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Regulation of the renal proximal tubule second sodium pump by angiotensins

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Abstract

For several years it was believed that angiotensin II (Ang II) alone mediated the effects of the renin-angiotensin system. However, it has been observed that other peptides of this system, such as angiotensin-(1-7) (Ang-(1-7)), present biological activity. The effect of Ang II and Ang-(1-7) on renal sodium excretion has been associated, at least in part, with modulation of proximal tubule sodium reabsorption. In the present review, we discuss the evidence for the involvement of Na^+-ATPase, called the second sodium pump, as a target for the actions of these compounds in the regulation of proximal tubule sodium reabsorption.

Key words
- Na^+-ATPase
- Angiotensin II
- Angiotensin-(1-7)
- Furosemide
- Proximal tubule

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The role of the renin-angiotensin system in extracellular volume regulation

Extracellular fluid volume depletion leads to the activation of several mechanisms involved in the conservation of water and electrolytes and in body fluid and arterial pressure homeostasis (1,2). In this context, the renin-angiotensin system (RAS) plays a crucial role in the regulation of extracellular volume and blood pressure (3).

The RAS is activated by the release of renin, an aspartyl-protease, from renal juxtaglomerular cells during hypovolemic and hypotensive states (4,5). This process is controlled basically by three pathways: a) macula densa, b) intrarenal baroreceptor, and c) ß-adrenergic receptor. The macula densa pathway is sensitive to the NaCl flux in the cortical thick ascending limb of Henle's loop (macula densa). Decreases in NaCl flux stimulate renin release and increases in NaCl flux across the macula densa result in renin inhibition. The second intrarenal mechanism controlling renin release is sensitive to variations in blood pressure in the preglomerular vessels where the increase or decrease in blood pressure inhibits or stimulates renin release, respectively. The latter mechanism involves the release of norepinephrine from postganglionic sympathetic nerve terminals, and activation of ß-adrenoreceptors on juxtaglomerular cells enhances renin secretion.

Angiotensinogen hydrolysis by renin forms the decapeptide angiotensin I (Ang I), which is the substrate for the formation of other angiotensin peptides (5). During several years, it was believed that the effects of the RAS were mediated only by angiotensin II (Ang II). More recently, it has been shown that the role of the RAS in the hydroelectrolytic balance involves other active peptides, such as Ang III, Ang IV and

angiotensin-(1-7) (Ang-(1-7)) (3,6-10).

Modulation of renal Na^+ excretion by angiotensin peptides

In general, the effects of the RAS during extracellular volume regulation involve the modulation of peripheral vascular resistance and renal Na^+ and water excretion (1). It is well accepted that low concentrations of Ang II cause marked antinatriuresis, whereas higher doses may lead to diuresis and natriuresis (11). Furthermore, it was observed that blockage of Ang II formation increases the capacity of renal Na^+ excretion in several physiological and pathophysiological conditions in which the RAS is activated (1,12). The antinatriuretic effect of Ang II is often associated with modifications in a) renal blood flow, b) glomerular filtration rate, c) aldosterone synthesis, and d) Na^+ reabsorption in different segments of the nephron such as proximal and distal tubules. Micropuncture and microperfusion studies performed on rat and rabbit renal proximal tubules demonstrated that Ang II modulates Na^+ reabsorption in a dose-dependent and biphasic manner (13,14). Physiological doses of Ang II from 1 pM to 100 pM stimulate Na^+ reabsorption, whereas higher Ang II concentrations from 0.1 μM to 10 μM are inhibitory. The stimulatory effect of Ang II on Na^+ reabsorption in proximal tubule has been associated with an increase in the activity of the Na^+/H^+ antiporter in the luminal membrane and of the Na^+/HCO_3^- cotransporter and Na^+,K^+-ATPase in the basolateral membrane (15). On the other hand, the natriuretic effect of Ang II was associated with an increase in renal arterial pressure, which decreases fractional Na^+ reabsorption in proximal and distal tubules and may cause increases in Na^+ delivery to the tubules (11). In addition, it was also observed that higher concentrations of Ang II inhibit the Na^+/H^+ antiporter and Na^+,K^+-ATPase activity in the basolateral membrane of proximal tubule cells (15,16).

