Functional Profile of the Isolated Uremic Nephron

IMPAIRED WATER PERMEABILITY AND ADENYLATE CYCLASE RESPONSIVENESS OF THE CORTICAL COLLECTING TUBULE TO VASOPRESSIN

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ABSTRACT Resistance of the chronically diseased kidney to vasopressin has been proposed as a possible explanation for the urinary concentrating defect of uremia. The present studies examined the water permeability and adenylate cyclase responsiveness of isolated cortical collecting tubules (CCT) from remnant kidneys of uremic rabbits to vasopressin. In the absence of vasopressin the CCTs of both normal and uremic rabbits were impermeable to water. At the same osmotic gradient, addition of a supramaximal concentration of vasopressin to the peritubular bathing medium led to a significantly lower net water flux per unit length (and per unit luminal surface area) in uremic CCTs than in normal CCTs. Transepithelial osmotic water permeability coefficient, P_f, was 0.0232 $\pm$0.0043 cm/s in normal CCTs and 0.0059$\pm$0.001 cm/s in uremic CCTs ($P < 0.001$). The impaired vasopressin responsiveness of the uremic CCTs was observed whether normal or uremic serum was present in the bath.

Basal adenylate cyclase activity per microgram protein was comparable in normal and uremic CCTs. Stimulation by NaF led to equivalent levels of activity in both, whereas vasopressin-stimulated activity was 50% lower in the uremic than in the normal CCTs ($P < 0.025$).

The cyclic AMP analogue, 8-bromo cyclic AMP, produced an increase in the P_f of normal CCTs closely comparable to that observed with vasopressin. In contrast, the P_f of uremic CCTs was only minimally increased by this analogue and was not further stimulated by theophylline.

These studies demonstrate an impaired responsiveness of the uremic CCT to vasopressin. This functional defect appears to be a result, at least in part, of a blunted responsiveness of adenylate cyclase to vasopressin. The data further suggest that an additional defect in the cellular response to vasopressin may exist, involving a step (or steps) subsequent to the formation of cyclic AMP.

A unifying concept of the urinary concentrating defect of uremia is proposed which incorporates a number of hitherto unexplained observations on the concentrating and diluting functions of the diseased kidney.

INTRODUCTION

Among the several mechanisms proposed to explain the urinary concentrating defect of uremia is an impaired responsiveness of the kidney to vasopressin (1, 2). The evidence supporting this proposed mechanism is indirect and has been derived from clearance-type studies in man. Nevertheless, certain features of end-stage renal disease suggest that some aspect of the uremic state per se rather than a series of separate defects, each specific for a different disease state, may account for the failure of the uremic patient to produce urine which is maximally concentrated in response to an appropriate stimulus. A large variety of renal diseases unrelated etiologically and vastly different anatomically are characterized by a urinary concentrating defect (1–5). Among these, disruption of the inner medullary structures (6, 7) or local alterations in medullary blood flow (8) are of only occasional importance

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as an explanation for the concentrating defect. Impaired sodium chloride transport out of the thick ascending limb of Henle's loop does not appear to play an important role in this pathophysiological aberration since the ability to dilute the urine persists long after the concentrating defect becomes manifest (3). Similarly, the obligatory solute diuresis per nephron characteristic of most forms of chronic renal disease is unlikely to be the main underlying cause of the defect since the maximal urine:plasma osmolality ratio is diminished even when values are corrected for the effects of increased solute excretion per nephron (3).

Recently we have demonstrated an impaired intrarenal recycling of urea and failure to concentrate solutes in the medullary interstitium in the pyelonephritic rat. This abnormality is associated with an inability to concentrate the urine normally with failure to conserve free-water maximally (9). Among the possible explanations for this phenomenon, and one which could not be ruled out by the existing data was a diminished responsiveness to vasopression in the cortical collecting tubule. Because this segment of the nephron is essentially impermeable to urea but permeable to water in the presence of vasopressin (10), the removal of water from this region serves to elevate the concentration of urea within the tubular fluid. Hence, a high concentration of urea is presented to the papillary collecting duct, a segment that is permeable to urea. By this mechanism, diffusion of urea into the interstitium occurs. Impaired vasopressin responsiveness would thus limit the ability of the kidney to concentrate the urine by two separate mechanisms: (*a*) impaired diffusion of water out of the collecting tubule, and (*b*) failure to recycle urea and accumulate interstitial solute normally.

In the present studies, the vasopressin responsiveness of the isolated cortical collecting tubule of normal and uremic rabbits was studied in vitro.

METHODS

Experimental animals

Experiments were performed on isolated cortical collecting tubules of white New Zealand female rabbits 2–3 kg in weight. The animals were maintained on an ad libitum diet of standard rabbit chow and tap water for 1–3 mo before the study. The tubule segments studied were obtained from (*a*) the intact kidneys of normal rabbits and (*b*) the solitary remnant kidneys of uremic rabbits. The surgical technique for the creation of the remnant kidney in the rabbit has been described elsewhere (11).

