Effects of angiotensin II on the CO2 dependence of HCO3− reabsorption by the rabbit S2 renal proximal tubule

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Zhou, Yuehan, Patrice Bouyer, and Walter F. Boron. Effects of angiotensin II on the CO2 dependence of HCO3− reabsorption by the rabbit S2 renal proximal tubule. Am J Physiol Renal Physiol 290: F666–F673, 2006. First published October 4, 2005; doi:10.1152/ajprenal.00287.2005.—Previous authors showed that, at low doses, both basolateral and luminal ANG II increase the proximal tubule’s HCO3− reabsorption rate (JHCO3−). Using out-of-equilibrium CO2/HCO3− solutions, we demonstrated that basolateral CO2 increases JHCO3−. Here, we examine interactions between ANG II and CO2 in isolated, perfused rabbit S2 segments. We first used equilibrated 5% CO2/22 mM HCO3−/pH 7.40 in bath and lumen. At 10−11 M, basolateral (BL) ANG II increased JHCO3− by 41%, and luminal ANG II increased JHCO3− by 35%. At 10−9 M, basolateral ANG II decreased JHCO3− by 43%, whereas luminal ANG II was without effect. Second, we varied [CO2]BL from 0 to 20% at fixed [HCO3−]L and pHL. Fractional stimulation produced by BL 10−9 M ANG II falls when [CO2]L exceeds 5%. Fractional inhibition produced by BL 10−9 M ANG II tends to rise when [CO2]L exceeds 5%. Regarding luminal ANG II, fractional stimulation produced by 10−11 M ANG II fell monotonically as [CO2]L rose from 0 to 20%. Fractional inhibition produced by 10−9 M ANG II rose monotonically with increasing [CO2]L. Viewed differently, ANG II at 10−11 M tended to reduce stimulation by CO2, and at 10−9 M, produced an even greater reduction. In conclusion, the mutual effects of 1) ANG II on the JHCO3− response to basolateral CO2 and 2) basolateral CO2 on the JHCO3− responses to ANG II suggest that the signal-transduction pathways for ANG II and basolateral CO2 intersect or merge.

ONE OF THE MAJOR tasks of the kidneys is to participate, along with the lungs, in the regulation of the acid-base balance of the extracellular fluid. For example, it has long been appreciated that acute respiratory acidosis (i.e., a rise in PaCO2 that causes a fall in pH) rapidly stimulates renal H+ secretion (8, 16). The proximal tubule (PT) plays a key role in this acid secretion. In addition to reabsorbing a near-isosmotic fluid that represents about two-thirds of the fluid filtered by the glomerulus, the PT reabsorbs ∼80% of the filtered HCO3− as follows. The PT cell actively secretes H+ into the tubule lumen (1, 6, 35) and uses this H+ to titrate filtered HCO3− in the lumen to CO2 and H2O, catalyzed by apical carbonic anhydrase IV (9, 36, 37). The newly formed CO2 and H2O then diffuse into the PT cells, where carbonic anhydrase II (36, 37) catalyzes the regeneration of H+ and HCO3−. The cell extrudes the H+ across the apical membrane via Na-H exchangers (4, 5, 30) and H+ pumps (20), while exporting the HCO3− across the basolateral membrane, mainly via the electrogenic Na/HCO3− cotransporter (7, 15, 32, 33).

Using out-of-equilibrium (OEE) CO2/HCO3− solutions to alter [CO2], [HCO3−], or [H+] one at a time (40, 41, 43), our laboratory demonstrated that, at least in regard to acute acid-base disturbances, H+ secretion by the PT responds not to changes in pH, but only to changes in basolateral [CO2] and [HCO3−] (43). Thus, in the case of acute respiratory acidosis, the PT cell senses the increase in blood [CO2] per se, which is a powerful stimulus for HCO3− reabsorption.

Perhaps the most powerful hormonal stimulus for HCO3− reabsorption is ANG II. The first report of the effects of ANG II on PT transport was in 1968 by Burg and Orloff (12) on isolated, perfused rabbit PTs. They noted that adding ~2×10−6 M ANG II to the basolateral solution had no detectable effect on the rate of fluid absorption (Jv). In a 1974 rat micropuncture study, Steven (38) reported that ~2×10−5 M basolateral ANG II lowered Jv, whereas 1×10−7 M had no effect. Harris and Young (23) later showed that basolateral ANG II has a biphasic effect on Na+ reabsorption in the rat PT, stimulating at low doses (10−12–10−10 M) and inhibiting at higher doses (3×10−7–3×10−6 M). Shuster and colleagues (34) in 1984 demonstrated a similar biphasic effect of basolateral ANG II on Jv in isolated, perfused rabbit PTs, ruling out a role of sympathetic innervation on the Jv response. In 1991, working in isolated, perfused rat proximal straight tubules (PSTs), Garvin (19) found that 10−6 M basolateral ANG II increases both JHCO3− and Jv. At about the same time, Chatshudhipong and Chan (14) found that high levels of basolateral ANG II reduce JHCO3− in rats and that these effects are blocked by saralasin, an antagonist of ANG II receptors.

