Interactions of Lysyl-Bradykinin and Antidiuretic Hormone in the Rabbit Cortical Collecting Tubule

Victor L. Schuster, Juha P. Kokko, and Harry R. Jacobson
Department of Internal Medicine, Division of Nephrology, The University of Texas Health Science Center, Southwestern Medical School, Dallas, Texas 75235; Department of Internal Medicine, Nephrology Division, University of Iowa, Iowa City, Iowa 52242

Abstract. Although intrarenal infusions of kinins produce diuresis, it is not clear to what extent this response is due to hemodynamically mediated medullary washout and/or to direct epithelial effects of kinins. Recent evidence has shown that bradykinin binds to collecting tubules in vitro. We therefore examined the interactions of lysyl-bradykinin and antidiuretic hormone (ADH) with respect to hydraulic conductivity (L_p) in the rabbit cortical collecting tubule perfused in vitro. To ensure adequate substrate for prostaglandin synthesis, the bath contained 2.5 μM arachidonic acid. Arachidonic acid produced no change in base-line L_p and had no effect on the subsequent response to a supramaximal dose of ADH (100 μU/ml). Therefore, all subsequent experiments were done in the presence of arachidonic acid. Lysyl-bradykinin (10^{-9} M) added to either the lumen or bath had no effect on base-line L_p. Collecting tubules which were exposed for 1 h to bath lysyl-bradykinin (10^{-9} M) had a significantly diminished subsequent L_p in response to ADH ($P < 0.02$). In tubules exposed to bath lysyl-bradykinin plus indomethacin (5 μM), the subsequent ADH response was normal. Lysyl-bradykinin (10^{-9} M) added to the lumen had no effect on subsequent ADH response.

We conclude that lysyl-bradykinin from the basolateral side inhibits the hydroosmotic response of the cortical collecting tubule to ADH, and that this inhibition is probably prostaglandin-mediated. Lysyl-bradykinin does not affect water flow from the luminal surface. These data indicate that the diuresis seen with kinin infusions may result, at least in part, from a direct epithelial effect. They also suggest a role of the renal kallikrein-kinin system in modulating water transport in vivo.

Introduction

The exact physiologic role of the renal kallikrein-kinin system remains unclear. Clearance studies have shown that infusion of kinins into the renal artery produces natriuresis and diuresis (1–4). Micropuncture and tissue-tonicity studies have further suggested that bradykinin-induced redistribution of blood flow toward the medulla and the resulting medullary washout could, at least in part, account for these effects (5, 6). On the other hand, recent enzymatic and anatomic data suggest an action of the kallikrein-kinin system within the cortex. In the rabbit and rat kidney, kallikrein and prekallikrein have been localized to the connecting segment (7, 8). Although it is clear that kallikrein is a protease which converts low molecular weight kininogen to lysyl-bradykinin (LBK)[1] (9), it is not known if the latter is generated in the lumen of the connecting segment, in the peritubular blood, or both. Bradykinin has also recently been shown to bind specifically to cortical and medullary collecting tubules of the rabbit (10). In concert, these findings suggest that the kallikrein-kinin system, at least within the cortex, may act to modulate tubule transport directly from the basolateral and/or apical membrane. We therefore studied the effects of LBK on base-line and antidiuretic hormone (ADH)-stimulated osmotic water flow in the rabbit cortical collecting tubule. The data show that LBK from the bath side, but not the lumen, inhibits water flow due to a supramaximal dose of ADH, and that this inhibition is probably mediated via prostaglandin synthesis.

Methods

General microperfusion methods. In vitro microperfusion of cortical collecting tubules followed the general methods of Burg and Orloff (11). Briefly, normal female New Zealand white rabbits weighing 1.5–2.5 kg

Dr. Schuster was a Roche Fellow of the National Kidney Foundation during part of this work. Please address correspondence to Dr. Jacobson, Department of Internal Medicine, University of Texas Science Center, Dallas, TX 75235.

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1. *Abbreviations used in this paper:* ADH, antidiuretic hormone; CVP, calibrated volume pipette; J_v, net volume flux; L_p, hydraulic conductivity; LBK, lysyl-bradykinin; V_i, perfusion rate.

were killed by decapitation. The left kidney was quickly removed and 1–2-mm coronal slices were cut and placed in chilled, oxygenated Ringers-bicarbonate at pH 7.4. Dissection of tubules was done in the same bath as was used for perfusion experiments (below).

