (Ref. 1 (and references in Ref. 1) and Refs. 24 and 27) were

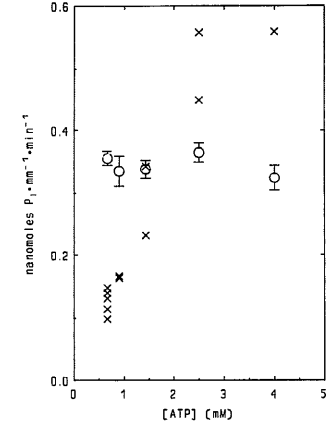

Fig. 6. Effect of medium and inhibitors. ATP hydrolysis rates obtained with intact vessels compared to rates obtained with released NTPase at varying ATP concentrations. Intact vessels (x): Freshly prepared vessels were incubated for 10 min in a volume of 500 μl of the assay medium proposed for the released system [1]. This has the following concentrations (in mM): histidine 30, NaCl 20, $CaCl_2$ 0.05, $MgCl_2$ 0.002, ouabain 1, vanadate 0.05, NEM 0.5 and oligomycin 0.006 with the addition of sucrose 193 mM for isotonicity. Each point corresponds to an individual assay. Released NTPase activity (○): Each point is the average of ATP hydrolysis rates measured with 2–4 enzyme sources, obtained as follows: 14 mm of vessel was submitted to 2 s of sonication in 300 μl of PSS on an ice bath [1]. The vessel was discarded, and assay of released ATPase activity was initiated adding 50 μl of the enzyme source ($\approx$ 14/6 mm of vessel) to 450 μl of PSS with the ATP concentrations indicated. Assay time 10 min, temperature 37 °C. The bars indicate S.E.

previously shown to hydrolyze ATP in the presence of only endogenous divalent cations and to be activated by micromolar concentrations of Ca^{2+} or Mg^{2+}.

4. Inhibitors

The incubation conditions chosen above for measuring the effect of Mg^{2+} and Ca^{2+} were chosen also for measuring the effect of inhibitors on the system (Fig. 6, see legend). In the simultaneous presence of the P-type ATPase inhibitors, ouabain and vanadate, (1 mM and 0.05 mM, respectively), a V-type ATPase inhibitor, NEM (0.5 mM) and an F-type ATPase inhibitor, oligomycin (6 μM) the ATP hydrolysis rate catalysed by the intact vessel (Fig. 6, crosses) was measured as a function of the ATP concentration, and the substrate curve obtained was not significantly different from the substrate curve determined in PSS (Fig. 4). The ATP hydrolysis rate in the histidine buffer with NaCl, sucrose, Mg^{2+} and Ca^{2+} in the absence of inhibitors (see Section 3) was not different from the hydrolysis rate in the presence of inhibitors. Time courses measured in PSS (Figs. 1a and b) and in the histidine buffer with inhibitors (used in Fig. 6) were not distinguishable from each other (not shown), indicating that the added inhibitors were without effect on the system. Lack of inhibition by these inhibitors has been reported for other ecto-ATPases [9,13,15,21–24,38] and for the Ca^{2+} or Mg^{2+}-dependent NTPase (Ref. 1 (and references in Ref. 1), and Ref. 27).

5. ATP hydrolysis catalysed by the intact vessel compared to the activity obtained with released NTPase

As described above (Section 4) the ATP hydrolysis catalysed by the intact vessel was measured in the medium proposed for the assay of the released Ca^{2+} or Mg^{2+}-dependent NTPase [1] (Fig. 6, crosses). Likewise, the released system was assayed in PSS (Fig. 6, circles). Here the hydrolysis rate was lower than the maximal velocity obtained under optimal conditions [1] as was expected due to the high concentrations of especially Mg^{2+} in PSS. However, saturation was observed at all substrate concentrations and no deviation from linearity was observed in time courses (not shown). This indicates that the ATP-induced modification does not occur in the released system, independent of the medium used for assay.

6. Quantity

When the NTP hydrolysing activity of the vessels was tested after functional removal of the endothelium (see Methods) no decrease in activity was observed (results not shown), indicating that the activity is situated mainly in the VSMC.

The number of VSMC per millimeter of resistance arteries from Wistar-Kyoto rats [39,40] with an internal diameter of 0.23 mm is 5100 VSMC per millimeter [39]. Therefore, a nucleotide hydrolysis rate of 0.7 nmol/min per mm (see Fig. 4) is equivalent to 0.14 μmol/min per 10^6 of VSMC. This is ten times more than the reported activity for cultured pig aortic VSMC [18], cultured pig aortic endothelial cells [16] and rat liver cells [23]. The activity of the VSMC of the vessel is, however, in agreement with the figure that was reported for bovine chromaffine cells [25], mammary gland cells [24] and rat pancreatic cells [22].

The maximal nucleotide hydrolysis rate obtained with the released Ca^{2+} or Mg^{2+}-dependent NTPase [1] was 1.32 μmol of nucleotide hydrolysed per min per mg of protein released. As 0.63 μg of protein were released per mm of vessel sonicated, the reported maximal velocity is equivalent to 0.83 nmol/min per mm of vessel and this value is in agreement with the values we have measured for NTP hydrolysing activity of the intact vessel.

For comparison, the Na,K-ATPase activity of the vessels may be calculated as follows: The number of ouabain binding sites was reported to be $3.4 \cdot 10^5$ sites per VSMC [41] and based on a turnover number of 8000 min^{-1} the activity of the Na,K-ATPase is $(3.4 \cdot 10^5/6.02 \cdot 10^{23}) \cdot 8000 \cdot 5100 = 0.023$ nmol/min per mm. If the Ca^{2+} or Mg^{2+}-dependent NTPase is concerned only with the regulation of agonist concentrations around the purinoceptors it is surprising that its activity is more than 30-times higher than the activity of the sodium pump.

As mentioned above identity between the published sequence of liver Ca^{2+} or Mg^{2+}-dependent ecto-NTPase and Cell-CAM 105 has been reported [30]. In the quoted work it was suggested that "Interactions between the Ig-domains of C-CAM may be the structural basis for the specific homophilic binding. Binding and hydrolysis of ATP may then modify this binding in a manner similar to ATP-regulated binding between actin and myosin". The substrate-induced modification described in Section 1 above would be in agreement with this idea, which is at present under investigation.

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