Effects of osmolality on bicarbonate absorption by medullary thick ascending limb of the rat.

D W Good

J Clin Invest. 1992;89(1):184-190. https://doi.org/10.1172/JCI115560.

Research Article

Previously we demonstrated that arginine vasopressin (AVP) directly inhibits bicarbonate absorption (JHCO3, pmol/min per mm) in the medullary thick ascending limb (MTAL) of the rat. To determine whether changes in osmolality also may affect bicarbonate absorption, MTAL were studied in vitro with 25 mM HCO3- solutions. Control osmolality was 290 mosmol/kg H2O. In the absence of AVP, increasing osmolality to 560 in perfusate and bath by addition of 150 mM NaCl reduced JHCO3 from 13.7 to 4.5. With 2 x 10(-10) M AVP in the bath, adding 150 mM NaCl to perfusate and bath reduced JHCO3 from 6.9 to 0.6, while adding NaCl to the bath alone reduced JHCO3 from 7.1 to 0.5. Adding 150 mM NaCl to perfusate and bath caused a similar inhibition of JHCO3 in MTAL perfused with furosemide to inhibit net NaCl absorption. In the presence of AVP, adding 600 mM urea to perfusate and bath inhibited JHCO3 by 55%; adding 300 or 600 mM mannitol to perfusate and bath inhibited JHCO3 by 75%. The effects on JHCO3 were reversible and dissociable from changes in transepithelial voltage. Conclusions: (1) osmolality is a factor capable of regulating renal tubule bicarbonate absorption; (2) hypertonicity produced with NaCl, urea, or mannitol markedly inhibits bicarbonate absorption in the MTAL; (3) this inhibition occurs independent of, and is additive to, inhibition […]

Effects of Osmolality on Bicarbonate Absorption by Medullary Thick Ascending Limb of the Rat

David W. Good
With the technical assistance of **Jose J. Molina**
Departments of Medicine and Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

Abstract

Previously we demonstrated that arginine vasopressin (AVP) directly inhibits bicarbonate absorption ($JHCO_3$, pmol/min per mm) in the medullary thick ascending limb (MTAL) of the rat. To determine whether changes in osmolality also may affect bicarbonate absorption, MTAL were studied in vitro with 25 mM HCO_3^- solutions. Control osmolality was 290 mosmol/kg H_2O. In the absence of AVP, increasing osmolality to 560 in perfusate and bath by addition of 150 mM NaCl reduced $JHCO_3$ from 13.7 to 4.5. With 2×10^{-10} M AVP in the bath, adding 150 mM NaCl to perfusate and bath reduced $JHCO_3$ from 6.9 to 0.6, while adding NaCl to the bath alone reduced $JHCO_3$ from 7.1 to 0.5. Adding 150 mM NaCl to perfusate and bath caused a similar inhibition of $JHCO_3$ in MTAL perfused with furosemide to inhibit net NaCl absorption. In the presence of AVP, adding 600 mM urea to perfusate and bath inhibited $JHCO_3$ by 55%; adding 300 or 600 mM mannitol to perfusate and bath inhibited $JHCO_3$ by 75%. The effects on $JHCO_3$ were reversible and dissociable from changes in transepithelial voltage. Conclusions: (1) osmolality is a factor capable of regulating renal tubule bicarbonate absorption; (2) hypertonicity produced with NaCl, urea, or mannitol markedly inhibits bicarbonate absorption in the MTAL; (3) this inhibition occurs independent of, and is additive to, inhibition by vasopressin. Hypertonicity may shift TAL HCO_3^- absorption from medulla to cortex, thereby limiting delivery of bicarbonate to the medullary interstitium during antidiuresis. (*J. Clin. Invest.* 1992. 89:184–190.) Key words: hypertonicity • loop of Henle • urinary acidification • vasopressin • antidiuresis

Introduction

In addition to its central role in the generation of a concentrated or dilute urine, the thick ascending limb of Henle also is an important site of renal bicarbonate absorption (1–3). Factors that influence bicarbonate absorption in the thick ascending limb include chronic metabolic acidosis and alkalosis (3, 4), peptide hormones (5), and dietary sodium intake (4). In particular, we have demonstrated recently that arginine vasopressin inhibits bicarbonate absorption by 40 to 50% in the isolated, perfused medullary thick ascending limb of the rat (5). Changes in vasopressin levels in vivo are accompanied by changes in medullary osmolality that may independently influence renal tubule ion transport. For example, in the mouse medullary thick ascending limb, hypertonicity and vasopressin have opposing effects on net NaCl absorption that may be components of a feedback system that regulates medullary interstitial osmolality (6–8). Whether changes in osmolality influence renal tubule bicarbonate absorption has not been investigated directly.

The purpose of the present experiments was to examine directly the effects of increasing osmolality on bicarbonate absorption in the medullary thick ascending limb of the rat. The aims were to determine whether hypertonicity influences net bicarbonate transport, and whether osmolality and vasopressin might interact to regulate bicarbonate absorption. The results show that an increase in osmolality markedly inhibits net bicarbonate absorption in the medullary thick ascending limb, and that this inhibition is both independent of and additive to inhibition by vasopressin.

