Role of urinary concentrating ability in the generation of toxic papillary necrosis

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Role of urinary concentrating ability in the generation of toxic papillary necrosis. We studied the pathogenesis of chemically induced papillary necrosis in six groups of rats. Papillary necrosis was produced by a single injection of 2-bromoethylamine hydrobromide (BEA), 50 mg, i.v.; the animals were followed for 7 to 10 days after the administration of the compound. Following BEA, heterozygous Brattleboro rats developed all the functional and morphologic lesions of papillary necrosis that we previously described in Sprague-Dawley rats. They were unable to maintain sodium balance when dietary sodium was withdrawn. Homozygous Brattleboro rats, on the other hand, developed none of the manifestations of papillary necrosis (that is, animals with central diabetes insipidus were protected completely from the nephrotoxic effects of BEA). They adapted normally to a zero sodium diet. Chronic administration of vasopressin to homozygous Brattleboro rats fully restored the toxic effects of BEA. Lowering urinary concentrating ability by inducing a water diuresis in Sprague-Dawley rats completely protected against BEA-induced papillary necrosis. Decreasing papillary solute concentration by furosemide or increasing urine flow after abrupt withdrawal of vasopressin to homozygous Brattleboro rats did not protect against BEA-induced papillary necrosis. We conclude that the combination, but not either alone, of increased urine flow and decreased papillary solute concentration protects against the development of BEA-induced papillary necrosis.

Rôle du pouvoir de concentration urinaire sur l'apparition d'une nécrose papillaire toxique. Nous avons étudié la physiopathologie d'une nécrose papillaire induite chimiquement chez six groupes de rats. La nécrose papillaire était produite par une injection unique de 2-bromoéthylamine hydrobromide (BEA), 50 mg, i.v.; les animaux étaient suivis pendant 7 à 10 jours après administration du produit. Après le BEA, des rats Brattleboro hétérozygotes ont développé toutes les lésions fonctionnelles et morphologiques d'une nécrose papillaire telles que nous les avions déjà décrites chez des rats Sprague-Dawley. Ils n'étaient pas capables d'assurer l'équilibre de la balance sodée lorsque le sodium alimentaire était supprimé. Par ailleurs, des rats Brattleboro homozygotes n'ont développé aucune des manifestations de nécrose papillaire (c'est-à-dire que les animaux ayant un diabète insipide central étaient complètement protégés des effets néphrotoxiques du BEA). Ils s'adaptaient normalement à un régime sans sodium. L'administration chronique de vasopressine à des rats Brattleboro homozygotes a totalement restauré l'effet toxique du BEA. Le fait d'abaisser la capacité de concentration des urines en induisant une diurèse aqueuse dans les rats Sprague-Dawley a complètement protégé contre la nécrose papillaire induite par le BEA. La diminution de la concentration de solutés papillaires par le furosémide ou l'augmentation du débit urinaire après arrêt brutal de la vasopressine chez des rats Brattleboro homozygotes ne protégeait pas contre la nécrose papillaire induite par le BEA. Nous concluons que la combinaison, mais non chaque facteur séparément, d'une augmentation du débit urinaire et d'une diminution de la concentration de solutés papillaires protègent contre le développement d'une nécrose papillaire induite par le BEA.

Papillary necrosis has been described repeatedly as a major feature of analgesic nephropathy in humans [1–3]. When studied in experimental animals, however, salicylates or acetaminophen produce papillary necrosis irregularly and only after large quantities are ingested [4]. Dehydration has been shown to increase the frequency with which papillary necrosis develops [5, 6], suggesting that the ability to concentrate the urine may be important in the generation of drug-induced papillary necrosis.

2-Bromoethylamine hydrobromide (BEA) is a low molecular weight halogeneated compound which produces complete papillary necrosis in rats. The histologic lesions caused by this compound were first described by Murray et al [7] and consist of necrosis of the thin limbs of Henle's loop and collecting duct as early as 6 hr. Complete papillary necrosis occurs in 7 to 14 days.

