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Determinants of Bumetanide Response in the Dog: Effect of Indomethacin

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Abstract □ Four male unanesthetized dogs each weighing 22.0–29.0 kg received 0.250 mg/kg iv of bumetanide before (treatment I) and after (treatment II) indomethacin pretreatment. Lactated Ringer's solution was administered intravenously throughout both treatments at a flow rate of 2 ml/min to avoid fluid and electrolyte depletion. Unchanged bumetanide and indomethacin concentrations were analyzed using high-performance liquid chromatography. Sodium was measured by flame photometry and creatinine by colorimetry. Indomethacin pretreatment did not significantly change the pharmacokinetics of bumetanide, affecting neither the total amount of drug nor time course of drug delivered into the urine. In contrast, indomethacin pretreatment resulted in a dramatic reduction in the 4-hr sodium excretion and urine volume. Therefore, a pharmacokinetic interaction may be eliminated as a possible mechanism for the attenuation, by indomethacin, of the natriuretic and diuretic response of bumetanide. Instead, it appears that indomethacin diminishes the response to bumetanide *via* prostaglandin inhibition.

Keyphrases □ Bumetanide—pharmacokinetics, sodium excretion, dogs, effect of indomethacin pretreatment □ Indomethacin—pretreatment, effect on sodium excretion, pharmacokinetics of bumetanide, dogs □ Pharmacokinetics—bumetanide, sodium excretion, pharmacokinetics in the dog, effect of indomethacin pretreatment

Bumetanide [3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid] is a high-ceiling diuretic with pharmacological action similar to that of furosemide (1–3). The diuretic appears to act primarily at the medullary portion of the ascending limb of the loop of Henle, where it inhibits solute reabsorption, although inhibition of sodium transport in the proximal nephron also occurs (4–7). In addition, bumetanide induces intrarenal hemodynamic changes (8–12). Since bumetanide is highly bound to plasma proteins (13, 14), the drug gains access to the kidney lumen predominantly at the pars recta of the proximal tubule *via* the nonspecific organic acid secretory pathway (1, 13).

Indomethacin has been shown recently to attenuate the natriuretic and diuretic response to bumetanide in experimental animals (12), healthy volunteers (15, 16), and patients (17). These authors proposed that indomethacin, a potent inhibitor of prostaglandin synthetase, interferes with the prostaglandin-mediated effect of bumetanide. However, it is also possible that indomethacin may compete with bumetanide (both drugs are weak organic acids) for active secretion into the lumen of the kidney tubule, thereby modifying either the total amount of diuretic delivered to its active site or the time course of drug delivery. Since previous investigators (12, 15–17) did not measure concentrations and/or amounts of bumetanide in the plasma and urine, this alternative hypothesis (pharmacokinetic interaction) cannot be eliminated. Therefore, the present investigation was undertaken to clarify the mechanism by which indomethacin diminishes the pharmacodynamic response to bumetanide.

EXPERIMENTAL

Materials—An aqueous solution dosage form of bumetanide[1] was prepared using 0.4 *N* NaOH immediately prior to use. Indomethacin capsules[2] were obtained commercially. Indomethacin powder[3] was used as received. All other chemicals and solvents were reagent grade or better, as previously reported (18).

Methods—Four male, mongrel, conditioned, unanesthetized dogs weighing 22.0–29.0 kg received 0.250 mg/kg of bumetanide before (treatment I) and after (treatment II) pretreatment with indomethacin. Each dog was fasted the night before and throughout the entire study period. Bumetanide was administered intravenously over a 3-min infusion[4] period, with the beginning of the infusion being considered as time zero. A 100-mg dose of indomethacin (two 50-mg capsules) was ingested the night before (11:00 to 11:30 p.m.) and on the study day (60 min prior to bumetanide administration). An interval of at least 1 week elapsed between studies, and identical lots for each drug were used throughout.

Heparinized scalp vein needles[5] were placed in the forelegs of each dog:

[1] Lot A-29; Hoffmann-La Roche, Inc., Nutley, N.J.
[2] Lot D2520; Merck Sharp and Dohme, West Point, Pa.
[3] Merck Sharp and Dohme, Rahway, N.J.
[4] Harvard Compact Infusion Pump; Harvard Apparatus Co., Inc., South Natick, Mass.
[5] E-Z Set—PRN Intermittent Infusion Set; The Deseret Co., Sandy, Utah.