Besides Ang II, both Ang IV and Ang-(1-7) modulate renal Na^+ excretion (17). Handa et al. (3) observed that Ang IV decreases Na^+ reabsorption in proximal tubules by modulating Na^+,K^+-ATPase activity. Furthermore, Ang IV potentiates the action of nitric oxide on renal cortical blood flow. 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 (18), but the effects of Ang-(1-7) on renal Na^+ excretion are controversial (8). In general, it is accepted that Ang-(1-7) is a natriuretic and diuretic compound (19). On the other hand, Baracho et al. (20) observed antidiuretic effects of Ang-(1-7) in water-loaded rats. These data agree with the observation that 10 nM Ang-(1-7) increases water conductivity about four-fold in inner medullary collecting ducts. Garcia and Garvin (21) observed that Ang-(1-7) exhibits a biphasic effect on water and bicarbonate transport in a perfused preparation of straight proximal tubules, similar to that of Ang II. A low concentration (1 pM) of Ang-(1-7) stimulates water transport, while a higher concentration (10 nM) inhibits fluid absorption. This effect has been associated with modulation of the Na^+/H^+ exchanger. Handa et al. (17) observed that Ang-(1-7) promotes a dose-dependent decrease in O_2 consumption over a large range of concentrations (0.1 nM to 10 nM), indicating that Ang-(1-7) also modulates the active transport in proximal tubules.

Taken together, the data indicate that modulation of renal Na^+ excretion by angiotensin peptides is due, in part, to modifications in Na^+ reabsorption along the nephron. However, the molecular mechanisms of action of angiotensin peptides are not completely understood. In this short review, we briefly discuss the effects of angiotensin peptides, in particularly Ang II and Ang-(1-7), on the Na^+-ATPase activity of renal proximal tubules.

Modulation of Na^+-ATPase of proximal tubule by angiotensin peptides

Most of the fluid formed during the ultrafiltration process at the glomerular level is reabsorbed in the proximal tubules. This step is modulated by several factors including angiotensin peptides (22). The activity of the ATPases and the process of Na^+ reabsorption are correlated. There is a prominent expression of Na^+,K^+-ATPase in renal proximal tubule cells which provides the energy gradient that supplies the transcellular Na^+ reabsorption in this segment of the nephron. In the past decades, a second Na^+-ATPase, which is insensitive to ouabain and sensitive to furosemide, was described in several animal tissues (23-28). This Na^+-ATPase transports Na^+ against an electrochemical gradient and is not stimulated by K^+. In proximal tubule cells this enzyme is localized in the basolateral membrane and is involved in the extrusion of sodium along with chloride and water (29). Recently, it was shown that Na^+-ATPase is a P-ATPase able to form a phosphorylated intermediate during the catalytic cycle, with a molecular weight of about 100,000. This phosphorylation is stimulated by furosemide and insensitive to K^+ (30).

Although several studies on this enzyme have been published, its physiological role remains to be elucidated. It was first suggested that Na^+-ATPase may be involved in cell volume regulation (27,29). The ratio between the Na^+-ATPase and Na^+, K^+-ATPase activities is about 1:10 (23,25, 26,29), compatible with a possible role of this enzyme in the fine tuning of Na^+ reabsorption in the proximal tubule, whereas Na^+,K^+-ATPase may be responsible for most of the Na^+ reabsorption. Furthermore, our laboratory proposed that the ouabain-insensitive Na^+-ATPase is a primary active transport target for compounds involved in the regulation of Na^+ reabsorption, such as adenosine, Ang II, Ang-(1-7) and bradykinin (9,23, 25,26).

The first evidence of the action of Ang II on the Na^+-ATPase activity of proximal tubules was obtained by Munday et al. (31) about 30 years ago. The authors observed that 1 pM Ang II increases the K^+- and ouabain-insensitive active Na^+ transport in slices of rat renal cortex. However, the interpretation of the data given by the authors in order to explain the effect of Ang II on the Na^+ reabsorption in the proximal tubule was based on the activation of Na^+,K^+-ATPase (15). Data obtained in our laboratory show that Ang II and Ang-(1-7), but not Ang III, modulate the Na^+-ATPase from porcine cortical proximal tubules (Figure 1).

