Angiotensin II inhibits NaCl absorption in the rat medullary thick ascending limb

Nicolas Lerolle,1,2 Soline Bourgeois,1,2 Françoise Level1,3,4 Gaëtan Lebrun,1 Michel Paillard,1,3,4 and Pascal Houiller1,3,4

1 Institut National de la Santé et de la Recherche Médicale U356, Institut Fédératif de Recherche 58, Universités 2 René Descartes et 3 Pierre et Marie Curie, and 4 Département de Physiologie, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, 75015 Paris, France

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ANGIOTENSIN II IS A POTENT regulator of extracellular fluid volume, mainly through its direct effects on renal tubular sodium reabsorption, as well as on aldosterone synthesis. Acute systemic infusion of low-dose angiotensin II stimulates overall tubular sodium reabsorption and decreases urine sodium excretion, independently of changes in renal or systemic hemodynamics (5, 15). In rat experiments using in vivo superficial tubule microperfusion, systemic angiotensin II infusion at 20 ng·kg⁻¹·min⁻¹, which achieves a subpressor, physiological plasma concentration (picomolar range), stimulates proximal water and NaCl reabsorption (24). Conversely, saralasin infusion, which suppresses endogenous angiotensin II activity, inhibits proximal tubule absorption (24). In addition, endogenously produced angiotensin II is responsible for a nanomolar intratubular angiotensin II concentration that stimulates proximal sodium reabsorption (33). In the more distal part of the nephron, angiotensin II also directly stimulates sodium reabsorption by activating Na⁺/H⁺ exchange and the amiloride-sensitive Na⁺ channel (2, 23, 37). Finally, angiotensin II stimulates aldosterone synthesis and thereby indirectly enhances sodium reabsorption via the epithelial sodium channel in the cortical collecting duct (26) and likely via the thiazide-sensitive sodium-chloride cotransporter in the distal convoluted tubule (21).

In the loop of Henle, available data from in vivo free-flow micropuncture studies of superficial tubules using acute angiotensin-converting enzyme (ACE) inhibition or systemic angiotensin II infusion did not reveal any change in the relationship between the rate of end proximal NaCl delivery and the rate of NaCl reabsorption by the loop of Henle as a whole (9, 17). In vivo perfusion of single loops with isotonic saline containing angiotensin II did not alter the fractional sodium reabsorption along Henle’s loop (16). However, no definitive conclusion could be derived from these in vivo experiments about angiotensin II-dependent NaCl reabsorption in the various segments that constitute the loop of Henle. In fact, the known stimulatory effect of angiotensin II on sodium reabsorption in pars recta (11) can be counterbalanced by a specific inhibitory effect of angiotensin II on sodium reabsorption in the thick ascending limb. Similarly, in vivo perfusion of the loop with a fluid containing 10⁻⁸ M angiotensin-(1–7), an angiotensin II metabolite that is produced by proximal tubule, elicits a modest increase in sodium and fluid reabsorption by the loop (36). For the above-mentioned reason, no definitive conclusion about the segmental effect of angiotensin-(1–7) could be derived from this data, although the parallel increase in sodium and fluid transport suggests an effect in the proximal straight tubule, in accordance with the previously reported effect of angiotensin-(1–7) via the angiotensin II subtype 1 (AT₁) receptor in this segment (10).

To date, the effect of angiotensin II on transepithelial NaCl reabsorption in the thick ascending limb of the loop of Henle has never been directly investigated. However, AT₁ receptors are present in the thick ascending limb (31). More recently, it has been shown in our laboratory that rat medullary thick ascending limb of Henle (MTALH) cells express AT₁ receptors at both the apical side and the basolateral side (32). In accordance with the presence of AT₁ receptors, Good et al.