We have previously reported the functional changes which occur over the first 3 days after administration of the compound [8, 9]. In brief those studies demonstrated that early papillary necrosis is associated with impaired urinary concentrating ability, sodium and chloride wastage, potassium wastage on a low potassium diet, and impaired ability to excrete potassium following chronic potassium adaptation. Urinary acidification was normal [8]. As assessed by the qualitative Hanssen's technique, the percent of filtering juxtamedullary nephrons was decreased [9].

The purpose of this study was to examine the role of urinary concentration on the generation of chemically induced papillary necrosis. A variety of animal models were selected in which urinary concentrating ability, urine flow rate, and papillary solute concentration were distinctly abnormal. Morphology and the adaptation to a zero sodium diet were studied for 7 to 10 days after the administration of BEA.

Methods

Balance studies

Group 1 - heterozygous Brattleboro rats. A pair of heterozygous Brattleboro rats was mated repeatedly at our institution

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over a 1-year period. Urine osmolality was measured on 4 consecutive days to separate the heterozygous Brattleboro rats from their homozygous litter mates. Animals with a urine osmolality greater than 1200 mOsm/kg H_2O were considered heterozygotes (group 1) while those with a urine osmolality less than 400 mOsm/kg H_2O were placed in the homozygous groups (groups 2, 3, and 5).

Twelve male heterozygotes, weighing 237 to 306 g, were placed in metabolic cages. The animals were allowed to adjust to the cages for 3 days prior to the beginning of the study. They were fed 20 g/day of regular powdered rat chow and were allowed free access to water. Three consecutive 24-hr urine collections were made for urine osmolality and excretion of sodium and creatinine. Fluid intake and weight were measured daily.

The animals were randomized and anesthetized with Ketamine® (10 mg/100 g body weight, i.p., Parke-Davis, Morris Plains, New Jersey). Papillary necrosis was produced by a single intravenous dose of 50 mg BEA in six animals. Six were sham injected with an equivalent volume of the diluent (0.9% NaCl). Three consecutive 24-hr urine collections were obtained as previously described. The animals were then placed on a zero sodium diet, and urine collections were obtained for the subsequent 6 days. The animals were sacrificed 9 days after BEA.

At the end of the experimental period the animals were again anesthetized with Ketamine®. Arterial blood was obtained for plasma electrolytes, osmolality, blood urea nitrogen, and creatinine. The kidneys were removed and fixed in formalin for light microscopy.

Group 2 - homozygous Brattleboro rats. Fourteen male homozygous Brattleboro rats were studied in a manner identical to their litter mate heterozygotes (that is, group 1). The animals were sacrificed 9 days after BEA.

Group 3 - homozygous Brattleboro rats, vasopressin replaced. Twelve male homozygous Brattleboro rats were given daily injections of vasopressin tannate in oil (1 mU, i.m.) for 9 days. Urine osmolality was checked prior to vasopressin and again after day 9 just prior to the beginning balance of the period. The animals were placed in metabolic cages, allowed free access to water, and the protocol outlined in group 1 was followed. Vasopressin was given as above for the remainder of the study. The animals were sacrificed 9 days after administration of BEA.

Group 4 - water diuresis in Sprague-Dawley rats. Fourteen male Sprague-Dawley rats weighing from 250 to 300 g were allowed free access to 5% glucose drinking solution for 9 days. Urine osmolality was checked prior to the administration of glucose and again 9 days later. The animals were then placed in metabolic cages and the protocol as outlined in group 1 was followed, except that 5% glucose was used as the drinking fluid. The animals were sacrificed 9 days after BEA.

Group 5 - abrupt vasopressin withdrawal in homozygous Brattleboro rats. Four male homozygous Brattleboro rats were treated with daily injections of vasopressin for 10 days as previously described. Urine osmolality increased to 1550 ± 43.8 mOsm/kg H_2O and urine volume was 7.6 ± 2.5 ml/day. Vasopressin was abruptly withdrawn, and urine volume was allowed to increase over a 36- to 48-hr period. The animals were followed as described for group 1 (H_2O as drinking fluid) except

Table 1. Group 1: Arterial electrolytes and cumulative sodium excretion in heterozygous Brattleboro rats

