Effect of catecholamines on rat medullary thick ascending limb chloride transport: interaction with angiotensin II

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ANGIOTENSIN II IS ONE OF THE factors responsible for increasing sodium absorption in the face of volume depletion. In addition to hemodynamic effects, ANG II has been shown to have a direct epithelial action to regulate sodium absorption along the nephron. Using in vivo micropuncture and in vitro micropерfusion, ANG II has been shown to increase sodium chloride and volume absorption in the proximal tubule (3, 8, 20, 26–28, 42, 45). Likewise, sodium transport has been shown to be stimulated in the macula densa by ANG II (5, 24, 36). In an in vitro micropерfusion study, ANG II was found to stimulate acidification and sodium absorption in the distal convoluted tubule (44). ANG II increased transcellular chloride transport and sodium transport across the epithelial sodium channel in the cortical collecting tubule (35, 37). Despite the increase in sodium absorption in almost every nephron segment, ANG II was found to decrease chloride transport in the medullary thick ascending limb (mTAL), a nephron segment responsible for a substantive amount of sodium chloride absorption (25).

Both the renin-angiotensin system and the renal nerves are stimulated by similar factors that result in a decrease in effective arteriolar pressure. The interdependence of the renin-angiotensin system and renal nerves can affect renal salt transport, as well as renal hemodynamics in not only a concordant but in a mutually dependent fashion (10, 11). As an example of this interdependence, circulating ANG II facilitated adrenergic transmission at the renal nerve-renal epithelial cell junction (18, 21, 22), so that renal nerve stimulation increased sodium transport in the presence of physiological circulating ANG II and was blunted by administration of captopril (21). We have previously shown that the stimulation in proximal tubule sodium absorption by the intrarenal renangiotensin system was regulated by and dependent upon renal nerves (40, 41).

Previous studies have shown that β-catecholamines stimulate sodium chloride transport in the mTAL (1, 29, 38). Since proximal and distal nephron segments have an increase in sodium absorption by the intrarenal angiotensin system was regulated by and dependent upon renal nerves (40, 41).

In vitro microperfusion flux studies. Segments of rat mTAL were dissected free hand and perfused with an ultrafiltrate-like solution that contained (in mM) 115 NaCl, 25 NaHCO3, 4.0 Na3HPO4, 10 Na acetate, 1.8 mM CaCl2, 1 MgSO4, 5 KCl, 8.3 glucose, 5 alanine, 2 lactate, and 2 glutamine at 4 or 5 nl/min. The tubules were bathed in a similar solution containing 6 g/dl of BSA. The osmolality of all solutions was adjusted to 300 mosmol/kg H2O. The bathing solution was heated to 38°C and exchanged at 0.5 ml/min to maintain a constant pH and osmolality.

The rate of chloride transport in the medullary thick ascending limbs (mTAL) was calculated as

\[ J_{Cl} = (V_L - V_L)/P_e \]

where \( V_L \) is the flow rate, \( V_L \) is the chloride concentration in the perfusate chloride concentration in millimoles and \( L \) is the tubular length in mm. The tubule lengths were measured with an eyepiece micrometer. The mean tubular length was 0.5 ± 0.1 mm. The perfusion solution contained

\[ 36Cl \]

at a concentration of 10 μCi/ml. A 30-nl constant-volume pipette was used to measure the collection rate. Tubules were perfused at about 5 nl/min. Tubules were incubated for about 15 min before initiation of the measurements for chloride absorption and between periods. There were at least three measurements per period, and the mean of the collections in each period was used to represent the chloride absorp-
chloride transport (271.1 pmol·mm⁻¹·min⁻¹) inhibited chloride transport from 317.8 pmol·mm⁻¹·min⁻¹ after the addition of ANG II, confirming that the plasma concentration of ANG II in a euvoletic animal does not affect mTAL transport (25). Similarly, 10⁻¹⁰ M ANG II had no effect on chloride transport (271.1 ± 40.5 pmol·mm⁻¹·min⁻¹ vs. 242.6 ± 98.1 pmol·mm⁻¹·min⁻¹) with 10⁻¹⁰ M ANG II pmol·mm⁻¹·min⁻¹), while 10⁻⁸ M ANG II inhibited chloride transport from 317.8 ± 68.6 to 258.7 ± 70.9 pmol·mm⁻¹·min⁻¹ (P < 0.05). The experiments shown in Fig. 1 confirmed previous findings that chloride transport was inhibited significantly by bath 10⁻⁸ M ANG II (25). Similarly, 10⁻⁶ M ANG II caused a reduction in the transepithelial potential difference from positive 4.6 ± 0.7 to 3.7 ± 0.7 mV, P < 0.01.

