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Angiotensin-(1–7) modulates the ouabain-insensitive Na^+-ATPase activity from basolateral membrane of the proximal tubule

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Abstract

Angiotensin-(1–7) (Ang-(1–7)) modulates the Na^+-ATPase, but not the Na^+,K^+-ATPase activity present in pig kidney proximal tubules. The Na^+-ATPase, insensitive to ouabain, but sensitive to furosemide, is stimulated by Ang-(1–7) (68% by 10^{-9} M), in a dose-dependent manner. This effect is due to an increase in V_{max}, while the apparent affinity of the enzyme for Na^+ is not modified. Saralasin, a general angiotensin receptor antagonist, abolishes the stimulation, demonstrating that the Ang-(1–7) effect is mediated by receptor. The Ang-(1–7) stimulatory effect is not changed by either PD 123319, an AT_2 receptor antagonist, or A779, an Ang-(1–7) receptor antagonist. On the other hand, increasing the concentration of the AT_1 receptor antagonist losartan from 10^{-11} to 10^{-9} M, reverses the Ang(1–7) stimulation completely. A further increase to 10^{-3} M losartan reverses the Na^+-ATPase activity to a level similar to that obtained with Ang-(1–7) (10^{-9} M) alone. The stimulatory effect of Ang-(1–7) at 10^{-9} M is similar to the effect of angiotensin II (AG II) alone. However, when the two peptides are both present, Na^+-ATPase activity is restored to control values. These data suggest that Ang-(1–7) selectively modulates the Na^+-ATPase activity present in basolateral membranes of kidney proximal tubules through a losartan-sensitive receptor. This receptor is probably different from the receptor involved in the stimulation of the Na^+-ATPase activity by angiotensin II.

Keywords: Na^+-ATPase; Angiotensin-(1–7); Furosemide; Proximal tubule

1. Introduction

The renin-angiotensin system (RAS) is fundamental in maintaining blood pressure [1]. The modulation of sodium transport in the kidney is the main long-term regulator of blood pressure. During several years, it was believed that angiotensin II (AG II) mediated the effects of the RAS. However, more recently it has been observed that other peptides, such as angiotensin-(1–7) (Ang-(1–7)), are also involved in this process. Ang-(1–7) can be formed either from AG II or directly through angiotensin I. It has been proposed that Ang-(1–7) is involved in hydromineral balance [2].

The renal actions of Ang-(1–7) are complex and involve both proximal and distal segments of the nephron [3]. In proximal tubules, Ang-(1–7) has a biphasic effect on fluid reabsorption: at lower concentrations (10^{-12} M), Ang-(1–7) increases fluid and

Abbreviations: EDTA, ethylenediaminetetraacetic acid; HEPES, (*N*-2-hydroxyethylpiperazine *N*′-2-ethanesulfonic acid); Tris, tris(trishydroxymethyl)-aminomethane; ATP, adenosine triphosphate (sodium salt); PMSF, phenylmethylsulfonyl fluoride

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bicarbonate reabsorption, while at higher concentrations (10^{-8} M), it decreases fluid reabsorption [4]. On the other hand, Handa et al. [5] observed that Ang-(1–7) promotes a dose-dependent decrease in O_2 consumption over a large range of concentrations (10^{-10} to 10^{-8} M), indicating that Ang-(1–7) modulates the active transport in proximal tubules.

Two primary active transporters are involved in Na^+ reabsorption in proximal tubules: the ouabain-sensitive Na^+,K^+-ATPase and the ouabain-insensitive, furosemide-sensitive Na^+-ATPase [6–9]. In spite of several papers published on this enzyme, its physiological role remains to be elucidated. It was first suggested that the Na^+-ATPase could be involved in cell volume regulation [7]. The Na^+-ATPase is about 10 times less activity than the Na^+,K^+-ATPase [6–8], which suggests that this enzyme may be involved in fine tuning, whereas the Na^+,K^+-ATPase is responsible for most of Na^+ reabsorption in the proximal tubule. Furthermore, our laboratory proposed that the ouabain-insensitive Na^+-ATPase is a primary active transport target for compounds that are involved in the regulation of Na^+ reabsorption, such as adenosine, AG II and bradykinin [10–12].

Considering the putative roles of the Na^+-ATPase and the Na^+,K^+-ATPase in proximal tubule Na^+ reabsorption, in this study the effects of Ang-(1–7) on the activities of these enzymes in proximal tubule cells of the pig kidney were investigated. The data indicate that Ang-(1–7) selectively modulates the Na^+-ATPase through a losartan-sensitive receptor.

2. Materials and methods

2.1. Materials

ATP, ouabain, furosemide, EGTA, HEPES, sodium azide, angiotensin-(1–7) (Asp-Arg-Val-Tyr-Ile-His-Pro), saralasin (Sar^1Ile^8-AG II) and angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) were purchased from Sigma, St. Louis, MO, USA. Percoll was from Pharmacia Biotech, Uppsala, Sweden. A779, an antagonist of Ang-(1–7), was donated by Dr. R.A.S. Santos (Departamento de Fisiologia e Biofísica of Universidade Federal de Minas Gerais, Belo Horizonte, BH, Brazil). All other reagents were of the highest purity available. [^{32}P]Pi was obtained from the Institute of Energetic and Nuclear Research, São Paulo, SP, Brazil.