Address for reprint requests and other correspondence: P. Houiller, Département de Physiologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France (E-mail: pascal.houillier@egp.ap-hop-paris.fr).
(13) have shown that peritubular angiotensin II (10^{-8} M) exerts an inhibitory effect on bicarbonate transport in MTALH. Therefore, a possible effect of luminal and/or peritubular angiotensin II on NaCl transport in the MTALH remains to be investigated.

The thick ascending limb of the loop of Henle is a major site of NaCl transport, reabsorbing up to 30% of the filtered load of NaCl. Moreover, NaCl reabsorbed in the MTALH contributes to the generation of the corticopapillary osmotic gradient that drives water reabsorption from the collecting duct in the presence of antidiuretic hormone. For these reasons, angiotensin II could participate in the control of NaCl transport and of water balance.

Therefore, we have investigated the effect of angiotensin II on NaCl reabsorption by the rat medullary thick ascending limb microperfused in vitro.

**MATERIALS AND METHODS**

*MATERIALS.* Angiotensin II was purchased from Sigma (Sigma-Aldrich, St. Quentin Fallavier, France), losartan, and PD-23319 were kindly provided by Merck (Rahway, NJ), PD-123319 by Pfizer (Paris, France), and furosemide by Hoechst Houdé (Paris la Défense, France). Angiotensin II, losartan, and PD-23319 were diluted into bath or perfusion solutions to the final requested concentration.

All other chemicals were of the highest purity available.

*Tubule perfusion.* Pathogen-free male Sprague-Dawley rats (60–75 g body wt, Ifla Credo, L’Arbresle, France) were allowed free access to autoclaved standard rat chow and distilled water until the time of the experiments. Rats were anesthetized with 50 mg/kg pentobarbital sodium intraperitoneally (ip) 10 min after the ip injection of 2 mg furosemide to limit oxygen consumption of the MTALH during the time of tubule dissection. Both kidneys were cooled in situ with control bath solution for 1 min and then removed and cut into thin coronal slices for tubule dissection. These maneuvers have been shown to improve the viability of renal tubules in vitro (12, 14). It is noteworthy that an in vivo ip furosemide injection does not prevent the ability of NaCl transport in the MTALH to be subsequently stimulated in vitro by 10^{−9} M AVP (Houillier P and Bourgeois S, unpublished observations). MTALHs were dissected from the inner stripe of the outer medulla at 4°C in the control bath solution of the experiment. The isolated tubule was transferred to the bath chamber on the stage of an inverted microscope (Axiovert 100, Carl Zeiss) and mounted on concentric glass pipettes for microperfusion at 37°C. The length of the perfused segments ranged from 0.45 to 0.8 mm. In all experiments, the perfusion (lumen) and bath solutions contained (in mM) 142 Na, 4 K, 1.2 Ca, 118 Cl, 23 HCO_{3}, 2 lactate, 5 HEPES, 1.2 SO_{4}, 1 citrate, 2 HPO_{4}, 5 glucose, and 5 alanine. The osmolality of the solution was 295 ± 5 mosmol/kg H_{2}O. All solutions were equilibrated with 95% O_{2}-5% CO_{2} and pH ranged from 7.38 to 7.43 at 37°C. The bath solution also contained 0.2% fraction V bovine serum albumin. Experimental agents were added to the bath and/or luminal solutions as described in the RESULTS. All investigations involving animals were conducted in conformity with APS Guiding Principles in the Care and Use of Animals.

*Study protocol.* The tubules were equilibrated for 20–30 min at 37°C in the initial perfusion and bath solutions, and the luminal flow was adjusted to 2.5–4.5 nL/min. In the experiments during which a change of the luminal solution was required, great care was taken not to dislocate the tube during the maneuver or alter the luminal flow. Two to three periods were successively performed on each tube: initial, experimental, and recovery. Four samples of tubular fluid were collected during each period. The collection time (t) for each sample was recorded, and the sample volume (V) was measured using volumetric pipettes to calculate the perfusion rate (V = Vt). Chloride concentration was determined in the perfusion solution ([Cl]_{p}) and in each collected sample ([Cl]_{c}) by microcoulometry (34). Tubule length (L) was measured using the optical scale of the microscope. Net transepithelial chloride flux (J_{Cl}) was calculated from the luminal flow and the difference between chloride concentrations measured in perfused and collected fluid: J_{Cl} = ([Cl]_{p} - [Cl]_{c}) × \dot{V}/L, expressed as picomoles per minute per millimeter of tubule length. An averaged J_{Cl} was calculated for each period in a given tubule.