Maximum urinary concentrating ability

Studies were performed on seven normal and nine uremic rabbits. The animals were placed in metabolic cages and deprived of food and water for 32 h. The urine formed during the first 24-h period was discarded. Each animal then received five units of Pitressin tannate in oil (Parke, Davis & Co., Detroit, Mich.) and all the urine passed during the ensuing 8 h was collected in a beaker under mineral oil. Urine osmolality was measured with a Wescor vapor pressure osmometer (Wescor Inc., Logan, Utah).

Preparation of sera and perfusion solutions

Uremic rabbit serum was obtained from the femoral artery of uremic rabbits as described elsewhere (11). Normal rabbit serum was obtained commercially (Microbiological Associates, Walkersville, Md.). Blood urea nitrogen (BUN)[1] was measured on all sera using a Beckman BUN analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Since the uremic sera tended to have higher osmolalities than the normal sera, all were adjusted to 330 mosmol/kg H_2O by the addition of the appropriate amount of urea. The perfusate used in all experiments was composed of (in millimoles per liter); NaCl 60; K_2HPO_4 2.5, $CaCl_2$ 1.0 and $MgSO_4$ 1.2. (Osmolality 130 mosmol/kg H_2O.) [*carboxy*-^{14}C]Inulin (50 μCi/ml) was added to the perfusate to act as an impermeant volume marker. The pH of all solutions was adjusted to 7.4.

Perfusion of cortical collecting tubules

Cortical collecting tubules (CCT) were dissected from normal or remnant kidneys and perfused in vitro as described previously from this laboratory (12, 13). CCTs from nine normal rabbits were studied; six were perfused in a bath of normal serum and three in uremic serum. CCTs from 13 uremic rabbits were studied; 9 in uremic sera and 3 in normal sera. Tubule lengths varied from 0.71 to 3.18 mm; there were no significant differences in the mean lengths of the segments perfused between the groups.

Experiments were conducted at 37°C at a transtubular osmotic gradient of 200 mosmol/kg H_2O. The bath was bubbled with 95% O_2–5% CO_2 and osmolality was maintained constant by monitoring the addition of deionized water to the bath (12, 13). Transepithelial potential difference (PD) was measured throughout the experiment as described previously (12, 13). Both ends of the tubule were insulated with Sylgard 184 (Dow Corning Corp., Midland, Mich.). The mean (±SE) perfusion rate was 7.55±1.03 nl/min in normal tubules and 7.07±0.74 nl/min in uremic tubules.

Influence of vasopressin. CCTs were generally mounted on the perfusion apparatus within 15–20 min after the death of the animal. An equilibration period of 2½ h was allowed, during which time transepithelial PD had stabilized and the tubules had become impermeable to water (see Results).

Four-five control collections of fluid emerging from the tubule were made. Vasopressin (Pitressin, Parke, Davis & Co.) 200 μU/ml was then added to the bath. Previous studies have demonstrated that this is a supramaximal dose of vasopressin (10, 14, 15). In five of the experiments on uremic tubules a dose of 2,000 μU/ml was used. After the addition of vasopressin, collections were made every 10 min for 1 h. Samples from each period were analyzed for ^{14}C activity and osmolality. The samples for radioactivity were pipetted directly into liquid scintillation fluid, whereas those used for osmolality measurements were deposited under oil and then transferred to the sample holder of a

[1] *Abbreviations used in this paper*: 8-bromo-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphoric acid; BUN, blood urea nitrogen; CCT, cortical collecting tubule; Jv, net water flux; PD, potential difference; P_f, transepithelial osmotic water permeability coefficient.

Clifton Nanoliter Osmometer (Clifton Technical Physics, Hartford, N. Y.).

Influence of 8-bromoadenosine 3',5'-cyclic monophosphoric acid (8-bromo-cAMP). Previous studies on isolated CCTs have demonstrated that only excessively high concentrations of cAMP (approximately 10 mM) can mimic the water permeability response of vasopressin (10, 15). In the present studies the influence of a more permeable analogue, 8-bromo-cAMP (16), was evaluated in four normal and five uremic CCTs. After the initial equilibration and control periods 8-bromo-cAMP was added to the bath solution in increasing concentrations varying from 10 μM to 1 mM. At each bath concentration of 8-bromo-cAMP, samples were collected every 10 min for 30 min and analyzed for ^{14}C and osmolality as described above. To evaluate the possibility that the decreased response to exogenous cAMP might be due to increased phosphodiesterase activity, the uremic CCTs were studied during an additional experimental period in which 1 mM 8-bromo-cAMP plus 8 mM theophylline were present in the bath.