All solutions were prepared with deionized glass-distilled water. [γ-^{32}P]ATP was prepared as described by Maia et al. [13].

2.2. Preparation of cortex homogenates and isolated basolateral membranes

The cortex homogenates and basolateral membranes were prepared from adult pig kidney. The kidneys were removed immediately after the animal's death and maintained in cold solution containing (mM): sucrose 250, HEPES–Tris (pH 7.6) 10, EDTA 2 and PMSF 1 [10–12]. Thin slices of the cortex (cortex-corticis) were removed with a scalpel. After dissection, slices were homogenized in the same cold solution with a Teflon and glass homogenizer. The homogenate was centrifuged during 10 min at 3000 rpm in a SCR20B centrifuge using an RP12–2 rotor at 4°C. The supernatant was collected and stored at −4°C. The fraction containing the basolateral membranes was isolated by the Percoll gradient method [14] modified from that described by Scarela et al. [15] and Sacktor et al. [16]. The membrane preparation was resuspended in 250 mM sucrose at a final concentration of 20–30 mg of protein ml^{-1} and stored at −4°C.

2.3. Measurement of ATPase activity

Except as noted in Section 3, the composition of the standard assay medium (0.2 ml) was: 10 mM $MgCl_2$; 5 mM [γ-^{32}P]ATP; 20 mM HEPES–Tris (pH 7.0); 5 mM azide; and 90 mM NaCl for the measurement of the Na^+-ATPase activity or 120 mM NaCl and 30 mM KCl for the measurement of the Na^+,K^+-ATPase activity.

The ATPase activity was measured according to the method described by Grubmeyer and Penefsky [17]. The reaction was started by the addition of cortex homogenate or isolated basolateral membranes to a final protein concentration of 0.3–0.5 mg/ml, and stopped after 20 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 in a clinical centrifuge.

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 from 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 [18,19], except as noted in legend of Fig. 2. The Na^+,K^+-ATPase activity measured in isolated basolateral membranes is five-fold higher than the activity in cortex homogenate. Protein concentrations were determined by the Folin phenol method [20] using bovine serum albumin as a standard. Each experiment was performed in at least four independent preparations of basolateral membranes or cortex homogenate. 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.

3. Results

3.1. Effect of Ang-(1–7) on the Na^+,K^+-ATPase and Na^+-ATPase activities

Although it is generally accepted that Ang-(1–7) modulates Na^+ reabsorption in the proximal tubule, the mechanism is not well established [3]. To determine whether one or both of the two sodium pumps are susceptible to Ang-(1–7), we measured both Na^+,K^+-ATPase activity and Na^+-ATPase activity in the presence of different Ang-(1–7) concentrations (Fig. 1). Ang-(1–7) had a biphasic effect on the Na^+-ATPase activity of both cortex homogenate and isolated basolateral membranes, with an increase in concentration from 10^{-11} to 10^{-9} M stimulating the enzyme activity (and returning to the control level at 10^{-6} M). The maximum stimulatory effect was observed with 10^{-9} M Ang-(1–7), in which situation the enzyme activity increased from 7.2 ± 1.1, in the control, to 12.1 ± 1.6 nmol Pi mg^{-1} min^{-1} in the presence of hormone (68% increase). The same effect of Ang-(1–7) on the Na^+-ATPase activity was observed in cortex homogenate, indicating that the signaling pathways involved are maintained during the isolation of basolateral membranes (Fig. 1). In contrast to its effect on the Na^+-ATPase, Ang-(1–7) did not change the Na^+,K^+-ATPase activity (Fig. 1). These data indicate that Ang-(1–7) modulates selectively the Na^+-ATPase activity. The observation that Ang-(1–7) did not alter Na^+,K^+-ATPase may mean that an intact tubular epithelium is required rather than membranes depleted of their intracellular components.

Fig. 2 shows the effect of 10^{-9} M Ang-(1–7) on the Na^+ concentration dependence of the Na^+-ATPase activity in the presence of 2 mM ouabain. The kinetic parameters were calculated using the equation:

$$v = (V_{max} \times [Na^+])/(K_{0.5} + [Na^+])$$

The Na^+ concentration that promotes half-maximal stimulation ($K_{0.5}$) was 7.8 ± 1.8 mM and the maximal rate (V_{max}) was 8.9 ± 0.7 nmol $Pi \times mg^{-1} \times min^{-1}$. 10^{-9} M Ang-(1–7) increased V_{max} to 13.6 ± 1.7 nmol Pi mg^{-1} min^{-1} ($P < 0.04$), but did not change the $K_{0.5}$ for Na^+ (9.8 ± 2.0, $P > 0.6$). The values of the kinetic parameters are means of those obtained by fitting the data for each experiment.