	BEA		$P<$	Sham	
P_{Na}, *mEq/liter*	132.5	± 7.4	NS	144.2	± 1.1
P_K, *mEq/liter*	4.88	± 0.49	NS	3.80	± 0.31
P_{Cl}, *mEq/liter*	90.5	± 8.7	NS	104.5	± 0.6
BUN, *mg%*	169.8	± 85.8	0.05	13.1	± 1.3
P_{Cr}, *mg%*	2.48	± 1.09	0.05	0.66	± 0.02
Cumulative $U_{Na}V$, *mEq/6 days*	5.21	± 0.72	0.05	2.85	± 0.26
Urine volume, *ml/day*	41.5	± 3.3	0.05	12.2	± 1.2
U_{osm}, *mOsm/kg H_2O*	631	± 31	0.01	1723	± 37
Initial weight, *g*	291	± 12	NS	300	± 25
% Change weight	−15.6	± 4.0	0.01	−3.0	± 1.6
N	6			6	

that all five animals in this group received BEA. The animals were sacrificed after 4 days on a zero sodium diet (7 days after BEA).

Group 6 - chronic furosemide administration to Sprague-Dawley rats. Group 6a. Twelve male rats were placed on chronic furosemide (15 mg/kg/day) and allowed free access to regular chow for 9 days. Urine osmolality decreased to 467 ± 51 mOsm/kg H_2O. The furosemide was withdrawn on the day prior to the administration of BEA, and water was administered as the drinking fluid. Six animals were given BEA and six animals received the diluent. The animals were placed in metabolic cages and treated as described in group 1. The animals were sacrificed after 4 days on a zero sodium diet (7 days after BEA).

Group 6b. Papillary tissue from five control animals and five chronic furosemide animals (treated as described in group 6a) was analyzed for osmolality and solute concentration after 9 days of regular diet and access to either H_2O or furosemide. On day 10 which was equivalent to the day of BEA administration they were sacrificed. Osmolality and solute concentration was determined according to the method described by Gilbert et al [10]. Osmolality in the papillary homogenate was calculated by the equation, Osm = 1.86 (Na + K) + urea.

Light microscopy

At the end of each experiment the kidneys from the six groups were coded and placed in buffered formalin for light microscopy. Sagittal sections from cortex to papilla, approximately 10-μ thick, were made and stained with hematoxylyn and eosin. The coded specimens were read by a renal pathologist.

Analytical methods

Plasma and urine electrolytes and creatinine were measured as previously described [11]. Urine osmolality was measured using a vapor pressure osmometer (Wescor, Inc., Logan, Utah).

All results are expressed as $\bar{X}$ ± SEM. Statistical analysis was performed using the unpaired t test with significance chosen at $P < 0.05$.

Diets

Powdered rat chow was obtained from the Biological Research Laboratory, the University of Illinois, Chicago, Illinois. The regular rat chow comprises a complete diet containing 27%
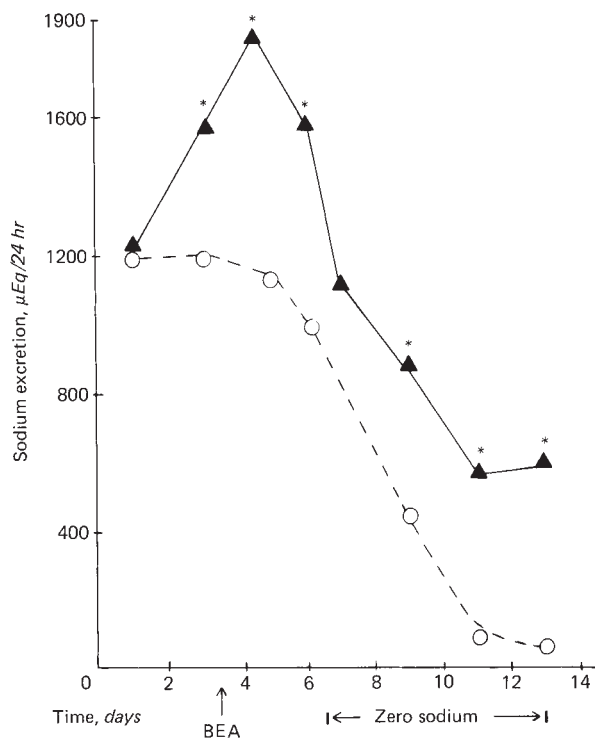
Fig. 1. *Mean sodium excretion in BEA-* (▲, closed triangles) *and sham-* (●, closed circles) *injected heterozygous Brattleboro rats (group 1).* BEA was administered after day 3 on regular rat chow; sodium was withdrawn after day 6. *Asterisks* (*) indicate a significant difference from control ($P < 0.05$).

