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Probenecid Inhibition of Bumetanide-Induced Natriuresis in the Dog (40540)[1]

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Bumetanide, 4-phenoxy-4-(*n*-butyl)-5-sulfomyl-*m*-anthanilic acid, is structurally related to the thiazide diuretics and furosemide and has been found to be a potent high-ceiling diuretic in dogs (1, 2) and in man (3). Although furosemide and bumetanide produce the same maximal diuretic–saluretic effects, bumetanide is 40 to 60 times more potent on a molar basis. Like furosemide, bumetanide appears to exert its major effect on the ascending limb of the loop of Henle (4) with perhaps an additional action on the proximal tubule (5).

Bumetanide is an organic acid and has a renal clearance which is similar to that of *p*-aminohippuric acid (2). Since bumetanide is highly bound to plasma proteins (2), its concentration in the lumen of the renal tubules would appear to be dependent primarily on renal tubular secretion. Thus, if the natriuretic action of bumetanide depends upon luminal concentration rather than on plasma concentration, agents which interfere with its transport into the tubular lumen should antagonize its natriuretic action. In previous studies, probenecid, an inhibitor of renal tubular secretion of organic acids, has been shown to inhibit the natriuretic actions of chlorothiazide (6), ethacrynic acid (7), and furosemide (8). However, conflicting reports have appeared in the literature concerning the ability of probenecid to block the natriuretic action of bumetanide. Lant (9) observed that probenecid decreased the bumetanide-induced increase in sodium excretion in humans, while Friedman and Roch-Ramel (10) observed that probenecid failed to block bumetanide-induced natriuresis in cats.

It is not clear why this apparent conflict concerning the ability of probenecid to block bumetanide-induced natriuresis exists. It is the purpose of this report to determine the effects of probenecid on bumetanide-induced natriuresis in the dog, since probenecid has been shown to decrease the natriuretic actions of chlorothiazide (6), ethacrynic acid (7), and furosemide (8) in this species.

Methods and materials. Mongrel dogs of either sex, weighing 13–19 kg were anesthetized with pentobarbital (30 mg/kg, intravenously). The animals were tracheostomized to maintain an airway. A femoral artery was cannulated with polyethylene tubing to monitor blood pressure. This was connected to a Statham arterial pressure transducer and blood pressure was recorded continuously on a Gilson polygraph. Blood samples were also obtained from this catheter. The ipsilateral femoral vein was cannulated for fluid infusion. Ureters were cannulated with polyethylene tubing either through a lower midline incision just above the bladder or the right ureter through a higher midline incision and the left ureter through a flank incision. The left renal artery (or right renal artery in dogs which had two separate left renal arteries) was exposed by a flank incision and a 24-gauge needle was inserted retrograde. Patency was maintained by an infusion of saline at 1.2 ml/min using a Harvard infusion pump.

Mannitol, 5%, and inulin, 0.2 or 0.4%, were infused intravenously to insure adequate urine flow and to monitor the glomerular filtration rate, respectively. Urine was collected from each ureter at 10-min intervals. Arterial blood samples were withdrawn every 20 min using a heparinized syringe. After the rate of urine flow had stabilized, four control urine samples were collected. The saline infusion into the renal artery was then replaced with a solution containing sufficient bumetanide to deliver 0.05 μg/kg/min at 1.2 ml/min. After four urine samples were collected from each kidney, the bumetanide solution was replaced with saline. When the rate of urine flow had returned to near control levels, pro-

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benecid (50 mg/kg, dissolved in 15 ml of 0.9% NaCl with the aid of NaOH) or the vehicle for probenecid was infused intravenously over a 7- to 10-min period. After 20 min, bumetanide was again infused into the renal artery and four urine samples collected.

Sodium and potassium concentrations were determined using a flame photometer (Instrumentation Laboratory 343). Inulin concentrations of plasma and urine were determined employing the method of Schreiner (11).

For statistical comparison, the values obtained from each animal for two 10-min collection intervals preceding the infusion of bumetanide were averaged, as were values from the last two periods of infusion of the drug. The differences between these averages were analyzed statistically using the Student *t* test, paired comparison (12). The 0.05 level of probability was used as the criterion of significance.

