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Angiotensin II stimulates both Na^+–H^+ exchange and Na^+/HCO_3^- cotransport in the rabbit proximal tubule

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ABSTRACT **Angiotensin II (AII) is a potent stimulus for HCO_3^- reabsorption in the rat proximal tubule *in vivo*. To determine the ionic mechanism of increased HCO_3^- reabsorption, we have examined the effect of AII on luminal Na^+–H^+ exchange and basolateral Na^+/HCO_3^- cotransport in perfused S1 proximal tubules isolated from superficial nephrons of the rabbit kidney. Transporter activity was assessed by removing Na^+ from both luminal and basolateral (i.e., bath) solutions and determining the rate at which intracellular pH (pH_i) increased after Na^+ was returned to only the lumen or only the bath. pH_i was measured with the pH-sensitive fluorescent dye 2′,7′-bis(2-carboxyethyl)-5(and 6)-carboxyfluorescein. We found that basolateral administration of 1 nM AII not only increased the rate of luminal Na^+–H^+ exchange ≈3.5-fold but also increased the rate of basolateral Na^+/HCO_3^- cotransport ≈2.5-fold. 5-(*N*-Ethyl-*N*-isopropyl)amiloride (50 μM) blocked luminal Na^+–H^+ exchange before and after stimulation by AII but had no effect on basolateral Na^+/HCO_3^- cotransport. Conversely, 4,4′-diisothiocyanato-2,2′-stilbenedisulfonate (50 μM) blocked basolateral Na^+/HCO_3^- cotransport before and after AII but had no effect on luminal Na^+–H^+ exchange. Our data thus indicate that, at least under the conditions of our assay, AII independently stimulates the transporters responsible for both the luminal and basolateral steps of transepithelial HCO_3^- reabsorption.**

The proximal tubule is the primary site of HCO_3^- reabsorption in the mammalian kidney. The work of others has shown that this HCO_3^- reabsorption can be under hormonal control. For example, parathyroid hormone inhibits both volume reabsorption (J_v) (1, 2) and HCO_3^- reabsorption (J_{HCO_3}) (3, 4). On the other hand, angiotensin II (AII) stimulates both J_v (5) and J_{HCO_3} (6, 7). In principle, this stimulation could reflect enhancement of either luminal acid-secretory mechanisms (e.g., Na^+–H^+ exchange) and/or basolateral HCO_3^--efflux mechanisms (e.g., Na^+/HCO_3^- cotransport). Therefore, in the present study we examined individually the effect of basolaterally applied AII (1 nM) on luminal Na^+–H^+ exchange and basolateral Na^+/HCO_3^- cotransport, assaying the transporters by their effect on intracellular pH (pH_i). The experiments were performed on isolated perfused superficial S1 segments of proximal tubules from rabbit kidney. We found that AII independently activates both luminal Na^+–H^+ exchange and basolateral Na^+/HCO_3^- cotransport. This synergistic effect on the two transporters is consistent with the stimulation of J_{HCO_3} by basolateral AII.

MATERIALS AND METHODS

The experiments were performed on female SPF (specific pathogen-free) New Zealand rabbits (3–4 kg). Single, superficial S1 segments of proximal tubules were dissected, perfused, and loaded with the pH-sensitive dye 2′,7′-bis(2-carboxyethyl)-5(and 6)-carboxyfluorescein (BCECF) as described (8). pH_i was measured microfluorometrically by alternately exciting the dye with a 10-μm-diameter spot of light at 440 and 490 nm while monitoring the emission at 532 nm (8, 9). The resulting fluorescence excitation ratios were converted to pH_i values as described (9), based on a high-$[K^+]$/nigericin-style calibration (10). Our extracellular solution was the standard HCO_3^- solution (11) and consisted of 145 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 1 mM $MgSO_4$, 1.4 mM $CaCl_2$, 1 mM Na_2HPO_4, 5 mM glucose, and 2 mM alanine. The solution was gassed with 5% CO_2/95% O_2. When Na^+ was removed, it was replaced mole-for-mole with *N*-methyl-D-glucammonium (Sigma); when Cl^- was removed, it was replaced with cyclamate (Sigma). In some experiments, we added 1 nM AII ([Asn^1, Val^5]AII; Sigma) and/or 50 μM 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA, from a 50 mM stock in dimethyl sulfoxide) or 50 μM 4,4′-diisothiocyanato-2,2′-stilbenedisulfonate (DIDS, added as a powder immediately before the experiment). Data are presented as mean ± SEM. We tested significance by using paired or unpaired *t* tests, as indicated. In averaging ratios, we assumed a log-normal distribution.