Cortical collecting tubules were freehand dissected at 4°C using sharpened forceps with the aid of a dissection microscope. The cortical ends of the tubules were cut just distal to their junction with a connecting segment. Tubules were not used if this junction occurred more than one-third of the way into the cortex from the cortical surface. Care was taken not to touch the tubules except at the ends. Tubules were transferred to a thermostatically-controlled chamber of 1 cm^3 volume containing ports for constant inflow and removal of bath. The tubules were sucked into holding pipettes containing elastomeric silicone resin (Sylgard 184, Dow Corning Corp., Midland, MI) and cannulated with an inner perfusion pipette. All tubules were perfused in an orthograde direction. Fluid from the tubules was collected under water-equilibrated mineral oil into a calibrated volume pipette (CVP). The volume of the CVP was determined by daily calibration: at the end of the experiment, the perfusion pipette was advanced into a second pipette containing a short (200 μm) column of Sylgard backed by mineral oil; perfusate was forced between the Sylgard and oil, and the CVP was filled in triplicate at 37°C from this perfusate.

Solutions. Tubules were always bathed in a bath containing (in mM): Na, 145; Cl, 112; HCO_3, 25; K, 5; Ca, 2.4; PO_4, 2.3; Mg, 1.0; SO_4, 1.0; acetate, 10; glucose, 8; alanine, 5; and bovine serum albumin, 6 g/100 ml (CRG-7, Armour Pharmaceutical Co., Tarrytown, NY). The osmolarity of the bath was 295 mOsM/kg. Tubules were initially perfused with a perfusate identical in composition to the bath except without bovine albumin. The tubules were warmed to 37°C and allowed to equilibrate for 60–90 min. The perfusate was then changed to one of identical composition except that the NaCl concentration was 15 mM (osmolarity = 132 mOsM/kg), and another 90 min were allowed. This second perfusate also contained [^{3}H]methoxy-inulin (New England Nuclear, Boston, MA) exhaustively dialyzed by the method of Schafer et al. (12). Baths were bubbled at 37°C with 95% O_2:5% CO_2 to pH 7.40, drawn anaerobically into syringes, and pumped through the perfusion chamber at ≥0.6 cm^3/min. Osmolarities of the bath and the 132 mOsM/kg perfusate containing [^{3}H]inulin were measured in duplicate daily.

Calculations. Net volume flux (J_v) was calculated from: $J_v = V_i - V_0/L$, where V_i = perfusion rate in nl/min, V_0 = collection rate in nl/min, and L = tubule length. V_0 was measured directly, and V_i was calculated from $V_i = V_0(cmp_0/cpm_i)$, where cpm_i and cpm_0 are perfusate and collected fluid [^{3}H]inulin counts/min per nl, respectively. Samples for cpm_0 were counted for 10 min. L was measured directly by eyepiece micrometer. Hydraulic conductivity, L_p (cm/atm per s) was determined from the J_v in response to an imposed osmotic gradient according to DuBois et al. (13): $L_p = (1/RTSC_b^2)\{C_b(V_i - V_0) + C_iV_i[\ln(C_b - C_i)V_i - \ln(C_bV_0 - C_iV_i)]\}$, where R is the gas constant, T is temperature (°K), S is the luminal surface area (assumed lumen diameter, 20 μm), C_b is bath osmolarity (mOsM/kg), C_i is perfusate osmolarity, and V_i and V_0 have the meanings previously described. It should be noted that the use of the method of DuBois (13) above, without measuring collected fluid osmolarity, rests on two assumptions: (*a*) that the reflection coefficient of the cortical collecting tubule for NaCl = 1.0, and (*b*) that no net transport of osmotically active substances (ions) occurs. The latter, of course, is untrue at 37°C. However, the net osmolar efflux rate due to ion transport in this segment is only ~20 pOsM/mm per min (14). With a perfusate of 130 mOsM/liter at a perfusion rate of 15 nl/min, the delivered osmolar load is 1950 pOsM/min. Even if bradykinin doubled or halved net ion flux rates, the effect on L_p measured in this way would only be ~1%.

Experimental protocols. Nine experimental protocols were used. The general format of these is as follows: Protocols 1–4: effect of various maneuvers on base-line L_p: (1) Addition of bath arachidonate (n = 5), (2) Time control (arachidonate present throughout) (n = 5), (3) Effect of bath LBK (arachidonate present throughout) (n = 6), (4) Effect of lumen LBK (arachidonate present throughout) (n = 8). Protocols 5–9: effect of various preexposures on the subsequent ADH response: (5) Unmodified bath (n = 5), (6) Arachidonate preexposure (n = 4), (7) Bath LBK plus arachidonate preexposure (n = 7), (8) Bath LBK plus indomethacin plus arachidonate preexposure (n = 5), (9) Lumen LBK plus arachidonate preexposure (n = 4). Specifically, in protocols 1–4, base-line L_p measurements were made after 180-min equilibration at 37°C (15), then a maneuver was performed, and then L_p was measured again over the next 30 min. In protocol 1, the maneuver performed was the addition of arachidonic acid (2.5 μM) to the bath. In protocol 2, the maneuver was a sham bath change, and thus, this protocol served as a time control. In protocol 3, the maneuver was the addition of LBK (10^{-9} M) to the bath, and in protocol 4, it was LBK addition to the perfusate. In protocols 2–4, arachidonic acid (2.5 μM) was present in the bath from the onset of perfusion.

