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Effect of adenosine on the ouabain-insensitive Na^+-ATPase activity from basolateral membrane of the proximal tubule

C. Caruso-Neves, L.G. Francisco-Pedro, L.P. Souza, C. Chagas, A.G. Lopes *

Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Bloco G, 21949 Rio de Janeiro, RJ, Brazil

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Abstract

The regulation of the furosemide-sensitive Na^+-ATPase activity and ouabain-sensitive $(Na^+ + K^+)$ATPase activities from proximal tubules by adenosine was investigated. When the concentration of adenosine was increased the furosemide-sensitive ATPase activity decreased with maximal inhibition at 10^{-8} M (56% of inhibition). However, the $(Na^+ + K^+)$ATPase activity was not affected by adenosine. Theophylline, an antagonist of P_1 adenosine receptor, completely reversed the effect of adenosine on the furosemide-sensitive ATPase activity in a dose-response manner. The adenosine effect was mimicked by N^6-cyclohexyladenosine (CHA), an agonist for A_1 adenosine receptor. 5′-*N*-ethylcarboxamide-adenosine (NECA), an agonist for A_2 adenosine receptor, did not affect the furosemide-sensitive ATPase activity. When adenosine was used in the presence of 1 μg ml^{-1} pertussis toxin, a Gi protein inhibitor, no change in the furosemide-sensitive ATPase activity was observed. The addition of 1 nM cholera toxin increased the Na^+-ATPase activity by 60%. Adenosine decreased the cholera toxin stimulated Na^+-ATPase in 42%, similar to the effect observed in the absence of cholera toxin. Dibutyryl-cAMP reversed the effect of adenosine in a dose dependent manner while the protein kinase A peptide inhibitor mimicked it. These data are compatible with a modulatory effect of adenosine on the Na^+-ATPase activity via A_1 subtype receptor. © 1997 Elsevier Science B.V.

Keywords: Na^+-ATPase; Adenosine; Furosemide; Proximal tubule

1. Introduction

Adenosine has a crucial role in cellular energy metabolism and is recognized as a cellular messenger that produces specific biochemical effects, which result in physiological response. It has been established that the effect of adenosine is mediated by interactions with receptors [1]. Two types of receptors were proposed to mediate the adenosine effects: P_1 (adenosine receptor) and P_2 (adenosine nucleotide receptors) [2,3]. The relative affinities for P_1 sites are adenosine > AMP > ADP, while for P_2 sites the potencies are ATP > ADP > AMP > adenosine [1]. P_1 receptors are further subdivided in two principal subtypes of receptors: A_1 and A_2, which can be differentiated on the basis of their affinities for adenosine and several adenosine analogues, and their effects on adenylyl cyclase [1]. The A_1 receptor has higher affinity for adenosine (0.5–100 nM) than the A_2 receptor (0.5–25 μM) [1]. A_1 receptors are involved with a decrease in adenylyl cyclase activity mediated by pertussis toxin-sensitive GTP binding

* Corresponding author. Fax: +55-21-2808193; E-mail: agilopes@chagas.biof.ufrj.br.

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protein, while A_2 receptors are involved with an increase in the adenylyl cyclase activity, mediated by cholera toxin-sensitive GTP binding protein [1,4,5].

It has been observed that the kidneys produce adenosine, and in some conditions, such as during ischemia, there is an increase in its production [6]. Palacios et al. [7] used autoradiographic methods to examine the A_1 receptor in guinea-pig kidney. They observed that A_1 receptors have a highly heterogeneous distribution. More recently, the cDNAs encoding A_1 and A_2 receptors have been cloned [8–10] and used to identify and localize adenosine receptors in the rat kidney [11]. A_1 receptor is more abundant in collecting duct.

Adenosine regulates several renal functions, among them the transport of solutes. Coulson and coworkers [12] found that adenosine increases the transport of phosphate and glucose in opossum kidney cells. Takeda and coworkers [13] showed that adenosine stimulates the Na^+-$3HCO_3^-$ cotransport in rabbit proximal convoluted tubule via an A_1-receptor-mediated mechanism. Furthermore, it has been reported that A_1 antagonists, such as DPCPX and FK-453, have diuretic and natriuretic action without affecting the glomerular flow rate (GFR) and renal blood flow (RBF) [14,15]. These effects were related to changes in proximal tubule transport of ions.

Two sodium pumps have been described in kidney: (1) The classic ouabain-sensitive $(Na^+ + K^+)$ATPase and (2) the ouabain-insensitive, furosemide-sensitive Na^+-ATPase. The furosemide-sensitive Na^+-ATPase is found in a variety of tissues from different animals including rat proximal tubule [16–18]. The Na^+-ATPase transports Na^+ against an electrochemical gradient and is not stimulated by K^+ [19,20]. This pump has a widespread and parallel distribution with the $(Na^+ + K^+)$ATPase, and its involvement in cell volume regulation has been proposed [16,18,20,21].

Since adenosine regulates the proximal sodium transport it is possible that it modulates the sodium pumps. In this work, we studied the effect of adenosine on both the ouabain-insensitive Na^+-ATPase and the ouabain-sensitive $(Na^+ + K^+)$ATPase activities and their signaling pathway. We observed that adenosine does not change the $(Na^+ + K^+)$ATPase activity but inhibits the furosemide-sensitive Na^+-ATPase activity via an A_1-receptor-mediated mechanism.