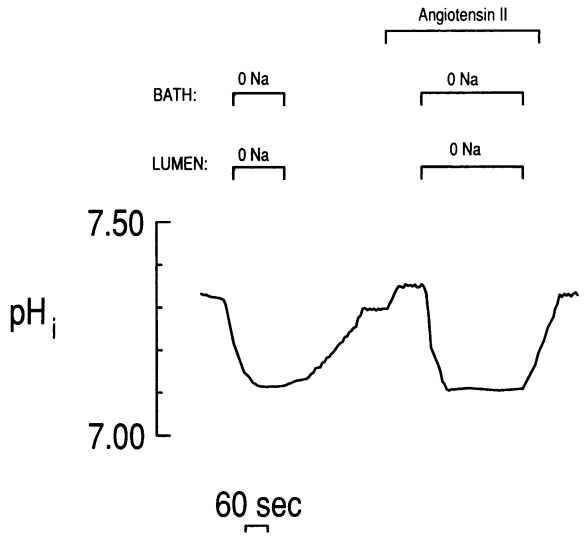

FIG. 1. Effect of 1 nM AII on the rate of recovery from an acid load imposed by bilateral Na^+ removal. Removal of Na^+ from both the lumen and bath caused a rapid decrease in pH_i, whereas bilateral readdition of Na^+ caused a rapid increase. Addition of AII to the bath elicited a prolonged alkalinization and greatly increased the rate of pH_i recovery from the acid load.

RESULTS

Effect of AII on pH_i. Simultaneous removal of Na^+ from lumen and bath caused a rapid and sustained decrease in pH_i

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Abbreviations: AII, angiotensin II; BCECF, 2′,7′-bis(2-carboxyethyl)-5(and 6)-carboxyfluorescein; DIDS, 4,4′-diisothiocyanato-2,2′-stilbenedisulfonate; EIPA, 5-(*N*-ethyl-*N*-isopropyl)amiloride; pH_i, intracellular pH.

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Table 1. Effect of AII on steady-state pH_i, and on the rate of pH_i increase (dpH_i/dt) following readdition of bilateral, luminal, or bath Na^+

Na^+ readdition	n	ΔpH_i caused by AII	$(dpH_i/dt) \times 10^4$, sec^{-1}		pH_i of dpH_i/dt measurement	dpH_i/dt AII/control ratio
			Control	AII		
Bath and lumen						
Presence of Cl^-	10	0.10 ± 0.02	308 ± 19	824 ± 25	6.79 ± 0.14	2.8
Bilateral 0 Cl^-	10	0.08 ± 0.01	282 ± 26	795 ± 37	6.73 ± 0.17	2.8
Lumen						
Presence of Cl^-	5	0.09 ± 0.02	183 ± 11	652 ± 14	6.68 ± 0.08	3.6
Bilateral 0 Cl^-	5	0.07 ± 0.03	180 ± 20	602 ± 31	6.76 ± 0.18	3.3
Bath						
Presence of Cl^-	5	0.09 ± 0.03	151 ± 17	380 ± 38	6.60 ± 0.08	2.5
Bilateral 0 Cl^-	5	0.08 ± 0.02	155 ± 24	392 ± 45	6.63 ± 0.08	2.5

