Effects of vasopressin and bradykinin on anion transport by the rat cortical collecting duct. Evidence for an electroneutral sodium chloride transport pathway.

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Research Article

Our previous studies in cortical collecting ducts isolated from rat kidneys have shown that vasopressin increases both sodium absorption and potassium secretion, while bradykinin inhibits sodium absorption without affecting potassium transport. To determine which anions are affected by these agents, we perfused cortical collecting ducts from rats treated with deoxycorticosterone and measured net chloride flux, net bicarbonate flux (measured as total CO2), transepithelial voltage, and the rate of fluid absorption. Arginine vasopressin (10(-10) M in the peritubular bath) caused a sustained sixfold increase in net chloride absorption and a two- to threefold increase in the magnitude of the lumen negative transepithelial voltage. Before addition of vasopressin, the tubules secreted bicarbonate. Vasopressin abolished the bicarbonate secretion, resulting in net bicarbonate absorption (presumably due to proton secretion) in many tubules. Bradykinin (10(-9) M added to the peritubular bath) caused a reversible 40% inhibition of net chloride absorption, but did not affect the transepithelial voltage or the bicarbonate flux. We concluded: (a) that arginine vasopressin stimulates absorption of chloride and inhibits bicarbonate secretion (or stimulates proton secretion) in the rat cortical collecting duct; and (b) that bradykinin inhibits net chloride absorption in the rat cortical collecting duct without affecting transepithelial voltage or bicarbonate flux. Combining these results with the previous observations on cation fluxes described above, we conclude that bradykinin inhibits electroneutral NaCl […]

Effects of Vasopressin and Bradykinin on Anion Transport by the Rat Cortical Collecting Duct

Evidence for an Electroneutral Sodium Chloride Transport Pathway

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Abstract

Our previous studies in cortical collecting ducts isolated from rat kidneys have shown that vasopressin increases both sodium absorption and potassium secretion, while bradykinin inhibits sodium absorption without affecting potassium transport. To determine which anions are affected by these agents, we perfused cortical collecting ducts from rats treated with deoxycorticosterone and measured net chloride flux, net bicarbonate flux (measured as total CO_2), transepithelial voltage, and the rate of fluid absorption. Arginine vasopressin (10^{-10} M in the peritubular bath) caused a sustained sixfold increase in net chloride absorption and a two- to threefold increase in the magnitude of the lumen negative transepithelial voltage. Before addition of vasopressin, the tubules secreted bicarbonate. Vasopressin abolished the bicarbonate secretion, resulting in net bicarbonate absorption (presumably due to proton secretion) in many tubules. Bradykinin (10^{-9} M added to the peritubular bath) caused a reversible 40% inhibition of net chloride absorption, but did not affect the transepithelial voltage or the bicarbonate flux. We concluded: (*a*) that arginine vasopressin stimulates absorption of chloride and inhibits bicarbonate secretion (or stimulates proton secretion) in the rat cortical collecting duct; and (*b*) that bradykinin inhibits net chloride absorption in the rat cortical collecting duct without affecting transepithelial voltage or bicarbonate flux. Combining these results with the previous observations on cation fluxes described above, we conclude that bradykinin inhibits electroneutral NaCl absorption (or stimulates electroneutral NaCl secretion) in the rat cortical collecting duct.

Introduction

It is generally recognized that the cortical collecting duct is an important site of control of renal electrolyte excretion. However, both the mechanisms of control and the transport pathways involved are incompletely understood. Previously, we reported that both bradykinin (BK)[1] and arginine vasopressin (AVP) have important effects on monovalent cation transport in isolated perfused cortical collecting ducts dissected from rats (1). Bradykinin in the peritubular bathing solution (but not in the lumen) inhibited net sodium absorption. Bradykinin did not affect net potassium transport or transepithelial potential difference, which indicates that it most likely inhibited electroneutral sodium absorption (or stimulated electroneutral sodium secretion). In contrast, arginine vasopressin not only markedly increased sodium absorption, but vasopressin also increased the lumen negative transepithelial potential difference and increased net potassium secretion. Therefore, vasopressin apparently stimulated electrogenic sodium absorption.