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one was used for administration of bumetanide and replacement fluids, the other for obtaining blood samples. Blood samples (3 ml) were collected just prior to bumetanide dose (blank) and at 3, 5, 10, 20, 30, 45, 60, 80, 100, 120, 150, 180, 210, and 240 min. Voided urine was collected *via* an indwelling bladder catheter[6] just prior to bumetanide dosing (blank) and at 20, 40, 80, 120, 180, and 240 min. The bladder was flushed with 2 × 5 ml of air at the end of each urine collection to ensure a complete catch. Lactated Ringer's solution ws administered intravenously throughout the entire study period of both treatments at a flow rate of 2 ml/min to avoid fluid and electrolyte depletion. All 4-hr plasma samples showed normal sodium concentrations.

Assays—Plasma and urine samples containing bumetanide, with and without indomethacin pretreatment, were analyzed by a high-performance liquid chromatographic (HPLC) method as described previously (18). Plasma samples (0.20 ml) containing indomethacin were prepared and analyzed in a similar fashion to that of bumetanide. A 50- μl aliquot of acetophenone (0.025 mg/ml) was used as the internal standard, and the solvent system (50% acetonitrile in 0.015 *M* phosphoric acid aqueous solution, adjusted to pH 5.0 with 4 *N* NaOH) was pumped isocratically at a flow rate of 2.0 ml/min at ambient temperature. Both indomethacin and acetophenone were measured using UV detection at 254 nm (0.01 AUFS). The voltage spans on the dual-pen recorder were set at 10 mV for indomethacin and at 50 mV for acetophenone. Using the mobile phase described above, indomethacin and acetophenone had retention times in plasma of 9.0 and 4.5 min, respectively (Fig. 1). A representative standard curve of indomethacin–acetophenone peak height ratio over the indomethacin plasma concentration range (0.25–5.00 μg/ml) resulted in the following linear least-squares regression equation: $y = 0.232x - 0.003$; $r^2 = 0.999$.

Plasma and urine samples were measured for sodium with a flame photometer[7]. Creatinine was determined colorimetrically using a commercial kit[8].

Calculations—Plasma concentration–time curves of bumetanide were fitted (equally weighted) to the general polyexponential equation for post constant-rate infusion data (19):

$$C_p = \sum_{i=1}^{n} Yi\, e^{-\lambda i t} \qquad \text{(Eq. 1)}$$

where C_p represents the plasma concentration at time t, Yi is the coefficient of the ith exponential term for post constant-rate intravenous infusion data, and λi is the exponent of the ith exponential term. The values of the coefficients and exponential terms in Eq. 1 were obtained using a nonlinear least-squares regression program[9] and a microcomputer[10]. Initial estimates were obtained using the RSTRIP[9] program. The number of exponents (n) needed for each data set were determined by the application of Akaike's information criterion (20).

Since:

$$Yi = \sum_{i=1}^{n} (1 - e^{\lambda i T}) Ci/(-\lambda i T) \qquad \text{(Eq. 2)}$$

and T is the constant-rate infusion time and Ci is the coefficient of the ith exponential term for bolus intravenous data, Eq. 1 can be rearranged (19) to:

$$C_p = \sum_{i=1}^{n} (1 - e^{\lambda i T})\, Ci\, e^{-\lambda i t}/(-\lambda i T) \qquad \text{(Eq. 3)}$$

Once the values of the coefficients and exponential terms in Eq. 1 are determined by computer fitting, the values of Ci in Eq. 3 can be calculated.

The following pharmacokinetic parameters were calculated using standard equations (19, 21):

$$V_1 = D/\sum_{i=1}^{n} Ci \qquad \text{(Eq. 4)}$$

$$Vd_{ss} = D \sum_{i=1}^{n} Ci/\lambda i^2 \Big/ \left(\sum_{i=1}^{n} Ci/\lambda i\right)^2 \qquad \text{(Eq. 5)}$$

$$Vd_{area} = D/\left(\lambda_1 \sum_{i=1}^{n} Ci/\lambda i\right) \qquad \text{(Eq. 6)}$$

[6] Swan-Ganz Flow-Directed Monitoring Catheter, Model 93-111-7F; American Edwards Laboratories, Santa Ana, Calif.
[7] Model 455; Corning Medical and Scientific, Medfield, Mass.
[8] Sigma Chemical Co., St. Louis, Mo.
[9] Personal communication, Dr. J. L. Fox, College of Pharmacy, The University of Michigan, Ann Arbor, Mich.
[10] Apple II Plus Computer; Apple Computer Inc., Cupertino, Calif.