Methods

Medullary thick ascending limbs from pathogen-free male Sprague-Dawley rats (60–90 g body wt) were isolated and perfused in vitro using methods described previously for this laboratory (1, 5, 9). The rats had free access to distilled H_2O and autoclaved food (NIH 31; Tecklad Premier Laboratory Diet, Madison, WI) up to the time of experiments. To preserve tubule function in vitro (1, 10), the rats were injected intraperitoneally with furosemide (2 mg) 5–10 min before being anesthetized. After anesthesia (50 mg/kg pentobarbital i.p.), the left kidney was bathed in situ for 1–2 min in ice-cold dissection solution and then removed for tubule dissection (9).

Medullary thick ascending limbs were dissected at 10°C from the inner stripe of the outer medulla and perfused in vitro at 37°C using concentric glass pipettes (1, 9). Tubule fluid emerging from the distal end of the tubules was collected for timed intervals into calibrated constriction pipettes for calculation of fluid collection rates and for analysis of total CO_2 concentrations. Four perfusion solutions were used for experiments: (1) control solution contained (in mM) 146 Na^+, 4 K^+, 122 Cl^-, 25 HCO_3^-, 2.0 Ca^{2+}, 1.5 Mg^{2+}, 2.0 phosphate, 1.2 SO_4^{2-}, 1.0 citrate, 2.0 lactate, and 5.5 glucose; (2) NaCl solution was identical to control solution except for addition of 150 mM NaCl; (3) urea solution was identical to control solution except for addition of 600 mM urea; (4) mannitol solution was identical to control solution except for addition of 300 or 600 mM mannitol. Corresponding bath solutions were identical except for addition of 0.2% BSA (fraction V, essentially fatty acid free, Sigma Chemical Co., St. Louis, MO). In most experiments the bath also contained 2×10^{-10} M arginine vasopressin (see Results). Also, in some experiments [^{3}H]inulin was added to the perfusion solution to serve as a volume marker for measurement of net fluid transport (see below). All solutions were equilibrated with 95% O_2, 5% CO_2 and were pH 7.47–7.48. The oxygen and CO_2 tensions in the bath

Results of these experiments were reported in preliminary form at the 21st Annual Meeting of the American Society of Nephrology (1990. *Kidney Int.* 37:538), the XIth International Congress of Nephrology (1990.P476A), and the Third International Vasopressin Conference (1991. *Colloqu. Inserm* (*Inst. Natl. Santé Rech. Med.* 208:460).

Address correspondence and reprint requests to David W. Good, Ph.D., Division of Nephrology, 4.200 John Sealy Hospital E-62, University of Texas Medical Branch, Galveston, TX 77550.

Received for publication 16 August 1991 and in revised form 6 September 1991.

J. Clin. Invest.

0021-9738/92/01/0184/07 $2.00
Volume 89, January 1992, 184–190

Table I. Net Fluid Transport

Perfusate/bath	Osm	J_V	Collection rate	V_{TE}	Tubule length
	mosmol/kg H_2O	*nl/min per mm*	*nl/min per mm*	*mV*	*mm*
A. Control/control (3/2)	290/290	0.03±0.01	1.3±0.04	12.1±1.1	0.60±0.01
B. NaCl/NaCl (10/3)	560/560	−0.01±0.01	1.2±0.07	7.5±0.7	0.64±0.02
C. Urea/urea (9/3)	890/890	−0.02±0.02	1.0±0.10	3.3±1.2	0.68±0.01
D. Control/NaCl (13/5)	290/560	0.17±0.03*	1.0±0.01	19.4±2.2	0.61±0.03

Values are means±SE for individual tubules. Numbers in parentheses are number of collected fluid samples/number of tubules. All bath solutions contained 2×10^{-10} M arginine vasopressin. Composition of solutions is given in Methods. J_V is net fluid transport rate. V_{TE} is transepithelial voltage, oriented lumen positive with respect to bath. * J_V significantly different from zero.

were maintained during the experiments by continuously flowing freshly gassed solutions into the perfusion chamber (1, 9). The control bath solution was used for tubule dissection.

The general protocol was similar in each experimental series. Tubules were equilibrated for 20–40 min with control perfusate and bath, following which, two to four tubule fluid samples were collected for determination of bicarbonate absorption rate (control period). The perfusion and/or bath solutions were then changed to one of the experimental solutions and the tubule was allowed to re-equilibrate for 5–20 min. Two to four additional tubule fluid samples were then collected (experimental period). Finally, the control solution was returned to the perfusate and bath, and control measurements were repeated (recovery period). Complete recovery generally was obtained within 20–40 min. In experiments in which net fluid transport was measured, additional tubule fluid samples were collected for measurement of inulin radioactivity. In some experiments, only two periods (control and experimental, or experimental and recovery) were studied. With one exception, solution changes involved symmetric replacement of both the perfusate and bath (see Results).

In previous studies, net fluid transport was absent in rat thick ascending limbs perfused and bathed with isosmotic (290 mosmol/kg H_2O) solutions in the presence or absence of vasopressin (1, 10–12). To confirm and extend these observations, we measured net fluid transport with isosmotic and hypertonic solutions under experimental conditions used in the present study (Table I). Net fluid transport was measured as described previously (1) using exhaustively dialyzed [methoxy-^{3}H]inulin (New England Nuclear, Boston, MA). In the presence of 2×10^{-10} M bath vasopressin, no significant net fluid transport was observed in tubules perfused and bathed symmetrically with either the control, NaCl, or urea solutions (conditions *A–C*, Table I). Thus, for tubules studied with symmetric solutions, absolute rates of bicarbonate absorption ($JTCO_2$, pmol/min per mm) were calculated as $JTCO_2 = V_l(C_o - C_l)/L$, where V_l is fluid collection rate (nl/min), C is total CO_2 concentration (mM) in perfused (o) and collected (l) fluid, and L is perfused tubule length (mm)[2]. In contrast, in tubules studied with an imposed transepithelial osmotic gradient (control perfusate/NaCl bath), significant net fluid absorption was observed (condition *D*, Table I). Accordingly, for this condition, the absolute bicarbonate absorption rate was calculated as $JTCO_2 = [(V_oC_o) - (V_lC_l)]/L$, where V_o is perfusion rate (nl/min) calculated from the fluid collection rate and the ratio of inulin radioactivities in perfused and collected fluids [$V_o = V_l([\text{inulin}]_l/[\text{inulin}]_o)$].