I next examined whether 10⁻⁶ M bath norepinephrine affected transport or influenced the effect of bath ANG II. These results are shown in Fig. 2. Bath norepinephrine caused a significant increase in chloride transport. This confirmed previous studies showing that this concentration of norepinephrine increases chloride transport in this nephron segment (38) in addition, when 10⁻⁸ M ANG II was added to the bathing solution in the third period in the presence of norepinephrine, the ANG II-mediated decrease in chloride transport was prevented. Norepinephrine caused an increase in the transepithelial potential difference from 9.0 ± 0.5 to 13.8 ± 1.1 mV, P < 0.001 and prevented the decrease in potential difference by ANG II (13.0 ± 0.9 mV P = not significant, ns) I also examined whether 2 × 10⁻¹¹ ANG II affected chloride transport in the presence of bath 10⁻⁶ M norepinephrine. Chloride transport was 325.8 ± 37.4 pmol·mm⁻¹·min⁻¹ in the presence of 10⁻⁸ M norepinephrine and 294.5 ± 36.6 pmol·mm⁻¹·min⁻¹ when 2 × 10⁻¹¹ M ANG II was added to the bathing solution in the presence of norepinephrine (P = ns). Thus, norepinephrine stimulates chloride transport and prevents the decrease in transport by 10⁻⁸ M ANG II.

The next experiments were designed to determine whether the stimulatory effect of norepinephrine on mTAL chloride transport and its effect to prevent the ANG II-mediated decrease in chloride transport was via an α- or β-effect of catecholamines. The effect of isoproterenol, a β-agonist, on chloride transport was next examined as shown in Fig. 3. Isoproterenol (10⁻⁶ M) stimulated chloride transport in the mTAL. These results are shown in Fig. 4. Furthermore, isoproterenol prevented the inhibition in chloride transport mediated by 10⁻⁸ M ANG II. Isoproterenol also increased the potential difference from 3.7 ± 1.0 to 5.7 ± 1.3 mV, P < 0.05 and prevented the decrease mediated by ANG II (5.4 ± 1.1 mV, P = ns). In the next series of experiments shown in Fig. 4, the effect of the α-agonist phenylephrine was examined. The addition of 10⁻⁶ M phenylephrine to the bathing solution resulted in no change in chloride transport compared with the control period in mTAL. However, phenylephrine also prevented the inhibition of chloride transport mediated by 10⁻⁸ M ANG II. Thus,
while the stimulation in chloride transport in the mTAL is mediated by a β-effect of catecholamines in this segment, both α- and β-effects of catecholamines prevent the inhibition by ANG II. Phenylephrine had no effect on the potential difference compared with control but prevented the decrease in potential difference by ANG II (3.7 ± 1.1 mV control vs. 4.3 ± 1.5 mV phenylephrine vs. 3.9 ± 0.8 mV phenylephrine plus ANG II, P = ns).

In the last series of experiments shown in Fig. 5, I show that 10⁻⁶ M propranolol blocked the increase in chloride transport by 10⁻⁶ M norepinephrine. Norepinephrine still prevented the inhibition of chloride transport by 10⁻⁸ M ANG II in the presence of propranolol. This is consistent with norepinephrine increasing chloride transport via a β-adrenergic effect and preventing the decrease in chloride transport by a non-β or -α effect. Similarly, norepinephrine did not significantly increase the potential difference in the presence of propranolol (3.6 ± 1.3 mV bath propranolol vs. 4.8 ± 2.7 mV bath propranolol plus norepinephrine, P = ns), but it prevented the inhibition of the potential difference by ANG II (5.0 ± 2.5 mV).

DISCUSSION
The present study confirms that ANG II by itself inhibits mTAL chloride transport. However, both α- and β-catecholamines prevent the inhibition of chloride transport by ANG II. Finally, norepinephrine stimulates chloride transport in the mTAL, an effect due entirely to its β-catecholamine effect.

While studies in the proximal tubule and distal convoluted tubule and collecting duct have all shown that ANG II stimulates sodium chloride transport consistent with its known regulatory role to limit sodium losses in the face of volume depletion (3, 5, 8, 20, 24–28, 35–37, 42, 45), the effect of ANG II on transport in the mTAL has not been clear. ANG II at 10⁻⁸ M has been shown previously to inhibit both chloride and bicarbonate transport in the mTAL perfused in vitro when added to the bathing solution (16, 25). Studies have also examined the effect of ANG II on loop of Henle sodium absorption in vivo, where ANG II resulted in an increase in proximal tubule reabsorption but had no effect on loop of Henle sodium absorption (12). However, when renal perfusion pressure was normalized to control values during ANG II infusion, there was an increase in proximal and loop of Henle reabsorption with ANG II infusion (12). While it is possible that sodium absorption in the proximal straight tubule was stimulated to such an extent that net sodium absorption in the
Perspectives and Significance

The present study shows that there is an interaction between norepinephrine and ANG II in their actions on chloride transport in the mTAL. This is another example of how different hormones and physiological conditions interact to affect transport. While there are advantages to studying the regulation of transport in vivo, some segments are not accessible, and examination of one factor in isolation of other hormones and renal nerves is often quite difficult. Whole animal clearance studies examine multiple nephron segments at one time and often do not provide mechanistic answers at the cellular level. Direct evaluation of an epithelium using in vitro approaches allows one to study the effect of a single perturbation on transport or signal transduction in a homogeneous cellular preparation. However, there are often interactions in vivo that are not directly obvious in vitro. Thus, it is not better to examine an issue using one approach or another but in vitro studies and in vivo studies should be complementary to examine mechanisms and significance of physiological perturbations.

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REFERENCES