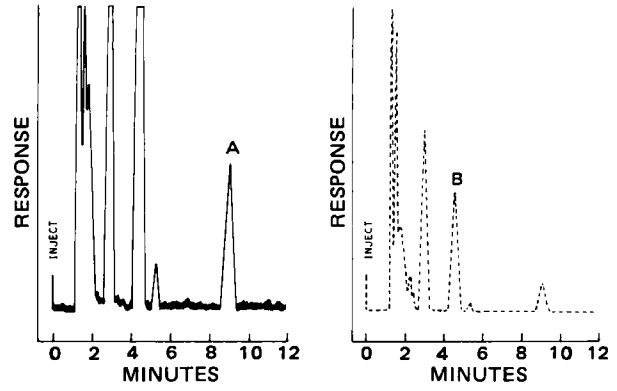

Figure 1—*Chromatograms for plasma spiked with indomethacin (A) and the internal standard (B), acetophenone, using UV detection (254 nm). Voltage spans on the dual-pen recorder were 10 mV (—) and 50 mV (- - - - -).*

$$CL_p = D/\sum_{i=1}^{n} Ci/\lambda i \qquad \text{(Eq. 7)}$$

$$CL_r = Ae^{\infty} \Big/ \sum_{i=1}^{n} Ci/\lambda i \qquad \text{(Eq. 8)}$$

$$CL_{nr} = CL_p - CL_r \qquad \text{(Eq. 9)}$$

$$t_{1/2} = 0.693/\lambda_1 \qquad \text{(Eq. 10)}$$

$$K_{10} = CL_p/V_1 \qquad \text{(Eq. 11)}$$

$$fe = Ae^{\infty}/D \qquad \text{(Eq. 12)}$$

In Eqs. 4–12, V_1 is the volume of the central compartment; Vd_{ss} is the volume of distribution at steady state; Vd_{area} is that volume which, when multiplied by C_p in the log-linear phase is equal to the amount of drug in the body; D is the intravenous dose (equal to the product of the zero-order infusion rate and the length of infusion); C_1 and λ_1 are the coefficient and exponent, respectively, such that λ_1 is the smallest of the λi values of the polyexponential equation; CL_p is the total plasma clearance; CL_r is the renal clearance; CL_{nr} is the nonrenal clearance; Ae^{∞} is the amount of unchanged drug recovered in the urine at time infinity; $t_{1/2}$ is the biological half-life; K_{10} is the first-order elimination rate constant from the central compartment; and fe is the fraction of the available dose excreted unchanged in the urine. Creatinine clearance (CL_{cr}) was calculated by dividing the urinary excretion rate of creatinine by its plasma concentration at the midpoint of the urine collection period.

Data throughout the study are expressed as mean ± *SD*, unless otherwise indicated. Statistical differences were determined by a paired t test. A p value of <0.05 was considered to be significant.

RESULTS

Plasma concentrations of bumetanide over 4 hr were fitted to a biexponential equation for six data sets and to a triexponential equation for two data sets (Table I). The goodness of the fit, as determined by R^2 and the correlation, was ≥0.991.

The pharmacokinetics of bumetanide before (treatment I) and after (treatment II) indomethacin pretreatment are presented in Table II. None of the pharmacokinetic parameters evaluated were statistically different between treatments. This is demonstrated by the virtually superimposable plasma concentration–time profiles (Fig. 2) and the urinary excretion rate–time profiles (Fig. 3) observed between treatments I and II. Mean plasma concentrations of indomethacin ranged from 0.62 to 4.36 μg/ml during the study period, well above the plasma concentrations of bumetanide (Fig. 2).