2. Materials and methods

2.1. Materials

ATP, ouabain, furosemide, oligomycin, EGTA, mannitol, pertussis toxin, cholera toxin, dibutyryl-cAMP, peptide (Thr–Thr–Tyr–Ala–Asp–Phe–Ile–Ala–Ser–Gly–Arg–Thr–Gly–Arg–Arg–Asn–Ala–Ile–His–Asp) inhibitor of protein kinase A (PKAi) and adenosine were purchased from Sigma. N^6-cyclohexyladenosine (CHA) and 5′-*N*-ethylcarboxamideadenosine (NECA) were purchased from Research Biochemicals International; Percoll was from Pharmacia. All chemical reagents were of the highest purity available. [^{32}Pi]Pi was obtained from the Institute of Energetic and Nuclear Research, Brazil.

All solutions were prepared with deionized glass-distilled water. [γ-^{32}P]ATP was prepared as described by Maia and coworkers [22].

2.2. Preparation of purified basolateral membrane vesicles

Basolateral membranes from pig kidney proximal tubule cells were isolated from renal cortex by the Percoll gradient method [23] modified from that described by Scalera et al. [24] and Sacktor et al. [25]. The membrane preparation was resuspended in 250 mM sucrose at a final concentration of 20–30 mg protein ml^{-1}.

2.3. Measurement of ATPase activity

Except as noted under Section 3, the composition of the standard assay medium (0.2 ml) contained: 10 mM $MgCl_2$; 5 mM [γ-^{32}P]ATP; 20 mM Hepes–Tris (pH 7.0); 2 $\mu g\ ml^{-1}$ oligomycin; 1 mM EGTA plus 6 mM NaCl for measurement of the Na^+-ATPase activity or 90 mM NaCl and 20 mM KCl for measurement of the $(Na^+ + K^+)$ATPase activity. The final osmolality was adjusted with mannitol to 300 mOsm kg^{-1}.

The ATPase activity was measured according to the method described by Grubmeyer and Penefsky [26]. The reaction was started by the addition of vesicles to a final protein concentration of 0.3–0.5 mg ml^{-1}. The reaction was stopped after 40 min by the addition of charcoal activated by HCl (0.1 N).

The [^{32}P]Pi released was measured in an aliquot of the supernatant obtained after centrifugation of the charcoal suspension for 20 min at 3000 rpm. Spontaneous hydrolysis of [γ-^{32}P]ATP was measured simultaneously in tubes where protein was added after the acid. The Na^+-ATPase activity was calculated by the difference between the [^{32}P]Pi released in the absence and in the presence of 2 mM furosemide, both in the presence of 1 mM ouabain [27,28]. The ($Na^+ + K^+$)ATPase activity was calculated by the difference between the [^{32}P]Pi released in the absence and in the presence of 1 mM ouabain [29]. The averages of the control values were 8.63 ± 1.80 and 73.01 ± 7.89 nmol ^{32}Pi mg^{-1} min^{-1} for ouabain-insensitive Na^+-ATPase and ouabain-sensitive ($Na^+ + K^+$)ATPase activities, respectively. The incubation time of the protein with the reagents is the same as for the reaction. Protein concentrations were determined using the Folin phenol reagent [30] and bovine serum albumin as a standard. Different preparations of basolateral vesicles were used for each experiment. The data were analyzed by two-way analysis of variance (ANOVA), considering the treatments as factors. The significance of the differences was verified by the Bonferroni *t*-test. Statistical analysis was made using absolute values and the results were expressed in percentage of the control.

3. Results

3.1. Effect of adenosine on the ($Na^+ + K^+$)ATPase and Na^+-ATPase activities

It has been observed that adenosine regulates several ion transport systems [12]. Recently, it was observed that adenosine regulates the Na^+-$3HCO_3^-$ cotransport in rabbit proximal convoluted tubule via A_1 receptors [13]. To determine whether exogenous adenosine regulates the two sodium pumps present in basolateral membrane from proximal tubule, we measured both the sodium-stimulated ouabain-sensitive ATPase activity and the sodium stimulated ouabain-insensitive furosemide-sensitive ATPase activity in the absence or in the presence of different adenosine concentrations (from 10^{-3} to 10^{-10} M). The results are shown in Fig. 1. The dependence on adenosine concentrations of the furosemide-sensitive ATPase activity showed a biphasic behavior (Fig. 1). Initially, the increase in adenosine concentration decreased the furosemide-sensitive ATPase activity by 56% ($P < 0.05$), with maximal inhibition at 10^{-8} M adenosine. Further increase in the concentration of adenosine abolished this inhibition, and complete reversal was obtained at a concentration of 10^{-3} M. In contrast, the increase in adenosine concentration did not affect the ouabain-sensitive ($Na^+ + K^+$)ATPase activity (Fig. 1).

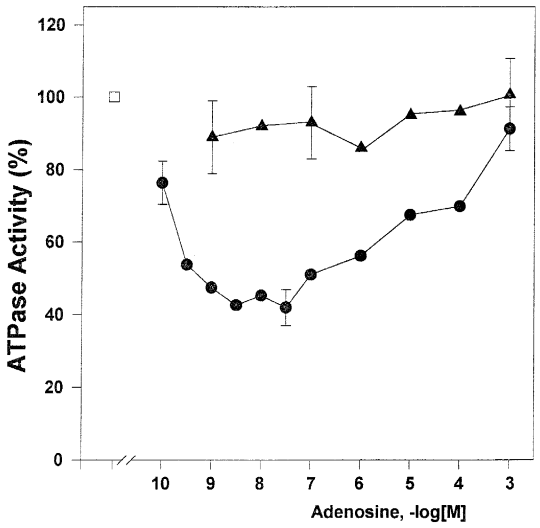

Fig. 1. Dependence of ouabain-insensitive Na^+-ATPase (●) and ouabain-sensitive ($Na^+ + K^+$)ATPase (▲) activities on adenosine concentration. The ATPase activity was measured as described in Section 2 ($n = 10$). The adenosine concentration was increased from 10^{-10} up to 10^{-3} M. Results are expressed as percentage of the control.