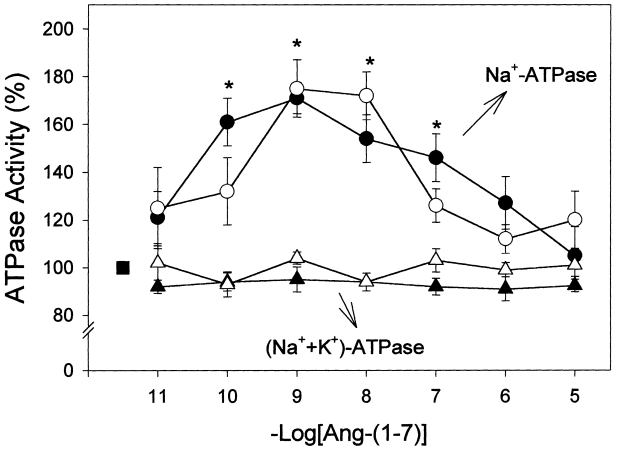

Fig. 1. Angiotensin-(1–7) (Ang-(1–7)) concentration dependence of Na^+-ATPase (circles) and Na^+,K^+-ATPase (triangles) activities in cortex homogenate (open symbols) and isolated basolateral membranes (closed symbols) of renal proximal tubules. ATPase activities were measured as described in Section 2. The Ang-(1–7) concentration was increased from 10^{-11} to 10^{-5} M. Results are expressed as percentage of the control, without Ang-(1–7) (square). The absolute values ± S.E. of the Na^+-ATPase and Na^+,K^+-ATPase activities were in nmol $Pi \times mg^{-1} \times min^{-1}$ 1.9 ± 0.1 and 12.5 ± 0.1 in cortex homogenate and 8.2 ± 0.6 and 69.2 ± 7.2 in isolated basolateral membranes, respectively. *Statistically significant when compared to control ($P < 0.05$) ($n = 6$).

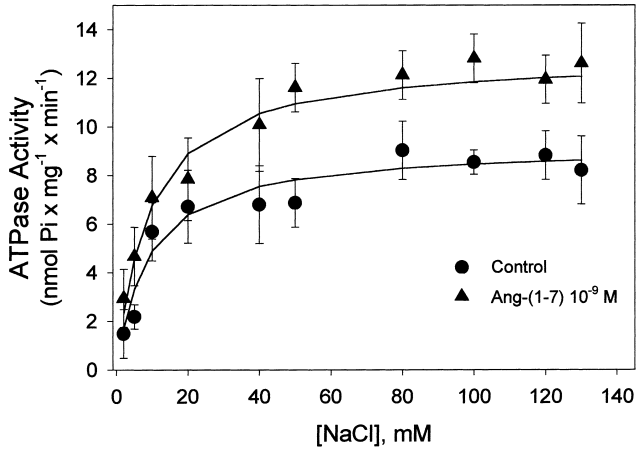

Fig. 2. Na^+ concentration dependence of Na^+-ATPase activity in isolated basolateral membranes of renal proximal tubule with (triangles) or without 10^{-9} M angiotensin-(1–7) (Ang-(1–7)) (circles). The Na^+-stimulated ATPase activity was calculated as the difference between the ATPase activity in the presence and absence of Na^+, both in the presence of ouabain 2 mM (see Section 2). Na^+ concentration was increased from 2 to 130 mM. Results are expressed as mean ± S.E. ($n = 5$).

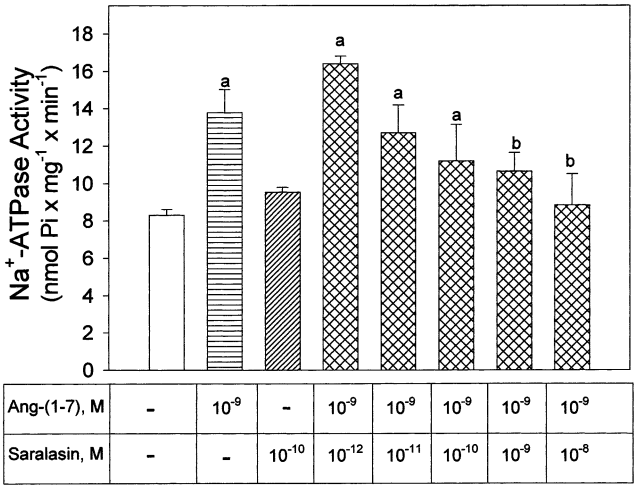

Fig. 3. Modulation of the effect of angiotensin-(1–7) (Ang-(1–7)) on Na^+-ATPase activity by saralasin. Saralasin concentration was increased from 10^{-12} to 10^{-8} M. When indicated, 10^{-9} M Ang-(1–7) was added. ATPase activity was measured as described in Section 2. Results are expressed as mean ± S.E. [a]Statistically significant when compared to control (in the absence of Ang-(1–7) and saralasin) and [b]statistically significant when compared to the Na^+-ATPase activity in the presence of Ang-(1–7) ($P < 0.05$) ($n = 6$).

3.2. *Identification of the receptor involved in the effect of Ang-(1–7) on the Na^+-ATPase activity*

Saralasin is a non-specific antagonist of angiotensin receptors, including those that bind Ang-(1–7) [21,22]. In Fig. 3, we tested the effect of saralasin on the stimulation of the Na^+-ATPase by 10^{-9} M Ang-(1–7). The addition of 10^{-9} M Ang-(1–7) increased the Na^+-ATPase activity by 61%, and this stimulatory effect was completely abolished by saralasin in a dose-dependent manner. Saralasin (10^{-10} M) alone did not change the enzyme activity, although it had a significant effect in the presence of Ang-(1–7). At 10^{-8}M, saralasin completely abolished the stimulation by Ang-(1–7).