The effects of indomethacin on bumetanide-induced diuresis and natriuresis are presented in Table III. Pharmacodynamic data are reported as electrolyte excretion rate and cumulative excretion (as opposed to fractional excretion), since sodium concentrations and creatinine clearances did not differ between treatments (CL_{cr} = 2.49 ± 0.24 ml/min·kg for treatment I *versus* 2.33 ± 0.32 ml/min·kg for treatment II; $p > 0.50$). Indomethacin pretreatment results in a dramatic reduction in urine volume (1060 ± 77 ml/4 hr for treatment I *versus* 543 ± 115 ml/4 hr for treatment II; $p < 0.005$) as well as sodium excretion (121 ± 17 meq/4 hr for treatment I *versus* 62.4 ± 21.3 meq/4 hr for treatment II; $p < 0.005$). Analyses of the sodium excretion rate over time show that the

Table I—Coefficients and Exponential Terms of Bumetanide Obtained Using Biexponential and Triexponential Equations

Dog	Treatment[a]	Biexponential Equation C_1, ng/ml	C_2, ng/ml	λ_1, min^{-1}	λ_2, min^{-1}	R^2 [b]	Correlation[c]
1	I	120	1855	0.0095	0.1150	0.991	0.994
2	I	119	1634	0.0102	0.1016	0.997	0.998
3	I	125	1978	0.0125	0.1166	0.996	0.997
	II	173	2289	0.0169	0.1028	0.992	0.995
4	I	164	1187	0.0134	0.1265	0.995	0.996
	II	110	1188	0.0122	0.1130	0.996	0.997

Dog	Treatment	Triexponential Equation C_1, ng/ml	C_2, ng/ml	C_3, ng/ml	λ_1, min^{-1}	λ_2, min^{-1}	λ_3, min^{-1}	R^2 [b]	Correlation[c]
1	II	49.5	529	1355	0.0081	0.0414	0.1604	0.999	0.999
2	II	106	647	1839	0.0102	0.0578	0.2020	0.999	0.999

[a] (I) bumetanide before indomethacin pretreatment; (II) bumetanide after indomethacin pretreatment. [b] $R^2 = [\Sigma(Obs)^2 - \Sigma(Dev)^2]/\Sigma(Obs)^2$. [c] Correlation between the calculated and observed plasma concentrations.

Table II—Pharmacokinetics of Bumetanide Before and After Indomethacin Pretreatment

Dog	Treatment[a]	Weight kg	CL_p, ml/min·kg	V_1, ml/kg	Vd_{ss}, ml/kg	Vd_{area}, ml/kg	$t_{1/2}$, min	K_{10}, min^{-1}	CL_r, ml/min·kg	CL_{nr}, ml/min·kg	fe
1	I	24.0	8.70	127	443	913	72.7	0.0685	3.81	4.89	0.438
	II	27.0	9.13	129	375	1134	86.0	0.0708	2.98	6.15	0.327
2	I	22.0	9.03	143	421	881	67.6	0.0631	4.12	4.91	0.456
	II	23.5	8.13	96	335	800	68.2	0.0847	3.55	4.58	0.437
3	I	22.5	9.28	119	324	741	55.3	0.0780	4.45	4.83	0.480
	II	24.0	7.69	102	195	455	41.0	0.0754	3.03	4.66	0.394
4	I	27.0	11.6	185	528	866	51.7	0.0627	5.82	5.78	0.504
	II	29.0	12.8	193	547	1053	57.0	0.0663	2.96	9.86	0.232
Mean (*SD*)	I	23.9 (2.2)	9.65 (1.32)	144 (29)	429 (84)	850 (75)	61.8 (9.9)	0.0681 (0.0071)	4.55 (0.89)	5.10 (0.45)	0.470 (0.029)
Mean (*SD*)	II	25.9 (2.6)	9.44 (2.32)	130 (44)	363 (145)	860 (305)	63.0 (18.9)	0.0743 (0.0079)	3.13 (0.28)	6.31 (2.47)	0.348 (0.089)
Level of Significance[b]		S ($p < 0.02$)	NS ($p < 0.50$)	NS ($p > 0.20$)	NS ($p > 0.10$)	NS ($p > 0.50$)	NS ($p > 0.50$)	NS ($p > 0.20$)	NS ($p > 0.05$)	NS ($p > 0.20$)	NS ($p > 0.10$)

[a] (I) bumetanide before indomethacin pretreatment; (II) bumetanide after indomethacin pretreatment. [b] (S) significant; (NS) not significant.