As far as the effects of luminal ANG II are concerned, in a 1988 micropuncture study, Liu and Cogan (26) showed that low-dose luminal ANG II (10−12–10−11 M) increases HCO3− reabsorption (JHCO3−) even in a denervated rat kidney. The 1990 study on the rat PT by Wang and Chan (39) extended the earlier observations by showing that increasing levels of luminal ANG II have biphasic effects on JHCO3− as well as Jv. Consistent with these last two papers, Morduchowicz et al. (29), working in brush-border membrane vesicles, showed that ANG II stimulates Na-H exchange. Li et al. (25) in 1994 and Du et al. (17) in 2003 extended the biphasic effects of ANG II on Jv to the lumen of the isolated, perfused rabbit PT. In 1997, working in isolated perfused rabbit proximal convoluted tubule (PCTs), Baum et al. (2) found that, in the presence but not in the absence of a luminal ACE inhibitor, low-dose luminal ANG II increased both Jv and JHCO3−.

The pattern that emerges from the above work is that, whether applied to the luminal or basolateral surface of the PT, low-dose ANG II generally increases Jv and JHCO3−, whereas high-dose ANG II generally has the opposite effect. In fact, until our observation that basolateral CO2 is also a powerful...
stimulus for $HCO_3^-$ reabsorption (43), low-dose ANG II was the single most powerful known stimulus. The purposes of the present study were to determine whether 1) the effects of basolateral $CO_2$ and low-dose ANG II, added to either the bath (i.e., basolateral solution) or lumen (i.e., luminal solution), are additive and 2) high-dose ANG II antagonizes the stimulatory effects of $CO_2$.

Our approach was to use OOE solutions to vary basolateral $[CO_2]$ from 0 to 20% while keeping basolateral [$HCO_3^-$] and pH fixed near their physiological values in isolated, perfused rabbit S2 PTs. We found that low-dose (i.e., $10^{-11}$ M) ANG II, whether added to the bath or lumen, maximally increased $J_{HCO_3}$ at low basolateral (BL) $[CO_2]$, but that the effects tended to wane at high values of $[CO_2]_{BL}$. High-dose ANG II (i.e., $10^{-9}$ M) added to the bath produced a uniform decrease in $J_{HCO_3}$ regardless of $[CO_2]_{BL}$. When added to the lumen, high-dose ANG II had no effect when $[CO_2]_{BL}$ was 0%, but increasingly larger inhibitor effects at high values of $[CO_2]_{BL}$.

**METHODS**

**Biological preparation.** All experiments were carried out in “pathogen-free” female rabbits (New Zealand White, Elite, Covance, Denver, PA) according to procedures that were approved by the Yale Animal Care and Use Committee. We perfused the PTs using methods that were similar to those originally described by Burg et al. (10) and later modified by Baum et al. (2) and also by our laboratory (31, 41). Briefly, a rabbit weighing 1.4–2.0 kg was euthanized by a single intravenous injection of pentobarbital sodium (i.e., “bath”) at 7 ml/min, with a solution at 37°C. The luminal perfusate always was solution 2, which contained dialyzed $H^+$-methoxymethyl (MW ~7146, catalogue no. NET-086L, PerkinElmer Life Sciences, Boston, MA) as the volume marker. Solution 3, which contained 2% albumin, flowed through the bath during a 20- to 30-min warm-up period at 37°C. Following the warm-up period, all experiments had two periods for the collection of luminal fluid. During the first of these, the bath contained solution 4, with or without ANG II. During the second collection period, the bath contained ANG II dissolved in solutions 4, 5, 6, 7, or 8. In experiments in which we perfused the lumen with ANG II, the hormone was present in the lumen throughout the experiment; however, in these experiments, ANG II was always absent from the bath during both collection periods. We generated OOE $CO_2/HCO_3^-$ solutions (solutions 5-8) by rapidly mixing streams of two dissimilar solutions (40) and delivering the newly mixed

