Effect of angiotensin II on calcium reabsorption by the luminal membranes of the nephron

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Charbonneau, A., M. Leclerc, and M. G. Brunette. Effect of angiotensin II on calcium reabsorption by the luminal membranes of the nephron. Am J Physiol Endocrinol Metab 280: E928–E936, 2001.—In the rat and the rabbit, a number of studies have reported the effects of angiotensin II (ANG II) on Na⁺ reabsorption by the proximal (PT) and distal (DT) convoluted tubules of the kidney. The aim of the present study was to examine the effect of ANG II on Ca²⁺ uptake by the luminal membranes of the PT and DT of the rabbit. Incubation of PT and DT with 10⁻¹² M ANG II enhanced the initial Ca²⁺ uptake in the two segments. Dose-response experiments revealed, for Ca²⁺ as well as for Na⁺ transport, a biphasic action with a maximal effect at 10⁻¹² M Ca²⁺ transport by the DT luminal membrane presents a dual kinetic. ANG II action influenced the high-affinity Ca²⁺ channel, increasing maximal velocity from 0.72 ± 0.03 to 0.90 ± 0.05 pmol·μg⁻¹·10⁻¹⁰ s⁻¹ (n = 3) and leaving the Michaelis-Menten constant unchanged. The effect of ANG II was abolished by losartan, suggesting that the hormone is acting through AT₁ receptors. In the PT, calphostin C inhibited the effect of the hormone. It is therefore probable that protein kinase C is involved as a messenger. In the DT, however, neither Rp cAMP, calphostin C, nor econazole (a phospholipase A inhibitor) influenced the hormone action. Therefore, the mechanisms involved in the hormone action remain undetermined. Finally, we questioned whether ANG II acts in the same DT segment as does parathyroid hormone on Ca²⁺ transport. The two hormones increased Ca²⁺ transport, but their actions were not additive, suggesting that they both influence the same channels in the same segment of the distal nephron, i.e., the segment responsible for the high-affinity calcium channel.

DURING THE LAST DECADES, a considerable amount of data has been accumulated concerning the activity of the renin-angiotensin system in the proximal tubule (PT) of the nephron. By use of several techniques [immunocytochemistry (20, 45), dosage of angiotensinogen and renin in the supernatant of PT cell cultures (39, 60), or detection of angiotensinogen mRNA by in situ hybridization (17, 28)], a number of studies clearly showed that this nephron segment contains a complete renin-angiotensin system able to synthesize renin and angiotensin-converting enzyme (ACE) (10, 49) and then secrete high concentrations of angiotensin II (ANG II) into the tubular fluid (5, 43, 52). This angiotensin synthesis is regulated by dietary Na⁺, as reported by Ingelfinger et al. (27), Fox et al. (16), Jo et al. (29), and Tank and colleagues (54, 55). Indeed, a low-sodium diet strongly increases both angiotensinogen and renin mRNA in the rat renal cortex.

Conversely, endogenous ANG II has been shown to control Na⁺ and water reabsorption in the PT through the presence of in situ receptors (29). This effect, however, depends on the hormone concentration; micropuncture and microperfusion experiments have demonstrated that physiological concentrations (from 10⁻¹² to 10⁻¹⁰ M) stimulate Na⁺ reabsorption, whereas higher doses (10⁻⁷ M) cause inhibition, a phenomenon which produces a bell-shaped dose-response curve (21, 22, 46, 50, 51, 53, 58).

Distal tubules (DT) are also the site of ANG II receptors, as shown in microdissection (40) and cell culture studies (11). In this segment, Stop-flow (57), microperfusion (25, 33, 34, 58), and micropuncture experiments (37) detected either a decrease (37, 57), an increase (33, 34, 58), or no change (25, 26) in Na⁺ transport, depending on the hormone concentration utilized.