Results. A typical experiment which illustrates the protocol employed is shown in Table I. The effects of bumetanide before and after systemic administration of probenecid were determined in eight dogs and are summarized in Table II. Infusion of bumetanide into one renal artery significantly increased urine flow and sodium excretion from the infused kidney. The increase in urine volume and sodium excretion in response to bumetanide at an infusion rate of 0.05 μg/kg/min was confined to the infused kidney. While the urine flow of the contralateral kidney did not change during bumetanide infusion, there was a small but significant decline in sodium excretion from that kidney. Urine flow and sodium excretion from the infused kidney increased within 10 min after the bumetanide infusion was initiated and declined to control values within one period after the bumetanide infusion was replaced with saline. The renal parameters measured prior to the initial infusion of bumetanide did not differ significantly from the saline infusion periods just after the probenecid injection (Table II). After the administration of probenecid, bumetanide infusion did not significantly increase urine flow or sodium excretion from the ipsilateral kidney. The rate of glomerular filtration, as estimated by inulin clearance, was not significantly altered by bumetanide. In experiments, which differed only by the omission of probenecid, the response to the second infusion of bumetamide did not differ from the first (Table III).

Discussion. Bumetanide is an organic acid which is highly bound to plasma proteins (2). It is excreted by the kidneys at a rate which corresponds to the clearance of *p*-aminohippuric acid and probenecid increases the half-

TABLE I. PROTOCOL OF AN EXPERIMENT DEMONSTRATING THE RENAL ACTIONS OF BUMETANIDE AND THEIR INHIBITION BY PROBENECID[a]

Collection period	Left renal artery infusion	Urine flow (ml/min)		Inulin clearance (ml/min)		Sodium excretion (μeq/min)	
		I[b]	C[c]	I	C	I	C
1	Saline	3.8	4.7	26	30	68	127
2		3.5	4.5	25	27	53	116
3	Bumetanide	3.6	4.5	23	27	79	99
4		5.3	4.2	22	24	254	88
5		6.0	4.2	25	25	294	97
6		5.4	4.1	23	27	243	90
7	Saline	5.0	4.1	23	25	195	98
8		3.5	3.8	20	24	63	84
	Probenecid (50 mg/kg iv)						
9		3.3	3.7	23	25	69	107
10		3.1	3.7	22	24	55	99
11	Bumetanide	3.4	4.0	23	24	58	120
12		3.3	4.0	23	23	59	116
13		3.3	3.8	18	21	65	103
14		3.4	3.7	18	21	75	100

[a] 13.5 kg female dog.
[b] Ipsilateral, infused kidney.
[c] Contralateral, control kidney.

TABLE II. INFUSION OF BUMETANIDE (0.05 μg/kg/min) INTO ONE RENAL ARTERY OF THE DOG BEFORE AND AFTER ADMINISTRATION OF PROBENECID (50 mg/kg, INTRAVENOUSLY)[a]

Unilateral renal artery infusion	Urine flow (ml/min)		Inulin clearance (ml/min)		Sodium excretion (μeq/min)	
	I[b]	C[c]	I	C	I	C
			Before probenecid			
Saline	3.0	3.2	21	22	77	89
Bumetanide	5.6	3.2	21	22	328	66
Difference	2.6[d]	0.0	0	0	251[d]	−23[d]
±SEM	0.6	0.2	2	0	44	9
			After probenecid			
Saline	3.2	3.7	19	21	63	78
Bumetanide	3.6	3.8	18	21	86	62
Difference	0.4	0.1	−1	0	22	−16
±SEM	0.3	0.2	1	1	10	13

[a] Saline values indicate excretions for 20 min just prior to bumetanide infusion, and bumetanide values indicate excretions for a 20-min interval beginning 20 min after starting the bumetanide infusion. Values represent means of eight dogs.
[b] Ipsilateral, infused kidney.
[c] Contralateral, control kidney.
[d] Significant difference ($P < 0.05$).