In contrast, protocols 5–9 examined the effect of various preexposure conditions on the L_p response to ADH (100 μU/ml). In each case, L_p was measured before, and for at least 45 min following, the addition of ADH (100 μU/ml) to the bath. In protocol 5, the ADH response was assessed in unmodified bath (no arachidonic acid present). In protocol 6, the ADH response was measured with arachidonic acid (2.5 μM) present from the onset of perfusion. In protocol 7, the bath contained LBK (10^{-9} M) for 1 h before L_p measurements. In protocol 8, the bath contained indomethacin (5 μM) from the start of perfusion, plus LBK (10^{-9} M) for 1 h before L_p measurements. In protocol 9, the perfusate contained LBK (10^{-9} M) for 1 h before L_p measurements. In protocols 6–9, arachidonic acid (2.5 μM) was present in the bath from the onset of perfusion.

Reagents. Arachidonic acid (99% purity) and LBK were purchased from Sigma Chemical Co. (St. Louis, MO). The arachidonic acid was kept frozen in the dark under inert gas as a 25 mM stock solution in 0.1 M Tris, pH 8. All bath infusion syringes containing arachidonic acid were kept covered with aluminum foil during the experiment to minimize oxidation. LBK was kept frozen as aliquoted 1 μg/μl stock in Ringers plus 2% bovine albumin and thawed daily as needed. ADH (Arg-Vasopressin, Calbiochem-Behring Corp., San Diego, CA) was kept refrigerated as an aqueous stock solution (0.5 mg/ml). Indomethacin (Sigma Chemical Co.) was aliquoted and kept frozen as a 1 mg/ml stock in 75 mM $NaCO_3$.

Statistics. Perfusion rates, J_v and L_p, from three to four collections on a given tubule were meaned; then, this value became the value for that tubule for that experimental period. The value "n" equals number of tubules. Since protocols 1–4 studied the effect of a given maneuver on base-line L_p in the same tubule, the two-tailed paired t test was used. In addition, protocols 1, 3, and 4 were compared with protocol 2 (time control) by two-way analysis of variance (16). In protocols 5–9, a control group of tubules was compared with each of several experimental groups, also by two-way analysis of variance. Differences were considered significant if $P < 0.05$.

Results

Base-line L_p: arachidonic acid. Arachidonic acid was present in the bath in experiments using LBK because of concern over the ability of cortical collecting tubules to adequately synthesize

prostaglandins in a bath containing only Ringers solution. Fetal calf serum was omitted because of likely kininase activity. Schlondorff et al. (17) reported that cortical collecting tubules incubated in Ringers alone have falling rates of prostaglandin synthesis with time and show lack of stimulation of prostaglandin synthesis by ADH, whereas Kirschenbaum et al. (18) reported that cortical collecting tubules incubated in 0.5 mM arachidonic acid do respond to ADH with prostaglandin synthesis. For reasons discussed later, we chose a much lower concentration of arachidonic acid (2.5 μM). In this first group of tubules, we examined the response of base-line water permeability to the addition of arachidonic acid. In five tubules, shown in Table I, base-line L_p was measured after 180 min; the bath was then changed to contain 2.5 μM arachidonic acid, and L_p was then measured again. The L_p changed by a mean of $-8.7\pm4.1 \times 10^{-7}$ cm/atm per s. This change was not significant (paired t test). Moreover, the magnitude of the fall in L_p was not different from that seen with time alone (next protocol, by analysis of variance).

Base-line L_p: time controls. In five tubules, L_p was measured after 180-min equilibration. The bath then underwent a sham change and L_p was measured again. Arachidonic acid, 2.5 μM, was present throughout. The results are shown in Table II. Base-line L_p changed by a mean paired difference of $-3.2\pm2.2 \times 10^{-7}$ cm/atm per s; this change was not significant (paired t test).