In contrast to this abundant literature concerning the action of ANG II on Na⁺ reabsorption, very few studies have questioned its role in Ca²⁺ transport. Romero et al. (46) suggested that the negative effect of high hormone concentrations on Na⁺ transport might be due to Ca²⁺ entry into the cell, but such a hypothesis has never been clearly confirmed. A study using voltage-clamp techniques in cardiac myocytes (13) showed that intracellular administration of ANG II increased Ca²⁺ cell content, but probably through a protein kinase C (PKC) activation rather than a change in membrane Ca²⁺ permeability. In adrenal glomerulosa cells, however, Maturana et al. (38) recently reported that high doses of ANG II (100 nM) induced a marked inhibition of L-type Ca²⁺ channel current.

The purpose of the present study was to examine the effect of ANG II on Ca²⁺ transport by the PT and DT

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luminal membranes of the rabbit kidney. Results indicate that ANG II influences Ca\(^{2+}\) uptake by both PT and DT membranes according to a bell-shaped curve. This effect occurs after a few minutes of incubation and involves the maximal velocity (\(V_{\text{max}}\)) kinetic parameter, supporting the hypothesis of an exocytosis mechanism at the origin of this action. Both parathyroid hormone (PTH) and ANG II act on the high-affinity component of Ca\(^{2+}\) uptake by the DT luminal membrane. Because their actions are not additive, it is probable that the two hormones act in the same segment of the DT.

**MATERIALS AND METHODS**

*Preparation of tubule suspensions.* PT and DT suspensions were prepared by means of collagenase digestion and Percoll-density gradient centrifugation techniques with fresh rabbit kidneys obtained directly from the slaughterhouse. Slices of cortex were incubated for 20 min at 37°C in a cell culture medium (DMEM) containing 1 mg/ml collagenase type V and equilibrated with 95% O\(_2\)-5% CO\(_2\). The suspensions were filtered through a tea strainer, and the filtrate was centrifuged at 200 g for a few seconds. The pellets were washed twice in Krebs-Henseleit buffer (KHB) containing 0.5% BSA suspended in 40% Percoll in the cell culture medium and centrifuged at 28,000 g for 30 min at 4°C. The DT and PT bands were separately collected, washed twice in KHB with BSA, and incubated at 37°C for 10 min in the cell culture medium containing 2% fetal bovine serum, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and ANG II at the indicated concentration. In the ANG II receptor or messenger inhibition experiments, these various inhibitors were added to the incubation medium. Incubation was stopped by rapid filtration technique. Uptake was initiated by adding 25 \(\mu\)l of incubation medium at 35°C to 5 \(\mu\)l of membrane suspension (~25 \(\mu\)g protein). The incubation medium contained either 120 mM NaCl and 20 mM choline chloride or 140 mM choline chloride, with 20 mM Tris-HEPES, pH 7.4, and, unless otherwise specified, 0.5 mM \(^{45}\text{CaCl}_2\). Uptake was stopped by the addition of 1 ml of ice-cold solution containing 150 mM KCl, 20 mM Tris-HEPES, pH 7.4, and 2 mM EGTA. The suspensions were then filtered through Millipore filters (0.45 \(\mu\)M), the filters were rinsed with an additional 5 ml of stop solution, and the retained radioactivity was counted. Nonspecific binding was measured at time 0 under the same conditions.

*Enzyme marker measurements.* The purity of the various preparations was monitored by measurement of the specific enzyme activities. Alkaline phosphatase activity was determined according to the technique of Kelly and Hamilton (31) and Na\(^+-K^+\)-ATPase to the technique of Post and Sen (44).

*Materials.* \(^{45}\text{CaCl}_2\) was purchased from Mandel (NEN Life Science Products, Boston, MA). Collagenase type V, ANG II, DBcAMP, econazole, and all the other chemicals were from Sigma (St. Louis, MO). Calphostin C was purchased from Calbiochem. Losartan and PD-123319 were generously given by Merck, Sharp and Dohme and by our colleague, Dr. John S. Chan, respectively.