Calculations

Perfusion rate (V_o) is calculated as $^{14}C_L/(^{14}C)_o \cdot t$ where $^{14}C_L$ is the total amount of isotope collected, $(^{14}C)_o$ is the concentration of isotope in the perfusate, and t is the duration of the collection. Net fluid reabsorption (net water flux), Jv, is equal to $V_o - V_L$ where V_L is the collection rate. Jv is expressed per millimeter length of tubule (nanoliters per millimeter per minute) or per unit luminal surface (nanoliters per square centimeter per second). Tubule length and internal diameter were measured during perfusion with a calibrated reticle in the ocular of the microscope. Transepithelial osmotic water permeability coefficient, P_f, (centimeters per second) was computed according to the expression derived by Al-Zahid and co-workers (17):

$$P_f = -\frac{V_0 C_0}{A \bar{V}w}\left[\frac{C_0 - C_L}{C_0 C_b C_L} + \frac{1}{(C_b)^2} \ln \frac{(C_L - C_b)\, C_0}{(C_0 - C_b)\, C_L}\right],$$

where V_o is the perfusion rate; C_o, C_b, and C_L are the osmolalities of the perfusate, bath, and collected fluids, respectively; A is the luminal surface area; and $\bar{V}w$ is the partial molar volume of water. This expression requires that the reflection coefficient of the solute driving osmotic flow be unity.[2]

After the addition of vasopressin to the bath, net water flux increased to maximal levels within 10 min and remained stable for at least 30 min. All results were therefore expressed as the mean of the values obtained during the first three 10-min periods after the addition of vasopressin.

Assays for adenylate cyclase on isolated cortical collecting tubules

Adenylate cyclase activity in isolated nephron segments was determined by a modification of the method of Imbert et al. (19) as previously described from this laboratory (20). After removal of the kidney, a cross section of cortex, 1–2 mm thick, was transferred to a dish of modified Hank's solution which served as the dissection medium. In contrast to the technique described by others (19, 21) no collagenase was used in the preparation of tubules. All subsequent steps were carried out at 4°C.

Segments of cortical collecting tubules were transferred into each of four wells of a Terasaki microtest plate (Falcon Plastics, Div. of BioQuest, Oxford, Calif.) containing 25 μl of dissection medium. Segments were pooled so that a total length of approximately 5 mm was used for each determination of adenylate cyclase under basal, NaF- and vasopressin-stimulated conditions.

The excess dissection solution was carefully aspirated under direct observation through an inverted microscope with a fine capillary tube and 2 μl of hypotonic preincubation solution, containing 8 mM Tris HCl buffer, pH 7.4, with 0.25 mM EDTA, 1 mM $MgCl_2$ and 0.1% bovine serum albumin, added. Each well was then photographed at ×50 through a calibrated eyepiece reticle and total tubule length was determined from the photographs. For each kidney studied, three wells were used for determination of basal, NaF- and vasopressin-stimulated adenylate cyclase activity and a fourth well (containing a total length of 10–15 mm of collecting tubule segments) was used for protein determination.

The wells used for assay of NaF and vasopressin stimulation contained the same preincubation solution but NaF and VP were added to result in respective concentrations of 10 mM and 20 mU/ml in the final assay.[3]

The wells were sealed with coverslips and preincubated on ice for 30 min. After this, the plates were frozen and thawed twice by applying them to a block of dry ice. (This maneuver together with the hypotonic preincubation solution causes disruption of the cell membranes.) Assays for adenylate cyclase were carried out in a final volume of 10 μl.

The reaction was initiated by the addition of 8 μl of a reaction mixture containing 25 mM Tris HCl, pH 7.71, 5 mM $Mg\ Cl_2$, 1.4 mM EDTA, 1 mg/ml creatine phosphokinase (155 U/mg), 17 mM phosphocreatine, 1 mM cAMP and 0.125 mM [α^{32}P]ATP (3–4 μCi/assay).

The wells were again sealed and incubations carried out at 30°C in a water bath. For both normal and uremic collecting tubules, the reaction product increases linearly for at least 30 min so that all incubations were carried out for 30 min. The reaction was terminated by the addition of 100 μl of "stopping solution" which contained 10 mM cAMP, 40 mM ATP, 1% sodium dodecyl sulfate, and [^{3}H]cAMP (approximately 400,000 cpm) for calculation of recovery.

Each total incubate was transferred to a test tube and the volume adjusted to 1 ml with water. The [^{32}P]cAMP generated was then chromatographed on Dowex AG 50W-X8 (chloride form) and neutral alumina columns and finally counted in 12 ml of Aquasol liquid scintillation fluid (New England Nuclear, Boston, Mass.) with a Packard liquid scintillation counter (Packard Instruments, Inc., Downers Grove, Ill.). Recovery for the [^{3}H]cAMP was 40–60%.