Total carbon dioxide concentrations in perfusion and bath solutions and in collected tubule fluid were measured by microcalorimetry as previously described (1, 2). As a technical note, we were unable to obtain reliable measurements of total CO_2 concentration in the NaCl solution using concentrated H_2SO_4 in the release chamber; however, reproducible and accurate results were obtained using H_2PO_4. The reason(s) for this difference were not investigated. Either acid gave reliable results for the control, urea, and mannitol solutions. Transepithelial voltage of the tubules was measured between calomel cells connected to perfusion and bath solutions by saline–agar bridges (1, 9). In one experimental condition in which the perfusate and bath differed in ionic composition (control perfusate/NaCl bath, Tables I and IV), the measured transepithelial voltage was corrected for the change in liquid junction voltage. The liquid junction voltage (calculated by use of the Henderson equation [2, 13]) was −2.7 mV, control vs. NaCl. This value was added to or subtracted from the measured voltages as appropriate to obtain the actual transepithelial voltages reported in Results. In each experiment, a mean transepithelial voltage and total CO_2 absorption rate was calculated for each period studied in that particular tubule. When control measurements were made at the beginning and end of an experiment, the control values were averaged. Single tubule values, presented in the Figures, were averaged to obtain the group means presented in the Tables (n = number of tubules). Differences between means were evaluated using the paired t test. $P < 0.05$ was considered statistically significant.

Results

Effects of NaCl on bicarbonate absorption. The effects of increasing NaCl concentration on bicarbonate absorption are shown in Table II (mean values) and Fig. 1 (individual data). Adding 150 mM NaCl to the perfusion and bathing solutions reduced net bicarbonate absorption by 67%. The inhibition of bicarbonate absorption was reversible (Fig. 1). Increasing the

Table II. Effect of Increasing NaCl Concentration in Perfusate and Bath on Bicarbonate Absorption

	V	[TCO_2]		$JTCO_2$	V_{TE}
		Perfusion	Collection		
	nl/min per mm	*mM*		*pmol/min per mm*	*mV*
Control	1.2±0.1	25.5±0.2	14.3±1.5*	13.7±2.0	8.0±1.3
NaCl	1.1±0.1	25.5±0.2	22.3±0.6*	4.5±0.7	5.0±0.9
P	NS	NS	<0.005	<0.025	<0.025

Values are means±SE. Number of experiments = 5. NaCl, 150 mM NaCl added to perfusate and bath. Mean tubule length = 0.64±0.06 mm. Total CO_2 concentration in bath solutions was 26.0±0.2 mM. V, fluid collection rate; [TCO_2], total carbon dioxide concentration; $JTCO_2$, absolute rate of total CO_2 absorption; V_{TE}, transepithelial voltage, oriented lumen positive with respect to bath. *P* values compare control vs. NaCl (paired t test). * Collected [TCO_2] different from perfused (paired t test).

1. *Abbreviation used in this paper:* $JTCO_2$, absolute rate of bicarbonate absorption.

2. In this paper, as in previous papers (1, 2, 5), total CO_2 absorption is referred to generally as bicarbonate absorption.

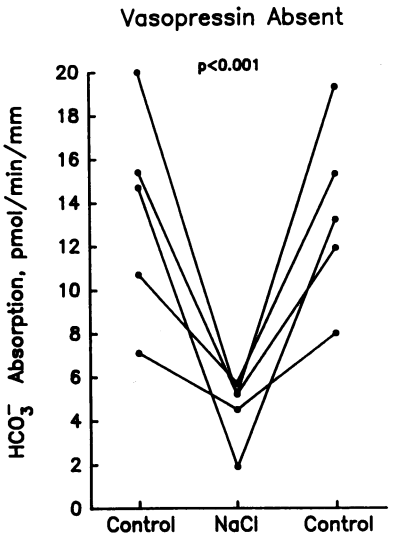

Figure 1. Effect of adding NaCl (150 mM) to perfusate and bath on bicarbonate absorption by rat medullary thick ascending limb. Arginine vasopressin was absent from the bathing solutions. Filled circles are mean values for single tubules; lines connect paired measurements made in the same tubule. *P* value is for paired *t* test.

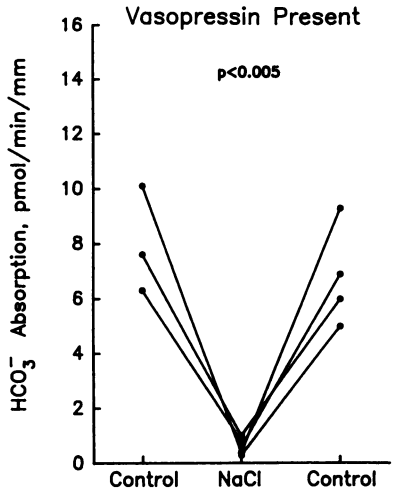

Figure 2. Effect of adding NaCl (150 mM) to perfusate and bath on bicarbonate absorption in the presence of vasopressin. Arginine vasopressin (2×10^{-10} M) was present in the bath throughout the experiments. Filled circles, lines, and *P* value as in Fig. 1.