**RESULTS**

*Effect of ANG II on the time course of Ca\(^{2+}\) uptake by PT luminal membranes.* The initial experiments were designed to evaluate the effect of PT and DT incubation.
with ANG II on 0.5 mM Ca^{2+} uptake by the corresponding luminal membranes. As shown in Fig. 1, 10^{-12} M ANG II significantly increased the initial Ca^{2+} transport by the PT membrane vesicles from 0.49 ± 0.03 to 0.66 ± 0.04 pmol·μg^{-1}·10 s^{-1} (P < 0.005, n = 4). This initial effect progressively decreased, to completely disappear after 180 s.

**Effect of ANG II on Ca^{2+} uptake by PT luminal membranes: dose-response curve.** When PT were incubated with increasing concentrations of ANG II, the rate of 0.5 mM Ca^{2+} uptake by the luminal membrane vesicles was progressively enhanced at hormone concentrations from 0 to 10^{-12} M and then returned to the control values with ANG II at 10^{-10} M and above, showing a bell-shaped curve (Fig. 2).

**Effect of ANG II on the time course of Ca^{2+} uptake by the DT luminal membranes in the presence or absence of Na^{+}.** We have previously shown (9) that the presence of Na^{+} in the incubation medium strongly decreases Ca^{2+} uptake by the luminal membrane of DT, whereas it has no effect on PT membranes. Then we were interested in investigating whether the hormone affects Ca^{2+} transport by the distal membrane, and if so, whether this action is influenced by the presence of Na^{+}. Ca^{2+} uptake was measured in two different incubation media containing either 140 mM choline chloride and no Na^{+} or 120 mM NaCl and 20 mM choline chloride. In the absence of Na^{+}, 10^{-12} M ANG II increased Ca^{2+} transport from 0.52 ± 0.02 to 0.96 ± 0.04 pmol·μg^{-1}·10 s^{-1} (P < 0.001, n = 4). The presence of Na^{+} curtailed Ca^{2+} uptake, but the hormone still enhanced this transport from 0.38 ± 0.02 to 0.63 ± 0.04 pmol·μg^{-1}·10 s^{-1} (P < 0.005, n = 4; Fig. 3).

**Dose-response curve in the DT luminal membranes.** In another series of experiments following the same protocol, we examined the possibility of a biphasic action of ANG II on Ca^{2+} uptake in DT as in PT. Indeed, a similar dose-response curve was observed in DT as in PT membrane vesicles (Fig. 4), again with a maximal response at ANG II concentration of 10^{-12} M.

**Effect of incubation time with ANG II on Ca^{2+} uptake by PT and DT luminal membranes.** The effect of tubule incubation with ANG II on Ca^{2+} uptake by their luminal membranes was relatively rapid. As shown in Fig. 5, a mere 10-min incubation sufficed to provoke the observed modification of Ca^{2+} transport.

**Effect of ANG II on kinetic parameters of Ca^{2+} uptake by the PT and DT membranes.** To further understand the mechanism involved in the effect of ANG II on Ca^{2+} uptake by the luminal membranes of the two nephron segments, we measured Ca^{2+} uptake using Ca^{2+} concentrations from 0.025 to 4.00 mM. Figure 6 shows the corresponding Eadie-Hofstee plot obtained in the PT and DT membranes. In PT, ANG II affected the V_{max} value, leaving the value of the Michaelis-Menten constant (K_{m}) intact. As we previously reported (9), Ca^{2+} uptake by DT luminal membranes presents a dual kinetic with a low-affinity component sensitive to calcitonin (61) and a high-affinity component sensitive to PTH (32) and calbindin 28K (4). Such a dual kinetic was again observed, with the ANG II targeting exclu-

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**Fig. 3.** Effect of 10^{-12} M ANG II on 0.5 mM Ca^{2+} uptake by distal tubule luminal membranes in the absence or presence of 100 mM NaCl. *P < 0.05, **P < 0.02, ***P < 0.005 vs. CTL values (unpaired Student’s t-test); n = 4.

**Fig. 4.** Dose-response curve of effect of ANG II on 0.5 Ca^{2+} uptake by distal tubule luminal membranes in the absence of Na^{+}. *P < 0.05, ***P < 0.005 vs. control values (unpaired Student’s t-test); n = 4–6.
sively the high-affinity component, increasing, as in PT, the $V_{\text{max}}$ value (Table 2).