**Measurement of transepithelial voltage.** Transepithelial voltage (V_{tr}) was measured with a DP-301 differential electrometer (Warner Instrument, Hamden, CT) by the use of an Ag-AgCl electrode connected to the perfusion pipette via a 0.15 M NaCl-agar bridge; a 0.15 M NaCl-agar bridge also connected the peritubular bath to an Ag-AgCl electrode. V_{tr} was measured during each period at the tip of the perfusion pipette.

**Statistical analysis.** Results are expressed as means ± SE. The difference between mean values was evaluated using Student’s t-test for paired data, with P < 0.05 considered as statistically significant. When repeated measurements were made at the beginning and the end of an experiment (initial and recovery periods), the values of the means were averaged and compared with the mean of the experimental period.

**RESULTS**

Effect of peritubular angiotensin II on J_{Cl}. The effects of peritubular angiotensin II on net chloride absorption and V_{tr} in the MTALH are shown in Fig. 1 and Table 1. We first tested the effect of 10^{-8} M angiotensin II. Under control conditions, J_{Cl} averaged 62.3 ± 7.4 pmol·min^{-1}·mm^{-1} of tubule length, a value similar to that previously reported by others (3).

![Fig. 1. Effect of peritubular addition of 10^{-8} M ANG II on net chloride reabsorption (J_{Cl}) in the medullary thick ascending limb of Henle. Individual results obtained with 7 independent tubules are displayed. Each tubule was studied during 2–3 successive periods (control, experimental, and recovery). Data points are average values for single tubules. Lines connect paired measurements made in same tubule. P values are for paired t-tests. Mean values of J_{Cl} appear in text. *P < 0.05](http://ajprenal.physiology.org/doi/abs/10.1152/ajprenal.00547.2004)
ANGIOTENSIN II AND MTAL NaCl TRANSPORT

Table 1. Effect of $10^{-11}$, $10^{-10}$, and $10^{-7}$ peritubular ANG II on net chloride absorption and transepithelial voltage in the rat medullary thick ascending limb

<table>
<thead>
<tr>
<th>JCl, pmol·min⁻¹·mm⁻¹</th>
<th>Transepithelial Voltage, mV</th>
<th>Tubular Flow, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$10^{-11}$ M</td>
<td>ANG II</td>
</tr>
<tr>
<td>47.0±6.3</td>
<td>49.6±10.7</td>
<td>55.5±11.3</td>
</tr>
<tr>
<td>Control</td>
<td>$10^{-10}$ M</td>
<td>ANG II</td>
</tr>
<tr>
<td>55.5±8.7</td>
<td>49.6±9.9</td>
<td>45.9±8.7</td>
</tr>
<tr>
<td>Control</td>
<td>$10^{-7}$ M</td>
<td>ANG II</td>
</tr>
<tr>
<td>72.0±13.4</td>
<td>39.9±6.8*</td>
<td>61.6±2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 tubules for $10^{-11}$ M, 5 tubules for $10^{-10}$ M, and 4 tubules for $10^{-7}$ M ANG II. JCl, net chloride absorption. *P < 0.01.