In each experiment a triplicate incubation was carried out in the absence of tubule segments to serve as a blank. The mean of these blanks did not exceed 10% of the lowest

[2] NaCl was the solute used to generate the osmotic gradient. $\sigma_{NaCl} = 1$ in the normal rabbit CCT (18). σ_{NaCl} was measured in two experiments on uremic CCTs where σ_{NaCl} is calculated as the ratio of the increment in net fluid movement (in the presence of vasopressin) caused by a given osmotic gradient of NaCl to that resulting from an equal osmotic gradient or raffinose. Values for σ_{NaCl} of 0.96 and 0.94 were obtained. These are regarded as being essentially equal to unity (σ = reflection coefficient).

[3] In preliminary studies it was established for both normal and uremic tubules that maximal stimulation occurred at 200 μU/ml of vasopressin. Doses up to 20 mU/ml resulted in no further stimulation or inhibition of enzyme activity and the latter dose was selected so that a comparison with studies on membrane preparations (20) could be made.

experimental counts in any experiment. Results were corrected for their respective blank value and the percent recovery was expressed as femtomoles cAMP generated per microgram tubule protein (or per millimeter tubule) per 30-min incubation. Protein content per millimeter tubule was detemined by the method of Lowry et al. (22). To minimize interassay variations, CCTs from normal and uremic animals were generally studied together in the same assay.

Materials

cAMP, 8-bromo-cAMP (sodium salt), ATP, creatine phosphokinase, phosphocreatine, and neutral alumina were obtained from the Sigma Chemical Co., St. Louis, Mo. [^{3}H]cAMP (20–40 Ci/nmol) and [α^{32}P]ATP (10–30 Ci/nmol) were obtained from New England Nuclear. Dowex AG-50W-Zi was obtained from Bio-Rad Laboratories, Richmond, Calif.

Statistics

The statistical significance of the differences between groups was assessed by Student *t* test.

RESULTS

Indices of renal function. Mean (±SE) BUN of normal rabbits was 8.8±0.9 mg/100 ml and of uremic rabbits was 62.5±10.8 mg/100 ml. Serum creatinine concentration was 1.00±0.06 mg/100 ml in normal and 3.13±0.38 mg/100 in uremic rabbits. Maximum urinary osmolality was 1293±92 mosmol/kg H_2O in normal and 659±49 mosmol/kg H_2O in uremic rabbits ($P < 0.005$).

Morphology. CCTs from remnant kidneys of uremic animals were identified according to the same criteria as were used for normal CCTs (12, 13). The remnant kidneys showed interstitial fibrosis making the dissection of intact CCTs relatively difficult. Only one-third of the attempts to dissect an adequate length of CCT for perfusion were successful. The internal diameters of normal and uremic CCTs are shown in Tables I and II. The mean internal diameter of the uremic tubules was increased by approximately 50% (32.3 μm).

Response of CCTs to vasopressin. In Fig. 1 the mean (±SE) Jv of 9 normal and 13 uremic CCTs are depicted for each of six 10-min periods after the addition of a supramaximal concentration of vasopressin to the bath. Under the conditions employed (37°C, 200 moSmol/kg gradient) there was a rapid increase in Jv followed by a gradual decline after 30 min in both groups of tubules. In almost all instances the maximum Jv was observed either in the first or second 10-min period. The values for the first three 10-min periods were not significantly different from each other and results are expressed as the mean of these values.

In Table I the results of each experiment conducted on CCTs from nine normal rabbits are listed. Table II lists the results for 13 uremic CCTs. Perfusion rates were comparable in the two groups of animals. The mean transepithelial PD of normal CCTs was −22±6 mV and of stage III CCTs was −21±7 mV (lumen negative). These results are not significantly different from each other. The influence of vasopressin on the transepithelial PD of normal and stage III CCTs is depicted in Fig. 2. In accordance with previous studies (14, 23, 24) vasopressin increased the luminal negativity of both normal CCTs (from −22±6 to −26·22±7 mV; $P < 0.02$) and of uremic CCTs (from −21±7 to −25±7 mV; $P < 0.02$). During the 60 min of observation, there was no tendency of PD to decrease to values below control after the initial increment.

TABLE I
Jv and P_f of CCTs from Normal Rabbit Kidneys

	BUN	Perfusion rate	Tubule internal diameter	Control PD	Jv		Jv		P_f
					Control	VP	Control	VP	(Post-vasopressin)
	mg/100 ml	*nl/min*	*μm*	*mV*	*nl/mm/min*		*nl/cm²/s*		*cm/s*
	11.2	11.65	23.0	−13	0.04	1.57	0.71	36.03	0.0357
	12.3	5.75	21.0	−4	0.14	0.67	3.45	16.93	0.0168
	5.8	3.98	15.5	−26	0.09	0.52	3.23	17.75	0.0271*
	5.7	7.64	22.2	−38	−0.05	0.52	−1.26	12.29	—
	10.3	6.86	24.4	−14	−0.07	1.65	−1.54	33.87	0.0239*
	10.7	13.58	22.2	−15	0.29	1.18	5.89	28.17	0.0437
	10.3	6.22	15.8	−61	0.23	0.32	7.67	9.19	0.0099
	8.1	5.22	26.6	−11	−0.18	0.94	−3.37	18.71	0.0094*
	4.8	7.09	17.8	−10	−0.15	1.40	−4.51	41.83	0.0191
Mean	8.8	7.55	20.9	−22	0.03	0.97	1.14	23.86	0.0232
±SE	0.9	1.03	1.3	6	0.05	0.17	1.40	3.82	0.0043