**Table 1. Physiological solutions**

<table>
<thead>
<tr>
<th>Component</th>
<th>1 Dissection</th>
<th>2 Lumen*</th>
<th>3 Bath†</th>
<th>4 Bath‡</th>
<th>5 Bath</th>
<th>6 Bath</th>
<th>7 Bath</th>
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<td>Standard,</td>
<td>OOE† Solution</td>
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<tr>
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<td>6.99</td>
<td>9.40</td>
<td>7.70</td>
<td>7.34</td>
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</table>

The concentrations are in mM except for $CO_2$ (given in both mM and %) and albumin (g/l). Except for solution 1, all solutions were titrated to the indicated pH at 37°C. Tris-HCl and HEPES were titrated with NaOH. *The solution was used as a luminal perfusate. †The solution was used as a basolateral perfusate. ‡Out-of-equilibrium (OOE) solutions were generated by rapidly mixing their respective A and B components in 1:1 ratio. Solutions 5A, 5B, 6B, 7B, and 8B were vigorously gassed with 100% $O_2$ to render them free of $CO_2$. 

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solution to the tubule within ~200 ms. All solutions had osmolalities of 300 ± 2 mosmol/kg H$_2$O.

**Measurement of $J_{HCO_3}$ and $J_V$** In each of the two collection periods, we allowed the tubule to stabilize in the appropriate bath solution for 5–8 min, removed and discarded the fluid that had accumulated in the holding pipette at the collection end of the tubule, and then began a series of three timed and calibrated collections. The first two were subsequently analyzed for $[^3]$H methoxyinulin for and then began a series of three timed and calibrated collections. The basolateral solution was solution 4 (open bar), solution 4 plus 10$^{-11}$ M ANG II (gray bar), or solution 4 plus 10$^{-9}$ M ANG II (filled bar). Values are means ± SE, with nos. of tubules in parentheses. As indicated, the difference between each of gray (or filled) bars and the corresponding open bar is statistically significant in a 1-way ANOVA for 5 groups, using Dunnett’s multiple comparison (***$P < 0.001$).

**RESULTS**

Effects of basolateral ANG II on $J_{HCO_3}$, and $J_V$ with an equilibrated CO$_2$/HCO$_3^-$ solution in the bath. In our first set of studies, we exposed the basolateral surface of S2 PTs to an equilibrated CO$_2$/HCO$_3^-$ solution, [CO$_2$]$_{BL}$ = 5%, [HCO$_3^-$]$_{BL}$ = 22 mM, and pH$_{BL}$ = 7.4 (solution 4), throughout the two collection periods of the experiment. During the first collection period, no hormone was present (Fig. 1A, open bar). During the second collection period, we added to the bath either 10$^{-11}$ M ANG II (gray bar) or 10$^{-9}$ M ANG II (filled bar). Thus we were able to compare the effects of ANG II on $J_{HCO_3}$ and $J_V$ in the same tubule. Because in our analysis of basolateral ANG II (Fig. 1A) we used the same control data as in our analysis of luminal ANG II (see Fig. 3A), we employed one-way ANOVA for five groups: (1) the identical control data for Figs. 1A and 3A; (2 and 3) the basolateral low- or high-dose ANG II data for Fig. 1A; and 4) and 5) the luminal low- or high-dose ANG II data for Fig. 3A. The overall $P$ value was <0.0001. Dunnett’s multiple comparison shows that, compared with the control condition in which no added ANG II was present in the bath, the presence of a “low” dose of 10$^{-11}$ M ANG II increased $J_{HCO_3}$ significantly from 56 ± 3 to 79 ± 5 pmol·min$^{-1}$·mm$^{-1}$ ($P < 0.0001$), as shown by the first two bars in Fig. 1A. In contrast, adding a “high” dose of 10$^{-9}$ M ANG II decreased $J_{HCO_3}$ significantly from 56 ± 3 to 33 ± 4 pmol·min$^{-1}$·mm$^{-1}$ ($P < 0.0001$).

Our control mean $J_{HCO_3}$, value of 56 pmol·min$^{-1}$·mm$^{-1}$ is similar to the values of 82 pmol·min$^{-1}$·mm$^{-1}$ reported by Burg and Green (11) and 62 pmol·min$^{-1}$·mm$^{-1}$ reported by Baum et al. (2), both from the isolated, perfused rabbit PCT. The 41% increase in $J_{HCO_3}$ that 10$^{-11}$ M ANG II produced in our experiments is somewhat higher than the 29% increase that 10$^{-10}$ M ANG II produced in the experiments in isolated, perfused rat PSTs by Garvin (19).