Table III—Effects of Indomethacin on Bumetanide Diuresis and Natriuresis

Dog	Treatment[a]	Urine Volume, ml/4 hr	Sodium Excretion, meq/4 hr
1	I	1131	123
	II	467	48.6
2	I	1028	116
	II	625	78.0
3	I	967	103
	II	424	40.0
4	I	1115	143
	II	657	83.1
Mean (*SD*)	I	1060 (77)	121 (17)
Mean (*SD*)	II	543 (115)	62.4 (21.3)
Level of Significance[b]		S ($p < 0.005$)	S ($p < 0.005$)

[a] (I) bumetanide before indomethacin pretreatment; (II) bumetanide after indomethacin pretreatment. [b] (S) significant.

inhibiting effect of indomethacin was most pronounced during the initial 40–60 min (Fig. 4).

DISCUSSION

Several studies have shown that indomethacin decreases the cumulative response to bumetanide in experimental animals (12), healthy volunteers, (15, 16), and patients (17). Mechanisms consistent with this attenuated response include the inhibition, by indomethacin, of prostaglandin-induced changes in renal hemodynamics and direct tubular effects, as well as competition between bumetanide and indomethacin for active tubular transport into the kidney lumen. Previous investigators (12, 15–17) only considered the mechanism involving prostaglandin inhibition, since concentrations and/or amounts of bumetanide in the plasma and urine were not determined.

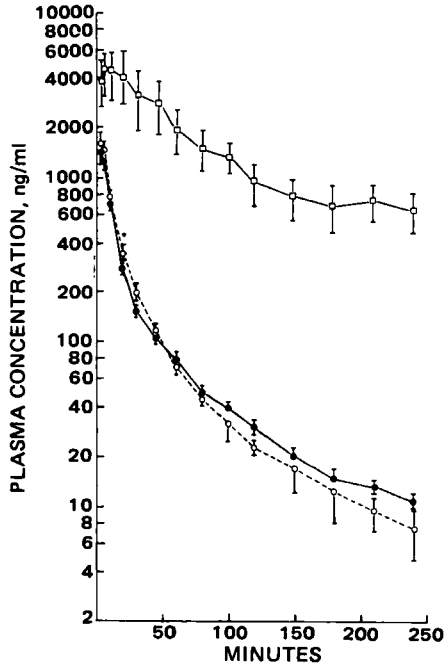

Figure 2—*Plasma concentration* versus *time plots of bumetanide alone (●), bumetanide after indomethacin pretreatment (○), and indomethacin (□). Data are expressed as the mean ± SEM (n = 4). Asterisks denote statistical differences between the treatments.*

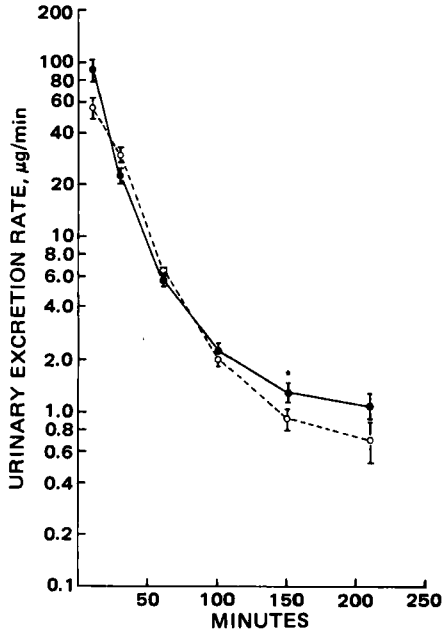

Figure 3—*Urinary excretion rate* versus *midpoint time plots of bumetanide alone (●) and bumetanide after indomethacin pretreatment (○). Data are expressed as the mean ± SEM (n = 4). Asterisks denote statistical differences between the treatments.*

In the present investigation, indomethacin pretreatment did not significantly change the pharmacokinetics of bumetanide (Table II), affecting neither the total amount of drug nor time course of drug delivered into the urine. Although *fe* was reduced ~25% in the presence of indomethacin, the extent of this change was minimal compared with the marked effect of indomethacin on bumetanide-induced natriuresis and diuresis (~50% reduction).