NaCl concentration significantly decreased the transepithelial voltage (Table II).

To examine possible interactions between hypertonicity and vasopressin, the effect of increasing NaCl concentration was studied in the presence of vasopressin. In tubules bathed with 2×10^{-10} M vasopressin, adding 150 mM NaCl to the perfusate and bath reduced net bicarbonate absorption reversibly from 6.9 to 0.6 pmol/min per mm (Table III, Fig. 2). Thus prior inhibition of HCO_3^- absorption by vasopressin did not prevent inhibition by NaCl, indicating that the inhibitory effects of hypertonicity and vasopressin are additive. In the presence of vasopressin, increasing NaCl concentration reduced the transepithelial voltage (Table III).

To determine whether the inhibition of bicarbonate absorption may have been the indirect result of an effect of NaCl concentration on transcellular NaCl transport, the effect of NaCl was studied in tubules perfused with furosemide to inhibit net NaCl absorption. The results of two such experiments are shown in Fig. 3. In these experiments, 2×10^{-10} M vasopressin was present in the bathing solutions and perfusion rate averaged 1.0±0.1 nl/min per mm. With 10^{-4} M furosemide in the luminal perfusate, adding 150 mM NaCl to perfusate and bath reduced net bicarbonate absorption nearly to zero. Thus, the effect of NaCl to inhibit bicarbonate absorption was similar in the presence or absence of luminal furosemide (Figs. 2 and 3). With furosemide, the transepithelial voltage averaged 1.3±0.1 mV and was unaffected by the increase in NaCl concentration.

The effects of increasing NaCl concentration in the bath alone are shown in Table IV and Fig. 4. The control and NaCl bath solutions contained vasopressin (2×10^{-10} M). Adding 150 mM NaCl to the bath reduced net bicarbonate absorption from 7.1 to 0.5 pmol/min per mm, an effect nearly identical to that observed when NaCl concentration was increased symmetrically (Table III). With NaCl added to the bath, bicarbonate concentration rose along the tubule lumen due to small net fluid absorption (0.17±0.03 nl/min per mm) driven by the transepithelial osmotic gradient (Table IV). Adding NaCl to the bath increased the transepithelial voltage (Table IV), presumably due to the diffusion voltage generated across the cation-selective paracellular pathway (14).

Effects of urea. In addition to NaCl, the other solute primarily responsible for hypertonicity in the renal medulla is urea. The effects of urea on bicarbonate absorption are shown in Table V and Fig. 5. In the presence of vasopressin (2×10^{-10} M in the bath), adding 600 mM urea to the perfusate and bath reduced net bicarbonate absorption by 55%. The inhibition of bicarbonate absorption was reversible (Fig. 5). Increasing osmolality with urea decreased the transepithelial voltage (Table V).

Effects of mannitol. The effects of increasing osmolality with a nonphysiologic solute are shown in Table VI and Fig. 6. Adding either 300 or 600 mM mannitol to the perfusate and bath inhibited net bicarbonate absorption (Table VI, Fig. 6). In four of the five experiments, vasopressin was present in the bath solutions; in one experiment, mannitol inhibited bicarbonate absorption in the absence of vasopressin (Fig. 6). Mannitol also decreased the transepithelial voltage (Table VI).

Table III. Effect of Increasing NaCl Concentration in Perfusate and Bath on Bicarbonate Absorption (Vasopressin Present)

	V	$[TCO_2]$		$JTCO_2$	V_{TE}
		Perfusion	Collection		
	nl/min per mm	*mM*		*pmol/min per mm*	*mV*
Control	1.0±0.1	26.0±0.3	18.8±0.9*	6.9±0.8	10.3±0.9
NaCl	1.0±0.1	25.8±0.3	25.1±0.4*	0.6±0.1	5.6±1.0
P	NS	NS	<0.005	<0.005	<0.05

Values are means±SE. Number of experiments = 5. NaCl, 150 mM NaCl added to perfusate and bath. Control and NaCl baths contained 2×10^{-10} M vasopressin. Mean tubule length = 0.60±0.03 mm. Total CO_2 concentration in bath solutions was 26.5±0.3 mM. V, $[TCO_2]$, $JTCO_2$, V_{TE}, and * as in Table II. *P* values compare control vs NaCl (paired *t* test).

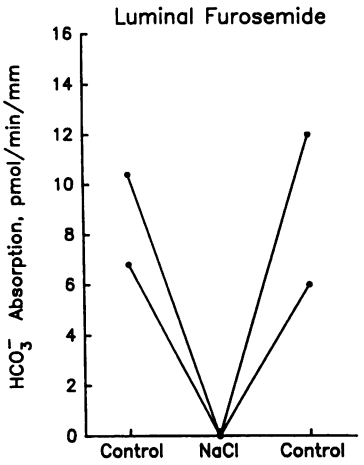

Figure 3. Effect of adding NaCl (150 mM) to perfusate and bath on bicarbonate absorption in the presence of furosemide. Furosemide (1×10^{-4} M) was present in the luminal perfusate and vasopressin (2×10^{-10} M) was present in the bath throughout the experiments. Filled circles and lines as in Fig. 1.