Identification of a specific receptor for Ang-(1–7) was achieved by use of a highly specific Ang-(1–7) receptor antagonist, D-[Ala^7]-Ang-(1–7) (A779) [23]. Fig. 4 shows the modulation of the Ang-(1–7) effect on the Na^+-ATPase activity by A779. The concentrations of DALA used in these experiments were based on data showing the effective concentration ratio between agonist/DALA is 1/1 [3]. Cesar et al. [24] also observed that the effect of angiotensin-(1–7) (10^{-9} M) was reversed by DALA (10^{-9} M) in collecting ducts. An increase in A779 concentration from 5×10^{-10} to 6×10^{-9} M did not change the stimulation of the Na^+-ATPase by 10^{-9} M Ang-(1–7). The addition of 5×10^{-8} M A779 alone did not change the Na^+-ATPase activity. The Na^+-ATPase

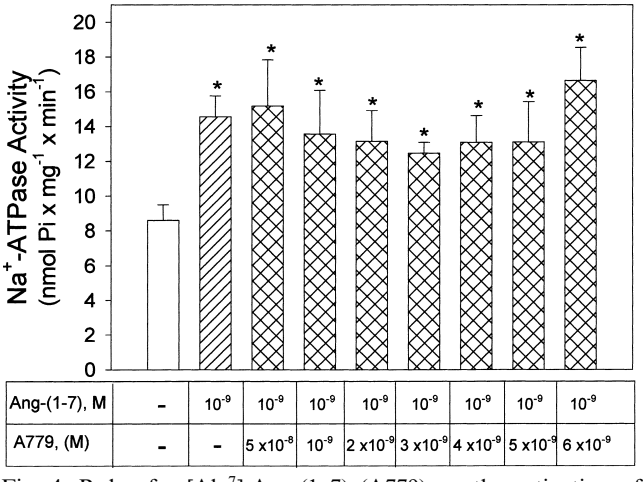

Fig. 4. Role of D-[Ala^7]-Ang-(1–7) (A779) on the activation of the Na^+-ATPase activity by Ang-(1–7). A779 concentration was increased from 5×10^{-10} M to 6×10^{-9} M. When indicated, 10^{-9} M Ang-(1–7) was added. ATPase activity was measured as described in Section 2. Results are expressed as mean ± S.E. *Statistically significant when compared to control ($P < 0.05$) ($n = 9$).

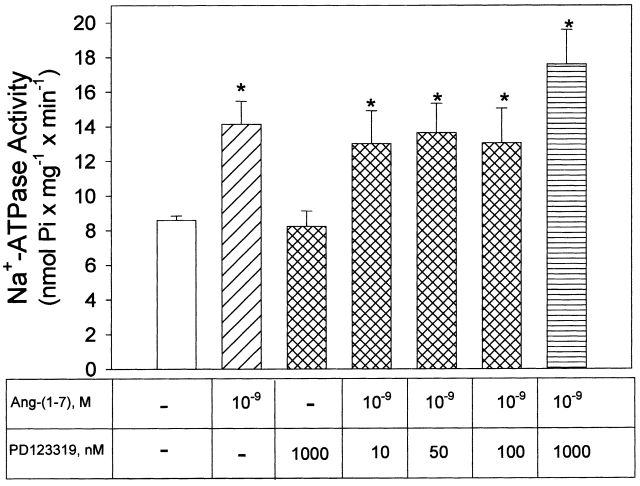

Fig. 5. Effect of PD 123319 on the activation of the Na^+-ATPase activity present in basolateral membranes of proximal tubules by angiotensin-(1–7) (Ang-(1–7)). PD 123319 was used in the concentrations of 10, 50, 100 and 1000 nM. When indicated, 10^{-9} M Ang-(1–7) was added. ATPase activity was measured as described in Section 2. Results are expressed as mean ± S.E. *Statistically significant when compared to control ($P < 0.05$) ($n = 4$).

activity in the presence of A779 (6×10^{-6} M) is statistically different ($P < 0.05$) from that obtained in the presence of Ang-(1–7) alone. These data indicate that the stimulation of the Na^+-ATPase activity by Ang-(1–7) is not mediated by the A779-sensitive receptor.

In kidney tubules cells, some of the effects of Ang-(1–7) are partially or completely abolished by losartan [25]. Furthermore, recently we observed that AG II activates the Na^+-ATPase activity in the same preparation through the AT_1 receptor [11]. To ascertain whether Ang-(1–7) modulates the Na^+-ATPase activity through AG II receptors, the following experiments were performed: (1) we compared the effects of PD123319, an antagonist of AT_2 receptors, and losartan, an antagonist of AT_1 receptors, on the stimulation of Na^+-ATPase activity by Ang-(1–7); and (2) we examined the interaction between Ang-(1–7) and AG II as modulators of the Na^+-ATPase activity.