To maintain electroneutrality, net transport of positive charges across an epithelium must be accompanied by net transport of an equal number of negative charges, independent of the transport mechanisms involved. Consequently, when vasopressin or bradykinin alters net cation transport across the rat cortical collecting duct, anion transport must also change. Learning which anions are affected by the peptides can shed light on the nature of the transport pathways regulated. Furthermore, the peptides could be important in the regulation of the balance of the anions themselves. Consequently, in this study we have investigated the effects of bradykinin and arginine vasopressin on bicarbonate and chloride transport by the cortical collecting duct of the rat.

Methods

Pathogen-free male Sprague-Dawley rats weighing 65–100 g (Small Animal Breeding Facility, National Institutes of Health, Bethesda, MD) were injected with deoxycorticosterone pivalate (5 mg i.m., Percorten pivalate suspension, Ciba Geigy Corp., Ardsley, NY) 7–15 d before experiments. This is a "depot" preparation, which gives a sustained release of the hormone for at least 4 wk. The rats were isolated in closed cages and received autoclaved food (NIH-31 diet, Ralston Purina Co., St. Louis, MO) and bedding. These procedures make possible the dissection of single nephron segments from rat kidneys without enzymatic treatment of the tissue (2).

Both kidneys were removed rapidly from decapitated rats and coronal slices were prepared. Cortical collecting ducts were dissected at 17°C from corticomedullary rays stripped from the slices. The dissection solution contained (in mM): NaCl, 118; $NaHCO_3$, 25; K_2HPO_4, 2.0; $MgSO_4$, 1.2; Ca lactate, 2.0; Na citrate, 1.0; l-alanine, 6.0; and glucose, 5.5. The dissection solution was gassed with 95% O_2 and 5% CO_2 before and during the dissection.

Tubules were mounted on concentric pipettes as described previously (3). The perfusion and bath solutions were identical to the dissection solution (described above) except that [^{14}C]inulin (New England Nuclear,

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; BK, bradykinin; C, control; J_{Cl}, chloride flux; J_{TCO_2}, total carbon dioxide flux; J_v, rate of fluid absorption; PD, transepithelial potential difference.

Boston, MA) was added to the perfusate (20 μCi/ml) to serve as a volume marker for the measurement of fluid absorption. Two recent modifications of the perfusion technique were employed. First, DC200 dielectric fluid (Dow Corning Corp., Midland, MI) was used instead of Sylgard 184 in the guard pipette at the collecting end (1). This material, like Sylgard, effectively prevents leaks between the bath and the tubule-holding pipette at the collection end, but because of its lower density, it has a lesser tendency to constrict the end of the tubule, allowing steady flow even at very low perfusion rates. Second, a continuously flowing bath exchange system with a gas-liquid mixer just before the input to the bath chamber was used as previously described (1). This system allowed continuous delivery to the bath chamber of fluid that was freshly equilibrated with 95% O_2/5% CO_2. The bath exchange rate was 0.4 ml/min. In addition, the perfusion chamber was suffused with 95% O_2/5% CO_2 throughout the experiments. Previously, it was shown that this system maintains the bath solution at pH 7.44–7.46 (1).

After they were mounted on the pipettes, the tubules were warmed to 37°C and then equilibrated for 20–30 min at that temperature while adjusting the perfusion rate to ~1 nl/min per mm. The lengths of the perfused segments ranged from 0.4 to 1.0 mm with a mean of 0.61 mm. Flow rates were determined by timing the filling of calibrated 10–13-nl constriction pipettes. Two or three collections were made for each experimental condition. Each collection was split into two aliquots, one for the determination of [^{14}C]inulin activity and the other for determination of either total carbon dioxide or chloride concentration.

The flux of total carbon dioxide was measured to assess bicarbonate transport. Total carbon dioxide concentrations in the collected fluid, perfusate, and bath were measured by microcalorimetry (4). In the range of sample sizes and pipette volumes used in this study, differences in total carbon dioxide concentration less than 1 mM can readily be resolved.

A continuous flow ultramicro-colorimeter (5) was used to measure chloride in perfusate, bath, and collected fluid. In this method, chloride displaces thiocyanate from mercuric thiocyanate, forming ferric thiocyanate, which absorbs light in the region 440–540 nM. The instrument was modified through the use of a focused incandescent light source (6) and two optical filters that isolate a 50% transmission band between 470 and 535 nM (7). A linear standard curve was obtained for standards containing up to 550 peq of chloride. In the range of sample sizes used in the present study, the coefficient of variation is 3%. The reagents for the chloride method were obtained as a kit (kit no. 460; Sigma Chemical Co., St. Louis, MO).