Base-line L_p: bath LBK. Six tubules were incubated in 2.5 μM arachidonic acid for the 180-min equilibration period, after which base-line L_p was measured. Then, the bath was changed to contain arachidonic acid plus LBK (10^{-9} M). As shown in Table III, LBK produced a mean change in L_p of $-7.2\pm3.6 \times 10^{-7}$ cm/atm per s. This change was not significant by the paired t test, nor was the magnitude of the fall in L_p significantly different from that seen in the time controls above (analysis of variance).

Base-line L_p: lumen LBK. In experiments analogous to the previous set, the effect of lumen LBK on base-line L_p was assessed in eight tubules. The mean change in L_p was $-0.7\pm2.0 \times 10^{-7}$ cm/atm per s, as shown in Table IV. Again, this change was not significant by paired analysis, and was not different from that seen in the time controls above (analysis of variance).

ADH response: no arachidonic acid. Five control tubules were incubated during the 180-min equilibration period without arachidonic acid. Then, the response to a supramaximal dose of ADH (100 μU/ml) was measured for 45 min. The L_p in the 25–35-min period post-ADH are shown in Table V. L_p for the 15–25 and 35–45-min periods were $132.6\pm11.6 \times 10^{-7}$ and $115.4\pm10.9 \times 10^{-7}$ cm/atm per s, respectively.

ADH response: arachidonic acid present. Four additional control tubules were incubated during the 180-min equilibration period in 2.5 μM arachidonic acid and their subsequent response to a supramaximal dose of ADH was assessed. These tubules are also shown in Table V. Mean L_p's for the 15–25 and 35–45-min post-ADH periods were $121.3\pm13.3 \times 10^{-7}$ and $130.6\pm17.1 \times 10^{-7}$ cm/atm per s. The two groups shown in Table V were not significantly different at any time period fol-

Table I. Effect of 2.5 μM Arachidonic Acid on Base-line Water Pemeability

	Pre-AA		Post-AA, 2.5 μM	
Tubule	V_i	L_p	V_i	L_p
1	18.6	7.7	19.7	−0.5
2	19.0	18.0	16.9	11.1
3	15.7	14.7	20.9	12.0
4	16.5	28.3	17.1	27.2
5	14.0	48.7	13.2	24.3
Mean	16.8	23.5	17.6*	14.8*
SE	0.9	7.1	1.3	4.9

AA, arachidonic acid.
*, NS compared with previous period (paired t test).

Table II. Base-line Water Permeability Time Controls with Arachidonic Acid

	Period 1		Period 2	
Tubule	V_i	L_p	V_i	L_p
1	12.9	36.5	16.9	25.0
2	14.7	32.4	16.6	28.7
3	14.4	21.3	14.7	22.0
4	13.8	20.5	17.1	20.0
5	15.5	15.3	12.4	14.3
Mean	14.3	25.2	15.1*	22.0*
SE	0.4	3.9	0.9	2.4

*, NS.

Table III. Effects of 10^{-9}M Bath LBK on Base-line Water Permeability

	Prebath LBK		Postbath LBK	
Tubule	V_i	L_p	V_i	L_p
1	17.9	52.8	16.8	31.1
2	17.1	27.2	19.5	18.2
3	17.8	24.8	14.6	18.5
4	15.3	1.1	16.0	0.5
5	13.0	19.0	15.8	9.0
6	14.7	6.3	13.9	10.6
Mean	15.9	21.9	16.1*	14.7*
SE	0.8	7.5	0.8	4.3

*, NS.

Table IV. Effect of 10^{-9}M Lumen LBK on Base-line Water Permeability

	Prelumen LBK		Postlumen LBK	
Tubule	V_i	L_p	V_i	L_p
1	13.3	18.7	11.7	16.0
2	11.3	9.8	8.6	10.5
3	10.9	24.0	8.8	12.0
4	11.3	9.8	8.6	11.8
5	13.3	15.3	13.4	10.3
6	15.5	20.3	12.4	23.0
7	14.0	9.0	12.4	12.8
8	16.5	11.8	15.5	16.8
Mean	13.3	14.8	11.4	14.2
SE	0.7	2.0	0.9	1.5

*, NS.

lowing ADH (by unpaired analysis). Therefore, for comparisons to subsequent experimental groups, all tubules in Table V were combined as a single control group with mean values as shown

Table V. Control Response to ADH (100 μU/ml) without and with Preincubation in 2.5 μM Arachidonic Acid

			25–35 min Post-ADH
Tubule	L	V_i	L_p
	mm		
No arachidonic acid			
1	1.90	11.8	85.0
2	2.10	10.1	130.0
3	2.30	11.3	149.0
4	1.60	17.0	144.5
5	1.50	16.3	128.5
Mean		13.3	127.4
SE		1.4	11.3
With arachidonic acid			
1	1.90	11.6	94.0
2	1.40	17.9	141.0
3	1.20	18.2	122.5
4	2.50	20.3	173.0
Mean		17.0	132.6
SE		1.91	6.6
P*		NS	NS
Combined means		14.9	129.7
SE		1.3	9.1

L, length.
*, compared with group without arachidonic acid (unpaired t test).