Tubules were studied in normal rabbit serum at 37°C with a 200-mosmol/kg H_2O transepithelial osmotic gradient.
* Indicates experiments with uremic rabbit serum in the bath. Vasopressin (VP) was added to the bath (200 μU/ml).

TABLE II

Jv and P_f of CCTs from Remnant Kidneys of Uremic Rabbits

	BUN	Perfusion rate	Tubule internal diameter	Control PD	Jv		Jv		P_f
					Control	VP	Control	VP	(Post-vasopressin)
	mg/100 ml	*nl/min*	*μm*	*mV*	*nl/mm/min*		*nl/cm²/s*		*cm/s*
	58.7	5.23	23.0	−7	0.23	0.06	5.37	1.38	0.0009
	35.1	10.06	22.2	−11	−0.02	0.59	−0.46	14.09	0.0063
	121.2	5.61	31.1	−21	−0.22	0.14	−3.70	2.35	0.0069
	148.6	9.11	26.7	−15	−0.15	1.36	−0.95	27.07	0.0157
	50.3	5.84	44.4	−13	−0.04	1.01	−0.52	12.16	0.0066*
	62.4	5.36	37.8	−12	−0.09	0.54	1.29	7.56	0.0057
	27.9	4.96	24.4	−93	−0.14	0.15	−3.07	3.29	0.0050
	24.5	9.723	33.3	−42	0.06	0.08	−0.95	1.27	0.0068
	47.2	5.06	33.3	−6	0.09	0.13	1.38	2.13	0.0035
	96.4	4.58	21.1	−4	−0.17	0.29	−4.19	7.35	0.0064*
	78.9	12.24	33.6	−25	0.27	0.27	4.28	10.53	0.0075
	35.8	7.09	46.6	−26	0.24	0.24	2.73	3.74	0.0018*
	25.5	3.94	42.2	−3	0.13	0.13	1.72	6.34	0.0037
Mean	62.5	6.83	32.3	−21	0.02	0.38	0.23	7.64	0.0059
±SE	10.8	0.72	2.4	−7	0.05	0.11	0.81	1.99	0.0010

Tubules were studied in uremic rabbit serum at 37°C with a 200-mosmol/kg H_2O transepithelial osmotic gradient.
* Indicates experiments with normal rabbit serum in the bath. Vasopressin (VP) was added to the bath (200–2,000 μU/ml).

In the control periods (i.e., before the addition of vasopressin) the CCTs of both groups of animals were impermeable to water and Jv was not significantly different from zero in either of the groups (Tables I and II).[4]

After the addition of a supramaximal dose of vasopression to the bath[5] mean±SE Jv increased to 0.97 ±0.17 nl/mm per min in normal CCTs and to 0.38±0.11 nl/mm per min in uremic CCTs (Tables I and II). Thus despite the larger luminal surface area of the tubules from the uremic rabbits, Jv was significantly lower than in the normal animals ($P < 0.01$). When results are expressed per unit luminal surface area, the differences are magnified even further, i.e., 23.86±3.82 nl/cm² per s in uremic CCTs ($P < 0.001$ vs. normal). The presence of normal or uremic rabbit serum in the bath did not influence the results which have consequently been pooled.

Values for the transepithelial osmotic water permeability coefficient, P_f, under the influence of vasopressin are listed in Tables I and II and depicted in Fig. 3. P_f was significantly lower ($P < 0.001$) in the uremic CCTs than in the normals.

Adenylate cyclase responsiveness of isolated CCTs. Values for adenylate cyclase activity under basal conditions and NaF and vasopressin stimulation in normal and uremic CCTs are listed for each experiment in Table III and the mean results are depicted in Fig. 4. Mean protein content for normal CCTs was 0.264±0.023 μg/mm and for uremic CCTs 0.298±0.056 μg/mm. Basal activity (expressed per unit tubule length or per microgram protein) in the uremic CCTs tended to be higher than in normal CCTs although this difference was not statistically significant (Table III). NaF-stimulated adenylate cyclase activity was not significantly different between the two groups. Activity of the enzyme after vasopressin stimulation reached levels which were only 50% of those obtained in normal CCTs

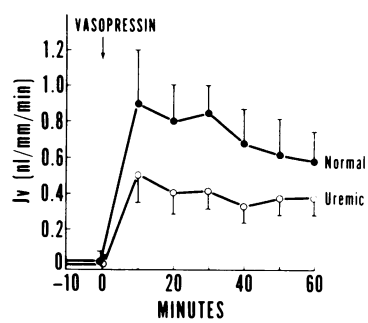

FIGURE 1 Jv of normal and uremic CCTs after the addition of vasopressin (200–2,000 μU/ml) to the peritubular bathing medium.