Table 2. Group 2: Arterial electrolytes and cumulative sodium excretion in homozygous Brattleboro rats

	BEA	$P<$	Sham
P_{Na}, *mEq/liter*	148.5 ± 1.1	NS	146.1 ± 3.2
P_K, *mEq/liter*	3.70 ± 0.66	NS	4.13 ± 0.23
P_{Cl}, *mEq/liter*	116.0 ± 1.1	NS	116.0 ± 3.5
BUN, *mg%*	23.9 ± 2.5	0.05	12.8 ± 2.0
P_{Cr}, *mg%*	0.78 ± 0.02	0.05	0.55 ± 0.06
Cumulative $U_{Na}V$, *mEq/6 days*	0.54 ± 0.063	NS	0.478 ± 0.058
Urine volume, *ml/day*	109.1 ± 9.8	NS	106.3 ± 20.1
U_{osm}, *mOsm/kg* H_2O	202 ± 15	NS	254 ± 52
Initial weight, *g*	279 ± 27	NS	268 ± 21
% Change weight	−10.5 ± 1.6	NS	−9.2 ± 2.2
N	6		5

Table 3. Group 3: Arterial electrolytes and cumulative sodium excretion in vasopressin-replaced homozygous Brattleboro rats

	BEA	$P<$	Sham
P_{Na}, *mEq/liter*	119.3 ± 3.9	0.01	138.4 ± 1.5
P_{Cl}, *mEq/liter*	85.8 ± 2.7	0.01	100.3 ± 2.3
BUN, *mg%*	174.0 ± 50.3	0.001	22.5 ± 2.5
P_{Cr}, *mg%*	2.40 ± 0.53	0.05	0.79 ± 0.15
Cumulative $U_{Na}V$, *mEq/6 days*	4.1 ± 0.4	0.05	1.8 ± 0.3
Urine volume, *ml/day*	37.1 ± 1.0	0.05	12.6 ± 1.7
U_{osm}, *mOsm/kg* H_2O	517 ± 46	0.01	1840 ± 106
Initial weight, *g*	244 ± 7	NS	237 ± 12
% Change weight	−24.0 ± 2.5	0.01	−2.6 ± 4.0
N	7		7

protein, 59% starch, 10% vegetable oil, and vitamins. The diet also contains 1.5 mEq sodium, 2.7 mEq potassium, and 1.5 mEq chloride per 10 g.

The zero sodium diet was obtained from ICN Nutritional Biochemical, Cleveland, Ohio. The powdered diet is a sucrose- and casein-based diet containing 5% of a sodium-free mineral mixture fortified with vitamins. The potassium and chloride content of the diet was similar to the regular rat chow.

Results

Group 1 - heterozygous Brattleboro rats. Table 1 shows the arterial electrolytes in the sham- and BEA-injected heterozygous Brattleboro rats at the end of the experimental period. Plasma sodium, potassium, and chloride were not different in the two groups of animals. Plasma creatinine and BUN was higher in BEA-treated animals.

Figure 1 illustrates the pattern of sodium excretion in the sham- (*open circles*) and BEA-treated animals (*closed circles*). Following the administration of BEA, cumulative sodium excretion was significantly higher than in sham-injected animals during the first 72 hr on a regular sodium diet as we have described previously [9]. As shown in Table 1, when placed on a zero sodium diet, sham-injected animals came into balance over the 6-day period excreting a virtually sodium-free urine. By contrast, cumulative sodium excretion was significantly higher than sham-injected controls.