Peritubular addition of $10^{-8}$ M angiotensin II reversibly decreased JCl by ~50% (to 32.2 ± 5.8 pmol·min⁻¹·mm⁻¹) of tubule length during the experimental period; P < 0.02; JCl returned to initial values (61.6 ± 10.6 pmol·min⁻¹·mm⁻¹) during the recovery period. The time course of transepithelial potential difference paralleled that of JCl; it reversibly decreased from 7.1 ± 1.3 to 3.8 ± 0.6 mV (P < 0.05) in the presence of peritubular angiotensin II (Fig. 1). The effect of a higher dose of angiotensin II ($10^{-7}$ M) has also been tested; the results are similar (Table 1).

In a separate set of experiments, we checked whether the acute in vivo pretreatment of animals with furosemide before anesthesia, to improve the subsequent viability of MTALH in vitro, could affect the results. Therefore, we measured the time course of transepithelial potential difference in tubules obtained from rats not injected with furosemide before anesthesia. The time course was similar to that observed with tubules obtained from rats pretreated with furosemide; Vse was 14.2 ± 2.2 mV under control conditions, 8.3 ± 0.8 mV in the presence of $10^{-8}$ M peritubular angiotensin II, and 10.9 ± 1.5 mV during recovery.

By contrast, lower doses of angiotensin II when added to the peritubular side of MTALH cells did not induce any significant change in JCl or in Vse (Table 1). In fact, during the initial control period, JCl was similar to that measured in the previous set of experiments described above (Fig. 1). The addition of either $10^{-10}$ or $10^{-11}$ M angiotensin II to the peritubular fluid did not alter JCl or Vse.

Effect of luminal angiotensin II on JCl. Because angiotensin II subtype 1 receptors have recently been reported to be present on the apical plasma membrane of MTALH cells (32), we tested whether the addition of angiotensin II in the lumen could alter JCl. The effect of luminal angiotensin II on JCl in the rat MTALH is shown in Fig. 2 and Table 2. Under control conditions, JCl was similar to that measured in the previous control periods (displayed in Fig. 1 and Table 1). The addition of $10^{-8}$ M angiotensin II in the perfusate elicited a reversible decrease in JCl (from 66.3 ± 3.7 to 42.9 ± 7.2 pmol·min⁻¹·mm⁻¹, a 35% decrease; P < 0.01). During the recovery period, JCl reincreased toward initial values (66.0 ± 16.0 pmol·min⁻¹·mm⁻¹) (Fig. 2).

We checked whether lower concentrations of angiotensin II could alter JCl when added in the perfusate. As was observed with peritubular angiotensin II, luminal $10^{-10}$ or $10^{-11}$ M angiotensin II did not change JCl (Table 2).

As a whole, $10^{-8}$ M angiotensin II inhibited JCl when added either to the peritubular or to the luminal side of the rat MTALH cells. In addition, there was no evidence of a biphasic

![Fig. 2. Effect of luminal addition of $10^{-8}$ M ANG II on JCl in medullary thick ascending limb of Henle. Individual results obtained with 5 independent tubules are displayed. Each tubule was studied during 2–3 successive periods (control, experimental, and recovery). Data points are average values for single tubules. Lines connect paired measurements made in same tubule. P values are for paired t-tests. Mean values of JCl appear in text.](http://ajprenal.physiology.org/)

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Table 2. Effect of 10^{-11} and 10^{-10} M luminal ANG II on J_{Cl} and transepithelial voltage in the rat medullary thick ascending limb

<table>
<thead>
<tr>
<th>J_{Cl}, pmol·min^{-1}·mm^{-1}</th>
<th>Transepithelial Voltage, mV</th>
<th>Tubular Flow, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10^{-11} M ANG II</td>
<td>Recovery</td>
</tr>
<tr>
<td>48.7±3.2</td>
<td>64.5±11.1</td>
<td>64.7±12.7</td>
</tr>
<tr>
<td>Control</td>
<td>10^{-10} M ANG II</td>
<td>Recovery</td>
</tr>
<tr>
<td>82.2±7.0</td>
<td>65.8±10.4</td>
<td>60.5±13.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 tubules for 10^{-11} and 10^{-10} M ANG II.