Does ANG II also act directly on the luminal membrane Ca$^{2+}$ channels? Unlike most hormone receptors that are exclusively expressed in basolateral membranes, ANG II receptors have been shown to be present on both the apical and basolateral membranes (6, 7). Because the ANG II concentration is relatively high in the tubular fluid, we questioned whether the hormone might have a direct effect on luminal Ca$^{2+}$ channels independent of the intracellular machinery. In these experiments, ANG II was added to the membrane suspensions either after the vesiculation step or in the incubation medium.

As shown in Fig. 7, Ca$^{2+}$ uptake by the two site membranes was not influenced by the presence of 10$^{-12}$ M ANG II in the incubation medium. Addition of the hormone to the vesicle suspension after vesiculization also had no effect (data not shown).

Which receptors are involved in the effect of ANG II on the PT and DT luminal membranes? To characterize the type of receptors through which ANG II exerts its enhancement of Ca$^{2+}$ transport by the DT membranes, a new series of experiments was performed using losartan and PD-123319 as AT$_1$- and AT$_2$-specific inhibitors. The results are presented in Fig. 8. As constantly observed, incubation of PT and DT with 10$^{-12}$ M ANG II significantly increased Ca$^{2+}$ uptake from 0.39 ± 0.020 to 0.55 ± 0.015 in PT and from 0.46 ± 0.027 to 0.75 ± 0.041 pmol·µg$^{-1}$·10 s$^{-1}$ in DT membranes. In the presence of 10$^{-6}$ M losartan, however, the effect of the hormone was completely abolished (0.39 ± 0.023 and 0.50 ± 0.037 pmol·µg$^{-1}$·10 s$^{-1}$ in PT and DT, respectively; not significant vs. control values). In contrast, the AT$_2$ inhibitor PD-123319 did not significantly interfere with the influence of ANG II on Ca$^{2+}$ uptake.

Is ANG II action influenced by nitrendipine? In previous unpublished experiments, we observed that the low-affinity Ca$^{2+}$ channel was inhibited predominantly by nitrendipine: 0.1 mM Ca$^{2+}$ uptake was 1.59 ± 0.08 and 1.51 ± 0.09 mmol·10 s$^{-1}$·µg protein$^{-1}$ in control and treated vesicles (NS) whereas 1.0 mM Ca$^{2+}$ uptake was 2.59 ± 0.25 vs. 1.96 ± 0.35 mmol·10 s$^{-1}$·µg protein$^{-1}$ in the two conditions, respectively ($P < 0.05$, $n = 3$; unpaired t-test). In the present experiments, addition of 10 µM nitrendipine to the vesicle suspensions again slightly decreased the total Ca$^{2+}$ uptake. However, this treatment did not prevent the response.
Table 2. Effect of 10^{-12} M ANG II on kinetic parameters of Ca^{2+} transport by PT and DT luminal membranes

<table>
<thead>
<tr>
<th></th>
<th><strong>K_{m}, mM</strong></th>
<th><strong>V_{max}, pmol \cdot \mu g^{-1} \cdot 10^{-1} s^{-1}</strong></th>
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<tbody>
<tr>
<td><strong>PT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.41 ± 0.02</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td>ANG II</td>
<td>0.40 ± 0.027</td>
<td>0.98 ± 0.035</td>
</tr>
<tr>
<td><strong>DT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low affinity</td>
<td>1.54 ± 0.39</td>
<td>2.34 ± 0.25</td>
</tr>
<tr>
<td>ANG II</td>
<td>1.34 ± 0.39</td>
<td>2.26 ± 0.25</td>
</tr>
<tr>
<td>High affinity</td>
<td>0.05 ± 0.01</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>ANG II</td>
<td>0.06 ± 0.003</td>
<td>0.90 ± 0.05</td>
</tr>
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</table>

Values are means ± SD. ANG, angiotensin. *P < 0.05, compared with the control values (unpaired Student's t-test); n = 3.