(Fig. 1). When Na^+ was returned bilaterally, pH_i rapidly returned to approximately its initial value (Table 1). Addition of 1 nM AII to the bath caused a sustained pH_i increase of ≈0.05; in 10 similar experiments the alkalinization averaged 0.10 (Table 1). After a second period of bilateral Na^+ removal in the continued presence of AII, bilateral Na^+ readdition elicited a pH_i recovery that was substantially faster than in the absence of AII. For each of 10 tubules, we compared the rate of alkalinization (dpH_i/dt) at the lowest pH_i (which averaged 6.79) common to both the control and AII pH_i recoveries; dpH_i/dt at this pH_i was 308×10^{-4} sec^{-1} under control conditions, but 824×10^{-4} sec^{-1} in the presence of AII (Table 1). The average stimulation was 2.8-fold.

Effect of AII on Luminal Na^+–H^+ Exchange. The above experiments demonstrate that a luminal and/or a basolateral Na^+-dependent acid–base transporter is stimulated by AII. Inasmuch as Liu and Cogan (7) suggested that the stimulation of J_{HCO_3} by AII is due to increased Na^+–H^+ exchange activity, we removed Na^+ bilaterally and then monitored the pH_i recovery after returning the Na^+ to only the lumen. Fig. 2*A* is a composite of three experiments in which we twice returned Na^+ to the lumen, first in the absence and then in the presence of AII. One experiment was conducted in the absence of inhibitors, a second in the presence of luminal DIDS (to block HCO_3^- transport), and a third in the presence of luminal EIPA (to block Na^+–H^+ exchange). In the absence of AII (Fig. 2*A Left*), returning Na^+ to only the lumen caused a rapid pH_i recovery (i.e., increase) when inhibitors were not present. Mean data are summarized in Table 2. If anything, 50 μM DIDS slightly accelerated this luminal-Na^+-dependent pH_i recovery, whereas 50 μM EIPA (added in the absence of Na^+) almost completely blocked it. In the presence of AII (Fig. 2*A Right*), returning Na^+ to only the lumen caused an even more rapid pH_i recovery under inhibitor-free conditions† ($P < 0.001$; Table 1, line 2). Once again DIDS had almost no effect, whereas EIPA virtually blocked the recovery. The lack of effect of DIDS and the near-complete blockade by EIPA suggest that the only acid-extrusion mechanism operative during this protocol was a luminal Na^+–H^+ exchanger. As summarized in Table 1, AII increased the EIPA-sensitive component of the pH_i recovery (i.e., Na^+–H^+ exchange) 3.6-fold.

As noted in the discussion of Fig. 1, addition of AII caused a sustained increase in pH_i. Not shown in Fig. 2*A* are the portions of experiments demonstrating that luminal EIPA failed to significantly reduce this AII-induced alkalinization, which averaged 0.10 ± 0.02 in the absence of EIPA and 0.05 ± 0.02 in the presence of EIPA ($P < 0.01$). Thus, it appears that the AII-induced alkalinization is not mediated by a Na^+–H^+ exchanger.

Effect of AII on Basolateral Na^+/HCO_3^- Cotransport. *Dependence on basolateral Na^+.* In a separate series of experiments, we removed Na^+ bilaterally and then returned Na^+ to only the bath. Mean data are summarized in Tables 1 and 2. In the absence of AII (Fig. 2*B Left*), returning Na^+ to only the bath caused a rapid pH_i recovery under inhibitor-free conditions. Addition of 50 μM EIPA to the bath had almost no effect on this basolateral-Na^+-dependent pH_i recovery, whereas addition of 50 μM DIDS to the bath almost completely blocked it. Thus, the pH_i increase is mediated by a transporter that is Na^+-dependent and DIDS-sensitive, presumably mediating the net influx of HCO_3^-. Two such transporters have been described, a Na-dependent Cl^-–HCO_3^- exchanger (which would be operating in its normal, acid-extruding direction) and an electrogenic Na^+/HCO_3^- cotransporter (which would be operating in reverse of its normal, acid-loading direction). In the presence of AII (Fig. 2*B Right*), returning Na^+ to only the bath caused an even more rapid pH_i recovery under inhibitor-free conditions‡ ($P < 0.001$; Table 1, line 3). As before, EIPA had almost no effect, whereas DIDS virtually blocked the pH_i increase. As summarized in Table 1, these results indicate that AII stimulates a basolateral-Na^+-dependent, DIDS-sensitive transporter ≈2.5-fold.