Light microscopy revealed papillary necrosis extending from the tip of the papilla to the base with virtually a total loss of cell architecture. Some areas of cellular infiltration were seen. The superficial cortex appeared normal.

Group 2 - homozygous Brattleboro rats. To further examine the role of urinary concentration and vasopressin on the generation of papillary necrosis, BEA was injected into six homozygous Brattleboro rats. Six animals were sham-injected. The balance protocol as outlined for group 1 was followed.

Table 2 shows the arterial electrolytes after 7 days on a zero sodium diet. There was no difference in plasma sodium, potassium, and chloride between the two groups. Plasma creatinine was 0.71 ± 0.02 versus 0.55 ± 0.06 mg% in BEA- and sham-injected animals, respectively ($P < 0.05$). BUN was slightly, though significantly, higher in BEA-treated animals. Urine osmolality was the same in both groups of animals. When placed on a zero sodium diet 3 days after BEA, there was no significant difference in the pattern of sodium excretion between the two groups of animals.

A saggital section of the papillary tip revealed that the epithelial lining of the papilla was intact. Except for mild leukocytic infiltration, a normal papilla was seen 10 days after the administration of BEA.

Group 3 - homozygous Brattleboro rats, vasopressin replaced. Daily injection of vasopressin to 14 homozygous Brattleboro rats caused an increase in urine osmolality from 222 ± 23 to 1898 ± 45 mOsm/kg H_2O ($P < 0.001$). The animals were then randomly divided into two groups and placed in metabolic cages. Urine sodium excretion was 1.5 ± 0.2 versus 1.3 ± 0.1 mEq/day for 3 days prior to injection of BEA in the sham and experimental groups, respectively (NS). BEA was given to one half of the animals and they were observed in the same manner as group 1.

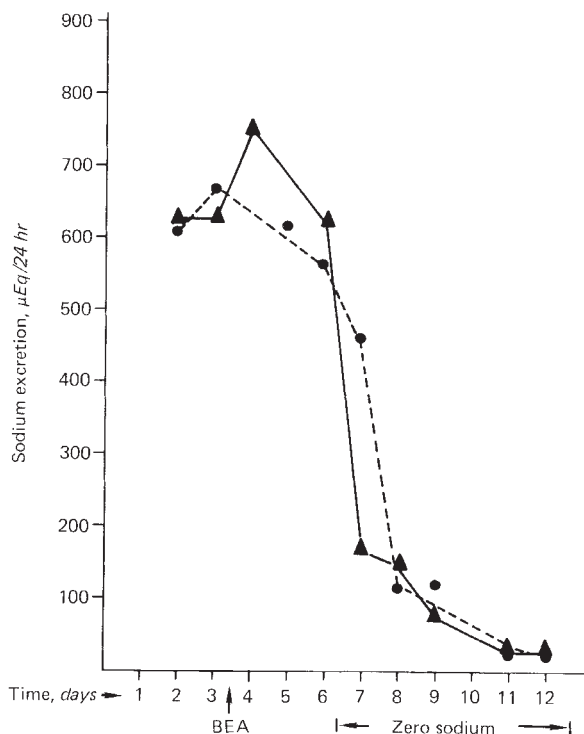Table 3 illustrates the arterial electrolytes, cumulative sodi-

Fig. 2. *Mean sodium excretion in BEA-* (▲, closed triangles) *and sham-* (●, closed circles) *injected Sprague-Dawley rats undergoing 5% glucose diuresis (group 4).* BEA was administered after day 3 on regular rat chow; sodium was withdrawn after day 6. There was no statistical difference between the two groups of animals.