Fig. 7. Direct effect of 10^{-12} M ANG II in the incubation medium of 0.5 Ca^{2+} uptake by the PT (left) and DT (right) luminal membranes.

econazole prevented the hormone effect, thus suggesting that PKC was the major, if not the only, messenger responsible for the increase in Ca^{2+} transport.

In the DT, however, different results were obtained. Inhibition of cAMP, PKC, or phospholipase A did not modify the response to ANG II. Because we had previously observed (23), as did others using different cell preparations (18), that Ca^{2+} transport in the DT was extremely sensitive to the association of cAMP and PKC, we questioned whether a combination of the two inhibitors Rp cAMP and calphostin C would interfere with ANG II action. Results of an additional experiment indicated that the combination of 10^{-5} M Rp-cAMP and 10^{-7} M calphostin C did not influence either 0.5 mM Ca^{2+} uptake (Ca^{2+} uptake: 0.44, 0.64 and 0.66 pmol \cdot \mu g^{-1} \cdot 10^{-1} s^{-1} in membranes from control tubules and tubules treated with ANG II or ANG II plus calphostin C and Rp cAMP, respectively).

Combined effect of ANG II and PTH. In the kidney, the main Ca^{2+}-regulating hormones are PTH, calcitomin (CT), and calbindin 28K. All of them increase Ca^{2+} uptake by the distal luminal membrane: CT opens the low-affinity (61), whereas PTH (32) and calbindin 28K (4) stimulate the high-affinity, channels. We have shown that 10^{-12} M ANG II influences the high-affinity component, as does PTH. Then we hypothesized that a lack of additivity of ANG II and PTH should confirm an identical site and mechanism of action of the two hormones. DT were incubated for 10 min with either the carrier ANG II alone, PTH alone, or ANG II in combination with PTH. Results are presented in Fig. 11. Both ANG II and PTH increased Ca^{2+} uptake by the DT luminal membranes. However, the combined action of the two hormones was not different from the effect of each of them: Ca^{2+} uptake: 0.49 ± 0.018, 0.61 ± 0.01, 0.71 ± 0.034, and 0.71 ± 0.032 pmol \cdot \mu g^{-1} \cdot 10^{-1} s^{-1} in the four experimental conditions, respectively. It is therefore probable that ANG II acts in the same segment as PTH, i.e., the late part of the distal cortical tubule (40).

Effect of ANG II on Na^{+} transport by the PT and DT luminal membranes: interrelationship of the two cat-
ions. Because Na\(^+\) and Ca\(^{2+}\) transports by the DT luminal membrane are tightly related (9), we performed a few additional experiments to investigate the effect of ANG II on Na\(^+\) uptake by PT and DT membranes. As already reported, low concentration of ANG II (10\(^{-12}\) M) significantly increased 1 mM Na\(^+\) transport by the PT brush border membrane (from 0.89 \(\pm\) 0.02 to 1.07 \(\pm\) 0.02 pmol \(\cdot \) mg\(^{-1}\) \cdot 10 s\(^{-1}\), \(P\), 0.01, \(n = 3\)). Unexpectedly, however, at the same concentration, the hormone had the opposite effect in the DT membranes: Na\(^+\) uptake fell from 0.60 \(\pm\) 0.03 to 0.44 \(\pm\) 0.05 pmol \(\cdot \) mg\(^{-1}\) \cdot 10 s\(^{-1}\) (\(P\), 0.05, \(n = 3\)).

DISCUSSION

ANG II influences Ca\(^{2+}\) transport by the PT and DT luminal membranes, but through which messengers and which mechanisms? The aim of the present study was to detect and characterize the effect of ANG II on Ca\(^{2+}\) transport through the luminal membranes of PT and DT. Results clearly showed that the hormone influences Ca\(^{2+}\) uptake by the two membranes according to a bell-shaped curve: physiological concentrations increase the cation transport with a maximal effect at 10\(^{-12}\) M, whereas at higher concentrations, such a stimulation progressively declines.