Lack of Cl^- involvement. To distinguish between a Na^+-dependent Cl^-–HCO_3^- exchanger and an electrogenic Na^+/HCO_3^- cotransporter, we repeated the protocols of Figs. 1 and 2*B* in the bilateral absence of Cl^-. The tubules were in a Cl^--free solution (Cl^- replaced with cyclamate) from the beginning of dissection and throughout the experiment, a minimum of 45 min to the time of assay. In one series of experiments, conducted in the absence of inhibitors, we determined the rate of pH_i recovery after returning Na^+ bilaterally before and after introduction of AII (Fig. 1 protocol). As summarized in Table 1, Cl^- removal had no significant effect on dpH_i/dt, either under control or under AII-stimulated conditions. In a second series of experiments, conducted in either the presence or absence of DIDS, we determined dpH_i/dt after returning Na^+ to only the bath (Fig. 2*B* protocol). As summarized in Table 3, Cl^- removal did not

†As discussed below under Na^+/HCO_3^- cotransport, we found that Na^+ readdition to the bath failed to produce a pH_i recovery in the presence of 50 μM bath DIDS. When Na^+ was then returned to the lumen, in the continued presence of bath Na^+ and DIDS, pH_i recovered rapidly. Under these conditions, the pH_i recovery rate in the presence of Na^+ was $173 \pm 19 \times 10^{-4}$ sec^{-1} in the absence of AII and $610 \pm 16 \times 10^4$ sec^{-1} in the presence of AII, at a lowest common pH_i of 6.76 ± 0.08 ($n = 5$). Thus, AII increased the pH_i recovery rate by a factor of 3.5.

‡As discussed above under Na^+–H^+ exchange, we found that Na^+ readdition to the lumen failed to produce a pH_i recovery in the presence of 50 μM luminal EIPA. When Na^+ was then returned to the bath, in the continued presence of luminal Na^+ and EIPA, pH_i recovered rapidly. Under these conditions, the pH_i recovery rate in the presence of Na^+ was $155 \pm 22 \times 10^{-4}$ sec^{-1} in the absence of AII and $395 \pm 18 \times 10^{-4}$ sec^{-1} in the presence of AII, at a lowest common pH_i of 6.66 ± 0.18 ($n = 5$). Thus, AII increased the pH_i recovery rate by a factor of 2.6.