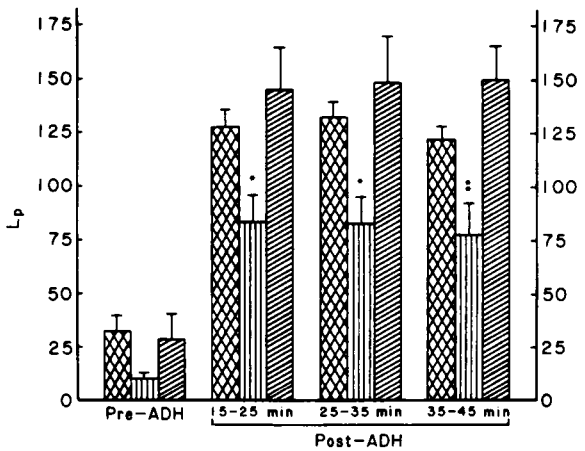

Figure 1. Base-line hydraulic conductivity (cm/atm per s × 10^7, *left*) and response to ADH (100 μU/ml, *right*) at 15–25, 25–35, and 35–45 min. The control group (cross-hatched bars) is the combined control group from Table V. A second group was exposed to bath LBK (10^{-9} M) for 1 h before ADH was added (vertical-line bars). A third group was exposed to bath LBK and indomethacin (5 μM) for 1 h before ADH (diagonal-line bars). *, $P < 0.02$; ** = $P < 0.01$ compared with control group, bath LBK plus indomethacin group, and lumen LBK group (Table VIII) by analysis of variance.

at the bottom of Table V. As shown in Fig. 1, the base-line L_p and three post-ADH period L_p's for this combined control group were 32.5±8.3 × 10^{-7}, 127.6±8.4 × 10^{-7}, 129.7±9.1 × 10^{-7}, and 122.2±9.4 × 10^{-7} cm/atm per s, respectively. Subsequent protocols had 2.5 μM arachidonic acid in the bath throughout.

ADH response: pre-incubation with bath LBK. Seven tubules were exposed for 1 h to 10^{-9} M bath LBK before ADH administration. As shown in Table VI and Fig. 1, this exposure resulted in a markedly blunted subsequent ADH response: the three periods following ADH had mean L_p of 83.4±13.3 × 10^{-7}, 82.7±13.1 × 10^{-7}, and 77.4±15.1 × 10^{-7} cm/atm per s, respectively. These differences were significantly different from the control group ADH response (see Fig. 1 for P values). The base-line L_p after 1-h incubation with 10^{-9} bath LBK was not different from the control period base-line L_p (10.6±2.2 × 10^{-7} vs. 32.5±8.3 × 10^{-7}, NS by analysis of variance).

Table VI gives the perfusion rates for the 25–35-min post-ADH period. These were not significantly different from the perfusion rates for the comparable control period. Likewise, perfusion rates for the 35–45-min period were not different. However, for the 15–25-min post-ADH period, perfusion rates for the bath LBK group were significantly higher than control (17.9±.8 vs. 15.1±.9 nl/min, $P < 0.03$). At these rapid perfusion rates, however, the collected fluid is far from being in osmotic equilibration with the bath. Moreover, if any error was introduced, it would have been in the direction of lessening the difference between the control and LBK L_p's (13).

ADH response: bath LBK plus indomethacin. Since available evidence implicates prostaglandins as mediators of bradykinin actions in several tissues, experiments using the cyclooxygenase

Table VI. Response to ADH (100 μU/ml) after Preexposure to 10^{-9} M Bath LBK

Tubule	L	V_i	25–35 min Post-ADH L_p
1	2.60	17.7	66.0
2	1.80	18.7	55.5
3	1.75	17.8	75.0
4	1.70	19.5	114.0
5	1.45	17.6	141.5
6	1.80	18.3	41.0
7	1.50	15.4	86.0
Means		17.9	82.7
SE		0.5	13.1
P*		NS	<0.02

* Compared with combined control group (Table V), bath LBK group plus indomethacin (Table VII), and lumen LBK group (Table VIII) by analysis of variance.