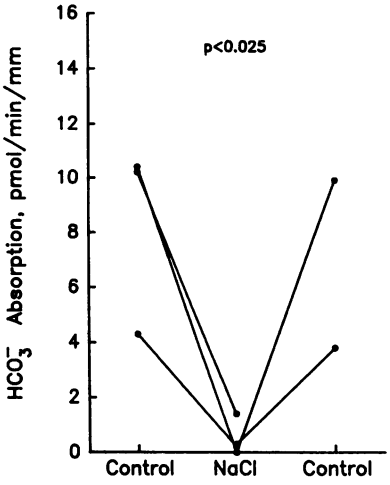

Figure 4. Effect of adding NaCl (150 mM) to the bath on bicarbonate absorption by rat medullary thick ascending limb. Vasopressin (2×10^{-10} M) was present in the bath throughout the experiments. Filled circles, lines, and *P* value as in Fig. 1.

Discussion

Although a variety of systemic and hormonal factors have been identified to regulate renal bicarbonate transport and urinary net acid excretion, direct studies of the effects of osmolality on renal tubule bicarbonate absorption are lacking. The present experiments were designed to examine directly the effects of increasing osmolality on bicarbonate absorption by the isolated, perfused medullary thick ascending limb of the rat. Because vasopressin directly inhibits bicarbonate absorption in the medullary thick ascending limb (5), possible interactions between osmolality and vasopressin also were examined. The results demonstrate that hypertonicity markedly inhibits bicarbonate absorption in the medullary thick ascending limb. The effect was observed with either NaCl, urea, or mannitol, indicating that the increase in osmolality, rather than the addition of a particular solute, was responsible for the inhibition. The inhibition of bicarbonate absorption by hypertonicity occurred independent of, and was additive to, inhibition by vasopressin. These findings indicate that osmolality may be an important factor regulating renal tubule bicarbonate transport, and that changes in medullary tonicity must be taken into account when evaluating changes in bicarbonate absorption in the medullary thick ascending limb in response to systemic acid–base or electrolyte disorders.

The inhibition of bicarbonate absorption by hypertonicity is likely to be of physiologic importance because it was observed with either of the two solutes primarily responsible for medullary tonicity, namely, NaCl and urea. The concentrations of NaCl (150 mM) and urea (600 mM) that were studied were chosen because they represent the upper end of the physiologic range of values achieved in the renal medulla in vivo, and because their effects on medullary thick ascending limb NaCl absorption previously had been studied in detail (6–8). Our observation that increasing bath osmolality inhibited bicarbonate absorption by an amount similar to that observed when osmolality was increased symmetrically (Tables III and IV), suggests that a change in peritubular osmolality is the proximate cause of the transport effect. In the rat in vivo, approximately 15% of filtered HCO_3^- is reabsorbed by the loop of Henle (1, 3). Because HCO_3^- absorption along the proximal straight tubule is limited in vivo by both a low luminal HCO_3^- concentration and a lack of functional luminal carbonic anhydrase (S3 segment) (15), it is likely that the major fraction of loop HCO_3^- absorption occurs along the thick ascending limb (3). Accordingly, the near-complete inhibition of HCO_3^- absorption in the medullary thick ascending limb by hypertonicity plus vasopressin suggests that reclamation of a substantial fraction of filtered HCO_3^- is under the control of these two regulatory factors. Whether changes in osmolality influence luminal acidification directly in other nephron segments has not been examined.

Although the mechanism(s) by which osmolality affects bicarbonate absorption cannot be determined from these experiments, it is possible to comment on some of the factors that might be expected to influence medullary thick ascending limb bicarbonate transport. First, the inhibition of bicarbonate absorption can be dissociated from effects of osmolality on net

Table IV. Effect of Increasing NaCl Concentration in Bath on Bicarbonate Absorption (Vasopressin Present)

	Flow rate		$[TCO_2]$			
	Perfusion	Collection	Perfusion	Collection	$JTCO_2$	V_{TE}
	nl/min per mm		*mM*		*pmol/min per mm*	*mV*
Control	1.1±0.1*	1.1±0.1	25.9±0.4	20.0±0.4‡	7.1±1.5	10.8±2.5
NaCl	1.3±0.1	1.1±0.1‡	25.9±0.4	30.2±0.3‡	0.5±0.3	17.4±2.3
P		NS		<0.001	<0.025	<0.01

Values are means±SE. Number of experiments = 4. NaCl, 150 mM NaCl added to bath. Control and NaCl baths contained 2×10^{-10} M vasopressin. Mean tubule length = 0.60±0.10 mm. Total CO_2 concentration in bath solutions was 25.6±0.5 mM. Perfusion and collection rates determined as described in Methods. * For control condition, perfusion rate is assumed to equal collection rate (Table I; references 1, 11, 12). ‡ Collected value different from perfused (paired *t* test). $[TCO_2]$, $JTCO_2$, V_{TE}, and *P* values as in Table II.