The mechanisms involved in these actions remain unclear. ANG II has been repeatedly reported to depress adenylate cyclase activity in several types of PT cell preparations (56, 59). The fact that ANG II decreases cAMP synthesis and increases Ca\(^{2+}\) transport by the luminal membrane suggests an inhibitory action of the messenger, a hypothesis which is in contradiction to data previously reported by our group (23). Indeed, we have been unable to clearly demonstrate any action of DB-cAMP on Ca\(^{2+}\) uptake by PT brush border membranes (23). Confirming the noninvolvement of adenylate cyclase in the effect of ANG II on Ca\(^{2+}\) transport by the PT, inhibition of the messenger delivery by Rp cAMP did not prevent the hormone effect. Phospholipase A is probably also not involved in the regulation of Ca\(^{2+}\) uptake by 10\(^{-12}\) angiotensin, since incubation with econazole did not inhibit the hormone action.

Our results rather support the role of PKC in the effect of ANG II on Ca\(^{2+}\) uptake by the PT. The hormone has been shown repeatedly to activate PKC to produce its effect in PT (30, 36, 47, 48) and the ascending limb of the loop of Henle (1). In the present study, addition of calphostin C to the incubation medium with ANG II abolished the influence of the hormone on Ca\(^{2+}\) transport, thus suggesting that PKC is the main, if not the only, messenger active at that site.

The mechanisms involved in DT membranes are no easier to understand. cAMP and PKC have been shown to interact with each other to stimulate Ca\(^{2+}\) transport by the luminal membranes (19, 23). Here again, therefore, a decrease in cAMP delivery should curtail Ca\(^{2+}\) uptake rather than the opposite, as observed in our study, confirming the nonimplication of cAMP in the distal effect, i.e., the lack of inhibitory effect of Rp cAMP on the ANG II-dependent Ca\(^{2+}\) uptake. However, at variance with what was observed in PT, calphostin C did not interfere with the hormone action and association of Rp cAMP, and calphostin C also failed to prevent the hormone action. Further experiments should explore other messenger systems such as mitogen-activated protein kinases, the various isoforms of phospholipases, the tyrosine kinases, or even cytochrome P-450.

Fig. 8. Effect of 10\(^{-6}\) M losartan and 10\(^{-6}\) M PD-123319 (AT\(_1\) and AT\(_2\) receptor inhibitors, respectively) on the ANG II-dependent (10\(^{-12}\) M) 0.5 mM Ca\(^{2+}\) transport by the PT and DT luminal membranes. ** \(P < 0.02\), *** \(P < 0.005\) vs. control values (unpaired t-test); \(n = 3\).

Fig. 9. Effect of 10 \(\mu\)M nitrendipine on the ANG II-dependent 0.1 mM Ca\(^{2+}\) uptake by the DT luminal membranes. ** \(P < 0.02\) (\(n = 3\)) vs. data obtained with nitrendipine alone.
Another aspect of the mechanism involved in the action of ANG II is the relatively short incubation time necessary to be efficient, i.e., 10 min in PT and 5 min in DT. The hypothesis of a direct action on the luminal membranes has been excluded by the lack of effect of ANG II added to the vesicle suspensions. It is possible, however, that the hormone influences the density of transport molecules through an indirect endo-/exo-cytosis mechanism independently of the synthesis of any new molecule. Indeed, a similar mechanism has been shown by Schelling and Linas (47) for Na⁺ transport by the PT luminal membranes. The fact that, in our kinetic experiments, the hormones increase the V\textsubscript{max} values without influencing the affinity (K\textsubscript{m}) is compatible with this hypothesis.

ANG II opens the high-affinity Ca\textsuperscript{2+} channels in the DT. Electrophysiology (43) and molecular biology experiments (2) showed the presence of several types of Ca\textsuperscript{2+} channels in the DT luminal membranes. Our laboratory clearly detected a dual kinetics of Ca\textsuperscript{2+} transport by the DT luminal membrane, a low-affinity channel sensitive to calcitonin (61), and a high-affinity channel sensitive to PTH (3) and the vitamin D-dependent calbindin 28K (4). The high-affinity channel has been further characterized by Hoenderop et al. (24), who identified the channel EC\textalpha{C} mRNA which was co-localized with the calbindin-D 28K and, therefore, probably the PTH receptors. In the present study, ANG II opened the high-affinity Ca\textsuperscript{2+} channel, i.e., the channel that is sensitive to PTH and calbindin 28K. The lack of additivity of PTH and ANG II actions on Ca\textsuperscript{2+} transport as shown in Fig. 9 strengthens this hypothesis.