inhibitor indomethacin during bath LBK exposure were performed. Preliminary attempts at using 5×10^{-5} M indomethacin resulted in tubule deterioration. Tubules remained viable, however, in 5×10^{-6} M indomethacin. In five tubules exposed to indomethacin from the onset of perfusion plus 10^{-9} M bath LBK for 1 h before ADH, the base-line and post-ADH L_p, as shown in Table VII and Fig. 1, were $28.6 \pm 7.6 \times 10^{-7}$, $144.8 \pm 20.5 \times 10^{-7}$, $148.1 \pm 22.5 \times 10^{-7}$, and $148.4 \pm 23.2 \times 10^{-7}$ cm/atm per s, respectively. The base-line L_p was not statistically different from either the control or bath LBK base-line L_p (analysis of variance). The post-ADH L_p, however, were significantly different from the bath LBK values, but not different from control. Thus, indomethacin reversed to normal the inhibition of ADH action seen with bath LBK alone.

The perfusion rates for the three post-ADH periods in this group (18.5±1.0, 17.4±1.1, and 17.9±0.7 nl/min, respectively) were not significantly different from those for the comparable bath LBK periods.

ADH response: lumen LBK. Because kallikrein and low molecular weight kininogen both can be found in the urine (9), it is possible that generation of LBK occurs within the lumen of the connecting segment and subsequently acts upon the cortical collecting tubule from the luminal surface. We, therefore, examined the effect of 10^{-9} M lumen LBK on the subsequent ADH response. The responses at 25–35-min post-ADH are shown in Table VIII, and are representative of the L_p values observed at 15–25 and 35–45 min. There was no significant difference between the ADH response in this group of tubules and that of the control or bath LBK plus indomethacin groups. The ADH response was, however, significantly higher than that for the bath LBK group (analysis of variance).

Discussion

These studies are important because they demonstrate, for the first time, a direct epithelial effect of bradykinin on nephron transport in vitro. Four aspects of the present studies deserve elaboration: (*a*) correlation with bradykinin effects in ADH-responsive anuran urinary bladders; (*b*) the role of prostaglandins in bradykinin's action; (*c*) the results with luminal LBK; and (*d*) the significance of these studies with respect to understanding the function of the renal kallikrein-kinin system.

The interactions of bradykinin and ADH-mediated water flow in the toad bladder have been previously addressed in two ways. First, exogenous bradykinin and LBK have been added to the mucosal and serosal surfaces. Furtado (19) found no effect of either of these kinins on basal water flow, but ADH action

Table VII. Response to ADH (100 μU/ml) after Preexposure to 10^{-9} M Bath LBK Plus Indomethacin (5 μM)

Tubule	L	V_i	25–35 min Post-ADH L_p
1	1.84	13.2	150.5
2	1.25	18.0	187.0
3	1.90	19.9	85.0
4	1.50	17.0	112.0
5	2.10	18.7	206.0
Means		17.4	148.1
SE		1.1	22.5
P*		NS	NS

* Compared with combined group (Table V) and lumen LBK group (Table VIII) by analysis of variance.

Table VIII. Response to ADH (100 μU/ml) after Preexposure to 10^{-9} M Lumen LBK

Tubule	L	V_i	25–35 min Post-ADH L_p
1	1.90	17.7	151.8
2	1.95	19.6	186.1
3	0.90	14.3	125.6
4	2.80	27.8	155.0
Mean		19.9	154.6
SE			12.4
P*			NS

* Compared with control group, and bath LBK group plus indomethacin (by analysis of variance).

was inhibited by both kinins. Second, since the toad bladder contains all components of the kallikrein-kinin cascade, another approach has been to inhibit kallikrein protease activity with aprotinin. Depending upon the species of toad, this maneuver has been reported to either have no effect (20) or to stimulate ADH-mediated water flow (21). Likewise, enhancement of endogenous kinin levels in the toad bladder by kininase inhibitors has led to inhibition of the osmotic response to ADH (21). In both the studies of Orce et al. (20) and Carvounis et al. (21), manipulation of the toad bladder endogenous kallikrein-kinin system produced no effect on non-ADH-stimulated (base-line) water flow. In concert, this evidence from the toad bladder has suggested that kinins inhibit ADH-mediated water flow, but do not alter basal water permeability. The present studies using isolated, perfused collecting tubules agree, at least qualitatively, with these previous toad bladder data.