Fig. 5 shows that (10^{-9} to 10^{-6} M) PD123319 did not change the effect of Ang-(1–7) on the Na^+-ATPase. The Na^+-ATPase activity in the presence of PD123319 (10^{-9} M) is statistically different ($P < 0.05$) from that obtained in the presence of Ang-(1–7) alone. On the other hand, an increase in the concentration of losartan from 10^{-11} to 10^{-3} M, in the presence of Ang-(1–7), promoted a biphasic response. Raising the concentration of losartan to 10^{-9} M completely reversed the stimulation of the Na^+-ATPase activity by Ang(1–7) (Fig. 6). Increasing the concentration of losartan to 10^{-7} M restored the Na^+-ATPase activity to a level similar to that obtained with 10^{-9} M Ang-(1–7) alone.

Fig. 7 shows the Na^+-ATPase activity in the presence of both Ang-(1–7) and AG II. The enzyme was initially pre-incubated with 10^{-8} M AG II. After this procedure, the reaction was performed in the absence or in the presence of different concentrations of Ang-(1–7) (10^{-12} to 10^{-7} M), with 10^{-8} M AG II added to the assay. AG II alone stimulated the enzyme activity by 61%, an effect that was completely blocked by losartan 10^{-8} M (data not shown). In addition, (10^{-12} to 10^{-7} M) Ang-(1–7) progressively reversed the stimulation of the enzyme by AG II. The maximal effect is obtained with 10^{-9} M Ang-(1–7).

4. Discussion

In the present work, we studied the regulation by Ang-(1–7) of the Na^+-ATPase and Na^+,K^+-ATPase

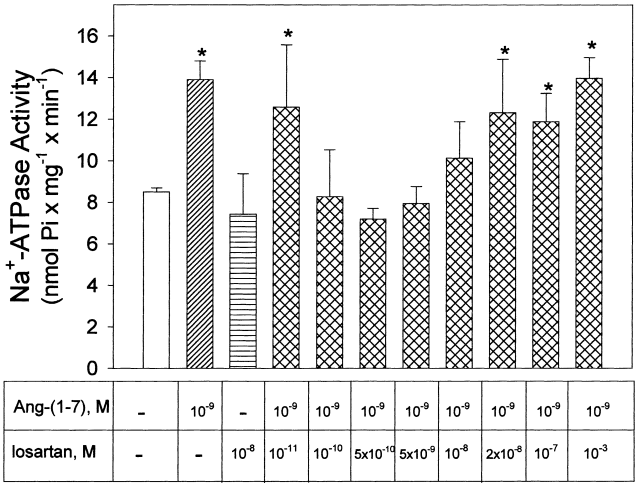

Fig. 6. Modulation of the effect of angiotensin-(1–7) (Ang-(1–7)) on Na^+-ATPase activity by losartan. Losartan concentration was increased from 10^{-11} M to 10^{-3} M. When indicated, 10^{-9} M Ang-(1–7) was added. ATPase activity was measured as described in Section 2. Results are expressed as mean ± S.E. *Statistically significant when compared to control ($P < 0.05$) ($n = 7$).

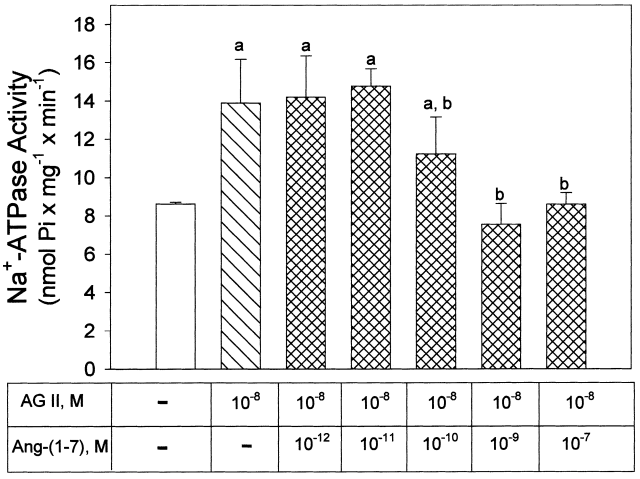

Fig. 7. Role of angiotensin II (AG II) on the modulation of the Na^+-ATPase activity by angiotensin-(1–7) (Ang-(1–7)). Ang-(1–7) concentration was increased from 10^{-12} to 10^{-7} M, in the presence of 10^{-8} M AG II. ATPase activity was measured as described in Section 2. Before assaying the ATPase activity, the enzyme was pre-incubated during 20 min in the presence or in the absence of AG II. The reaction was carried out in the presence of AG II 10^{-8} M. Results are expressed as mean ± S.E. [a]Statistically significant when compared to control (in the absence of Ang-(1–7) and AG II) and [b]statistically significant when compared to the Na^+-ATPase activity in the presence of AG II ($P < 0.05$) ($n = 5$).

activities present in proximal tubule basolateral membranes from pig kidney. It was observed that Ang-(1–7) modulates the Na^+-ATPase activity but does not regulate, at least under the conditions used, the Na^+,K^+-ATPase activity.