Collected fluid and perfusate samples were mixed with Aquasol for measurement of ^{14}C radioactivity by liquid scintillation counting (model 6872; G. D. Searle & Co., Skokie, IL). The perfusion rate (V_0) was calculated from the equation $V_0 = V_L (X_L/X_0)$, where V_L is the collection rate, X_0 is the activity (cpm) of ^{14}C in the perfusate, and X_L is the activity of ^{14}C in the collected fluid. The rate of fluid absorption (J_v) was calculated as $J_v = (V_0 - V_L)/L$, where L is the tubule length.

The rate of chloride or total CO_2 transport (J_i) was calculated as $J_i = (C_0V_0 - C_LV_L)/L$, where C_0 is the concentration of total CO_2 or chloride in the perfusion fluid and C_L is the concentration in the collected fluid. As defined by this equation, a positive flux indicates absorption and a negative flux indicates secretion.

The transepithelial potential difference was measured as previously described (8).

BK (Peninsula Laboratories, Inc., Belmont, CA) or AVP (Sigma Chemical Co.) were introduced into the bath chamber via the bath exchange system.

Differences between means were tested for statistical significance using the *t* test for paired data. A P value < 0.05 was considered statistically significant.

Results

Effect of AVP on chloride flux (Fig. 1). The effects of AVP (10^{-10} M in the peritubular bath) on the chloride flux (J_{Cl}), the rate of spontaneous fluid absorption (J_v), and the transepithelial potential difference (PD) in five tubules are shown in Fig. 1. In initial control measurements, chloride was absorbed at a low rate (7.1±2.4 pmol/min per mm). AVP in the bath markedly increased the rate of chloride absorption (40.1±7.7 pmol/min per mm). This effect was reversed upon removal of AVP from the bath.

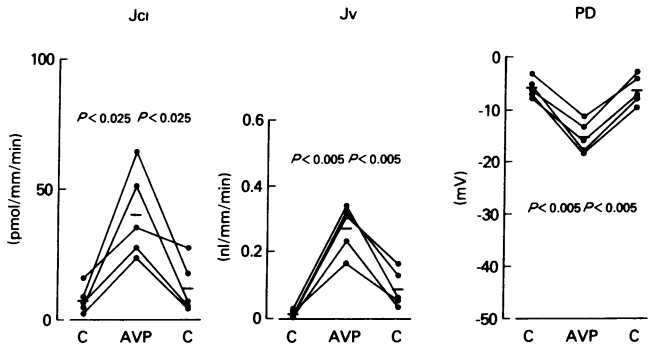

Figure 1. Effect of 10^{-10} M arginine vasopressin in bath on net chloride flux (J_{Cl}), rate of fluid transport (J_V), and transepithelial potential difference (*PD*). Negative flux indicates net secretion; positive flux, net absorption. *C*, control period; *AVP*, experimental period with vasopressin in bath. Horizontal bars indicate mean values. Statistical comparisons by paired *t* test.

There was no fluid absorption in the absence of AVP. Addition of AVP induced a significant rate of fluid absorption (0.27±0.03 nl/min per mm).

The transepithelial potential difference (Fig. 1, right) was lumen-negative (−6.1±0.8 mV) in the absence of AVP. As in previous studies (1, 9, 10), the potential difference was increased by AVP addition to the bath (−15.4±1.2 mV).

Effect of AVP on total CO_2 flux (Figs. 2 and 3). The effects of AVP (10^{-10} M in the peritubular bath) on the total CO_2 flux (J_{TCO_2}), the rate of spontaneous fluid absorption, and the transepithelial potential difference in five tubules are shown in Fig. 2. In initial control measurements, total CO_2 was secreted (−7.3±1.8 pmol/min per mm). Addition of AVP to the bath inhibited the total CO_2 secretion (2.4±3.2 pmol/min per mm) and resulted in net total CO_2 absorption in most tubules. The effect partially reversed upon removal of AVP. As in Fig. 1, AVP increased the rate of spontaneous fluid absorption (Fig. 2, middle)