Table 3. Effect of peritubular and luminal 10^{-6} M losartan on the effect of 10^{-8} M ANG II on J_{Cl} and transepithelial voltage in the rat medullary thick ascending limb

<table>
<thead>
<tr>
<th>J_{Cl}, pmol·min^{-1}·mm^{-1}</th>
<th>Transepithelial Voltage, mV</th>
<th>Tubular Flow, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losartan</td>
<td>Losartan + ANG II</td>
<td>Losartan</td>
</tr>
<tr>
<td>Peritubular</td>
<td>47.1±3.2</td>
<td>50.0±10.7</td>
</tr>
<tr>
<td>Luminal</td>
<td>53.0±7.0</td>
<td>51.0±4.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 tubules for peritubular and 5 tubules for luminal 10^{-6} M losartan.

**DISCUSSION**

The role of angiotensin II in the control of NaCl reabsorption by the thick ascending limb is poorly understood; in fact, the effect of angiotensin II on net transepithelial NaCl flux in this segment has never been directly assessed until now. The present study demonstrates that angiotensin II directly inhibits J_{Cl} in the rat MTALH. This inhibition involves AT_{1} receptors and is observed with either peritubular or luminal angiotensin II. As discussed below, the effect of angiotensin II to decrease NaCl reabsorption in the MTALH may play an important role in the preservation of water balance during alterations in dietary NaCl intake.

Whereas angiotensin II consistently stimulates overall tubular NaCl reabsorption, in vivo free-flow micropuncture studies
of rat superficial tubules using acute ACE inhibition have concluded that angiotensin II does not alter NaCl reabsorption in the loop of Henle (9, 17). Indeed, in these experiments, during which renal perfusion pressure was carefully controlled, angiotensin II infusion or ACE inhibition did not alter the linear relationship between the reabsorption rate of NaCl in Henle’s loop and end proximal delivery observed under control conditions (9, 17). However, because the loop of Henle is composed of distinct tubule segments, it remained possible that the lack of an effect of angiotensin II or ACE inhibition in the whole loop was the consequence of opposite effects on distinct parts of the loop. Garvin (11) reported that angiotensin II increases fluid (and therefore NaCl) absorption in the rat straight proximal tubule perfused in vitro. Our study demonstrates that, in vitro, angiotensin II specifically inhibits NaCl absorption in the MTALH, thereby providing an explanation for the lack of an effect of this hormone in the entire loop. The present study establishes that the angiotensin II-dependent decrease in MTALH NaCl absorption is due to an alteration in the activity of the transporters involved in transcellular NaCl transport. At this point, we do not know whether angiotensin II acts directly on the apical Na-K-2Cl cotransporter or whether angiotensin II may act indirectly to reduce the activity of the cotransporter through effects on other transporters involved in transepithelial NaCl absorption. Surprisingly, high concentrations of angiotensin II have been suggested to stimulate apical Na-K-2Cl cotransporter activity in MTALH cell suspensions (1). In this study, Na-K-2Cl cotransporter activity has been indirectly assessed by measuring the cell acidification rate after addition to the medium of NH$_4^+$, which substitutes for K$^+$ on the cotransporter. However, one cannot exclude that a high concentration of angiotensin II, which inhibits HCO$_3^-$ absorption in vitro (13), may inhibit apical Na/H exchange and thus acidify cells independently of NH$_4^+$ entry, which would render the NH$_4^+$ technique inappropriate to assess Na-K-2Cl cotransporter activity. Consistently, high concentrations of angiotensin II decrease ouabain-sensitive $^{86}$Rb uptake, used as an index of ion transport in MTALH suspensions (7). Angiotensin II in the nanomolar range has been shown to increase the activity of the apical 70-pS K$^+$ channel in MTALH (25). However, to date, from studies showing that loss-of-function mutations of the ROMK channel can lead to Bartter’s syndrome (19), only the ROMK channel is thought to control the activity of the apical Na-K-2Cl cotransporter. Finally, no data are available on the effect of angiotensin II on K$^+$ or Cl$^-$ channels, the K-Cl cotransporter, or Na-K-ATPase in the basolateral membrane of the MTALH cells, which might indirectly affect transcellular NaCl transport. In the present study, no attempt was made to identify the primary target of angiotensin II in MTALH cells, and answering this question will require further experiments that were beyond the scope of the present study.