3.2. Adenosine receptor agonists and antagonists

Since P_1 receptor is sensitive to methylxanthines such as theophylline and P_2 receptor is not, this antagonist was used to clarify the receptor type involved in the adenosine effect [1]. Fig. 2 shows the modulation of the adenosine effect on the furosemide-sensitive ATPase activity by theophylline. The increase in theophylline concentration from 10^{-9} to 10^{-4} M reversed the inhibition promoted by 10^{-8} M adenosine. The maximal effect was obtained with 10^{-6} M theophylline. The addition of 10^{-6} M theophylline alone did not change significantly the ATPase activity ($14.1 \pm 13.2\%$, $P > 0.05$).

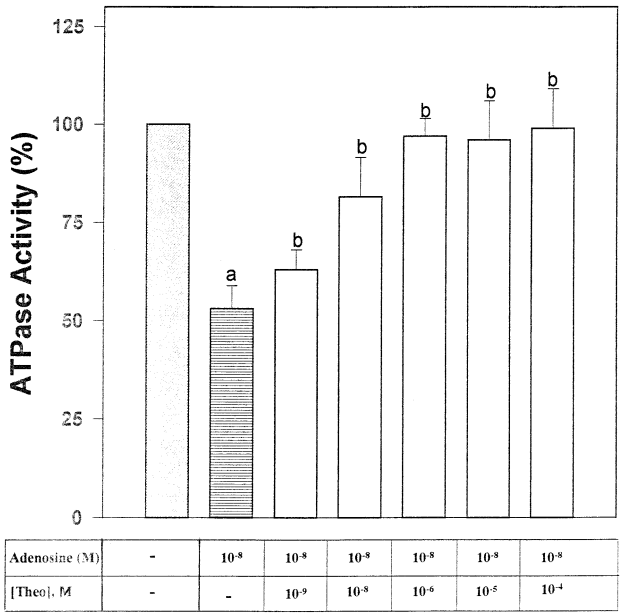

Fig. 2. Modulation of the effect of adenosine on ouabain-insensitive Na^+-ATPase activity by theophylline (Theo). The ATPase activity was measured as described in Section 2 ($n = 9$). The theophylline concentration was increased from 10^{-9} up to 10^{-4} M. 10^{-8} M adenosine (Ade) was added where indicated. [a] Statistically significant when compared to control ($P < 0.05$). [b] Statistically significant when compared to the level of the ATPase activity in the presence of 10^{-8} M adenosine ($P < 0.05$). Results are expressed as percentage of the control.

The P_1 receptor can be subdivided into A_1 and A_2 receptors [5]. These receptors can be activated by different agonists. The adenosine analog N^6-cyclohexyladenosine (CHA) has higher affinity for A_1 receptor while 5′-*N*-ethyl-carboxamide adenosine (NECA) has higher affinity for the A_2 receptor [5,31,32].

Fig. 3 shows the effect of an increase in CHA concentration from 10^{-11} to 10^{-6} M, on the Na^+-ATPase and $(Na^+ + K^+)$ATPase activities. The increase of CHA concentration up to 10^{-8} M decreased the Na^+-ATPase activity in 56%. Further increase in CHA concentration increased the enzyme activity. Similarly to adenosine, CHA did not change the $(Na^+ + K^+)$ATPase activity.

The increase of NECA concentration from 10^{-8} to 10^{-5} M did not change the Na^+-ATPase activity (Fig. 4). Similarly, the addition of 10^{-6} M NECA did not modify the $(Na^+ + K^+)$ATPase activity as shown in the inset of Fig. 4.

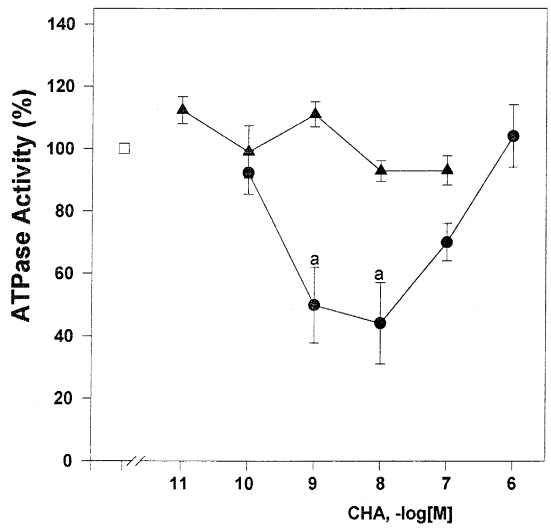

Fig. 3. Dependence of ouabain-insensitive Na^+-ATPase (●) and ouabain-sensitive $(Na^+ + K^+)$ATPase (▲) activities on CHA concentration. The ATPase activity was measured as described in Section 2 ($n = 7$). The CHA concentration was increased from 10^{-11} up to 10^{-6} M. [a] Statistically significant when compared to control ($P < 0.05$). Results are expressed as percentage of the control.