Table V. Effect of Adding Urea to Perfusate and Bath on Bicarbonate Absorption (Vasopressin Present)

	V	$[TCO_2]$		$JTCO_2$	V_{TE}
		Perfusion	Collection		
	nl/min per mm	*mM*		*pmol/min per mm*	*mV*
Control	1.1±0.1	25.5±0.1	17.3±0.8*	9.3±1.7	7.4±0.6
Urea	1.1±0.1	25.4±0.3	21.6±0.7*	4.1±1.1	1.9±0.6
P	NS	NS	<0.025	<0.05	<0.005

Values are means±SE. Number of experiments = 6. Urea, 600 mM urea added to perfusate and bath. Control and urea baths contained 2×10^{-10} M vasopressin. Mean tubule length = 0.61±0.06 mm. Total CO_2 concentration in bath solutions was 25.4±0.3 mM. V, $[TCO_2]$, $JTCO_2$, V_{TE}, and * as in Table II. *P* values compare control vs urea (paired *t* test).

NaCl absorption and transepithelial voltage. Peritubular hypertonicity markedly inhibits NaCl absorption in the medullary thick ascending limb (6–8), an effect that could indirectly influence HCO_3^- absorption through effects on cell ion activities, membrane voltages, or intracellular pH. The results show, however, that the effect of hypertonicity on bicarbonate transport was similar in untreated tubules (Fig. 2) and in tubules perfused with furosemide to inhibit transcellular NaCl transport (Fig. 3). Thus, the inhibition of bicarbonate absorption occurs independent of effects of osmolality on net NaCl absorption. The results also demonstrate that the effect of hypertonicity to inhibit bicarbonate absorption was observed with an increase (Table IV), a decrease (Tables II, III, and V), or no change (furosemide experiments, Fig. 3) in transepithelial voltage, consistent with previous studies demonstrating independence of bicarbonate absorption rate and V_{TE} (2, 4, 5).

Second, the inhibition of bicarbonate absorption by hypertonicity occurs independent of inhibition by vasopressin. This is evident from the observations that NaCl and mannitol inhibited bicarbonate absorption in the absence of vasopressin, and that the inhibitory effects of hypertonicity and vasopressin were additive. Inhibition of bicarbonate absorption by vasopressin is mediated by an increase in intracellular production of cyclic AMP (5). At the concentration of vasopressin used in the present study (2×10^{-10} M), cyclic-AMP-dependent inhibition of bicarbonate absorption should have been maximal. This conclusion is based on the observations that a high concentration (10^{-3} M) of exogenous 8-bromo-cyclic AMP inhibited bicarbonate absorption by an amount similar to that observed with vasopressin, and that the inhibitory effects of 8-bromo-cyclic AMP and vasopressin were not additive (5). Thus, the additivity of the effects of hypertonicity and vasopressin suggests that the inhibition by hypertonicity involves a signal transduction mechanism other than cyclic AMP. This view is supported by the observation that hypertonicity produced with a variety of solutes, including NaCl and urea, had no effect on baseline cyclic AMP production in the rat medullary thick ascending limb (16). Urea also had no effect on cyclic AMP production in the presence of 10^{-8} M vasopressin (16).

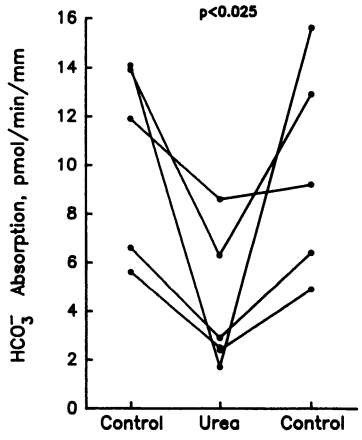

Figure 5. Effect of adding urea (600 mM) to perfusate and bath on bicarbonate absorption by rat medullary thick ascending limb. Vasopressin (2×10^{-10} M) was present in the bath throughout the experiments. Filled circles, lines, and *P* value as in Fig. 1.

Finally, the inhibition of bicarbonate absorption could be the indirect result of activation of transport mechanisms necessary for cell volume regulation. Cell volume regulation has not been studied directly in the thick ascending limb of the rat. However, several features of hypertonic cell volume regulation described for the medullary thick ascending limb of the mouse suggest that the inhibition of bicarbonate absorption can be dissociated from cell volume. First, vasopressin was required for hypertonic cell volume regulation (17), but is not necessary for inhibition of bicarbonate absorption (Figs. 2 and 6). Second, cell volume was decreased by addition of NaCl or mannitol but not by addition of urea (6), whereas all three agents inhibit HCO_3^- absorption. Third, the vasopressin-dependent volume regulatory increase required parallel activation of basolateral membrane Na^+–H^+ and Cl^-–HCO_3^- exchangers (18), a mechanism that should have no net effect on basolateral base efflux or intracellular pH. It is possible that preferential activation of basolateral Na^+–H^+ exchange in the rat medullary thick ascending limb could inhibit transcellular bicarbonate absorption by reducing net base efflux. Other possible mechanisms for the inhibition of HCO_3^- absorption include inhibition of apical membrane Na^+–H^+ exchange and/or inhibition of basolateral membrane HCO_3^- transport (3). Further direct studies of the effects of hypertonicity on apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters will be necessary to determine the cellular mechanism(s) of the bicarbonate transport inhibition.