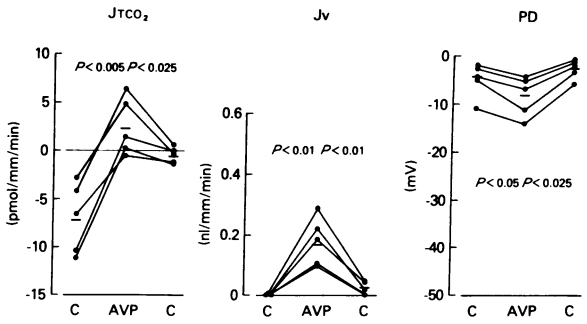

Figure 2. Effect of 10^{-10} M arginine vasopressin in bath on net total CO_2 flux (J_{CO_2}), rate of fluid transport (J_V), and transepithelial potential difference (*PD*). Negative flux indicates net secretion; positive flux, net absorption, *C*, control period; *AVP*, experimental period with vasopressin in bath. Horizontal bars indicate mean values. Statistical comparisons by paired *t* test.

and increased the lumen-negative potential difference (Fig. 2, right).

Time control experiments in the absence of AVP (11, 12) revealed a gradual decline in bicarbonate secretion with time in rat cortical collecting ducts. To distinguish more clearly between the effect of vasopressin itself and the spontaneous decline in bicarbonate secretion, we repeated the measurements with the order of control and experimental measurements reversed (Fig. 3). Initially, with AVP in the bath, total CO_2 was absorbed (left panel). Removal of vasopressin from the bath resulted in a marked decrease in the total CO_2 flux, and re-addition of vasopressin increased the rate of total CO_2 absorption. The effects of AVP on fluid absorption and potential difference were similar to those described above.

Effect of BK on chloride flux (Fig. 4). All BK experiments were done in the presence of AVP (10^{-10} M in the peritubular bath), which maximized the initial fluxes during control periods. The effects of BK (10^{-9} M in the peritubular bath) on the chloride flux, the rate of spontaneous fluid absorption, and the transepithelial potential difference in seven tubules are shown in Fig. 4. In initial periods with only AVP in the bath the rate of chloride absorption was relatively high (48.3±9.2 pmol/min per mm). Addition of BK to the bath caused a significant decrease in the flux of chloride to 29.2±6.2 pmol/min per mm. This effect was reversed upon removal of BK from the bath.

BK also inhibited the rate of fluid absorption (Fig. 4, middle) as shown in prior studies (1).

Addition of BK to the bath had no effect on the transepithelial potential difference (Fig. 4, right), as also found in our previous study (1). The transepithelial potential difference was −19.1±5.6 mV before the addition of BK and −18.5±5.6 mV with BK in the bath. After removal of BK from the bath the potential difference was −17.3±5.2 mV.

Effect of BK on total CO_2 flux (Fig. 5). The effects of 10^{-9} M BK in the peritubular bath on the total CO_2 flux, the rate of spontaneous fluid absorption, and the transepithelial potential difference in five tubules are shown in Fig. 5. In initial control measurements with only AVP in the bath, total CO_2 was absorbed (12.2±1.4 pmol/min per mm). The addition of BK to the bath did not significantly affect the rate of total CO_2 transport (14.2±2.2 pmol/min per mm). Net bicarbonate absorption increased with time as is evident from a comparison of initial and final control periods with AVP alone in the bath.

The effects of BK on fluid absorption (Fig. 5, middle) and potential difference (Fig. 5, right) were similar to those described in Fig. 4.

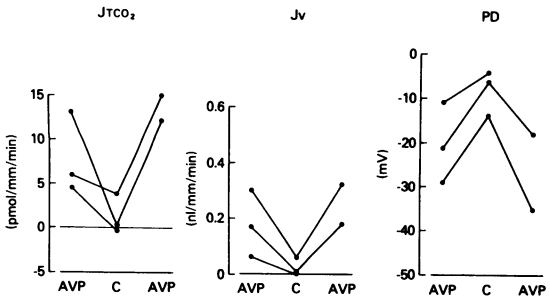

Figure 3. Effect of 10^{-10} M arginine vasopressin in bath on net total CO_2 flux (J_{CO_2}), rate of fluid transport (J_V), and transepithelial potential difference (*PD*). Same as Fig. 2 except that the order of experimental and control measurements was reversed.