Figure 1B summarizes the $J_V$ data, which we analyzed using the same ANOVA approach summarized in the previous paragraph. The overall $P$ value was 0.00013. The effect of 10$^{-11}$ M basolateral ANG II on $J_V$ was stimulatory, as it was for $J_{HCO_3}$. The low dose of the peptide increased the mean $J_V$ from 0.46 ± 0.03 to 0.62 ± 0.05 nl·min$^{-1}$·mm$^{-1}$ ($P = 0.0087$). On the other hand, adding 10$^{-9}$ M basolateral ANG II did not have a statistically significant effect on $J_V$, which changed from 0.46 ± 0.03 under control conditions to 0.41 ± 0.05 nl·min$^{-1}$·mm$^{-1}$ in the presence of the high dose of the peptide ($P = 0.8033$).

Our control $J_V$ value of 0.46 nl·min$^{-1}$·mm$^{-1}$ is about two-thirds as great as the value reported by Schuster et al. (34), who worked with a combination of S1 and S2 segments from midcortical and juxtamedullary nephrons. On the other hand, our observed 36% increase of $J_V$ with 10$^{-11}$ M ANG II is about twice as great as that observed by the earlier investigators. Our $J_V$ data confirm the earlier observations of others, made in a combination of rat (14, 23, 38) and rabbit (34) PTs, that increasing levels of basolateral ANG II have a biphasic effect on $J_V$. In addition, we observed that increasing levels of basolateral ANG II have a biphasic effect on $J_{HCO_3}$. Our $J_{HCO_3}$ data are consistent with those made by Garvin (19) in rat PSTs.
used a t-test to analyze the data at \([\text{CO}_2]_{\text{BL}}\) values of 2.5 and 10% in Fig. 2A. Compared with the control condition, \(10^{-11}\) M basolateral ANG II produced a statistically significant increase in \(J_{\text{HCO}_3}\) at 0 \((P = 0.041)\) and 5% \(\text{CO}_2\) \((P < 0.0001)\). The difference was not statistically significant at either 2.5 \((P = 0.092)\) or 10% \(\text{CO}_2\) \((P = 0.51)\). Basolateral \(10^{-11}\) M ANG II produced a small but statistically significant decrease in \(J_{\text{HCO}_3}\) at 20% \(\text{CO}_2\) \((P = 0.032)\). Viewed differently, low-dose basolateral ANG II steepens relationship between \(J_{\text{HCO}_3}\) and \([\text{CO}_2]_{\text{BL}}\) at low levels of \([\text{CO}_2]_{\text{BL}}\) (i.e., 0–5%), but eliminated the stimulation by \(\text{CO}_2\) at higher \([\text{CO}_2]_{\text{BL}}\) levels.

We analyzed the \(J_V\) data the same way we analyzed the \(J_{\text{HCO}_3}\) data. For the ANOVA, the overall P values were 0.00088 for 0% \(\text{CO}_2\) and 0.063 for 20% \(\text{CO}_2\). Compared with the control situation with no added hormone (circles in Fig. 2B), low-dose basolateral ANG II (diamonds) tended to increase \(J_V\), although the effect was statistically significant only at a \([\text{CO}_2]_{\text{BL}}\) value of 5%.

**Effect of high-dose ANG II.** The squares in Fig. 2A summarize \(J_{\text{HCO}_3}\) data obtained in the presence of \(10^{-9}\) M basolateral ANG II as we increased \([\text{CO}_2]_{\text{BL}}\) from 0% \(\text{CO}_2\) to 20% \(\text{CO}_2\) at a fixed \([\text{HCO}_3]_{\text{BL}}\) of 22 mM and a fixed \(\text{pH}_{\text{BL}}\) of 7.4. The statistical analysis of these \(J_{\text{HCO}_3}\) data was part of the same ANOVA discussed two paragraphs above. Compared with the control condition, high-dose basolateral ANG II produced a statistically significant decrease in \(J_{\text{HCO}_3}\) at all three levels of \([\text{CO}_2]_{\text{BL}}\): 0% \((P = 0.0049)\), 5% \((P < 0.0001)\), and 20% \((P < 0.0001)\). Viewed differently, high-dose basolateral ANG II flattens the relationship between \(J_{\text{HCO}_3}\) and \([\text{CO}_2]_{\text{BL}}\) at low levels of \([\text{CO}_2]_{\text{BL}}\) (i.e., 0–5%) and eliminates \(\text{CO}_2\) sensitivity at higher \([\text{CO}_2]_{\text{BL}}\) levels.

for low-dose ANG II, and with those made in rats by Chat-suadhthipong and Chan (14) for high-dose ANG II.