Nitrendipine does not curtail the action of ANG II. As aforementioned, nitrendipine is an L-type Ca\textsuperscript{2+} channel blocker that inhibits the low-affinity channel in the DT luminal membrane. The inhibitor did not prevent the effect of ANG II on Ca\textsuperscript{2+} transport. This observation is quite logical, because ANG II rather opens the high-affinity component of Ca\textsuperscript{2+} uptake. It further confirms that ANG II and nitrendipine act in two different segments of the distal nephron.

ANG II activates the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in PT and curtails the exchanger activity in the DT. In PT, the observed stimulation of the exchanger by ANG II first confirms other data previously reported. In DT, however, the hormone has an opposite effect, a finding which was unexpected. This is another observation of a certain antagonist behavior of Ca\textsuperscript{2+} and Na\textsuperscript{+} transports through the distal luminal membranes. Na\textsuperscript{+} decreases Ca\textsuperscript{2+} uptake (23); conversely, Ca\textsuperscript{2+} partially blocks Na\textsuperscript{+}/H\textsuperscript{+} exchange (12). The Ca\textsuperscript{2+} regulating hormones PTH (4), calcitonin (61), and calbindin 28K (8), all of which open the distal Ca\textsuperscript{2+} channels, also curtail the Na\textsuperscript{+} entry by this membrane. Hypothetically, it is therefore possible that these Ca\textsuperscript{2+} channels are, in fact, cation channels through which Ca\textsuperscript{2+} competes with Na\textsuperscript{+} to be transported.

In summary, the present studies investigated the action of ANG II on Ca\textsuperscript{2+} uptake by the PT and DT luminal membranes. At both sites, ANG II provoked a biphasic response, with a stimulation of Ca\textsuperscript{2+} trans-

Table 3. Effect of messenger inhibitors on the ANG II-dependent 0.5 mM Ca\textsuperscript{2+} uptake

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ANG II</th>
<th>ANG II + Rp cAMP</th>
<th>ANG II + Calphostin C</th>
<th>ANG II + Econazole</th>
</tr>
</thead>
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<tr>
<td>PT (n = 3), pmol·μg⁻¹·10⁻⁸ s⁻¹</td>
<td>0.24 ± 0.02</td>
<td>0.35 ± 0.02**</td>
<td>0.35 ± 0.007***</td>
<td>0.27 ± 0.00</td>
<td>0.35 ± 0.012**</td>
</tr>
<tr>
<td>DT (n = 5), pmol·μg⁻¹·10⁻¹ s⁻¹</td>
<td>0.29 ± 0.03</td>
<td>0.49 ± 0.05***</td>
<td>0.47 ± 0.09</td>
<td>0.46 ± 0.06*</td>
<td>0.48 ± 0.05***</td>
</tr>
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Values are means ± SD. *P < 0.05; **P < 0.02; ***P < 0.01 vs. control (Student’s unpaired t-test).
port of hormone concentrations below 10^-12 M and a return to control values at higher concentrations. In the DT, the presence of 100 mM NaCl in the incubation medium decreased Ca^{2+} uptake but did not prevent the effect of ANG II. The hormone increased the V_max without changing the K_m values. Addition of 10^-6 M losartan to the incubation medium prevented the effect of the hormone. Inhibition of PKC stimulation curtailed the effect in the PT but not in the DT. Finally, PTH, like ANG II, enhances Ca^{2+} transport; however, the effects of the two hormones were not additive, suggesting that they act on the same Ca^{2+} transport molecule, i.e., the high-affinity Ca^{2+} channel previously described in the DT luminal membrane.

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