Figures 5 and 6 demonstrate the effect of indomethacin on the dose–response curves of bumetanide. Indomethacin decreased the maximal response (sodium excretion rate) to bumetanide when the dose was expressed as either plasma concentration (Fig. 5) or urinary excretion rate (Fig. 6). These effects are consistent with those of a noncompetitive inhibition, presumably that of prostaglandin synthesis. The effect of indomethacin on bumetanide-induced natriuresis and diuresis could not be explained by normalizing the response to creatinine clearance. This finding is in agreement with previous studies by Brater *et al.* (15) and Olsen (12). Brater *et al.* (15) reported that unlike furosemide, indomethacin decreased the increment in fractional sodium excretion due to bumetanide in healthy volunteers. Olsen (12) observed that absolute

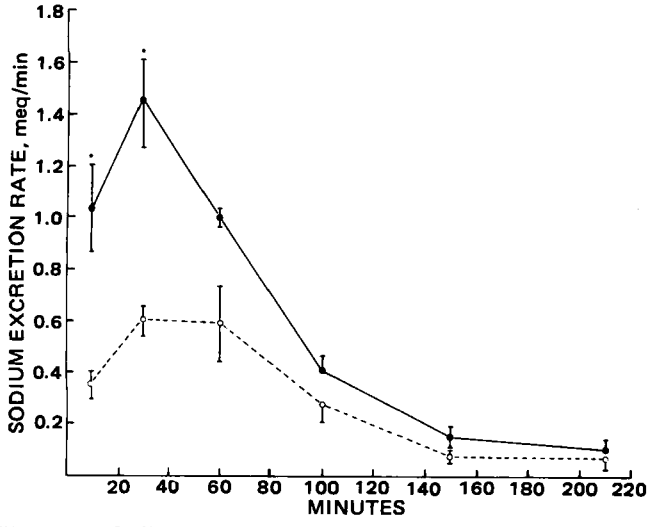

Figure 4—*Sodium excretion rate* versus *midpoint time plots of bumetanide alone (●) and bumetanide after indomethacin pretreatment (○). Data are expressed as the mean ± SEM (n = 4). Asterisks denote statistical differences between the treatments.*

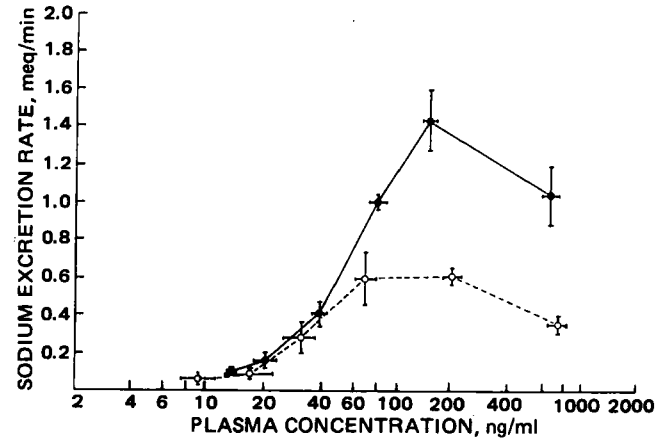

Figure 5—*Sodium excretion rate* versus *plasma concentration plots of bumetanide alone (●) and bumetanide after indomethacin pretreatment (○). Data are expressed as the mean ± SEM (n = 4).*

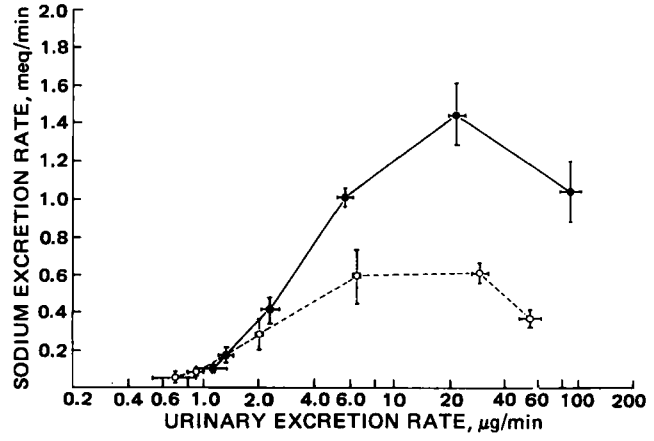

Figure 6—*Sodium excretion rate* versus *urinary excretion rate plots of bumetanide alone (●) and bumetanide after indomethacin pretreatment (○). Data are expressed as the mean ± SEM (n = 4).*