**Effects of basolateral ANG II on the basolateral \(\text{CO}_2\) dependence of \(J_{\text{HCO}_3}\) and \(J_V\).** A previous study from our laboratory demonstrated that increasing \([\text{CO}_2]_{\text{BL}}\) from 0 to 20%, using OOE technology to fix \([\text{HCO}_3]_{\text{BL}}\) at 22 mM and fix \(\text{pH}_{\text{BL}}\) at 7.4, substantially stimulated bicarbonate reabsorption by the rabbit S2 PT (43). In the present study, we examined the effects of low- or high-dose basolateral ANG II on the basolateral \(\text{CO}_2\) dependence of \(J_{\text{HCO}_3}\). In this series of experiments, the bath contained \(10^{-11}\) or \(10^{-9}\) M ANG II plus an equilibrated 5% \(\text{CO}_2/22\) mM \(\text{HCO}_3/\text{pH} 7.40\) solution during the first collection period, and an OOE solution containing the same level of ANG II and the same 22 mM \(\text{HCO}_3/\text{pH} 7.40\) but a variable level of \(\text{CO}_2\) during the second collection period.

**Effect of low-dose ANG II.** The diamonds in Fig. 2A summarize \(J_{\text{HCO}_3}\) data obtained in the presence of \(10^{-11}\) M basolateral ANG II as we increased \([\text{CO}_2]_{\text{BL}}\) from 0% \(\text{CO}_2\) to 20% \(\text{CO}_2\) (solution 5) to 20% \(\text{CO}_2\) (solution 8) at a fixed \([\text{HCO}_3]_{\text{BL}}\) of 22 mM and a fixed \(\text{pH}_{\text{BL}}\) of 7.4. The circles summarize comparable control data in the absence of added ANG II. These control data are from an earlier study (43), augmented by 12 additional experiments at 5% \(\text{CO}_2\) from Fig. 1A. In the previous section, we discussed the use of one-way ANOVA for five groups to analyze the data at \([\text{CO}_2]_{\text{BL}}\) values of 0 and 20%. The overall \(P\) values were <0.0001 for both the 0 and 20% data. In addition, we
The statistical analysis of the \(J_V\) data was part of the same \(J_V\) ANOVA discussed two paragraphs above. Compared with the control situation with no added hormone (circles in Fig. 2B), high-dose basolateral ANG II (squares) had no significant effect on \(J_V\) (Fig. 2B).

**Effects of luminal ANG II on \(J_{HCO_3}\) and \(J_V\) with equilibrated \(CO_2/HCO_3\) solutions in the bath.** In our next set of studies, all of the data came from the first collection period of experiments. The open bar in Fig. 3A repeats the control \(J_{HCO_3}\) data from Fig. 1A. The gray bar in Fig. 3A represents the effect on \(J_{HCO_3}\) of perfusing the lumen with \(10^{-11}\) M ANG II, and the filled bar represents the effect on \(J_{HCO_3}\) of perfusing the lumen with \(10^{-9}\) M ANG II. The statistical analysis of these \(J_{HCO_3}\) data was part of the same \(J_{HCO_3}\) ANOVA discussed in conjunction with Fig. 1A. Luminal \(10^{-11}\) M ANG II significantly increased \(J_{HCO_3}\), from \(56 \pm 3\) to \(76 \pm 7\) pmol·min\(^{-1}\)·mm\(^{-1}\) (\(P = 0.011\)). In contrast, adding a “high dose” of \(10^{-9}\) M ANG II caused \(J_{HCO_3}\) to change by a statistically insignificant amount, from \(56 \pm 3\) to \(46 \pm 4\) pmol·min\(^{-1}\)·mm\(^{-1}\) (\(P = 0.34\)).

The stimulation by \(10^{-11}\) M ANG II that we observed confirms the observation by others in rat PCTs by Wang and Chan (39). These same authors found that \(10^{-8}\) M luminal ANG II reduced \(J_{HCO_3}\), in rat, whereas we observed no significant effect at \(10^{-9}\) M. In rabbit PCTs, Baum et al. (2) found no effect of luminal ANG II at concentrations from \(10^{-11}\) to \(2 \times 10^{-8}\) M. However, in the presence of luminal enalaprilat, these authors found that \(10^{-10}\) M luminal ANG II did indeed increase \(J_{HCO_3}\). Two technical differences between our study and that of Baum et al. is that they perfused the bath at 0.5 ml/min (vs. 7.0 ml/min) and added 6 g/dl albumin to the bath throughout the experiment (vs. 2 g/dl only during the warm-up period).