Table 4. Group 4: Arterial electrolytes and cumulative sodium excretion Sprague-Dawley rats given 5% glucose

	BEA	$P<$	Sham
P_{Na}, *mEq/liter*	147.3 ± 2.3	NS	146.5 ± 1.8
P_K, *mEq/liter*	3.80 ± 0.25	NS	4.08 ± 0.11
P_{Cl}, *mEq/liter*	109.5 ± 3.5	NS	108.3 ± 2.5
$P_{glucose}$, *mg%*	148.3 ± 3.3	NS	172.3 ± 12.2
BUN, *mg%*	19.6 ± 3.8	NS	12.0 ± 1.2
U_{osm}, *mOsm/kg H_2O*	170 ± 45	NS	266 ± 23
Cumulative $U_{Na}V$, *mEq/6 day*	0.8 ± 0.3	NS	1.1 ± 0.2
Initial weight, *g*	300 ± 11	NS	306 ± 11
% Change weight	−15.5 ± 4.8	NS	−7.2 ± 3.1
Urine volume, *ml/day*	83.3 ± 7.5	NS	76 ± 12.6
N	6		6

Table 5. Group 5: Plasma electrolytes and urinary sodium excretion following abrupt withdrawal of vasopressin to Brattleboro rats

P_{Na}, *mEq/liter*	140.6 ± 11.4
P_K, *mEq/liter*	7.2 ± 2.1
P_{Cl}, *mEq/liter*	102.3 ± 9.8
U_{osm}, *mOsm/kg H_2O*	313 ± 64
BUN, *mg%*	183.8 ± 86.8
P_{Cr}, *mg%*	2.67 ± 0.83
Cumulative $U_{Na}V$, *mEq/4 days*	4.1 ± 0.6
N	4

um excretion, and urine osmolality in the BEA- and sham-injected animals followed for 6 days after the withdrawal of dietary sodium. BEA-injected animals had severe hyponatremia and hypochloremia as compared to sham-injected animals. This is probably due to a combination of excessive water retention secondary to vasopressin, as well as the renal failure induced by BEA. Plasma creatinine was significantly higher in BEA-injected animals as compared to controls. BEA-injected animals develop a concentrating defect as we have described previously [8, 9]. After placing all the animals on a sodium deficient diet, cumulative sodium excretion was higher in BEA-treated animals. Thus, vasopressin-replaced homozygous Brattleboro rats, when given BEA, were unable to reach sodium balance.

Light microscopy performed at the end of the study revealed severe papillary necrosis in every BEA-injected animal. The juxtamedullary region revealed total loss of the papillary structures, cellular infiltration, vacuolization, and fibrinous debris in the lumen of the thin limbs of Henle's loop.

Group 4 - Sprague-Dawley rats with water diuresis. To further evaluate the role of urinary concentrating ability on the generation of BEA-induced papillary necrosis, 12 Sprague-Dawley rats were given 5% glucose as their drinking fluid 9 days prior to study. Urine osmolality was 1250 ± 161 mOsm/kg H_2O prior to initiating a water diuresis ($N = 12$).

Figure 2 illustrates the pattern of sodium excretion following the withdrawal of dietary sodium. Over the 6 experimental days both groups of animals (BEA- and sham-injected) were able to reach sodium balance in a completely normal fashion. This

pattern was similar to that seen with the homozygous Brattleboro rats given BEA (that is, group 2).

Table 4 shows the arterial electrolytes, blood urea nitrogen, and cumulative sodium excretion in the two groups of animals at the end of the study. There was no difference in plasma sodium, potassium, glucose, or blood urea nitrogen. Urine osmolality, following BEA was unchanged from control. Cumulative sodium excretion was the same in both groups following the withdrawal of dietary sodium.

Light microscopy performed at the end of the study revealed an intact papilla in every animal. There was some evidence of cellular infiltration noted; however, otherwise the histology was completely normal.

Group 5 - abrupt withdrawal of vasopressin in homozygous Brattleboro rats. Vasopressin was given to four rats to restore urine concentration and urine flow to normal. This maneuver has been shown to also restore papillary solute concentration [12, 13]. Vasopressin was then abruptly withdrawn, and after the urine flow rate had increased (36 to 48 hr later), the animals were given BEA. Prior to administration of BEA, urine osmolality was 1550.2 ± 43.8 mOsm/kg H_2O, and urine volume was 7.6 ± 2.5 ml/24 hr. Urine volume increased to 52.0 ± 8.4 ml/24 hr when the BEA was given. Plasma electrolytes of the four animals are shown in Table 5. Plasma creatinine and BUN rose in a manner similar to group 1. Furthermore, these animals were unable to excrete a sodium-free urine when placed on a sodium-deficient diet. Weight loss in these animals was 41.5 ± 3.2%.