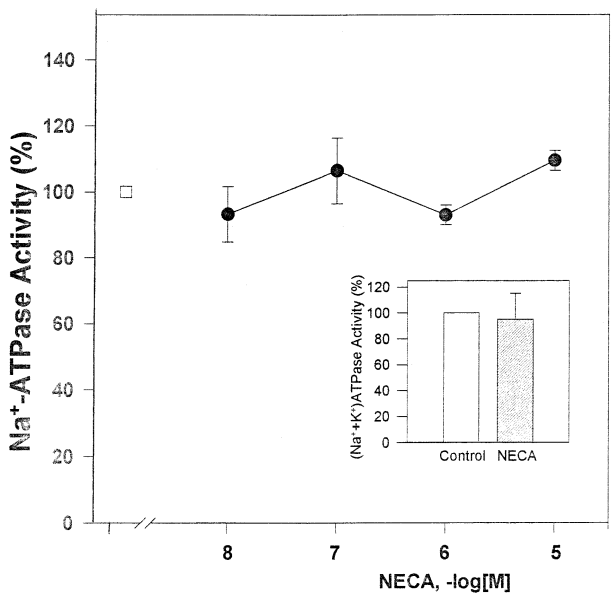

Fig. 4. Dependence of ouabain-insensitive Na^+-ATPase and ouabain-sensitive $(Na^+ + K^+)$ATPase activities (inset) on NECA concentration. The ATPase activity was measured as described in Section 2 ($n = 7$). The NECA concentration was increased from 10^{-8} up to 10^{-5} M. The conditions of the experiments shown in the inset were described in Section 2. Results are expressed as percentage of the control.

3.3. G-protein involvement

It has been described that A_1 receptors are coupled to pertussis toxin-sensitive GTP binding protein (Gi protein) [1,4,5]. Thus, if the adenosine effect on the furosemide-sensitive ATPase activity is mediated by activation of the Gi protein, addition of pertussis toxin, an inhibitor of Gi protein, should reverse the effect of adenosine. Addition of 1 μg ml^{-1} pertussis toxin completely reversed the inhibition of the furosemide-sensitive ATPase activity by adenosine (Fig. 5). The addition of pertussis toxin alone did not affect the furosemide-sensitive ATPase activity.

Addition of 10^{-9} M cholera toxin alone, a Gs protein stimulator, increased the Na^+-ATPase activity by 60%. When 10^{-8} M adenosine was added simultaneously with cholera toxin, the furosemide-sensitive ATPase activity was decreased 42% ($P > 0.05$), when compared to the level obtained with cholera toxin alone (Fig. 6).

3.4. cAMP involvement

The data obtained so far suggest that the effect of adenosine involves the activation of Gi protein. So, if

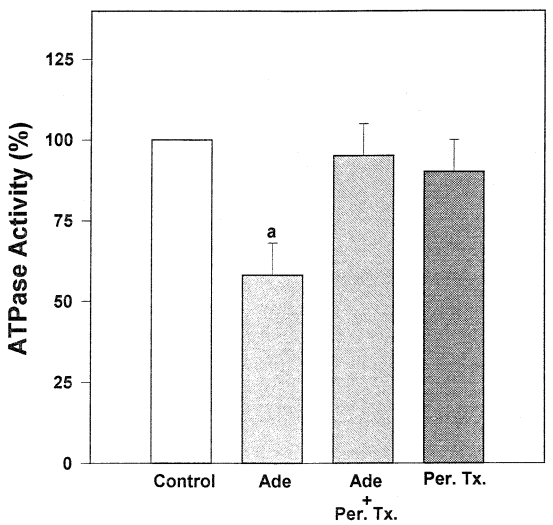

Fig. 5. Inhibition of the effect of adenosine on ouabain-insensitive Na^+-ATPase activity by 1 μg ml^{-1} Pertussis toxin (Per. Tx.). The ATPase activity was measured as described in Section 2 ($n = 10$). 10^{-8} M adenosine (Ade) was added where indicated. [a] Statistically significant when compared to control ($P < 0.05$). Results are expressed as percentage of the control.

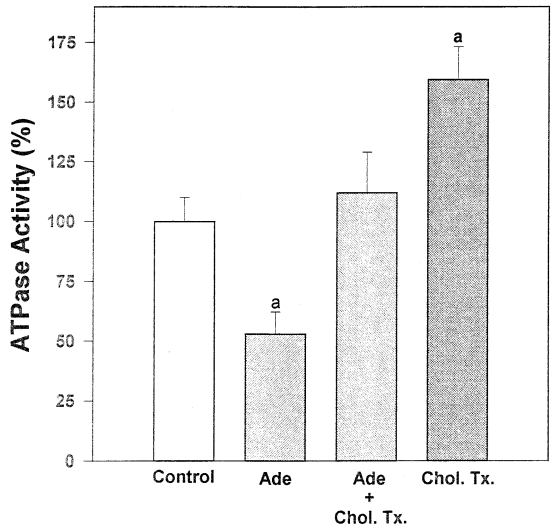

Fig. 6. Modulation of the effect of adenosine on ouabain-insensitive Na^+-ATPase activity by 1 nM Cholera toxin (Chol. Tx.). The ATPase activity was measured as described in Section 2 ($n = 10$). 10^{-8} M adenosine (Ade) was added where indicated. [a] Statistically significant when compared to control ($P < 0.05$). Results are expressed as percentage of the control.