The statistical analysis of the \(J_V\) data in Fig. 3B was part of the same \(J_V\) ANOVA discussed in conjunction with Fig. 1B. Luminal \(10^{-11}\) M ANG II changed the mean \(J_V\) by a statistically insignificant amount from \(0.46 \pm 0.03\) to \(0.60 \pm 0.06\) nl·min\(^{-1}\)·mm\(^{-1}\) (\(P = 0.14\)). Others had observed a stimulation by low-dose ANG II on rabbit PCTs (17, 25). We found that adding \(10^{-9}\) M luminal ANG II significantly increased \(J_V\) from \(0.46 \pm 0.03\) to \(0.67 \pm 0.06\) nl·min\(^{-1}\)·mm\(^{-1}\) (\(P = 0.014\)), which is in a direction opposite that seen by others in the rabbit (17, 25) or rat (39).

**Effects of luminal ANG II on the basolateral \(CO_2\) dependence of \(J_{HCO_3}\) and \(J_V\).** In this series of studies, the lumen contained \(10^{-11}\) or \(10^{-9}\) M ANG II throughout the experiment. During the first collection period, the bath contained equilibrated 5% \(CO_2/22\) mM \(HCO_3/\text{pH 7.40}\), while during the second collection period the bath contained an OSE solution with the same 22 mM \(HCO_3/\text{pH 7.40}\) but a variable level of \(CO_2\).

**Effect of low-dose ANG II.** The diamonds in Fig. 4A summarize \(J_{HCO_3}\) data obtained in the presence of \(10^{-11}\) M luminal ANG II as we increased \([CO_2]_{BL}\) from 0% \(CO_2\) (solution 5) to 20% \(CO_2\) (solution 8) at a fixed \([HCO_3]_{BL}\) of 22 mM and a fixed \(pH_{BL}\) of 7.4. The diamond at 5% \(CO_2\) represents the same data that we already presented in Fig. 3A (see bar labeled the \(10^{-11}\) M). The circles summarize the control data in the absence of added ANG II. These control data are the same as those presented in Fig. 2A. The statistical analysis of these \(J_{HCO_3}\) data was part of the same \(J_{HCO_3}\) ANOVA discussed in conjunction with Fig. 2A. Compared with the control condition, \(10^{-11}\) M luminal ANG II produced a statistically significant increase in \(J_{HCO_3}\), at 0% \((P = 0.0023)\) and 5% \(CO_2\) \((P = 0.011)\). However, the difference was not statistically significant at a \([CO_2]_{BL}\) of 20% \((P = 0.055)\). Viewed differently, low-dose luminal ANG II produces a modest upward shift of the relationship between \(J_{HCO_3}\) and \([CO_2]_{BL}\) and low \([CO_2]_{BL}\) values but eliminated the stimulation by 20% \(CO_2\).

The statistical analysis of the \(J_V\) data was part of the same \(J_V\) ANOVA discussed in conjunction with Fig. 2B. Compared with the control situation with no added hormone (circles in Fig. 4B), low-dose luminal ANG II (diamonds) produced a statistically significant increase in \(J_V\) (diamonds) at 0% \(CO_2\) \((P = 0.0020)\) but did not have a significant effect at 5 \((P = 0.14)\) or 20% \(CO_2\) \((P = 0.99)\).

**Effect of high-dose ANG II.** The squares in Fig. 4A summarize \(J_{HCO_3}\), data obtained in the presence of \(10^{-9}\) M luminal ANG II as we increased \([CO_2]_{BL}\) from 0% \(CO_2\) to 20% \(CO_2\) at a fixed \([HCO_3]_{BL}\) of 22 mM and a fixed \(pH_{BL}\) of 7.4. The statistical analysis of these \(J_{HCO_3}\) data was part of the same \(J_{HCO_3}\) ANOVA discussed in conjunction with Fig. 2A and the diamonds in Fig. 4A. Compared with the control condition, high-dose luminal ANG II had no effect on \(J_{HCO_3}\), at 0% \(CO_2\) \((P = 1.00)\) or 5% \(CO_2\) \((P = 0.34)\), but produced a statistically significant decrease at 20% \(CO_2\) \((P < 0.0001)\). Viewed differently, high-dose basolateral ANG II flattened the relationship between \(J_{HCO_3}\) and \([CO_2]_{BL}\).