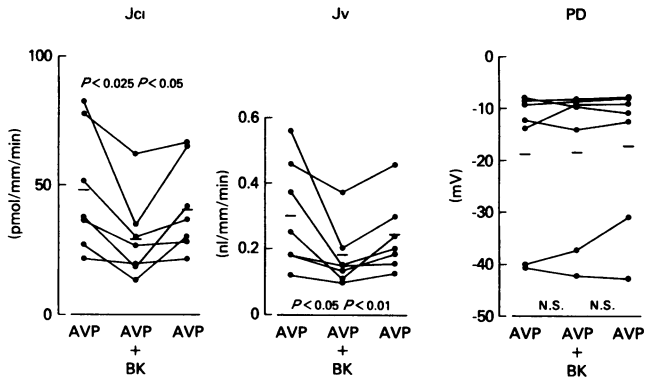

Figure 4. Effect of 10^{-9} M bradykinin in the bath on net chloride flux (J_{Cl}), rate of fluid transport (J_V), and transepithelial potential difference (*PD*). Vasopressin (10^{-10} M) was present in bath throughout the experiments. *AVP*, control period with vasopressin alone in bath; *AVP + BK*, experimental period with vasopressin and bradykinin in bath; *N.S.*, not statistically significant. Horizontal bars indicate mean values.

Discussion

Vasopressin

Table I summarizes the effects of vasopressin on fluid and electrolyte transport in isolated rat cortical collecting ducts observed in this and in our previous (1) study. Vasopressin increased both net sodium absorption and net potassium secretion (1). The increase in net sodium absorption was greater than the increase in net potassium secretion, which indicates that vasopressin increased net cation absorption. To preserve electroneutrality, net anion absorption must have also increased in response to vasopressin. One objective of the present study was to identify the anion or anions whose transport is affected by vasopressin. We found that vasopressin altered the transport of both chloride and bicarbonate. It increased chloride absorption, and it either inhibited bicarbonate secretion, stimulated bicarbonate absorption, or possibly did both.

Consistent with previous studies of cation transport and water permeability in the rat cortical collecting duct (1, 9, 10), the

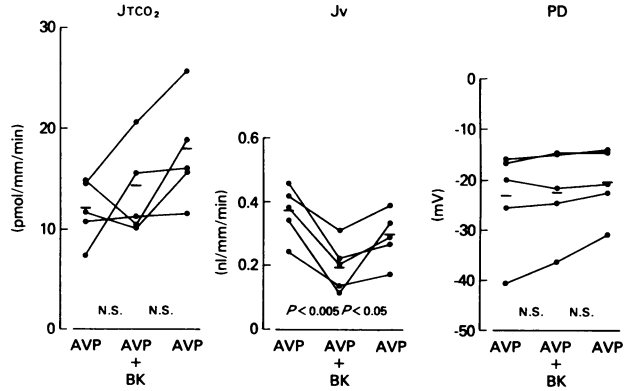

Figure 5. Effect of 10^{-9} M bradykinin in bath on net total CO_2 flux (J_{CO_2}), rate of fluid transport (J_V), and transepithelial potential difference (*PD*). Vasopressin (10^{-10} M) was present in bath throughout the experiments. *AVP*, control period with vasopressin alone in bath; *AVP + BK*, experimental period with vasopressin and bradykinin in bath; N.S., not statistically significant. Horizontal bars indicate mean values.

Table I. Effects of Arginine Vasopressin and Bradykinin on Fluid and Electrolyte Transport by Rat Cortical Collecting Ducts

	Arginine Vasopressin	Bradykinin
Cation transport (ref. 1)		
Sodium absorption	Increased	Decreased
Potassium secretion	Increased	No effect
Anion transport (present study)		
Chloride absorption	Increased	Decreased
Bicarbonate transport	Secretion → absorption	No effect
Other measurements (ref. 1 and present study)		
Fluid absorption	Increased	Decreased
Transepithelial potential difference	Increased	No effect

effects of vasopressin on anion fluxes were found to be sustained for as long as the vasopressin remained in the bath. The full effect of vasopressin generally occurred within 10–15 min and reversed rapidly upon removal of the vasopressin.

In our previous study, treatment of rats with deoxycorticosterone increased sodium and potassium transport rates in isolated, perfused cortical collecting ducts (1). In this study, we treated the rats with deoxycorticosterone anticipating that anion fluxes would also be increased and therefore more accurately measured. Tubules from untreated rats were not studied. However, the effects of vasopressin on cation transport and voltage are qualitatively the same with (1) and without (9, 10) deoxycorticosterone treatment of the rats.