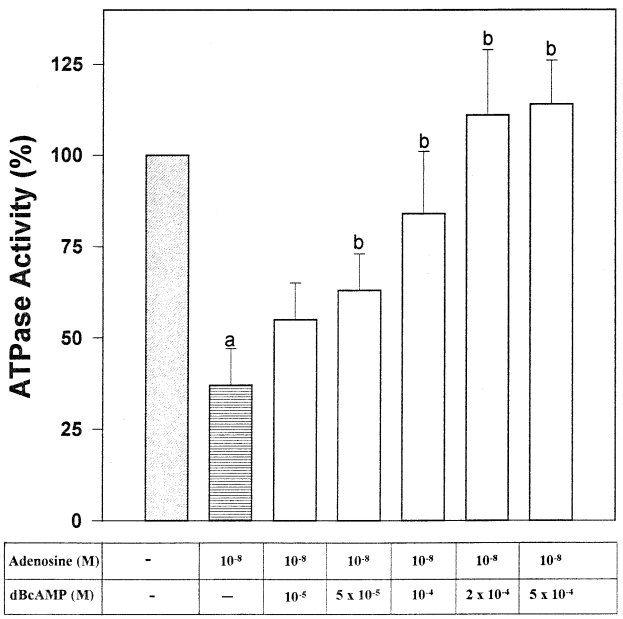

Fig. 7. Reversal of the effect of adenosine on ouabain-insensitive Na^+-ATPase activity by increasing dibutyryl-AMPc concentration. The ATPase activity was measured as described in Section 2 ($n = 10$). 10^{-8} M adenosine (Ade) was added where indicated. [a] Statistically significant when compared to control ($P < 0.05$). [b] Statistically significant when compared to the level of the ATPase activity in the presence of 10^{-8} M adenosine ($P < 0.05$). Results are expressed as percentage of the control.

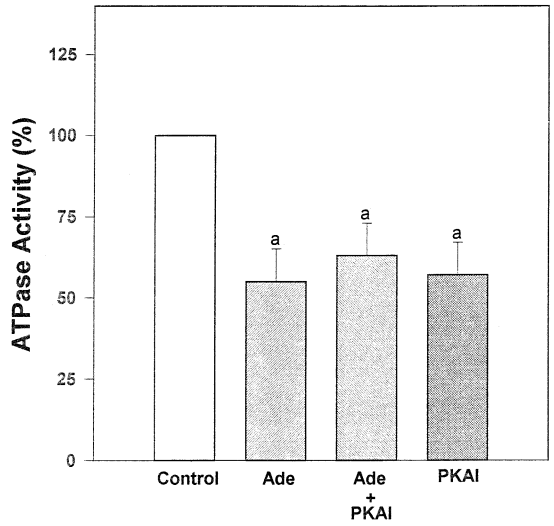

Fig. 8. Modulation of the effect of adenosine on ouabain-insensitive Na^+-ATPase activity by addition of 10 nM protein kinase A inhibitor (PKAI). The ATPase activity was measured as described in Section 2 ($n = 10$). 10^{-8} M adenosine (Ade) was added where indicated. [a] Statistically significant when compared to control ($P < 0.05$). Results are expressed as percentage of the control.

the Gi protein decreases the adenylyl cyclase activity it is possible that an increase in the cAMP level reverses the inhibition promoted by adenosine. Fig. 7 shows that an increase in dibutyryl-cAMP, permeant analog of cAMP, concentration from 10 to 500 μM increased the furosemide-sensitive ATPase activity in the presence of adenosine. Complete reversal of the adenosine inhibition of the furosemide-sensitive ATPase activity was obtained at 2×10^{-4} M dibutyryl-cAMP. The same results were obtained with 10^{-4} M cAMP indicating that the site of action of cAMP is located on the outside of the vesicle (data not shown). However, we can not rule out the presence of leaky vesicles. The addition of 10^{-4} M cAMP alone did not change significantly the ATPase activity ($2.7 \pm 2.0\%$, $P > 0.05$).

cAMP effects are mediated through the activation of protein kinase A. If the effect of adenosine is related to a decrease in cAMP levels, protein kinase A activity should be decreased. To determine if protein kinase A is involved in adenosine inhibition of Na^+-ATPase activity, we carried out experiments using the protein kinase A inhibitor peptide alone (Fig. 8). Addition of 200 nM protein kinase A inhibitor peptide inhibited the furosemide-sensitive Na^+-ATPase activity by 50%. The simultaneous addition of adenosine and protein kinase A inhibitor peptide were not additive.

4. Discussion

In the present work, we studied the regulation of the ouabain-sensitive $(Na^+ + K^+)$ATPase activity and ouabain-insensitive, furosemide-sensitive Na^+-ATPase activity by adenosine. Proverbio et al. [33] described two forms of Na^+-stimulated ATPase activity which were present in aged microsomal fractions from guinea-pig kidney cortex. One was the well known ouabain-sensitive $(Na^+ + K^+)$ATPase, the other was ouabain-insensitive, Na^+-stimulated ATPase activity which was preferentially sensitive to ethacrynic acid and furosemide. Proverbio and Del Castillo [34] showed that the ouabain-insensitive Na^+-stimulated ATPase is present in basolateral membranes of proximal tubular cells of guinea-pigs. Several papers have shown the differences between ouabain-sensitive $(Na^+ + K^+)$ATPase and ouabain-insensitive, furosemide-sensitive Na^+-ATPase [16,18–20,27,35,36].