Two features of the inhibitory effect of angiotensin II deserve specific comment: the effect is observed whether angiotensin II is added to the peritubular or the luminal fluid, and it is observed with $10^{-8}$ M but not with lower ($10^{-11}$ or $10^{-10}$ M) concentrations.

The bilateral action of angiotensin II has previously been recognized in the proximal tubule where both luminal and basolateral angiotensin II stimulate fluid, sodium, and HCO$_3^-$ reabsorption. Indeed, picomolar concentrations of systemic angiotensin II stimulate proximal tubule transport (24); in addition, endogenously produced angiotensin II also stimulates proximal fluid reabsorption in vivo, an effect that is blunted when losartan is added to the luminal fluid (33). Furthermore, in this segment, basolateral and apical angiotensin II receptors are not coupled to the same transduction pathways because, in the proximal tubule brush-border membrane, angiotensin II does not activate PLC activity but activates PLA$_2$ independently of the presence of calcium. In the rat MTALH, our group has recently demonstrated the presence of AT$_1$ receptors in apical and basolateral membranes (32). In addition, these receptors appeared to be differentially coupled to intracellular signaling pathways because binding of angiotensin II to the peritubular receptor elicited an increase in free cytosolic calcium concentration, whereas binding of angiotensin II to the apical receptor did not. Our present data expand these findings by demonstrating that both apical and basolateral angiotensin II receptors are functional because binding of angiotensin II to either receptor induces a decrement in NaCl reabsorption. Furthermore, we observe that the effects of luminal and peritubular angiotensin II are not additive, suggesting that the signaling pathways that couple apical and basolateral angiotensin II receptors to the regulation of NaCl reabsorption are, at least in part, common. Finally, angiotensin II decreases NaCl.

Table 4. Effect of peritubular $10^{-6}$ M PD-123319 on the effect of $10^{-8}$ M peritubular ANG II on $J_{Cl}$ and transepithelial voltage in the rat medullary thick ascending limb

<table>
<thead>
<tr>
<th>$J_{Cl}$, pmol·min$^{-1}$·mm$^{-1}$</th>
<th>Transepithelial Voltage, mV</th>
<th>Tubular Flow, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-123319</td>
<td>PD-123319 + ANG II</td>
<td>PD-123319 + ANG II</td>
</tr>
<tr>
<td></td>
<td>157.9 ± 20.9</td>
<td>111.2 ± 18.1*</td>
</tr>
<tr>
<td></td>
<td>18.1 ± 1.3</td>
<td>140.8 ± 31.5</td>
</tr>
<tr>
<td></td>
<td>14.6 ± 1.7</td>
<td>18.1 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>4.5 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 4$ tubules.

Table 5. Effect of $10^{-8}$ M peritubular ANG II on $J_{Cl}$ and transepithelial voltage in the presence of $10^{-8}$ M luminal ANG II in the rat medullary thick ascending limb