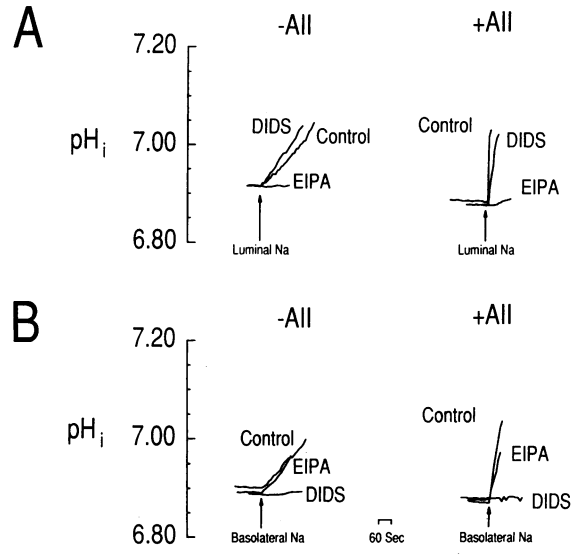

FIG. 2. Effects of AII on the rates of recovery from an acid load in the presence and absence of the inhibitors EIPA and DIDS. (*A*) Effect on the pH_i increase caused by readdition of Na^+ to the lumen. AII greatly increased the rate of pH_i recovery. Both in the presence (*Right*) and in the absence (*Left*) of AII, luminal EIPA virtually blocked the recovery, whereas luminal DIDS had a minimal effect. This figure is a composite of three experiments, in which only the portions immediately before and after readdition of luminal Na^+ are shown. Data from the same three tubules are used in *Right* and *Left*. (*B*) Effect on the pH_i increase caused by readdition of Na^+ to the bath. As in *A*, this is a composite of three experiments. AII greatly increased the pH_i recovery. Both in the presence (*Right*) and in the absence (*Left*) of AII, bath DIDS virtually blocked the recovery, whereas bath EIPA had a minimal effect.

significantly affect the DIDS-sensitive component of the pH_i recovery. Thus, if we assume that the period of Cl^- washout was sufficient to block Na^+-dependent Cl^-–HCO_3^- exchange, these results indicate that Na^+/HCO_3^- cotransport is the dominant Na^+-dependent acid–base transporter at the basolateral membrane of the superficial S1 segment and that this is stimulated ≈2.5-fold by AII.

DISCUSSION

Luminal Na^+–H^+ Exchange. There is substantial evidence that AII directly affects proximal-tubule transport functions (12). In particular, AII increases the rate of both Na^+ and volume reabsorption in the rat (13, 14) and rabbit (5) proximal convoluted tubule. More recently, Liu and Cogan (6) confirmed that AII increases volume reabsorption in the S1 and S2 segments of the rat proximal tubule. Moreover, they showed that AII increases HCO_3^- reabsorption 1.5-fold in the early segment of the proximal tubule (6). It is reasonable to expect that this effect of AII could at least in part be due to stimulation of luminal Na^+–H^+ exchange, inasmuch as this transporter has been identified in a variety of mammalian proximal-tubule preparations (15, 16), though not yet in rabbit superficial S1. In the present study, conducted on rabbit superficial S1 segments, we have demonstrated the presence of a luminal EIPA-sensitive Na^+–H^+ exchanger and found that it is stimulated ≈250% by AII. Note that we assayed Na^+–H^+ exchange activity at an average pH_i of 6.68, below the pH_i normally prevailing in the presence of CO_2/HCO_3^-, 7.33. It remains to be seen how the activity of the luminal Na^+–H^+ exchanger in superficial S1 segments depends upon pH_i, and how this pH_i dependence is affected by AII. If AII also stimulates the Na^+–H^+ exchanger in the physiological pH_i range, then this effect could contribute to the stimulation of HCO_3^- reabsorption by AII.

Table 2. Effect of bath DIDS, luminal EIPA, and Cl^--free solutions on the rate of pH_i increase following readdition of bilateral, luminal, or bath Na^+ in the absence and presence of AII

Condition	*n*	ΔpH_i caused by EIPA or DIDS	ΔpH_i caused by AII*	With Na^+ returned to lumen						With Na^+ returned to bath					
				Control		AII		dpH_i/dt AII/control ratio		Control		AII		dpH_i/dt AII/control ratio
				dpH_i/dt†	pH_i	dpH_i/dt†	pH_i			dpH_i/dt†	pH_i	dpH_i/dt†	pH_i	
50 μM EIPA in lumen	5	−0.01 ± 0.01	0.05 ± 0.02	20 ± 8	6.66 ± 0.18	20 ± 8	6.67 ± 0.05	1.0		155 ± 22	6.77 ± 0.07	395 ± 18	6.77 ± 0.07	2.6
50 μM DIDS in bath	5	0.03 ± 0.01	0.07 ± 0.02	173 ± 19	6.81 ± 0.07	610 ± 16	6.81 ± 0.07	3.5		24 ± 8	6.76 ± 0.08	25 ± 15	6.76 ± 0.08	1.0
Bilateral 0 Cl^-, 50 μM DIDS in bath	5	0.03 ± 0.01	0.07 ± 0.02	165 ± 25	6.83 ± 0.12	595 ± 35	6.83 ± 0.12	3.4		26 ± 9	6.70 ± 0.15	27 ± 8	6.73 ± 0.15	1.0

*pH_i change caused by AII in the presence of the inhibitor condition (e.g., 50 μM DIDS).
†Value × 10^4, sec^{-1}.