The Na^+-ATPase was initially described by Proverbio et al. [26,27] in aged microsomal fractions from guinea-pig kidney cortex. They described two forms of Na^+-stimulated ATPase activity: (a) the classical ouabain-sensitive Na^+,K^+-ATPase, and (b) the ouabain-insensitive Na^+-ATPase, which was sensitive to ethacrynic acid and furosemide. Later it was observed that this enzyme is located in basolateral membranes of the proximal tubule and it is also expressed in different cells of several species [8,10,12]. Recently, it was shown that the Na^+-ATPase is a P-ATPase, able to form a phosphorylated intermediate during the catalytic cycle, with a molecular weight of about 100 000, and phosphorylation stimulated by furosemide and insensitive to K^+ [28]. In the same membrane preparation used in this paper, we have shown that an increase in Na^+ concentration in the presence of 1 mM ouabain, enhances the ATPase activity that is susceptible to inhibition by 2 mM furosemide [29]. This ATPase activity is not changed by 30 mM K^+ and it is 10 times lower than the Na^+,K^+-ATPase activity. These data indicate that the basolateral membranes of the proximal tubules cells from pig kidney express both Na^+-ATPase and Na^+,K^+-ATPase activities.

The Na^+-ATPase activity is measured either as a furosemide-sensitive ATPase activity or as a Na^+-stimulated ATPase activity, both in the presence of ouabain [7,10,11,30]. Here we show that Ang-(1–7) stimulates both to a similar extent, indicating that this peptide is specific for the Na^+-ATPase (Figs. 1 and 6). In addition, the Mg^{2+}-ATPase activity is not changed by Ang-(1–7) (data not shown).

It has been described that the effects of Ang-(1–7) are mediated by different receptors, including AT_1 and AT_2 receptors for AG II, and A779-sensitive receptor(s) [31,32]. These receptors have a common characteristic, i.e. sensitivity to saralasin [33]. The fact that the stimulatory effect of Ang-(1–7) on the Na^+-ATPase activity is completely reversed by saralasin (Fig. 3) indicates that its action is mediated by a receptor and is not due to a direct interaction with the enzyme. In proximal tubules, the effects of Ang-(1–7) are associated with a losartan-sensitive receptor. On the other hand, the inhibitory effect of Ang-(1–7) in collecting ducts is blocked by the antagonist D-[Ala^7]-Ang-(1–7) (A779) [34]. We observed that the stimulation of the Na^+-ATPase activity of proximal tubules by Ang-(1–7) is not modified by either PD123319, an antagonist of AT_2 receptors, or A779, but it is completely reversed by losartan, a specific antagonist of AT_1 receptors (Figs. 4–6). Taken together, these data suggest that the stimulation by Ang-(1–7) of the Na^+-ATPase activity of proximal tubules is mediated by an AT_1 receptor or other losartan-sensitive receptor.

Recently, it was observed in our laboratory that AG II stimulates the Na^+-ATPase activity through an AT_1 receptor in the same preparation of membranes [11]. However, we show here that there are several differences between the effects of AG II and Ang-(1–7) on the Na^+-ATPase activity: (1) the effect of AG II is monophasic, whereas that of Ang-(1–7) is biphasic; (2) the maximal effect of AG II occurs at 10^{-8} M, a ten-fold higher concentration than that required for the maximal stimulation of the Ang-

(1–7); (3) the effect of Ang-(1–7) is completely reversed by 10^{-10} M losartan, while reversal of the effect of AG II requires 10^{-8} M losartan. We postulate that Ang-(1–7) modulates the Na^+-ATPase activity through a different AT_1 receptor subtype than AG II. This possibility of Ang-(1–7) acting through multiple AT_1 receptor sites has been suggested previously based on the observation that its actions on kidney tubules are either partially or completely blocked by AT_1 antagonists, but that it lacks vasoconstrictive effects that are characteristic of agonists at AT_1 receptor sites [35–37]. Another possibility is the existence of a losartan-sensitive Ang-(1–7) receptor subtype that is not an AT_1 receptor [3]. This last hypothesis is in accordance with: (a) the observation that Ang-(1–7) changes the V_{max} of the Na^+-ATPase activity, but does not change the apparent affinity of enzyme for Na^+ (Fig. 2), while AG II increases the apparent affinity for Na^+, but it does not change the V_{max} (unpublished data); (b) losartan has a biphasic effect on the Na^+-ATPase activity in the presence of Ang-(1–7) and a monophasic behavior in the presence of AG II [11]; (c) the effect of AG II is completely blocked by Ang-(1–7), indicating that they do not act through the same route. Maximal stimulation of the Na^+-ATPase activity by Ang-(1–7) is observed at the same concentration (10^{-9} M) that it is most effective in reversing the action of AG II. Thus Ang-(1–7) possibly acts through a receptor other than AT_1.