Chloride transport. Studies of isolated cortical collecting ducts from rabbits have shown a relatively high paracellular chloride conductance (13–16). This finding is consistent with the view that chloride absorption in this segment normally occurs largely by passive paracellular diffusion driven by the lumen-negative voltage. If so, it is predictable that an increase in lumen negativity caused by vasopressin should be associated with an increase in chloride absorption. This was, in fact, observed (Fig. 1). However, a more thorough analysis of the data reveals that the mechanism of chloride absorption may be more complex.

An equation describing voltage-driven diffusion of chloride across a single barrier pathway (17) is: $J_{Cl} = -C_{Cl}A_sP_{Cl}(F/RT)\Delta\psi$, where J_{Cl} is the chloride flux, C_{Cl} is the mean chloride concentration across the epithelium, A_s is the surface area per unit length of the tubule, P_{Cl} is the permeability of the tubule to chloride, F/RT is Faraday's constant divided by the product of the gas constant and the absolute temperature, and $\Delta\psi$ is the transepithelial potential difference. This equation ignores passive fluxes driven by concentration differences across epithelium, since the luminal chloride concentration fell only by a few milliequivalents per liter in our experiments (see below). According to this equation, if the paracellular pathway is the sole route of chloride transport, the increase in chloride absorption should be proportional to the increase in voltage (assuming that vasopressin does not affect the chloride conductance of the paracellular pathway). However, in the present study, vasopressin caused a sixfold increase in chloride absorption and only a two- to threefold increase in transepithelial voltage (Fig. 1). This result raises the possibility that the increment in chloride absorption caused by vasopressin is not solely due to increased chloride transport through the paracellular pathway. It is consistent with the conclusion from the BK studies (discussed below) that a portion of the chloride absorption occurs via an electroneutral transcellular pathway.

Fluid absorption. Vasopressin addition resulted in significant fluid absorption (Table I) even though the bath and perfusate had the same osmolality. The stimulation of fluid absorption required two distinct effects of vasopressin, namely, stimulation of solute absorption and increased water permeability. Solute absorption (mostly sodium chloride) by itself tends to decrease the osmolality of the luminal fluid, creating an osmotic driving force for water absorption. The increase in osmotic water permeability caused by vasopressin in the rat cortical collecting duct is sustained for as long as the vasopressin remains in the peritubular bath (9), which contrasts with prior observations at 37°C in rabbit cortical collecting ducts. The maximum osmotic water permeability in the presence of vasopressin is approximately threefold greater in rat than in rabbit cortical collecting ducts (9). Because of this high water permeability, only a small osmolality gradient is needed to drive water absorption. Consequently, in these in vitro experiments, fluid absorption by the rat cortical collecting duct was a result of net solute absorption and did not depend on an imposed transepithelial osmotic gradient. In our experiments, the chloride concentration in the reabsorbed fluid (calculated as the rate of chloride absorption divided by the rate of fluid absorption) was 150–160 mM with vasopressin present, compared with 118 mM in the perfusate and bath. The luminal chloride concentration fell only 8–10 meq/liter below the perfusate despite the absorption of 20% or more of the perfused chloride.

In micropuncture studies, the tubule fluid present in early distal tubule of rats was hypotonic to plasma during antidiuresis (18, 19). However, osmotic equilibration with the plasma occurred before the end of the distal tubule (18, 19), i.e., in the initial collecting tubule (20). Thus, the tubule fluid entering the rat cortical collecting duct in vivo is normally isotonic to plasma during antidiuresis. Consequently, as in our in vitro experiments, water absorption in the cortical collecting duct in vivo is most likely dependent on solute absorption by the cortical collecting duct, not on a transepithelial osmolality gradient present at the beginning of the segment.

Bicarbonate transport. Vasopressin consistently altered bicarbonate transport. In some experiments net bicarbonate secretion was inhibited and in others net bicarbonate absorption increased. The interpretation of these results is complicated by the fact that the cortical collecting duct possesses two independent bicarbonate transport systems, namely, direct bicarbonate secretion and bicarbonate absorption mediated by proton secretion (11, 21, 22). We cannot conclude from these experiments whether vasopressin inhibits bicarbonate secretion, stimulates proton secretion, or both. Recently, Schuster (23) reported that there was no significant effect of vasopressin on net bicarbonate transport by cortical collecting ducts from another species, namely, the rabbit. Apparently, rat cortical collecting ducts differ from rabbit cortical collecting ducts in this respect as they do in a number of other respects, (1, 9, 10, 12).