The statistical analysis of these \(J_V\) data was part of the same \(J_V\) ANOVA discussed in conjunction with Fig. 2B and the diamonds in Fig. 4B. Compared with the control situation with...
no added hormone (circles in Fig. 4B), high-dose luminal ANG II (diamonds) produced a statistically significant increase in Jv at 0% (P = 0.0075) and 5% CO2 (P = 0.014). The hormone did not have a statistically effect at 20% CO2 (P = 0.55).

**DISCUSSION**

**Effects of basolateral or luminal ANG II with equilibrated CO2/HCO3− solutions in the bath.** It is well established that ANG II is a potent modulator of volume and HCO3− reabsorption by the PT. ANG II, regardless of whether it is added to the basolateral or to the luminal solution, tends at low doses to increase Jv and JHCO3, but tends at high doses to decrease both parameters. Low-dose ANG II (pM range) appears to act via AT1 receptors (22, 24). Indeed, an AT1 antagonist blocks the effect of low-dose luminal ANG II on Jv and JHCO3, in rabbit PTs (2). Moreover, low-dose luminal ANG II fails to increase JHCO3, in AT1A-deficient mice (42). The AT1 receptors apparently stimulate phospholipase C (PLC), which releases diacylglycerols that in turn activate protein kinase C. The role of inositol-1,4,5-trisphosphate (IP3), which ought to be released together with diacylglycerols, is not clear. Although it has also been suggested that ANG II enhances HCO3− reabsorption by lowering intracellular levels of cAMP (27), the consensus seems to be that ANG II produces its physiological effects without altering [cAMP]. (13, 18).

High-dose ANG II (nM-μM range), like low-dose ANG II, appears to act via AT1A receptors: high-dose luminal ANG II fails to reduce JHCO3, in AT1A-deficient mice (42). Cumulative evidence suggests that, at least in part, high-dose ANG II acts via PLA2 to release arachidonic acid (AA). In the epoxygenase pathway, a cytochrome P-450 enzyme then converts this AA to a metabolite such as 5,6-EET (3, 21, 24).

Our data obtained with the equilibrated CO2/HCO3− solution, [CO2]BL = 5%, [HCO3−]BL = 22 mM, and pHBL 7.4, generally confirm earlier observations that both basolateral and luminal ANG II have biphasic effects on JHCO3. We believe that ours is the first study to examine the effects of low- or high-dose basolateral ANG II on JHCO3, in a rabbit PT.

**Mutual interdependence of the effects of basolateral ANG II and basolateral CO2 on JHCO3.** As shown in Fig. 2A, 10−11 M basolateral ANG II tends to stimulate HCO3− reabsorption at low values of [CO2]BL, but actually produces a small inhibition at the highest [CO2]BL. The upper curve in Fig. 5A is a replot of these data and confirms the general trend that, as [CO2]BL rises, the fractional stimulation produced by low-dose ANG II tends to fall, eventually turning into a small inhibition. Returning to Fig. 2A, we recall that 10−9 M basolateral ANG II inhibits HCO3− reabsorption at all values of [CO2]BL. The lower curve in Fig. 5A, a replot of these data, suggests that, as [CO2]BL rises, the fractional inhibition produced by high-dose ANG II is at first stable and then tends to increase at the highest [CO2]BL. In other words, under conditions in which the JHCO3 response to the CO2-sensing mechanism is greatest, low-dose ANG II produces the least stimulation and high-dose ANG II produces the greatest inhibition.

Another way to analyze our data is to ask how changes in [CO2]BL affect the tubule’s response to ANG II. The black curve in Fig. 6A is a replot of the data at [CO2]BL = 5% in Fig. 2A and confirms the well-known biphasic effect of basolateral ANG II under “control” basolateral acid-base conditions. The red curve in Fig. 6A is a replot of the data at [CO2]BL = 0% in Fig. 2A and shows that, even with minimal stimulation of the basolateral CO2-sensing mechanism, basolateral ANG II also has a biphasic effect on JHCO3. Finally, the green curve in Fig.

![Figure 5](http://ajprenal.physiology.org/)

**Fig. 5.** Stimulatory or inhibitory effect of ANG II on HCO3− reabsorption as a function of basolateral [CO2]. **A:** effect of basolateral ANG II. The 2 curves are replots of data from Fig. 2, with ♦ representing 10−11 M basolateral ANG II and ■ representing 10−9 M basolateral ANG II. **B:** effect of luminal ANG II. The 2 curves are replots of data from Fig. 4, with ○ representing 10−11 M luminal ANG II and □ representing 10−9 M luminal ANG II.