Table 3. Inhibitor (EIPA or DIDS)-sensitive components of pH_i recovery following readdition of luminal or bath Na^+ in the absence and presence of AII

			$(dpH_i/dt) \times 10^4$, sec^{-1}					
			Without AII			With AII		
Condition	Inhibitor	*n*	Without inhibitor	With inhibitor	Inhibitor-sensitive	Without inhibitor	With inhibitor	Inhibitor-sensitive
Na^+ returned to lumen	EIPA	5	183	20	163	652	20	632
Na^+ returned to bath	DIDS	5	151	24	127	380	20	355
Bilateral 0 Cl^-, Na^+ returned to bath	DIDS	5	155	26	129	392	27	365

Basolateral Na^+/HCO_3^- Cotransport. Basolateral Na^+/HCO_3^- cotransport (17) also has been identified in a variety of proximal-tubule preparations, including those from the rat (18, 19) and rabbit (15, 16, 20–22). An earlier study (8) conducted on the rabbit S1 in the nominal absence of HCO_3^- provided evidence for a basolateral acid–base transporter that was dependent on Na^+, blocked by DIDS, and independent of Cl^-. The most plausible explanation for these earlier data is that Na^+/HCO_3^- cotransport was supported by ambient and or metabolically generated HCO_3^-. We have now extended these observations by conducting experiments in the presence of HCO_3^-. Thus, although data on voltage and $[Na^+]_i$ transients are still lacking, it is likely that a potent Na^+/HCO_3^- cotransporter exists at the basolateral membrane of the rabbit superficial S1. Our data also indicate that when the cotransporter is assayed by returning Na^+ to the bath, it is stimulated ≈150% by AII. However, it should be pointed out that the transporter was running backwards during the assay and that the prevailing pH_i (≈6.60) was below the normal pH_i. It remains to be seen how the activity of the forward-running Na^+/HCO_3^- cotransporter depends upon pH_i, and how this pH_i dependence is affected by AII. If the forward-running cotransporter is also enhanced by AII at a physiological pH_i, then this effect could contribute to the stimulation of HCO_3^- reabsorption by AII.

Steady-State pH_i. The observation by Liu and Cogan (6) that AII enhances HCO_3^- reabsorption implies that, in the presence of the hormone, acid–base transporters at both the luminal membrane (Na^+–H^+ exchange) and the basolateral membrane (Na^+/HCO_3^- cotransport) must be operating more rapidly than normal. However, the hormone need not affect both transporters directly. For example, AII could alter the kinetics of Na^+–H^+ exchange so as to stimulate the exchanger and raise pH_i. This alkalinization might secondarily enhance Na^+/HCO_3^- cotransport, even though the kinetics of this cotransporter would not be fundamentally altered. Conversely, AII could directly stimulate Na^+/HCO_3^- cotransport, thereby lowering pH_i. This might secondarily stimulate Na^+–H^+ exchange, without altering its kinetics. We found that 1 nM AII elicits a modest increase in steady-state pH_i. Although this observation is consistent with the hypothesis that only the Na^+–H^+ exchanger is stimulated by the hormone, this explanation is unlikely, given the observation that blockade of Na^+–H^+ exchange by luminal EIPA fails to eliminate the AII-induced pH_i increase. As to the mechanism of this AII-induced alkalinization, it is possible that the hormone stimulates another acid-extruding process (e.g., a luminal H^+ pump) or inhibits an acid-loading process (e.g., metabolic production of acid).