All animals had histologic evidence of papillary necrosis. Thus, it appears that a rise in urine flow rate alone is not protective against this form of papillary necrosis.

Group 6 - chronic furosemide administration to Sprague-Dawley rats. This group of animals was studied to assess

Table 6. Papillary solute concentration

	Measured Osm (mOsm/kg H₂O)	Na mEq/liter	K mEq/liter	Urea mmoles/kg	Calculated Osm (mOsm/kg H₂O)
Control N = 5	987.2 ±64.4	295.8 ±35.4	59.3 ±6.1	149.1 ±18.2	809.6[a] ±51.8
P<	0.05	0.05	0.05	0.05	0.05
Furosemide-treated N = 5	284.8 ±27.3	41.0 ±11.2	16.6 ±2.3	97.6 ±23.0	204.5[b] ±39.6

[a] Control = 174.1 ± 45.4 unmeasured osmoles.
[b] Furosemide = 82.1 ± 16.5 unmeasured osmoles.

Table 7. Group 6: Arterial electrolytes and cumulative sodium excretion in chronic furosemide-treated Sprague-Dawley rats with and without BEA

	BEA	P<	Sham
P$_{Na}$, mEq/liter	140.1 ± 6.1	NS	144.6 ± 0.8
P$_{K}$, mEq/liter	4.7 ± 0.6	0.05	3.0 ± 0.2
P$_{Cl}$, mEq/liter	93.3 ± 4.4	NS	94.6 ± 2.6
BUN, mg%	159.3 ± 55.1	0.05	31.8 ± 3.8
P$_{Cr}$, mg%	3.3 ± 0.0	0.05	0.63 ± 0.05
Cumulative U$_{Na}$V, mEq/4 day	3.1 ± 0.5	0.05	1.0 ± 0.2
U$_{osm}$, mOsm/kg H₂O	379 ± 47	NS	401 ± 10
Initial weight, g	316 ± 18	NS	320 ± 11
% Weight	−15.4 ± 1.4	NS	−12.8 ± 1.2

whether or not a fall in papillary solute concentration with normal urinary flow rate would affect the generation of papillary necrosis. Five animals were sacrificed at the same time that BEA was given to group 6a animals to determine the papillary solute concentration (group 6b). These data are shown in Table 6. As shown, there is a low papillary concentration of sodium, potassium, and urea as compared to control. Twenty-four hour urine volume was 6.8 ± 1.2 versus 10.6 ± 3.3 ml in control and furosemide-treated animals, respectively (NS). After zero sodium diet the BEA-treated animals (group 6a) continued to waste sodium as compared to controls (Table 7). Cumulative sodium excretion, plasma BUN, and creatinine was higher in the BEA-treated animals.

Each animal in the BEA-treated group had histologic evidence of papillary necrosis. Thus, a loss of papillary solute alone does not protect against this form of papillary necrosis.

Discussion

The administration of a single dose of BEA causes papillary necrosis in virtually 100% of the treated animals [7–9]. The mechanism responsible for this effect has not been elucidated clearly, but it may be secondary to a direct toxic effect of this compound on the renal tubular cells or renal interstitium. Since this effect is selective for the renal papilla it is also likely that the agent is concentrated in the renal papilla where it achieves concentrations high enough to exert its toxic effect. If this was the mechanism responsible for the papillary necrosis seen following administration of this agent, then maneuvers which alter the concentrating capacity of the kidney might exert a protective effect.

The initial observation made in this study was that administration of the usual toxic dose of BEA to animals with central diabetes insipidus, that is, homozygous Brattleboro rats, resulted in minimal or no toxicity. These animals did not develop a typical histologic picture of papillary necrosis and were also able to conserve sodium when placed on a zero sodium diet. This is in sharp contradistinction to the observations made in heterozygous Brattleboro rats identically treated. These animals, rats which have endogenous vasopressin and are able to concentrate the urine normally, developed the full blown picture of papillary necrosis and its functional counterpart, sodium wastage. It is possible, however, that a larger dose of BEA might induce papillary necrosis in rats with central diabetes insipidus.