Recently, it was reported that the highly selective A_1 receptor antagonists FK-453 and DPCPX have diuretic and natriuretic action, although these compounds induced little or no increase in renal plasma flow and glomerular filtration rate [14,15]. FK-453 also increases urinary excretion of phosphate and HCO_3^- in humans in addition to natriuresis [14]. Takeda and coworkers [13] showed that FK-453 inhibited the Na^+-HCO_3^- cotransporter in rabbit convolute proximal tubule.

Adenosine has multiple actions in the kidney [4,6]. In this paper, we show that adenosine inhibits the furosemide-sensitive Na^+-ATPase activity in concentrations as low as 5×10^{-8} M. When the adenosine concentration was increased to 10^{-3} M the inhibitory effect was reversed. These data suggest that adenosine binds to different sites but more experiments are needed to elucidate this observation. In the present work, we studied only the inhibitory effect of adenosine. Adenosine is a normal constituent of all body fluids including the extracellular space [37–39]. It

has been calculated that the total renal adenosine concentration is 7 μM in rats and 9.5 μM in dogs [40], whereas the extracellular concentration ranges from 0.1 to 1 μM [14]. Furthermore, it was observed that adenosine concentration increases several fold in special situations such as ischemia [41]. We observed that the maximal effect of adenosine on furosemide-sensitive ATPase activity was obtained at 10^{-8} M. Therefore, the effects of adenosine observed in this work may be of potential significance in vivo.

The inhibitory effect of adenosine on the furosemide-sensitive Na^+-ATPase activity is mediated by the P_1 type adenosine receptor. This is supported by the observation that the effect of adenosine is abolished by addition of theophylline (Fig. 2). Methylxanthines, such as theophylline, are antagonists of the P_1-receptor but not of P_2 receptors [5]. The P_1-receptor is subdivided into A_1 and A_2 receptors [5] which are both present in the kidney [11]. In addition, it has also been shown that adenosine regulates the Na^+-$3HCO_3^-$ cotransport in rabbit proximal convoluted tubules via A_1 receptors [13]. The affinities of A_1 and A_2 receptors for adenosine are 0.5–100 nM and 0.5–25 μM, respectively [5]. Fig. 1 shows that the adenosine concentration that inhibits the Na^+-ATPase activity is close to the adenosine concentration that binds to the A_1 receptor, suggesting that the effect of adenosine could be mediated by the A_1 receptor. This hypothesis is favored by the observation that adenosine analogues with higher affinity for A_1 receptor such as N^6-cyclohexyladenosine (CHA) inhibit the Na^+-ATPase activity at low concentrations (Fig. 3). Furthermore, the addition of 5′-*N*-ethylcarboxamideadenosine (NECA), a selective agonist for the A_2 receptor, did not change neither Na^+-ATPase activity nor $(Na^+ + K^+)$ATPase activity (Fig. 4). In some tissues it has been described that A_1 and A_2 receptors have the same affinity for NECA. The absence of effect of NECA on the Na^+-ATPase could be due the simultaneous interaction of NECA with both A_1 and A_2 receptors. Taken together, these data indicate that adenosine inhibits Na^+-ATPase activity through its interaction with the A_1-subtype of adenosine receptor. However, the observation that high concentrations of adenosine and CHA ($> 10^{-7}$ M) promotes an increase is Na^+-ATPase activity when compared to that obtained in presence of 10^{-8} M adenosine or CHA (Figs. 1 and 3) suggests that the binding of adenosine to A_2 receptors modulates Na^+-ATPase activity.

All subtypes of adenosine receptors belong to the family of rhodopsin-like G protein-coupled receptors [1,5,39]. The A_1 receptor couples to members of the pertussis toxin-sensitive GTP binding protein family [5]. These G proteins may inhibit adenylyl cyclase. Our results suggest that the pertussis toxin-sensitive GTP binding protein (inhibitory G protein; Gi protein) is involved in adenosine inhibition of the Na^+-ATPase activity. This is supported by the observation that the effect of adenosine is reversed by addition of pertussis toxin, an inhibitor of Gi protein (Fig. 5). The different levels observed with adenosine plus cholera toxin and adenosine alone could be explained because cholera toxin per se stimulated the ATPase activity. Probably this effect is not correlated to stimulation of the cAMP cascade since the addition of 5×10^{-4} M cAMP plus 10^{-8} M adenosine did not change the ATPase activity (Fig. 7). We then propose that the cholera toxin and adenosine modulate the Na^+-ATPase activity by different, non-interactive ways, suggesting that Gs protein is not involved in the effect of adenosine on the Na^+-ATPase activity.

If the effect of adenosine on Na^+-ATPase activity is due to a decreased adenylyl cyclase activity, the addition of dibutyryl-cAMP should reverse this inhibition. In Fig. 7, we show that increasing dibutyryl-cAMP concentration completely reverses the effect of adenosine on the Na^+-ATPase activity. The reversal of the effect of adenosine by cAMP has been observed in different systems from different preparations [4,13,42,43].

cAMP effects are described as being mediated by stimulation of protein kinase A which in turn promotes phosphorylation of specific substrates. Thus, it is possible that inhibition of Na^+-ATPase activity by adenosine could be due to inhibition of protein kinase A. This hypothesis is supported by the observation that the addition of the specific inhibitor peptide of protein kinase A had an effect on the Na^+-ATPase activity similar to that of adenosine.