[4] Negative values for Jv reflect random variation of experimental results about zero.

[5] In normal tubules a bath concentration of 200 μU/ml was used; in uremic tubules 200 μU/ml was used in eight experiments and 2,000 μU/ml in five experiments. Increasing the concentrations by an order of magnitude did not influence the results.

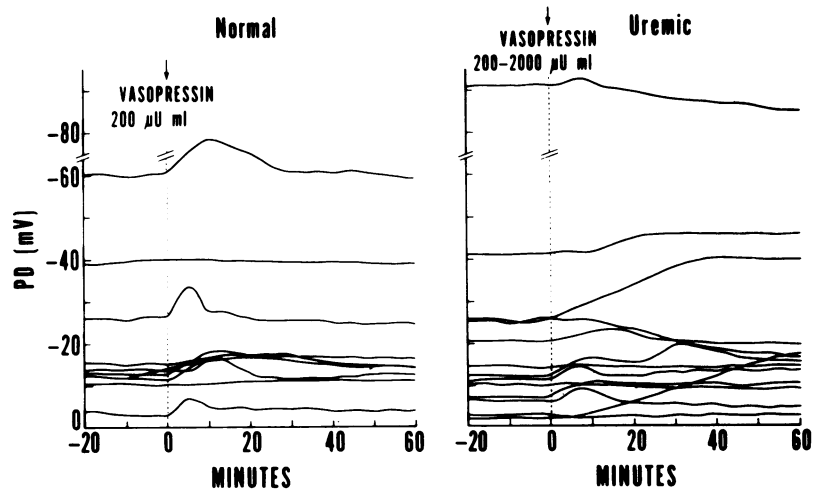

FIGURE 2 Influence of vasopressin on the transepithelial PD of normal and uremic CCTs.

($P < 0.025$) (Table III). Comparisons between normal and uremic CCTs under all three conditions are depicted in Fig. 4.

Response of CCTs to 8-bromo-cAMP. To establish whether the decreased vasopressin responsiveness of uremic tubules was explicable solely on the basis of the reduced adenylate cyclase activity (resulting in a decreased production of cAMP), the effects of the cAMP analogue, 8-bromo-cAMP were evaluated. The results for four normal and five uremic CCTs are shown in Fig. 5. In the normal CCTs, at a bath concentration of 0.1 mM 8-bromo-cAMP, P_f approached values obtained with vasopressin (i.e., 0.0202±0.0026 cm/s). In contrast, at a bath concentration of 1 mM 8-bromo-cAMO, the P_f of the uremic CCTs was only 0.007±0.0007 cm/s. Addition of 8 mM theophylline to the bath did not increase P_f further.

DISCUSSION

The present study provides documentation of the first direct observations on the function of the CCT in uremia. The experimental model in which isolated segments of renal tubules from uremic animals are perfused in vitro has been described elsewhere (11). The present observations indicate that CCTs derived from remnant kidneys of uremic animals show a marked hyporesponsiveness to vasopressin. Under basal conditions, in the absence of vasopressin, these nephron segments are essentially impermeable to water.

The CCTs studied showed evidence of compensatory hypertrophy similar to that seen in the proximal straight tubules studied in the same experimental model (11). However, the degree of hypertrophy was more variable and some tubules were of normal size. The mean luminal diameter of uremic CCTs perfused at flow rates comparable to those used in normal

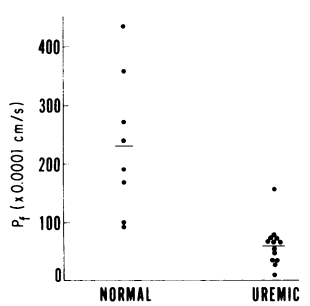

FIGURE 3 P_f of normal and uremic CCTs under the influence of vasopressin.

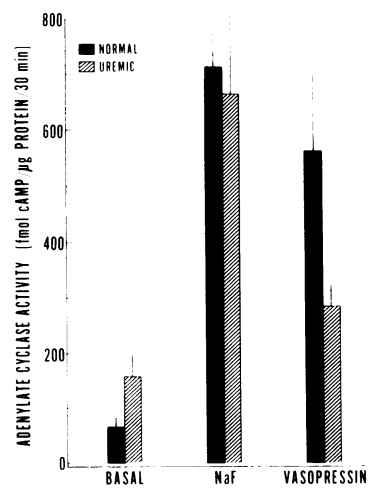

FIGURE 4 Adenylate cyclase activity of normal and uremic CCTs measured under basal, NaF-stimulated, and vasopressin-stimulated conditions.