Ang-(1-7) has a biphasic effect on the Na^+-ATPase activity of both cortex homogenate and isolated basolateral membranes from cortical proximal tubules. The maximum stimulatory effect on the enzyme activity is observed at the concentration of 1 nM, corresponding to a 68% increase in the enzyme activity. Similar to Ang-(1-7), Ang II enhances Na^+-ATPase activity in a dose-dependent manner. At the concentration of 1 µM, Ang II increases the Na^+-ATPase activity of basolateral membrane from cortical proximal tubule cells by 139%. These effects of Ang II and Ang-(1-7) are consistent with their effects on fluid reabsorption in proximal tubules as discussed above. So, it is acceptable to propose that Na^+-ATPase participates as an important effector mechan-

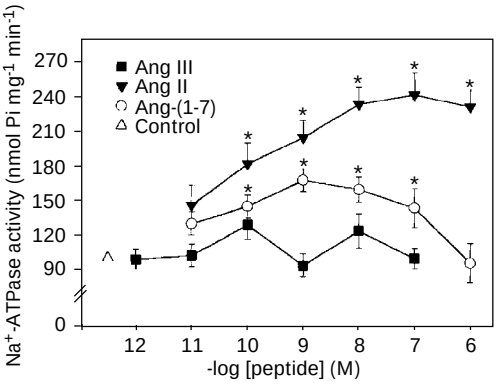

Figure 1. Dependence of Na^+-ATPase activity in the isolated basolateral membrane of renal proximal tubules on Ang-(1-7), Ang II and Ang III. ATPase activity was measured as described by Caruso-Neves et al. (9). Each experiment was performed in an independent preparation of basolateral membrane or cortex homogenate. The data were analyzed by two-way analysis of variance (ANOVA), considering the treatments as factors. The significance of the differences was determined by the Bonferroni *t*-test. Statistical analysis was performed using absolute values and the results are expressed as percentage of the control. *P<0.05 compared to control (N = 7).

ism in the modulation of fluid reabsorption in the proximal tubule cells by Ang II and Ang-(1-7).

Angiotensin receptors mediate the modulation of Na^+-ATPase

The presence of Ang II receptors in the cortical nephron segments, mainly in the convoluted proximal tubules, was demonstrated in rat kidney by autoradiographic studies using I^{125}-Ang II (32). In the proximal tubule, Ang II receptors are uniformly distributed in both luminal and basolateral membranes (15,33). Burns et al. (34) showed that over 80% of the Ang II receptors found in the basolateral membrane of proximal tubules from rat and rabbit kidneys are of the AT_1 type, with the other 20% being of the AT_2 type. In general, it is accepted that the stimulatory effect of Ang II on proximal tubule Na^+ reabsorption is mediated by losartan-sensitive AT_1 receptors located in both luminal and basolateral membranes (15,35).

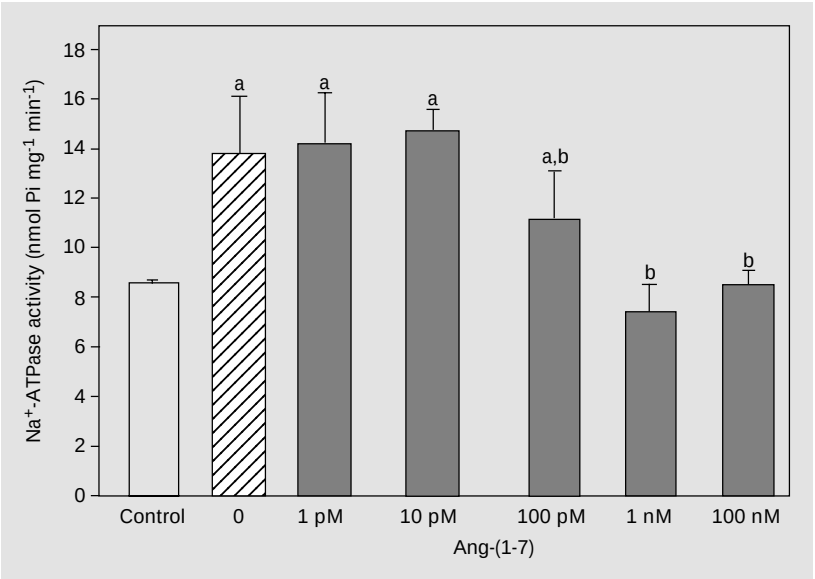

Figure 2. Role of Ang II in the modulation of Na^+-ATPase activity by Ang-(1-7). Ang-(1-7) concentration was increased from 1 pM to 100 nM in the presence of 10 nM Ang II. ATPase activity was measured as described by Caruso-Neves et al. (9). Before assaying ATPase activity, the enzyme was preincubated for 20 min in the presence or in the absence of Ang II. The reaction was carried out in the presence of 10 nM Ang II. The data were analyzed as described in Figure 1. Results are reported as mean ± SEM. $^aP<0.05$ compared to control (in the absence of Ang-(1-7) and Ang II) and $^bP<0.05$ compared to the Na^+-ATPase activity in the presence of Ang II (N = 5).