The possible role for furosemide-sensitive Na^+-ATPase is unknown. Borgatti and coworkers [21] proposed a widespread and parallel distribution of the furosemide-sensitive Na^+-ATPase and ouabain-sensitive $(Na^+ + K^+)$ATPase. Moretti and coworkers [16]

showed that the furosemide-sensitive Na^+-ATPase was found in a variety of tissues from different animals and is activated by hyposmotic shock and inhibited by hyperosmotic shock. It has been proposed that the furosemide-sensitive Na^+-ATPase may be involved in the extrusion of Na^+ from the intracellular compartment in order to decrease its osmolality and regulate the cellular volume in hyposmotic medium [16,18,20]. Thus, it is possible that adenosine is involved in cell volume regulation of the proximal tubule during hyperosmotic shock by regulation of the furosemide-sensitive Na^+-ATPase. On the other hand, the possible involvement of furosemide-sensitive Na^+-ATPase in proximal tubule sodium reabsorption cannot be discarded, which is compatible with the adenosine effect on sodium excretion described by several workers [14,15,36].

References

[1] M.E. Olah, G.L. Stiles, Adenosine receptors, Annu. Rev. Physiol. 54 (1992) 211–225.

[2] G. Burnstock, In: L. Bolis, R.W. Straub (Eds.), Cell Membrane Receptors for Drugs and Hormones, Raven Press, New York, 1978, pp. 107–118.

[3] G. Burnstock, C. Kennedy, Is there a basis for distinguishing two types of P_2-purinoceptor?, Gen. Pharmacol. 16 (1985) 433–440.

[4] R.M. Edwards, W.S. Spielman, Adenosine A_1 receptor-mediated inhibition of vasopressin action in inner medullary collecting duct, Am. J. Physiol. 266 (1994) F791–F796.

[5] B.B. Fredholm, M.P. Abbracchio, G. Burnstock, J.W. Daly, T.K. Harden, K.A. Jacobson, P. Leff, M. Williams, VI. Nomenclature and classification of purinoceptors, Pharmacol. Rev. 46 (1994) 143–156.

[6] S.W. Spielman, C.I. Thompson, A proposed role of adenosine in the regulation of renal hemodynamics and renin release, Am. J. Physiol. 242 (1982) F423–F435.

[7] J.M. Palacios, J. Fastbom, K.H. Wiederhold, A. Probst, Visualization of adenosine A_1 receptors in the human and the guinea-pig kidney, Eur. J. Pharmacol. 138 (1987) 273–276.

[8] L.C. Mahan, L.D. McVittie, E.M. Smyk-Randy, H. Nakata, F.J. Monsma, C.R. Gerfen, D. Sibley, Cloning and expression of an A_1 adenosine receptor from rat brain, Mol. Pharmacol. 40 (1991) 1–7.

[9] S.M. Reppert, D.R. Weaver, J.H. Stehle, S. Rivkees, Molecular cloning of a rat A_1-adenosine receptor that widely expressed in brain and spinal cord, Mol. Endocrinol. 5 (1991) 1037–1048.

[10] J.S. Fink, D.R. Weaver, S.A. Rivkees, R. Peterfreund, A.E. Pollack, E.M. Adler, S.M. Reppert, Molecular cloning of the rat A_2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum, Mol. Brain. Res. 14 (1992) 186–195.

[11] D. Weaver, S. Reppert, Adenosine receptor gene expression in rat kidney, Am. J. Physiol. 263 (1992) F991–F995.

[12] R. Coulson, R.A. Johnson, R.A. Olsson, D.R. Cooper, S.J. Scheinman, Adenosine stimulates phosphate and glucose transport in opossum kidney epithelial cells, Am. J. Physiol. 260 (1991) F921–F928.

[13] M. Takeda, K. Yoshitomi, M. Imai, Regulation of Na^+–$3HCO_3^-$ cotransport in rabbit proximal convoluted tubule via adenosine A_1 receptor, Am. J. Physiol. 265 (1993) F511–F519.

[14] V.S. Balakrishnan, G.A. Coles, J.D. Williams, Renal response to an A-selective adenosine receptor antagonist (Abstract) Nephrol, Dial. Transplant. 7 (1992) 1152.

[15] K.A. Munger, E.K. Jackson, Direct evidence that afferent arteriolar resistance and sodium excretion is regulated by endogenous adenosine/A1 receptor interactions in vivo (Abstract) J, Am. Soc. Nephrol. 3 (1992) 567.

[16] R. Moretti, M. Martín, T. Proverbio, F. Proverbio, R. Marín, Ouabain-insensitive Na–ATPase activity in homogenates from different animal tissues, Comp. Biochem. Physiol. 98B (1991) 623–626.

[17] G. el Mernissi, C. Barlet-Bas, C. Khadouri, S. Marsy, L. Cheval, A. Doucet, Characterization and localization of ouabain-insensitive Na-dependent ATPase activities along the rat nephron, Biochim. Biophys. Acta 1064 (1991) 205–2111.

[18] I.R. Arenstein, C. Caruso-Neves, L.F. Onuchic, A.G. Lopes, Mechanisms of cell volume regulation in the proximal segment of the Malpighian tubule of *Rhodnius neglectus*, J. Membr. Biol. 146 (1995) 47–57.