TABLE III

Adenylate Cyclase Activity of Isolated CCTs from Normal Kidneys and Uremic Remnant Kidneys: Response to Sodium Fluoride and Vasopressin Stimulation

	Basal	NaF		Vasopressin	
	fmols cAMP/μg protein/30 min	*fmols cAMP/μg protein/30 min*	*% basal*	*fmols cAMP/μg protein/30 min*	*% basal*
Normal CCTs	96.2	726.0	754	893.8	929
	26.4	484.6	1,835	253.1	960
	60.6	843.0	1,391	1,048.8	1,732
	21.3	735.8	3,453	256.5	1,204
	66.8	593.5	888	444.3	665
	142.8	903.4	632	616.3	431
Mean±SE	68.9±18.5	714.4±63.3	1,492±432	585.1±135.2	986±184
Uremic CCTs	68.9	630.6	914	249.5	362
	53.7	128.1	238	197.3	386
	86.4	507.3	588	444.7	516
	13.7	379.2	2,767	105.5	777
	395.7	1,577.5	398	338.5	85
	300.6	478.9	159	310.4	103
	70.0	414.6	591	277.5	638
	297.3	1,052.7	354	256.4	86
	188.3	831.4	441	372.0	198
Mean±SE	163.8±45.5	666.7±144.5	722±266	283.0±33	352±86

tubules was increased by approximately 50%. However, despite this increase in internal surface area, in the presence of the same osmotic gradient and supramaximal levels of vasopressin, Jv per unit length was significantly reduced in uremic vs. normal CCTs. When expressed per unit of luminal surface area, the difference between normal and uremic CCTs is magnified even further. The reduced osmotic water permeability coefficient of the uremic CCTs in response to vasopressin was an almost consistent finding.

To determine the possible biochemical mechanisms involved in this functional alteration, studies of adenylate cyclase activity were performed on isolated collecting tubules of normal and uremic animals (19, 20). This technique offers the opportunity of correlating functional measurements with biochemical or enzymatic events in the same nephron segment.

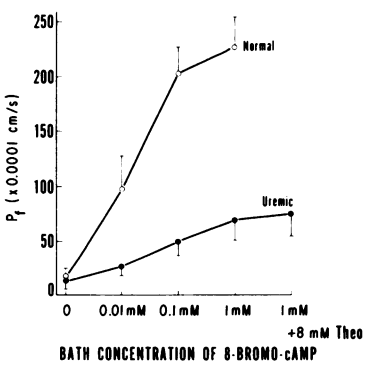

FIGURE 5 P_f of normal and uremic CCTs exposed to increasing bath concentrations of 8-bromo-cAMP. Uremic tubules were also exposed to theophyline (Theo).

Activation of membrane-bound adenylate cyclase by vasopressin at the peritubular surface of the nephron appears to be the initiating event in increasing the water permeability of the luminal membrane of the collecting tubule (25, 26). This leads to the generation of increased amounts of intracellular cAMP which, through the activation of protein kinase, membrane protein phosphorylation, and microtubule formation (25), ultimately effect a physical change in the luminal membrane which increases its permeability to water.

In the present studies basal adenylate cyclase specific activity was higher in uremic than in normal CCTs although the difference did not achieve statistical significance due to a large standard error in the former values. Stimulation by NaF led to comparable levels of absolute activity in both groups. However, under maximal vasopressin stimulation the uremic tubules showed only 50% of the activity of normal tubules. Although it is possible that this defect is nonspecific, it is of interest that parathyroid hormone stimulation of adenylate cyclase activity in uremic proximal convoluted tubules is not different from normal[6] suggesting that the present finding represents a decreased responsivity to vasopressin per se. It is possible, however, that stimulation of renal adenylate

[6] Fine, L. G., and D. Schlondorff. Unpublished observations.

cyclase by other hormones may be similarly impaired in uremia.