![Figure 6](http://ajprenal.physiology.org/)

**Fig. 6.** Effect of basolateral CO2 on the ANG II dependence of JHCO3. **A:** basolateral ANG II. The 3 curves are replots of data from Fig. 2, with the red lines representing an out-of-equilibrium solution (OOE) 0% CO2/22 mM HCO3− in the basolateral solution, the black lines representing equilibrated 5% CO2/22 mM HCO3−, and the green lines representing OOE 20% CO2/22 mM HCO3−. **B:** luminal ANG II. The 3 curves are replots of data from Fig. 4, with the colors of the lines having the same meaning as in A.
6A is a replot of the data at [CO₂]BL = 20% in Fig. 2A and shows that, with maximal stimulation of the basolateral CO₂-sensing mechanism, basolateral ANG II no longer has a biphasic effect on J₇HCO₃, both low-dose and high-dose ANG II now inhibit HCO₃⁻ reabsorption, with the effect being substantially more pronounced at 10⁻⁹ M.

**Mutual interdependence of the effects of luminal ANG II and basolateral CO₂ on J₇HCO₃.** We found that luminal ANG II tends to produce effects similar to those of basolateral ANG II, although the details are different. In Fig. 4A we saw that 10⁻¹¹ M luminal ANG II stimulates HCO₃⁻ reabsorption at 0 and 5% CO₂. The upper curve in Fig. 5B, a replot of these data, confirms that, as [CO₂]BL rises, low-dose ANG II produces a smaller fractional stimulation of HCO₃⁻ reabsorption. Focusing again on Fig. 4A, we see that 10⁻⁹ M luminal ANG II has no effect on J₇HCO₃ at the two lowest [CO₂]BL but reduces J₇HCO₃ at 20% [CO₂]BL. Indeed, the lower curve in Fig. 5B confirms that the fractional inhibition produced by high-dose ANG II progressively increases as [CO₂]BL increases. Thus, as was the case for basolateral ANG II (see Fig. 5A), low-dose luminal ANG II produces its smallest stimulation of HCO₃⁻ reabsorption, and high-dose ANG II produces its greatest inhibition, when the J₇HCO₃ response to the CO₂-sensing mechanism is maximal.

In Fig. 6B, the black curve is a replot of the data at [CO₂]BL = 5% in Fig. 4A. These results verify the biphasic effect of luminal ANG II under “control” basolateral acid-base conditions. The red curve in Fig. 6B, a replot of the data at [CO₂]BL = 0% in Fig. 4A, shows that, with minimal stimulation of the basolateral CO₂-sensing mechanism, luminal ANG II has a blunted biphasic effect on J₇HCO₃. That is, low-dose ANG II increases J₇HCO₃, but high-dose ANG II has no effect. This result contrasts to that with basolateral ANG II (see red curve in Fig. 6A), which produces a full biphasic effect (i.e., inhibition at high-dose ANG II) at [CO₂]BL = 0%. The green curve in Fig. 6B, a replot of the data at [CO₂]BL = 20% in Fig. 4A, shows that, with maximal stimulation of the basolateral CO₂-sensing mechanism, luminal ANG II still has a biphasic effect on J₇HCO₃. This result contrasts to that with basolateral ANG II (see green curve in Fig. 6A), which loses its biphasic effect at [CO₂]BL = 20%.

In conclusion, the mutual effects of low- vs. high-dose ANG II. Obviously, the distinction between a “low” stimulatory and a “high” inhibitory dose of ANG II is somewhat arbitrary and may differ according to the species studied and experimental preparation employed. In addition, our data demonstrate that the distinction between low and high also depends on whether one is examining J₇HCO₃ or J₇V. For example, a low [CO₂]BL of 20%, 10⁻⁹ M luminal ANG II lowered J₇HCO₃ (Fig. 3A) but had no significant effect on J₇V (Fig. 3B). Thus, increasing levels of [ANG II] may produce decreases in J₇HCO₃ earlier than they produce decreases in J₇V. Finally, our data indicate that the distinction between low and high depends on [CO₂]BL. Thus, at a [CO₂]BL of 20%, 10⁻¹¹ M basolateral ANG II actually inhibited HCO₃⁻ reabsorption (green curve in Fig. 6A).

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