In our studies, the inhibition of ADH action by preexposure to bath LBK was completely reversed by indomethacin, suggesting a role for prostaglandins in this bradykinin effect. Bradykinin has been reported to stimulate prostaglandin E_2 synthesis in cultured dog cortical collecting duct cells (22) and in MDCK cells (which have some properties in common with collecting ducts) (23) at the concentration (10^{-9} M) used here. Also, it has been known since the studies of Grantham and Orloff (24) in the cortical collecting tubule and Orloff et al. (25) in the toad bladder that prostaglandin E_1 can inhibit the hydroosmotic response to ADH, at least in part (26) by inhibiting the generation of cyclic AMP. Our experiments are therefore consistent with a model in which bradykinin stimulates endogenous prostaglandin synthesis in cortical collecting tubules, such that the subsequent hydroosmotic action of ADH is, in turn, inhibited by these prostaglandins.

Two important points should be made concerning this interpretation, however. First, it is possible that part of the increase in L_p seen in the indomethacin-treated tubules was due to phosphodiesterase inhibition. Flores and Sharp (27) reported about 15–20% inhibition of phosphodiesterase activity in the toad urinary bladder by indomethacin at the concentration (5 μM) used here. It is unclear, however, whether this action of the drug produces a quantitatively significant effect on water flow. Indomethacin alone does not alter basal water flow in the toad bladder (28, 29). Moreover, even when toad bladder phosphodiesterase activity is inhibited virtually 100% by theophylline (27), the resulting water flow is still only a fraction of that seen at low doses of ADH (30). Indomethacin does not alter rat renal medullary phosphodiesterase activity (31, 32), and in contrast to its effects in the toad bladder, in the case of isolated, perfused cortical collecting tubules bathed in arachidonic acid, indomethacin does not augment L_p in response to submaximal ADH (18). As far as we are aware, the effect of indomethacin on phosphodiesterase activity in rabbit cortical collecting tubules has not been directly determined. On the whole, however, we feel that the most likely mechanism for indomethacin-induced reversal of the bradykinin effect reported here is inhibition of prostaglandin synthesis.

The second point concerning the apparent prostaglandin mediation of bradykinin's effect concerns the temporal sequence of these experiments. Our data show that preexposure of tubules to bradykinin blunts the subsequent ADH response. It is not clear that bradykinin added after ADH would have had an identical effect. We performed these experiments at 37°C because, based on the data of Hall and Grantham (15), we were unsure whether or not cortical collecting tubules can synthesize prostaglandins normally at room temperature. If, at 37°C, the decline in L_p which begins about 45 min post-ADH (15) is due to prostaglandin synthesis, then the blunted L_p seen at 30-min post-ADH in our bradykinin-exposed tubules may have represented an experimental condition in which ADH-mediated prostaglandin synthesis had not yet peaked. The sequential exposure to the two peptides which we employed may not necessarily occur in vivo; rather, the epithelium might be exposed to bradykinin during maximal ADH-induced prostaglandin synthesis. If this were the case, a further reduction in L_p by bradykinin might depend on whether or not bradykinin and ADH stimulate the same prostaglandin end products and/or share a common intracellular pool of arachidonic acid. We have not addressed this reverse sequence of peptide exposure because of the difficulties inherent in interpreting falling L_p at 37°C.

We used arachidonic acid in the bathing medium to ensure optimal conditions for hormone-mediated prostaglandin synthesis. Moreover, we sought a concentration of arachidonic acid which would minimize nonspecific augmentation of prostaglandin synthesis but still allow hormone-mediated stimulation to occur. In this regard, Schwartzman et al. (33) found, in the isolated perfused rabbit kidney, that 6.6 μM arachidonic acid produced only a 50% increase in prostaglandin synthesis, whereas bradykinin produced a nearly 1,200% increase. Similarly, in the colon, even an arachidonic acid concentration of 0.5 mM (200-fold the present dose) failed to quantitatively mimic the action of bradykinin on chloride transport (34). Kirschenbaum et al. (18) found that 50 μM arachidonic acid in the bath inhibited the hydroosmotic response of in vitro cortical collecting tubules to 2.5 μU/ml ADH. In contrast, Stokes (35) found that neither 10 nor 5 μM arachidonic acid altered base-line water permeability or the response to 1 μU/ml ADH. These differences underscore the dose-dependent effect of exogenously added arachidonic acid on apparent endogenous prostaglandin synthesis rates. Our own data suggest that 2.5 μM arachidonic acid did not stimulate nonspecific prostaglandin production to an extent which would inhibit maximal ADH action, since the osmotic permeabilities with and without this dose of arachidonic acid were virtually identical (Table V). Base-line water permeabilities were also not altered by this dose of arachidonic acid.