TABLE III. INFUSION OF BUMETANIDE (0.05 μg/kg/min) INTO ONE RENAL ARTERY OF THE DOG BEFORE AND AFTER ADMINISTRATION OF PROBENECID VEHICLE INTRAVENOUSLY[a]

Unilateral renal artery infusion	Urine flow (ml/min)		Inulin clearance (ml/min)		Sodium excretion (μeq/min)	
	I[b]	C[c]	I	C	I	C
			Before probenecid vehicle			
Saline	3.3	3.2	22	22	60	41
Bumetanide	6.1	3.0	23	20	325	49
Mean difference	2.8[d]	0.2	1	2	265[d]	7
±SEM	0.5	0.3	1	1	36	9
			After probenecid vehicle			
Saline	4.3	4.0	21	20	76	64
Bumetanide	6.0	3.6	22	20	282	58
Mean difference	1.6[d]	0.3	1	0	206[d]	6
±SEM	0.3	0.2	1	1	13	4

[a] Saline values indicate excretions for 20 min just prior to bumetanide infusion, and bumetanide values indicate excretion for a 20-min interval beginning 20 min after starting the bumetanide infusion. Values represent means of four dogs.
[b] Ipsilateral, infused kidney.
[c] Contralateral, control kidney.
[d] Significant difference ($P < 0.05$).

life of the drug in plasma (2). Because it is highly bound, its renal clearance is highly dependent upon secretion. If the natriuretic action of bumetanide depends upon attaining adequate luminal concentration rather than an adequate plasma concentration, then blocking tubular transport should block the natriuretic response. In the experiments presented in this paper, a dose of probenecid (50 mg/kg) which has been shown to block tubular secretion of *p*-aminohippuric acid was employed (13). This dose of probenecid has also been shown previously to block the natriuretic effects of furosemide (8). As shown in the Results section, probenecid was found to completely block the natriuretic and diuretic actions of bumetanide. Thus, in the dog, adequate luminal concentration appears to be necessary for the natriuretic and diuretic actions of bumetanide. In this respect, bumetanide is similar to other diuretic agents in the dog (6–8). The slight decline in sodium excretion by the contralateral kidney seen when bumetanide was infused into one renal

artery is probably the result of physiological compensation.

Conflicting reports have appeared in the literature concerning the ability of probenecid to block the natriuretic action of bumetanide (9, 10). Lant (9) observed that increases in sodium excretion induced by bumetanide (0.5 mg, intravenously) in humans were markedly decreased when probenecid (500 mg, orally every 6 hr for nine doses) was given prior to bumetanide. Probenecid decreased the rate of bumetanide clearance, but did not block the natriuretic action of a 3 mg/kg iv dose of bumetanide in other experiments. Thus, in man, probenecid antagonizes renal excretion of bumetanide and inhibits the natriuretic action of low doses of bumetanide but not of higher doses. It is likely that at higher plasma levels, sufficient drug enters the tubular lumen by filtration to produce a natriuretic action.

The lack of effect of probenecid on bumetanide-induced natriuresis reported by Friedman and Roch-Ramel (10) in the cat is difficult to reconcile with the study reported here. Their experimental design was similar to that used in this study. Also, they observed that probenecid abolished PAH secretion and blocked furosemide-induced natriuresis. It is possible that the acid transport system in the cat may differ from that of dogs or humans. Bumetanide could also have a higher affinity for the transport system in cat, or perhaps utilizes a separate acid transport system which probenecid does not block. It is also possible that bumetanide may not be as highly protein bound in plasma in cat, thus allowing effective concentrations of bumetanide to reach the tubular lumen by glomerular filtration even at the low infusion rate (0.025 μg/kg/min) employed in their studies.

Summary. Bumetanide, when infused into one renal artery at 0.05 μg/kg/min in the dog, produced a significant ipsilateral increase in the excretion of sodium and water without affecting the rate of glomerular filtration. At this dose, these responses were limited to the infused kidney. When probenecid (50 mg/kg, iv) was given prior to bumetanide to inhibit tubular secretion of this drug, the natriuretic and diurectic actions of bumetanide were blocked. The inhibition of bumetanide-induced natriuresis and diuresis by probenecid indicates that adequate luminal concentration is a prerequisite to the natriuretic–diuretic actions of this drug.

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