If the failure of vasopressin to stimulate adenylate cyclase activity normally were the only cellular defect underlying the hyporesponsiveness of the uremic CCT to vasopressin (as measured by Jv and osmotic water permeability), it should be possible to correct the defect with exogenous cAMP or one of its analogues. cAMP itself simulates the effect of vasopressin on the isolated CCT only in very high concentrations (10 mM) due to its low permeability across cell membranes (10). However, it has recently been shown that 8-(*p*-Cl-phenylthio)cAMP at a concentration of 10 μM mimics the effect of vasopressin on the hydraulic conductivity of the isolated CCT (15). In the present study, 8-bromo-cAMP, another analogue of cAMP (16), was used. When added to the peritubular bathing medium at a concentration of 0.1 mM, the P_f of normal CCTs increased to a level comparable to that which obtained with maximal vasopressin stimulation. The same analogue at a concentration of 1 mM failed to increase the P_f of uremic CCTs. To exclude the possibility that increased phosphodiesterase activity was responsible for this blunted response, theophylline was added to the bath solution in the presence of 1 mM 8-bromo-cAMP. No additional increase in P_f was observed. Although it is difficult to interpret these results with certainty, failure of the cAMP analogue to induce a normal water permeability response in uremic CCTs suggests that more than one biochemical alteration is responsible for the observed functional difference between normal and uremic CCTs. Such an additional defect would have to reside in a step or steps subsequent to the formation of cAMP. These studies do not, however, exclude the possibility that 8-bromo-cAMP failed to permeate the cells of the uremic tubules to the same extent as it does in normal tubules.

The mechanisms underlying the functional and biochemical alterations described above are unclear. It is possible that CCTs from remnant kidneys may demonstrate "memory" effects of uremia in vitro induced either by unidentified inhibitory substances (27) or by alterations in circulating hormone levels.[7]

The results of these studies may help to clarify a number of hitherto unexplained aspects of the urinary concentrating defect of uremia. The clinical entity of vasopressin resistant hyposthenuria has been described in a number of unrelated forms of renal disease (1–4). The possibility that resistance to the effect of vasopressin might be a more common event than was generally supposed was advanced by Holliday et al. (2) and Tannen et al. (1). Additional studies by Schrier and Regal illustrated the uniform impairment of renal concentrating ability in patients with renal disease, a significant proportion of whom had vasopressin-resistant hyposthenuria (4). The difference between an absolute inability to concentrate the urine above the osmolality of plasma (hyposthenuria) and the ability to raise the urinary osmolality to a value slightly greater than that of plasma is a quantitative one only. If it is accepted that the kidney in uremia can generate hypotonic tubular fluid (3, 9) the absolute amount of water removed by the distal tubule and collecting tubule will determine the final urine osmolality which may vary from hypo- to hypertonic. In the present study the resistance to vasopressin was not complete.

One possible explanation for the resistance of uremic subjects to vasopressin is the existence of a circulating inhibitor to vasopressin (1). The present studies provide no support for this. The impaired responsiveness of the uremic collecting tubule was observed when the tubules were studied in normal or uremic serum and these observations suggest that the defect lies at the level of the tubular epithelial cell per se. They would also minimize the role of other circulating ionic or hormonal influences as important pathogenetic factors.

An additional observation of importance relates to the fact that the uremic collecting tubules under basal conditions (i.e., in the absence of vasopressin) were essentially impermeable to water. This finding provides an important explanation for the observation that the diluting ability of the diseased kidney is well preserved long after the concentrating defect becomes overtly manifest (3, 9). The ability of the diseased kidney to dilute the urine requires that the generation of hypotonic fluid by the ascending limb is intact and that little or no equilibration occurs between this fluid and the hypertonic medullary interstitium in the collecting tubule.

The vasopressin unresponsiveness of the uremic collecting tubule provides a unifying framework within which a number of observations on the concentrating and diluting functions of the diseased kidney can be explained. First, as pointed out above, it explains how diluting functions can be maintained in the absence of normal concentrating ability. Second, it explains the inability of uremic subjects to respond to maximal doses of vasopressin and since the defect is intrinsic to the renal epithelial cell rather than to a circulating inhibitor, accounts for the reported failure of hemodialysis to correct this abnormality (1). Failure of water removal from the collecting tubule under the influence of vasopressin not only directly limits the ability of the kidney to concentrate the urine but indirectly influences its ability to generate a hypertonic medullary interstitium via an influence on urea re-

[7] We have recently obtained plasma arginine vasopressin (pAVP) levels on six uremic and four normal rabbits. Radioimmunoassay of pAVP was performed by Dr. L. C. Keil, Ames Research Center, Calif. pAVP was 5.7±0.4 pg/ml in the normal and 23.6±6.9 pg/ml in the uremic rabbits.

cycling. In the presence or absence of vasopressin, the CCT is impermeable to urea (10). Water abstraction from the CCT under the influence of vasopressin consequently leads to a progressive increase in urea concentration of the fluid coursing through the collecting tubules leading to a high tubular fluid urea concentration in the terminal papillary collecting tubules. The relative high urea permeability of these latter nephron segments (28) allows urea to diffuse into the papillary interstitium down its concentration gradient and thereby facilitates concentration of other solutes in the interstitium according to the "passive theory" of countercurrent multiplication (29). We have recently described a disturbance of such urea recycling and failure to accumulate interstitial solute in rats with chronic pyelonephritis (9). If vasopressin unresponsiveness is a component of the uremic state, it is highly likely that failure to abstract water from the cortical and outer medullary collecting tubules plays an important role in the failure of the diseased kidney to concentrate the urine normally.

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