It should be noted that, even though intrarenal infusions of bradykinin produce renal prostaglandin release (33, 36), several clearance studies have been unable to implicate prostaglandins in the whole-kidney diuretic response to bradykinin (4, 37, 38). As discussed in the introduction, however, such peptide infusions produce multiple intrarenal effects. It is possible that medullary washout persists in the face of cyclooxygenase inhibitors to an

extent such that the normalized collecting duct water permeability cannot be expressed in the final urine osmolarity.

Our results with luminal addition of LBK were negative. These studies are thus at variance with those of Furtado (19) who found equivalent inhibition of ADH action in the toad bladder by bradykinin added to either the mucosal or serosal side. In addition, in a preliminary report, Garcia-Perez and Smith (39) have suggested that bradykinin stimulates prostaglandin release from cultured canine collecting tubule cells when applied to the apical side of the cell monolayer, but not the basolateral side. It is not clear why basolateral bradykinin would produce no prostaglandin synthesis in cultured cells, but would inhibit ADH action via an indomethacin-sensitive mechanism in intact perfused tubules. Species differences may play a role. It is also possible that intact tubules and cultured cells behave differently with respect to prostaglandin synthesis. For example, 33 μM arachidonic acid produces sevenfold stimulation of prostaglandin synthesis in intact rabbit cortical collecting tubules, but 250-fold stimulation in cultured collecting duct cells from the same species (40). The reason(s) for this quantitative difference are not clear. Our studies with luminal LBK may be at variance with the toad bladder studies of Furtado (19) because of differences in the dose of LBK used. It is possible that the high dose (10^{-6} M) of LBK employed by Furtado would also act from the lumen in collecting tubules. We have not examined this issue. However, we chose the LBK dose of 10^{-9} M because this concentration stimulates prostaglandin synthesis at just above the half-maximal rate in cultured collecting duct cells (22), and it is quite close to the normal human urine LBK concentration (41). The apparent sidedness (basolateral) for bradykinin's action demonstrated here appears inconsistent with immunohistochemical studies showing an apical location of distal nephron kallikrein (42). It should be noted, however, that in the isolated perfused kidney, more kallikrein-like activity is spontaneously released into the venous effluent than into the urine per unit time (43). Moreover, Yamada and Erdos (44) have recently reported isolation of a basolateral kallikrein from rat kidney with properties which are different from urinary kallikrein. Taken together, these studies and our data suggest that an action of bradykinin from the basolateral side in vivo is possible. Our negative luminal studies do not rule out the potential for bradykinin action from this membrane under certain conditions, however.

Finally, the present results help to further define the physiologic role of the renal kallikrein-kinin system. Water loading increases kallikrein excretion (9, 45), suggesting activation of the kallikrein-kinin system. In addition, examination of circadian rhythms of urine volume and urinary kallikrein excretion during spontaneous drinking shows that these vary linearly with each other (46). These studies thus suggest that kinin activation occurs during water diuresis when ADH levels are either declining or are suppressed. In this context, we have interpreted our data as showing no effect of kinins on osmotic water permeability in the absence of ADH; thus, it makes a regulatory role for bradykinin in the absence of ADH unlikely. However, we cannot absolutely rule out an LBK effect on base-line L_p. In percentage terms, bath LBK addition over 30 min appeared to decrease base-line L_p more (33%) than either lumen addition (5%) or time controls (13%), but this decrease was not statistically different from that seen in the time controls. After exposure to bath LBK for 1 h, the pre-ADH L_p (a "base-line" L_p term) was not significantly lower than similar measurements in control, lumen LBK, or bath LBK plus indomethacin groups, all of which were compared by analysis of variance ($P = 0.053$). However, analysis of variance is weighted toward avoiding an "alpha" error, i.e. concluding that two groups are different when they are not. Particularly with the relatively small sample size we employed, analysis of variance may commit a beta type II error; that is, it may conclude that no difference exists between two groups when such a difference does exist (16). Therefore, it is possible that exposure to bath LBK for 1 h does indeed lower base-line L_p. Studies utilizing a large number of tubules would probably be needed in order to demonstrate such a difference.

On the other hand, administration of ADH to dogs and rats results in enhanced urinary kallikrein excretion (47, 48) and since infusions of bradykinin antagonize the action of ADH in clearance experiments (1), these studies have suggested that the kallikrein-kinin system may serve as a feedback inhibitor of ADH action. Although the examination of kallikrein excretion rates suffers the drawback that these determinations may not reflect rates of intraluminal or peritubular generation of LBK, urinary kinins have been correlated with diuresis in at least one study (49). Thus, this second group of studies suggests activation of the kallikrein-kinin system when ADH levels are high. If our data represent a physiologic event, then the inhibition of ADH action by LBK would be consistent with activation of the kallikrein-kinin system at high ADH levels.

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