[19] R. Marín, T. Proverbio, F. Proverbio, Active sodium transport in basolateral plasma membrane vesicles from rat kidney proximal tubular cells, Biochim. Biophys. Acta 814 (1985) 363–373.

[20] F. Proverbio, R. Marín, T. Proverbio, The 'second' sodium pump and cell volume, Curr. Topics Membranes Transport 34 (1989) 105–120.

[21] A.R. Borgatti, G. Trigari, A. Pagliarani, V. Ventrella, Ouabain-insensitive Na^+-stimulation of a microsomal Mg^{2+}–ATPase in gills of sea bass (*Dicentrarchus labrax L.*), Comp. Biochem. Physiol. 81A (1985) 127–135.

[22] J.C.C. Maia, S.L. Gomes, M.H. Juliani, In: C.M. Morel (Ed.), Genes of Antigenes of Parasites, A Laboratory Manual. Ed. Fundação Oswaldo Cruz, Rio de Janeiro, 1983, pp. 146–157.

[23] M.S. Grassl, P.S. Aronson, Na^+/HCO_3^- co-transport in basolateral membrane vesicles isolated from rabbit renal cortex, J. Biol. Chem. 261 (1986) 8778–8783.

[24] V. Scalera, C. Storelli, C. Storelli-Joss, W. Haase, H. Murer, A simple and fast method for isolation of basolateral plasma membranes from rat small-intestinal epithelial cells, Biochem. J. 186 (1980) 177–181.

[25] B. Sacktor, I.L. Rosenbloom, C.T. Liang, L. Cheng, Sodium

gradient - and sodium plus potassium gradient — dependent glutamate uptake in renal basolateral membrane vesicles, J. Membr. Biol. 60 (1981) 63–71.
[26] C. Grubmeyer, H.S. Penefsky, The presence of two hydrolytic sites on beef heart mitochondrial adenosine triphosphatase, J. Biol. Chem. 256 (1981) 3718–3727.
[27] J.R. Del Castillo, R. Marín, T. Proverbio, F. Proverbio, Partial characterization of the ouabain-insensitive, Na^+-stimulated ATPase activity of kidney basal–lateral plasma membranes, Biochim. Biophys. Acta 692 (1982) 61–68.
[28] F. Proverbio, R. Marín, T. Proverbio, Na^+–ATPase is a different entity from the $(Na^+ + K^+)$ATPase in rat kidney basolateral plasma membranes, Biochim. Biophys. Acta 858 (1986) 202–205.
[29] P.L. Jørgensen, J.C. Skou, Purification and characterization of $(Na^+ + K^+)$ATPase, Biochim. Biophys. Acta 233 (1971) 366–380.
[30] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[31] C. Londos, D.M.F. Cooper, J. Wolff, Subclasses of external adenosine receptors, Proc. Natl. Acad. Sci. USA 77 (1980) 2551–2554.
[32] T.P. Kenakin, R.A. Bond, T.I. Bonner, II. Definition of pharmacological receptors, Pharmacol. Rev. 44 (1992) 351–362.
[33] F. Proverbio, M. Condrescu-Guidi, G. Whittembury, Ouabain-insensitive Na^+ stimulation of an Mg^{2+}-dependent ATPase in kidney tissue, Biochim. Biophys. Acta 394 (1975) 281–292.
[34] F. Proverbio, J.R. Del Castillo, Na^+-stimulated ATPase activities in kidney basal–lateral plasma membranes, Biochim. Biophy. Acta 646 (1981) 99–108.
[35] F. Proverbio, T. Proverbio, R. Marín, Ouabain-insensitive Na^+-stimulated ATPase activity of basolateral plasma membranes from guinea-pig kidney cortex cells, Biochim. Biophys. Acta 688 (1982) 757–763.
[36] F. Proverbio, J.A. Duque, T. Proverbio, R. Marín, Cell volume-sensitive Na^+–ATPase activity in rat cortex cell membranes, Biochim. Biophys. Acta 941 (1988) 107–110.
[37] F.K. Okwuasaba, J.T. Hamilton, M.A. Cook, Antagonism by methylxanthines of purine-nucleotide- and diipyridamole-induced inhibition of peristaltic activity of guinea-pig ileum, Eur. J. Pharmacol. 43 (1977) 181–194.
[38] S. Nees, A.L. Gerbes, B. Willershausen-Zonnchen, E. Gerlach, In: Purine metabolism in man III. Plenum Press, New York, 1980, 122B, pp. 25–30.
[39] B.B. Fredholm, Adenosine receptores in the central nervous system, NIPS 10 (1995) 122–128.
[40] S.S. William, C.I. Thompson, A proposed role for adenosine in the regulation of renal hemodanemics and renin release, Am. J. Physiol. 242 (1982) F423–F435.
[41] H. Osswald, H.J. Schmitz, R. Kemper, Tissue content of adenosine, inosine, and hypoxanthine in the rat kidney after ischemia and postischemic recirculation, Pfluegrs. Arch. 371 (1977) 45–49.
[42] L.J. Arend, W.K. Sonnenburg, W.L. Smith, W.S. Spielman, A_1 and A_2 adenosine receptors in rabbit cortical collecting tubule cells, J. Clin. Invest. 79 (1987) 710–714.
[43] Y. Yagil, Interaction of adenosine with vasopressin in the inner medullary collecting duct, Am. J. Physiol. 259 (1990) F679–F687.