That the protective effect observed in the homozygous Brattleboro rats was the consequence of vasopressin deficiency was demonstrated by the experiments in which vasopressin was administered to these animals. Prolonged vasopressin administration resulted in a complete restoration of normal concentrating capacity. At this point the administration of BEA produced papillary necrosis. Furthermore, the administration of glucose-containing drinking water to Spraque-Dawley rats resulted in a high rate of urine flow and a low urine osmolarity when compared to control animals. This functional state is similar to that observed in central diabetes insipidus and was also associated with virtually complete protection from the nephrotoxic effect of BEA. Thus, it seems quite clear that animals elaborating a dilute urine in increased amounts do not develop papillary necrosis when BEA is given.

The animals with central diabetes insipidus and the glucose-drinking rats have a functional alteration, when compared to normal hydropenic rats, which consists of two parts. The elaboration of a dilute urine for prolonged periods of time is associated with a decrease in the papillary solute gradient [12, 13]. Furthermore, these animals have a high rate of urine flow. Thus, it is possible that the protective effect we observed was the consequence of a high rate of urine flow, a decrease in papillary osmolarity, or combination of the two. To dissect these possibilities we performed additional experiments. Furosemide was given on a chronic basis. This resulted in a decrease in papillary osmolarity (Table 6). At this point the furosemide was withdrawn and BEA was administered. In other words, the compound was given to animals with a decreased papillary osmotic gradient but with a normal rate of urine flow. As is clearly demonstrated in Table 7, the presence of a reduced osmotic gradient in the renal papilla exerted no protective effect.

To examine the other variable, an increase in urine flow without a prolonged decrease in papillary tonicity, we studied

the effect of abrupt withdrawal of vasopressin to vasopressin-replaced Brattleboro rats. This maneuver resulted in a sudden increase in urine volume on the background of a previously normal papillary osmotic gradient. Again, as seen in Table 5, no protective effect was observed. Thus, it is clear that to prevent the nephrotoxic effect of BEA, both a prolonged increase in urine flow and a decreased papillary solute gradient must be present. Or, for BEA to exert its toxic effect either a reduced (in essence, a normal) rate of urine flow must be present or a normal papillary osmotic gradient must be present. The exact interrelationship between these two variables in the causation or prevention of this lesion remains to be elucidated. In addition, vasopressin may play a role in the pathogenesis of this lesion through a mechanism unrelated to urine flow or papillary tonicity.

While there are obvious differences between BEA-induced papillary necrosis and its human counterpart, there may be some relationship between the results reported in this study and analgesic nephropathy. Several investigators have reported that analgesic-induced papillary necrosis was more severe in experimental animals that were volume-contracted [5, 6]. Clinical anecdotes and epidemiologic studies have long held that well hydrated patients are less prone to the nephrotoxic properties of analgesics than their less well hydrated counterparts [14]. These observations in humans combined with ours using the BEA model in rats suggest that a concentrated urine is important, or indeed required, in the pathogenesis of drug-induced papillary necrosis. Furthermore, salicylates, compounds implicated as causing papillary necrosis in humans, are concentrated preferentially in the innermost portions of the kidney [15, 18]. Likewise, acetaminophen is concentrated in the urine during antidiuresis [19]. Concentrations within the renal medulla under these conditions are five to seven times higher than those seen in the cortex. It has been postulated that the intrarenal gradient, allowing concentration of these drugs in the deeper structures of the kidney, is the mechanism responsible for the toxic effects of these compounds (for review, see [20]).

Regardless of the relationship between our studies using BEA and analgesic nephropathy in humans, it is quite clear that an intact concentrating mechanism in the rat kidney is required for the expression of BEA nephrotoxicity using a single dose. The presence of either intact papillary osmotic gradient or a relatively reduced rate of urine flow allows the compound to cause papillary necrosis. The combination of a high rate of urine flow and reduced papillary tonicity prevents BEA nephrotoxicity most likely because it prevents accumulation of a sufficient amount of the agent within the papilla to cause necrosis. These observations may have clinical relevance.

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