One possible explanation for the effect of vasopressin on bicarbonate transport is that vasopressin causes changes in electrical potential gradients across the cells that directly affect bicarbonate or proton transport. Based on studies in turtle bladders,

bicarbonate absorption is thought to be mediated by an electrogenic proton pump in the apical membrane (24), and bicarbonate secretion is thought to be driven by a similar pump located on the basolateral membrane of a different cell type (25). A change in the voltage across either membrane, accompanying the change in transepithelial voltage, could in principle affect bicarbonate transport by affecting the proton pump. Voltage and proton flux measurements across the individual membranes are required to establish whether the changes in voltage and bicarbonate transport are related. Because the transepithelial bicarbonate permeability is small (16, 23), a substantial direct effect of the transepithelial voltage on passive bicarbonate transport is unlikely.

We are not aware of direct evidence for or against effects of vasopressin on renal bicarbonate transport in vivo. The late distal tubule, or initial collecting tubule, is morphologically similar to the cortical collecting duct (20, 26) and the two may function similarly. In vivo studies of bicarbonate transport in the superficial distal tubule using micropuncture have not, however, consistently shown either net bicarbonate secretion or absorption. Some investigators have reported little or no net bicarbonate transport in the distal tubule of normal rats (27, 28), while others have concluded that substantial bicarbonate absorption occurs (29, 30). The effect of vasopressin was not tested, however, and considering the striking effect of vasopressin on bicarbonate transport in cortical collecting ducts in vitro, the differing results in vivo could conceivably be due in part to differences in circulating vasopressin levels in the rats.

Bradykinin

Table I summarizes the effects of bradykinin on fluid and electrolyte transport in isolated rat cortical collecting ducts. Previously, bradykinin was found to inhibit net sodium absorption with no significant effect on either transepithelial voltage or potassium transport (1). Based on these results, we concluded that bradykinin inhibited net sodium transport by affecting an electroneutral sodium transport process. The present studies were done to determine the anion whose transport is affected by bradykinin and thus to further identify the electroneutral sodium transport process. We confirmed the lack of effect of bradykinin on transepithelial voltage, and showed that bradykinin in the bath at a concentration of 10^{-9} M eliminated ~40% of the chloride absorption (Fig. 4) without a demonstrable effect on bicarbonate transport. Thus, bradykinin had a selective effect on sodium and chloride absorption. Accordingly, the electroneutral transport process affected by bradykinin most likely involves coupled transport of sodium and chloride.

Several issues remain unresolved: (*a*) From the results thus far, we cannot determine whether bradykinin inhibits a sodium chloride absorptive process or stimulates a sodium chloride secretory process. Both would have the same effect on net sodium chloride absorption. (*b*) We do not yet know whether the coupled transport process involves only sodium and chloride as described in the urinary bladder of the flounder (31), or also involves other ions. For example, in the medullary thick ascending limb of the rabbit, coupled sodium chloride transport requires potassium (32). In mouse cortical thick ascending limb, coupled sodium chloride transport requires bicarbonate, and it has been concluded that the overall process involves dual ion exchangers, Na^+-H^+ and Cl^--HCO_3^- (33). (*c*) Active absorption of chloride has been demonstrated in rabbit cortical collecting ducts (7, 34). It is presently unclear whether active chloride absorption in the cortical collecting duct of the rabbit is related to the electroneutral chloride transport process found in this study. (*d*) Coupled sodium chloride transport has recently been demonstrated in the rat distal tubule by in vivo microperfusion (35). It is unknown whether this process may be the same as that found in the present study.

Previously, we argued that the direct inhibition of sodium and fluid absorption by bradykinin is involved in the regulation of extracellular fluid volume, particularly during extracellular fluid volume expansion (1). The finding that the anion whose transport is inhibited by bradykinin is chloride and not bicarbonate further supports a possible role for bradykinin in the regulation of extracellular fluid volume, and indicates that a direct role in the regulation of acid-base balance is unlikely.

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