Actually, the question of which receptor(s) mediate the actions of Ang-(1-7) is still unsettled. It has been reported that the effects of Ang-(1-7) are mediated by different receptors, including AT_1 receptors for Ang II, D-Ala^7-Ang-(1-7) (A-779)-sensitive receptors, PD123319-sensitive receptors and losartan-sensitive receptors (7). These receptors have a common characteristic, i.e., their sensitivity to saralasin (8). In the kidney, most of the effects of Ang-(1-7) are associated with a losartan-sensitive receptor including the effects of Ang-(1-7) on the proximal tubule (17,21,36). However, other receptor types have been associated with the actions of Ang-(1-7) on the kidney. The stimulatory effect of Ang-(1-7) on water permeability in the inner medullary collecting ducts of rats is blocked by A-779 and also by an AVP-V_2 receptor antagonist (18,37). More recently, Handa (38) observed that Ang-(1-7) can also bind with high affinity to the AT_4 receptor in bovine kidney epithelial cells regulating the MAP kinase/Erk signaling pathway.

The fact that the stimulatory effect of Ang-(1-7) and Ang II on Na^+-ATPase activity is completely reversed by saralasin indicates that their actions are mediated by a receptor and are not due to a direct interaction with the enzyme (9,26). Recently, we observed that stimulation of the Na^+-ATPase activity of proximal tubules by Ang-(1-7) and Ang II is not modified by PD123319, an antagonist of AT_2 receptors, or A-779, but is completely reversed by losartan, a specific antagonist of AT_1 receptors (9,26). Taken together, these data suggest that stimulation of the Na^+-ATPase activity of proximal tubules by Ang-(1-7) and Ang II is mediated by an AT_1 receptor or some other losartan-sensitive receptor. These data agree with a recent observation that Ang-(1-7) competes with high affinity with Ang II in rat renal cortex (39). However, we showed several

differences between the effects of Ang II and Ang-(1-7) on Na^+-ATPase activity (9,26): 1) the effect of Ang II is monophasic, whereas that of Ang-(1-7) is biphasic; 2) the effect of Ang-(1-7) is completely reversed by 0.1 nM losartan, while reversal of the effect of Ang II requires 10 nM losartan, and 3) at high losartan concentration (100 μM) Ang-(1-7) stimulates Na^+-ATPase in a manner similar to that observed in the absence of Ang II. We postulate that Ang-(1-7) modulates Na^+-ATPase activity through a different AT_1 receptor subtype than Ang II. This possibility of Ang-(1-7) acting at multiple AT_1 receptor sites has been suggested previously based on the observation that its actions on renal tubules are either partially or completely blocked by AT_1 antagonists, but that it lacks the vasoconstrictive effects characteristic of agonists at AT_1 receptor sites (18,40,41). Another possibility is the existence of a losartan-sensitive Ang-(1-7) receptor subtype that is not an AT_1 receptor (7,8). This last hypothesis is in accordance with a) the observation that Ang-(1-7) changes the V_{max} of Na^+-ATPase activity but does not change the apparent affinity for Na^+, while Ang II increases the apparent affinity for Na^+, but does not change the V_{max} (9), b) losartan has a biphasic effect on Na^+-ATPase activity in the presence of Ang-(1-7) and a monophasic behavior in the presence of Ang II (9,26), and c) the maximal effect of Ang II on Na^+-ATPase activity occurs at 10 nM, a ten-fold higher concentration than that required for maximal stimulation with Ang-(1-7) (Figure 1).

Interactions between the effects of Ang II and Ang-(1-7) on Na^+-ATPase activity

It has been proposed that Ang-(1-7) may help counteract the actions of Ang II (8). Recently, we observed that Ang-(1-7) progressively reversed the stimulation of the Na^+-ATPase activity of proximal tubule by Ang II, with a maximal effect observed at 1 nM (Figure 2). On the other hand, Ang II does not change the stimulation of Na^+-ATPase activity by Ang-(1-7) (9). So, under conditions in which Ang II promotes maximal Na^+ reabsorption in proximal tubules, Ang-(1-7) may down-regulate this process, leading to a fine tuning of the regulatory mechanism of Na^+ excretion.

Conclusions

In summary, Na^+-ATPase may play an important role in the short-term regulation of Na^+ reabsorption in the proximal tubule, being the target of angiotensin peptides such as Ang-(1-7) and Ang II. Furthermore, Ang-(1-7) could act as an Ang II agonist or antagonist depending on its concentration which represents an important physiological mechanism of extracellular volume regulation.

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