Inasmuch as it appeared that AII does not stimulate only Na^+–H^+ exchange, we designed experiments to assay each transporter independently of the activity of the contralateral transporter. For example, when we removed Na^+ bilaterally and then returned it to only the lumen, the luminal Na^+–H^+ exchanger was presumably stimulated both by the low pH_i and the low$[Na^+]_i$. Thus, the EIPA-sensitive component of the initial pH_i recovery state (i.e., Na^+–H^+ exchange rate) should have been independent of the activity of the basolateral Na^+/HCO_3^- cotransporter. In addition, we found that basolateral DIDS had no significant effect on the rate of pH_i increase elicited by returning Na^+ to the lumen (Table 2, line 3). Conversely, luminal EIPA did not significantly affect the rate of pH_i recovery upon return of Na^+ to the bath. Our results thus indicate that, at least under the conditions of our assays, AII causes a direct and substantial stimulation of both the Na^+–H^+ exchanger and the Na^+/HCO_3^- cotransporter.

In conclusion, AII stimulates both the luminal and basolateral steps of HCO_3^- reabsorption in a coordinated fashion. The net effect of stimulating both an intracellular acid extruder (luminal Na^+–H^+ exchange) and an intracellular acid loader (basolateral Na^+/HCO_3^- cotransport) would thus be to increase greatly the transepithelial of HCO_3^-, with only a modest change in pH_i.

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1. Morel, F. & Doucet, A. (1986) *Physiol. Rev.* **66,** 377–468.
2. Hamburger, R, Jr., Lawson, N. & Schwartz, J. (1976) *Am. J. Physiol.* **230,** 286–290.
3. Dennis, V. & Brazy, P. C. (1979) *J. Clin. Invest.* **64,** 387–397.
4. Gullans, S. R., Brazy, P. C., Dennis, V. W. & Mandel, L. J. (1984) *Am. J. Physiol.* **246,** F859–F869.
5. Schuster, V. L., Kokko, J. P. & Jacobson, H. R. (1984) *J. Clin. Invest.* **73,** 507–515.
6. Liu, F.-Y. & Cogan, M. G. (1987) *J. Clin. Invest.* **80,** 272–275.
7. Liu, F.-Y. & Cogan, M. G. (1988) *J. Clin. Invest.* **82,** 601–607.
8. Geibel, J. P., Giebisch, G. & Boron, W. F. (1989) *Am. J. Physiol.* **257,** F790–F797.
9. Boyarsky, G., Ganz, M. B., Sterzel, B. & Boron, W. F. (1988) *Am. J. Physiol.* **255,** C844–C856.
10. Thomas, J. A., Buchsbaum, R. N., Zimniak, A. & Racker, E. (1979) *Biochemistry* **81,** 2210–2218.
11. Geibel, J. P., Giebisch, G. & Boron, W. F. (1989) *Am. J. Physiol.* **257,** F586–F594.
12. Harris, P. J. & Navar, L. (1985) *Am. J. Physiol.* **248,** 621–630.
13. Harris, P. J. & Young, J. (1977) *Pflügers Arch.* **367,** 295–297.
14. Harris, P. J., Navar, L. G. & Ploth, D. W. (1984) *Clin. Sci.* **66,** 541–544.
15. Krapf, F., Alpern, R. J., Rector, F. C., Jr., & Berry, C. A. (1987) *J. Gen. Physiol.* **90,** 833–853.
16. Kurtz, I. (1987) *J. Clin. Invest.* **80,** 928–935.
17. Boron, W. F. & Boulpaep, E. L. (1983) *J. Gen. Physiol.* **81,** 53–94.
18. Yoshitomi, K., Burckhardt, B.-C.h. & Fromter, E. (1985) *Pflügers Arch.* **405,** 360–366.
19. Alpern, R. J. (1985) *J. Gen. Physiol.* **86,** 613–636.
20. Kondo, Y. & Fromter, E. (1987) *Pflügers Arch.* **410,** 481–486.
21. Nakhoul, H. L., Chen, L. K. & Boron, W. F. (1990) *Am. J. Physiol.* **258,** F371–F381.
22. Krapf, R., Berry, C. A., Alpern, R. J. & Rector, F. C., Jr. (1988) *J. Clin. Invest.* **81,** 381–389.