It is of interest to consider briefly the possible physiologic significance of osmolality as a factor controlling bicarbonate absorption in the medullary thick ascending limb. As discussed earlier, hypertonicity and vasopressin have opposing effects on medullary thick ascending limb NaCl absorption, suggesting the existence of a feedback system for the control of medullary interstitial osmolality (6–8). A similar feedback mechanism clearly is not present for the control of medullary thick ascending limb bicarbonate transport because hypertonicity and vasopressin act cooperatively to inhibit bicarbonate absorption. The induction of antidiuresis in vivo is associated with an increase in urinary net acid excretion, due in part to direct stimu-

Table VI. Effect of Adding Mannitol to Perfusate and Bath on Bicarbonate Absorption (Vasopressin Present)

	V	$[TCO_2]$		$JTCO_2$	V_{TE}
		Perfusion	Collection		
	nl/min per mm	*mM*		*pmol/min per mm*	*mV*
Control	1.2±0.1	25.3±0.2	19.2±0.6*	7.1±0.7	7.7±1.0
Mannitol	1.1±0.1	25.1±0.2	23.6±0.6*	1.8±0.8	5.5±1.0
P	NS	NS	<0.01	<0.001	<0.05

Values are means±SE and are combined results of experiments with 300 or 600 mM mannitol added to perfusate and bath. Total number of experiments = 5 (n = 3 with 300 mM mannitol; n = 2 with 600 mM mannitol). 2×10^{-10} M vasopressin was present in bath in four of five experiments (see Fig. 6). Mean tubule length = 0.61±0.07 mm. Total CO_2 concentration in bath solutions was 25.4±0.4 mM. V, $[TCO_2]$, $JTCO_2$, V_{TE}, and * as in Table II. *P* values compare control vs mannitol (paired *t* test).

lation by vasopressin of acidification in the distal tubules and collecting ducts (19–21). This stimulation of urinary acidification may serve to prevent a fall in plasma bicarbonate concentration due to water retention during antidiuresis and/or to maintain net acid excretion under conditions in which urine flow rate is reduced (19). The effect of hypertonicity to inhibit bicarbonate absorption in the medullary thick ascending limb may contribute to the stimulation of urinary acidification in a manner similar to that proposed recently for vasopressin (5). During antidiuresis, osmolality would increase in the interstitial fluid of the renal medulla but would remain relatively unchanged in the renal cortex. Consequently, bicarbonate absorption would be inhibited in the medullary portion but not in the cortical portion of the thick ascending limb. The net result would be a shift in thick ascending limb bicarbonate absorption from the medulla to the cortex, maintaining constant net bicarbonate absorption by the loop segment as a whole. Such a shift would have the important effect of reducing delivery of bicarbonate to the interstitial fluid surrounding the outer medullary collecting ducts. This could limit accumulation of bicarbonate in the peritubular fluid adjacent to the collecting ducts, thus contributing to the increased collecting duct acidification observed during antidiuresis.

Finally, the results of this study also may provide insight into the urinary-acidifying effects of loop diuretics. The administration of furosemide causes a marked decrease in urine pH and an increase in urinary net acid excretion (22–26). This effect is due in part to a direct stimulation by furosemide of bicarbonate absorption in the cortical thick ascending limb (2). Results of the present experiments indicate that furosemide also may stimulate bicarbonate absorption indirectly in the medullary thick ascending limb as a result of its action to diminish medullary tonicity.

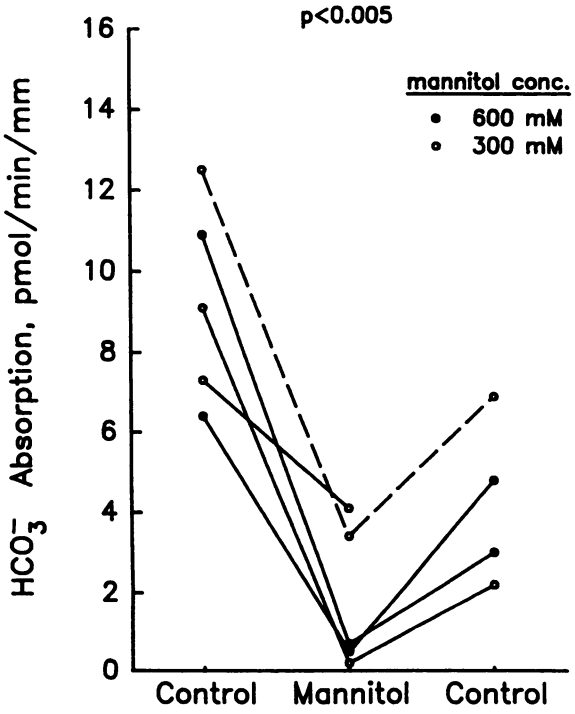

Figure 6. Effects of adding mannitol to perfusate and bath on bicarbonate absorption by rat medullary thick ascending limb. Open circles, 300 mM mannitol; filled circles, 600 mM mannitol. Solid lines, vasopressin (2×10^{-10} M) present in the bath throughout the experiments; broken line, vasopression absent from bath solutions. *P* value as in Fig. 1.

Acknowledgments

The author thanks Ofilia Chlamon for preparation of the manuscript.

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant DK-38217 and Research Career Development Award DK-01745.

References

1. Good, D. W., M. A. Knepper, and M. B. Burg. 1984. Ammonia and bicarbonate transport by thick ascending limb of rat kidney. *Am. J. Physiol.* 247(*Renal Fluid Electrolyte Physiol. 16*):F35–F44.

2. Good, D. W. 1985. Sodium-dependent bicarbonate absorption by cortical thick ascending limb of rat kidney. *Am. J. Physiol.* 248(*Renal Fluid Electrolyte Physiol. 17*):F821–F829.