It is well established that Ang-(1–7) plays an important role in maintaining body fluid and electrolyte balance and long-term blood pressure homeostasis [35]. In general, it is accepted that Ang-(1–7) is a natriuretic and diuretic compound [38]. However, Baracho et al. [39,40] observed anti-diuretic effects of Ang-(1–7) in water-loaded rats. In this paper, we have observed that Ang-(1–7) stimulates the Na^+-ATPase activity at low concentrations (10^{-9} M), while it inhibits it at higher concentrations (10^{-6} M). This hypothesis is in accordance with the observation that Ang-(1–7) exhibits biphasic effects on water and bicarbonate transport in a perfused preparation of straight proximal tubules, similar to that for AG II [4]. Low concentrations (10^{-12} M) of Ang-(1–7) stimulate water transport, while higher concentrations (10^{-8} M) inhibit fluid absorption. This effect of Ang-(1–7) has been associated with modulation of the Na^+/H^+ exchanger. Our data suggest that the biphasic effect of Ang-(1–7) observed in a perfused preparation of straight proximal tubules must also be due, at least in part, to modulation of the Na^+-ATPase activity. Furthermore, we cannot rule out the possibility that loss of the effect of Ang-(1–7) on the Na^+-ATPase activity, observed at higher concentrations of the peptide may be due to receptor down-regulation.

Inhibition of the Na^+-ATPase activity at higher concentrations of Ang-(1–7) indicates that Ang-(1–7) mediates the enzyme activity through two different receptors: one stimulatory and another inhibitory. It has been proposed that Ang-(1–7) may help counteract the actions of AG II. So, under conditions where AG II promotes maximal reabsorption of Na^+ in proximal tubules, Ang-(1–7) may serve to down-regulate this process, leading to a fine tuning of the regulatory mechanism of Na^+ excretion. Further experiments are necessary to clarify this issue.

Recently, we observed that adenosine and bradykinin, both natriuretic compounds, inhibit the ouabain-insensitive Na^+-ATPase activity, but not the Na^+,K^+-ATPase activity of the proximal tubule from pig kidney [10,12]. In this paper, we observe that Ang-(1–7) modulates the Na^+-ATPase activity but not the Na^+,K^+-ATPase activity. The data presented here suggest that the Na^+-ATPase, but not the ouabain-sensitive Na^+,K^+-ATPase, may play an important role in the fast regulation of sodium reabsorption in the proximal tubule by natriuretic compounds.

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References

[1] D.J. Campbell, A.C. Lawrence, A. Towrie, A. Kladis, A.J. Valentijn, Differential regulation of angiotensin peptide levels in plasma and kidney of the rat, Hypertension 18 (1991) 763–773.

[2] R.A.S. Santos, N.C.V. Baracho, Angiotensin-(1–7) is a potent antidiuretic peptide in rats, Braz. J. Med. Biol. Res. 25 (1992) 651–654.

[3] A.C. Simões-e-Silva, N.C.V. Baracho, K.T. Passaglio, R.A.S. Santos, Renal actions of angiotensin-(1–7), Braz. J. Med. Biol. Res. 30 (1997) 503–513.

[4] N.H. Garcia, J.L. Garvin, Angiotensin 1–7 has a biphasic effect on fluid absorption in the proximal straight tubule, J. Am. Soc. Nephrol. 5 (1994) 1133–1138.

[5] R.K. Handa, C.M. Ferrario, J.W. Strandhoy, Renal actions of angiotensin-(1–7): in vivo and in vitro studies, Am. J. Physiol. 270 (1996) F141–F147.

[6] G. Whittembury, F. Proverbio, Two modes of Na extrusion in cells from guinea-pig kidney slices, Pflugers Arch. 316 (1970) 1–25.

[7] F. Proverbio, R. Marín, T. Proverbio, The 'second' sodium pump and cell volume, Curr. Membr. Transp. 34 (1989) 105–119.

[8] 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.

[9] 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.

[10] C. Caruso-Neves, L.G. Francisco-Pedro, L.P. Souza, C. Chagas, A.G. Lopes, Effect of adenosine on the ouabain-insensitive Na^+-ATPase activity from basolateral membrane of the proximal tubule, Biochim. Biophys. Acta 1329 (1997) 336–344.

[11] L.B.A. Rangel, C. Caruso-Neves, L.S. Lara, F.L. Brasil, A.G. Lopes, Angiotensin II activates the ouabain-insensitive Na^+-ATPase from renal proximal tubules through a G-protein, Biochim. Biophys. Acta 1416 (1999) 309–319.

[12] C. Caruso-Neves, A.S.E. Siqueira, G. Iso-Cohen, A.G. Lopes, Bradykinin modulates the ouabain-insensitive Na^+-ATPase activity from basolateral membrane of the proximal tubule, Biochim. Biophys. Acta 1431 (1999) 483–491.

[13] 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, 1993, pp. 146–157.

[14] 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.

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

[16] B. Sacktor, I.L. Rosemblom, 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.

[17] C. Grubmeyer, H.S. Penefsky, The presence of two hydrolytic sites on beef heart mitochondrial adenosine triphosphate, J. Biol. Chem. 256 (1981) 3718–3727.

[18] 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.

[19] 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.

[20] 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.

[21] D.I. Diz, D.A. Goldfarb, R.R. Tubbs, C.M. Ferrario, A.C. Novick, Angiotensin-(1–7) shows selectivity for AT_2 receptors in human renal cortex (Abstract), Hypertension 20 (1992) 417.

[22] P. Ernsberger, J. Zhou, T.H. Damon, J.G. Douglas, Angiotensin II receptor subtypes in culture rat renal mesangial cells, Am. J. Physiol. 263 (1992) F411–F416.