<table>
<thead>
<tr>
<th>$J_{Cl}$, pmol·min$^{-1}$·mm$^{-1}$</th>
<th>Transepithelial Voltage, mV</th>
<th>Tubular Flow, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal</td>
<td>Luminal and peritubular ANG II</td>
<td>Luminal and peritubular ANG II</td>
</tr>
<tr>
<td>ANG II</td>
<td>ANG II</td>
<td>ANG II</td>
</tr>
<tr>
<td></td>
<td>45.9 ± 7.1</td>
<td>45.5 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>9.5 ± 0.8</td>
<td>8.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>11.5 ± 0.8</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2.8 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 4$ tubules.
reabsorption both through the basolateral receptor, which is coupled to the intracellular calcium pathway, and through the apical receptor, which is not, suggesting that the angiotensin II-dependent release of intracellular calcium is probably not a critical event in the regulation of NaCl reabsorption. It should be noted that luminal angiotensin II has been reported to have no effect on HCO₃⁻ reabsorption by the rat MTALH (13). One possible explanation is that apical AT₁ receptors could be coupled to signaling pathways altering NaCl but not HCO₃⁻ reabsorption.

We observed that angiotensin II concentrations in the picomolar range do not alter transepithelial NaCl reabsorption in the MTALH, whereas they stimulate fluid, sodium, and bicarbonate reabsorption in the proximal tubule (24). In the present work, only nanomolar concentrations of angiotensin II are able to decrease NaCl absorption, and we found no evidence for a biphasic regulation of NaCl transport. These results are reminiscent of what was observed by Good et al. (13) in the study of the regulation of HCO₃⁻ reabsorption by the rat MTALH. It is likely that this observation is physiologically relevant. Indeed, compared with plasma levels, angiotensin II tissue contents are much higher (30) because of intrarenal formation of angiotensin II and accumulation from the circulation via an AT₁-receptor-mediated process. In addition, the intrarenal content of angiotensin II is not distributed in a homogeneous manner but is compartmentalized. Medullary angiotensin II levels are higher than cortical levels (27). At the end of the proximal tubule, intratubular angiotensin II concentration has been measured in the 10⁻⁹–10⁻⁸ M range (29, 35). The angiotensin II concentration in the tubular fluid of other segments of the nephron remains unknown, but because angiotensin II levels in final urine have also been reported to be in the nanomolar range (27) and because intratubular angiotensin II is mainly produced in the proximal tubule, it is likely that luminal angiotensin II concentration in the MTALH is also much higher than the plasma concentration. Interstitial fluid angiotensin II concentration has also been repeatedly measured in the nanomolar range and is also much greater than plasma concentration (28). Taken together, the inhibitory effect of angiotensin II observed in this study could well be also observed in vivo because physiological interstitial and luminal angiotensin II concentrations are in the nanomolar range (29, 30). In addition, intrarenal angiotensin II concentrations change when dietary NaCl intake is altered (8, 18): a low-sodium intake increases, and a high-sodium intake decreases, intrarenal angiotensin II concentrations, which could, in turn, decrease or increase, respectively, NaCl reabsorption in the MTALH. Consistently, a recent study in rats fed a high-NaCl diet for 5 days shows that outer medullary Na-K-2Cl cotransporter abundance is increased under this condition (20). It should be noted that, in a recent study in rats, prolonged systemic infusion of angiotensin II has been shown to be paradoxically responsible for an increase in Na-K-2Cl cotransporter abundance in the inner stripe of the outer medulla (22). However, infusion rates of angiotensin II used in this study were supraphysiologic and known to induce increased blood pressure (4).

An adaptive increase in MTALH NaCl reabsorption in response to NaCl loading and decreased intrarenal angiotensin II concentration may seem contrary to the requirements for the regulation of sodium balance. However, we can speculate that this response is appropriate as a means of maintaining water balance. NaCl loading inhibits NaCl reabsorption in segments distal to the TAL (6) and markedly increases urine NaCl excretion. The large increase in NaCl excretion during NaCl loading would tend to increase water excretion via an osmotic effect. However, increased medullary NaCl accumulation, secondary to an increase in NaCl absorption by the MTALH, would tend to balance the osmotic effect of high NaCl levels in the collecting duct fluid and allow NaCl to be excreted without obligating large amounts of water, in turn allowing independent regulation of NaCl and water balances.

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References