3. Good, D. W. 1990. Bicarbonate absorption by the thick ascending limb of Henle's loop. *Semin. Nephrol.* 10:132–138.

4. Good, D. W. 1990. Adaptation of HCO_3^- and NH_4^+ transport in rat MTAL: effects of chronic metabolic acidosis and Na^+ intake. *Am. J. Physiol.* 258(*Renal Fluid Electrolyte Physiol. 27*):F1345–F1353.

5. Good, D. W. 1990. Inhibition of bicarbonate absorption by peptide hormones and cyclic adenosine monophosphate in rat medullary thick ascending limb. *J. Clin. Invest.* 85:1006–1013.

6. Hebert, S. C., R. M. Culpepper, and T. E. Andreoli. 1981. NaCl transport in mouse medullary thick ascending limbs. III. Modulation of the ADH effect by peritubular osmolality. *Am. J. Physiol.* 241(*Renal Fluid Electrolyte Physiol. 10*):F443–F451.

7. Hebert, S. C., W. B. Reeves, D. A. Molony, and T. E. Andreoli. 1987. The medullary thick ascending limb: function and modulation of the single-effect multiplier. *Kidney Int.* 31:580–588.

8. Winters, C. J., W. B. Reeves, and T. E. Andreoli. 1991. A survey of transport properties of the thick ascending limb. *Semin. Nephrol.* 11:236–247.

9. Good, D. W. 1987. Effects of potassium on ammonia transport by medullary thick ascending limb of the rat. *J. Clin. Invest.* 80:1358–1365.

10. Knepper, M. A. 1983. Urea transport in isolated, perfused thick ascending limbs and collecting ducts from rats. *Am. J. Physiol.* 245(*Renal Fluid Electrolyte Physiol. 14*):F634–F639.

11. Sasaki, S., and M. Imai. 1980. Effects of vasopressin on water and NaCl transport across the in vitro perfused medullary thick ascending limb of Henle's loop of mouse, rat and rabbit kidneys. *Pfluegers Arch. Eur. J. Physiol.* 383:215–221.

12. Work, J., J. H. Galla, B. B. Booker, J. A. Schafer, and R. G. Luke. 1985. Effect of ADH on chloride reabsorption in the loop of Henle of the Brattleboro rat. *Am. J. Physiol.* 249(*Renal Fluid Electrolyte Physiol. 18*):F698–F703.

13. Good, D. W., and F. S. Wright. 1980. Luminal influences on potassium secretion: transepithelial voltage. *Am. J. Physiol.* 239(*Renal Fluid Electrolyte Physiol. 8*):F289–F298.

14. Greger, R. Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol. Rev.* 65:760–797.

15. Mackovic-Basic, M., and I. Kurtz. 1990. H^+/base transport in the proximal straight tubule and thin descending limb of Henle. *Semin. Nephrol.* 10:122–131.

16. Torikai, S., and M. Imai. 1984. Effects of solute concentration on vasopressin stimulated cyclic AMP generation in the rat medullary thick ascending limb of Henle's loop. *Pfluegers Arch. Eur. J. Physiol.* 400:306–308.

17. Hebert, S. C. 1986. Hypertonic cell volume regulation in mouse thick limbs I. ADH dependency and nephron heterogeneity. *Am. J. Physiol.* 250 (*Cell Physiol. 19*):C907–C919.

18. Hebert, S. 1986. Hypertonic cell volume regulation in mouse thick limbs II. Na^+–H^+ and Cl^-–HCO_3^- exchange in basolateral membranes. *Am. J. Physiol.* 250(*Cell Physiol. 19*):C920–C931.

19. Paillard, M., and M. Bichara. 1989. Peptide hormone effects on urinary acidification and acid-base balance: PTH, ADH and glucagon. *Am. J. Physiol.* 256(*Renal Fluid Electrolyte Physiol. 25*):F973–F985.

20. Tomita, K., J. J. Pisano, M. B. Burg, and M. A. Knepper. 1986. Effects of vasopressin and bradykinin on anion transport by the rat cortical collecting duct. Evidence for an electroneutral sodium chloride transport pathway. *J. Clin. Invest.* 77:136–141.

21. Wall, S. M., J. M. Sands, M. F. Flessner, H. Nonoguchi, K. R. Spring, and M. A. Knepper. 1990. Net acid transport by isolated perfused inner medullary collecting ducts. *Am. J. Physiol.* 258(*Renal Fluid Electrolyte Physiol. 27*):F75–F84.

22. Bosch, J. P., M. H. Goldstein, M. F. Levitt, and T. Kahn. 1977. Effect of chronic furosemide administration on hydrogen and sodium excretion in the dog. *Am. J. Physiol.* 232:F397–F404.

23. Sebastian, A., M. Schambelan, and J. M. Sutton. 1984. Amelioration of hyperchloremic acidosis with furosemide therapy in patients with chronic renal insufficiency and Type 4 renal tubular acidosis. *Am. J. Nephrol.* 4:287–300.

24. Hropot, M., N. Fowler, B. Karlmark, and G. Giebisch. 1985. Tubular actions of diuretics: Distal effects on electrolyte transport and acidification. *Kidney Int.* 28:477–489.

25. Rastogi, S., J. M. Bayliss, L. Nascimento, and J. A. L. Arruda. 1985. Hyperkalemic renal tubular acidosis: effect of furosemide in humans and rats. *Kidney Int.* 28:801–807.

26. DuBose, T. D., Jr., and D. W. Good. 1988. Effects of diuretics on renal acid-base transport. *Semin. Nephrol.* 8:282–294.