[23] R.A.S. Santos, M.J. Campagnole-Santos, N.C.V. Baracho, M.A.P. Fontes, L.C.S. Silva, L.A.A. Neves, D.R. Oliveira, S.M. Caligiorne, A.R.V. Rodrigues, C. Gropen Jr., W.S. Carvalho, A.C. Simões-e-Silva, M.C. Kosla, Characterization of a new angiotensin antagonist selective for angiotensin-(1–7) Evidence that the actions of angiotensin-(1–7) are mediated by specific angiotensin receptors, Brain Res. Bull. 35 (1994) 293–298.

[24] K.R. Cesar, R.A.S. Santos, M.C. Khosla, A.J. Magaldi, Angiotensin-(1–7) increases water reabsorption in rat inner medullary collecting duct, Hypertension 25 (1995) 1408.

[25] M.C. Chappell, D.I. Diz, C. Yunis, C.M. Ferrario, Differential actions of angiotensin-(1–7) in the kidney, Kidney Int. 54 ((Suppl. 68)) (1998) S3–S6.

[26] F. Proverbio, M. Condrescu-Guidi, G. Wittembury, Ouabain-insensitive Na^+ stimulation of Mg^{+2}-dependent ATPase in kidney tissue, Biochim. Biophys. Acta 394 (1975) 281–292.

[27] F. Proverbio, J.R. Del Castillo, Na^+-stimulated ATPase activities in kidney basal-lateral plasma membranes, Biochim. Biophys. Acta 646 (1981) 99–108.

[28] R. Marín, F. Proverbio, T. Proverbio, N. Ventrella, A. Pagliarane, F. Trombette, G. Trigari, A. R. Borgatti, Phosphorylated intermediate associated with ouabain-insensitive Na^+-ATPase, Satellite Symposium 'Cell homeostasis: Channels and transporters' in XVth International Congress of Nephrology and XIth Latin-American Congress of Nephrology, 1999, p. 9.

[29] C. Caruso-Neves, S. Coelho-Souza, G. Goes, A.G. Lopes, Modulation of the ouabain-insensitive Na^+-ATPase activity of the proximal tubule by pH, in preparation.

[30] C. Caruso-Neves, J.R. Meyer-Fernandes, J. Saad-Nehme, F.

Proverbio, R. Marín, A.G. Lopes, Ouabain-insensitive Na^+-ATPase activity of Malpighian tubules from *Rhodnius prolixus*, Comp. Biochem. Physiol. 119B (1998) 807–811.

[31] A. Abbas, G. Gorelik, L.A. Carbini, A.G. Scicli, Angiotensin-(1–7) induces bradykinin-mediated hypotensive responses in anesthetized rats, Hypertension 30 (1997) 217–221.

[32] C.M. Ferrario, M.C. Chappell, E.A. Tallant, K.B. Brosnihan, D.I. Diz, Counterregulatory actions of angiotensin-(1–7), Hypertension 30 (1997) 535–541.

[33] J.G. Douglas, Angiotensin receptor subtypes of the kidney cortex, Am. J. Physiol. 253 (1987) F1–F7.

[34] I.F. Benter, C.M. Ferrario, M. Morris, D.I. Diz, Antihypertensive actions of angiotensin-(1–7) in spontaneously hypertensive rats, Am. J. Physiol. 269 (1995) H313–H319.

[35] R.A.S. Santos, A.C.S. Silva, A.J. Magaldi, M.C. Kosla, K.R. Ceasr, K.T. Passaglio, N.C.V. Baracho, Evidence for a physiological role of angiotensin-(1–7) in the control of hydroelectrolyte balance, Hypertension 27 (1996) 875–884.

[36] R.A.S. Santos, M.J. Campagnole-Santos, N.C.V. Baracho, M.A.P. Fontes, L.C.S. Silva, L.A.A. Neves, D.R. Oliveira, S.M. Caligiorne, A.R.V. Rodrigues, C. Gropen Jr., W.S. Carvalho, A.C.S. Silva, M.C. Kosla, Characterization of a new angiotensin antagonist selective for angiotensin-(1–7): evidence that the actions of angiotensin-(1–7) are mediated by specific angiotensin receptors, Brain Res. Bull. 35 (1994) 293–398.

[37] M. Burnier, A. Pechere-Bertschi, J. Nussberger, B. Waeber, H.R. Brunner, Studies of the renal effects of angiotensin II receptor blockade: the confounding factor of the acute water loading on the action of vasoactive systems, Am. J. Kidney Dis. 26 (1995) 108–115.

[38] A.M. DelliPizzi, S.D. Hilchey, C.B. Bell-Quilley, Natriuretic action of angiotensin-(1–7), Br. J. Pharmacol. 111 (1994) 1–3.

[39] N.V.C. Baracho, A.C.S. Silva, M.C. Kosla, R.A.S. Santos, Characterization of the antidiuretic action of angiotensin-(1–7) in water-loaded rats (abstract), Hypertension 6 (1995) 1408.

[40] R.A.S. Santos, N.C.V. Baracho, Angiotensin-(1–7) is a potent antidiuretic peptide in rats, Braz. J. Med. Biol. Res. 25 (1992) 651–654.