and fractional sodium excretions after bumetanide were significantly lower in indomethacin-pretreated dogs compared with nonpretreated dogs at a time when neither renal blood flow nor glomerular filtration rate were significantly different.

The present study and that of previous investigators (12, 15) suggest that indomethacin affects the pharmacodynamic response to bumetanide by a mechanism other than prostaglandin-mediated changes in renal hemodynamics. Although speculative, renal prostaglandins may be involved in the regulation of medullary tonicity and solute excretion at a tubular level. This hypothesis is supported by an *in vitro* study in the isolated, perfused segments of rabbit nephrons (22), which demonstrated that dinoprostone inhibits net chloride transport across the medullary thick ascending limb of the loop of Henle, but had no effect on the cortical segment. This is consistent with the mechanism of action of bumetanide, which is inhibition of active chloride reabsorption in the ascending limb of the loop of Henle.

The present study demonstrated that indomethacin had no significant effect on the disposition of bumetanide. Therefore, a pharmacokinetic interaction may be eliminated as a possible mechanism for the attenuation, by indomethacin, of the natriuretic and diuretic response of bumetanide. Instead, it appears that indomethacin diminishes the response to bumetanide *via* prostaglandin inhibition, although the precise nature of this interaction remains unclear.

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Contamination of Injectable Solutions with 2-Mercaptobenzothiazole Leached from Rubber Closures

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Abstract □ An impurity, discovered in a sample of digoxin injectable solution commercially packaged in a syringe for single-dose delivery, was found to originate from the rubber closure of the syringe and was identified as 2-mercaptobenzothiazole, a common accelerator for rubber vulcanization. Several similarly packaged injectable solutions of a variety of drugs from various manufacturers were examined and over half contained 2-mercaptobenzothiazole. The compound was identified by UV spectrophotometry (including a pH-dependent shift in its absorbance maximum), by mass spectrometry, and by comparison with standard 2-mercaptobenzothiazole using silica gel and reverse-phase high-performance liquid chromatography (HPLC). The presence of this impurity in injectable solutions may have implications with regard to toxicity and may interfere with the assay of digoxin injectable solution by HPLC.

Keyphrases □ Injectable formulations—contamination by 2-mercaptobenzothiazole leached from rubber closures, single-dose syringes, syringe cartridges □ 2-Mercaptobenzothiazole—contaminant of injectable solutions, leached from rubber closures, single-dose syringes, syringe cartridges □ Drug packaging—injectable solutions, single-dose syringes, and syringe cartridges, contamination by 2-mercaptobenzothiazole leached from rubber closures

During the assay for digoxin in injectable solutions by reverse-phase high-performance liquid chromatography (HPLC) conducted according to the USP method (1), an impurity was discovered in a sample commercially packaged in a syringe for a single-dose delivery. The small variation in mobile phase compositions permitted by the method produced considerable differences in resolution of digoxin from its contaminant and differences in the digoxin assays. When the mobile phase composition was varied, a significant difference was observed between the change in retention time of digoxin and that of the impurity, which implied that the impurity was structurally unrelated to digoxin. The origin, identification, and significance of this impurity are discussed in this report.

EXPERIMENTAL

Reverse-Phase HPLC—For the analysis of digoxin injectable solutions, the HPLC system consisted of a liquid chromatograph[1], a variable-wavelength detector[2] set at 218 nm and 0.2 AUFS, a recorder–integrator[3] with a chart speed of 0.5 cm/min, and an automatic injector[4] set to inject 20 μl. A reverse-phase C18 column[5] and a mobile phase of 30% aqueous acetonitrile[6] were used; the flow rate was 2.0 ml/min. The digoxin injectable solution samples were used undiluted (0.25 mg/ml). To determine if the contaminant in the digoxin injectable solution was a cardiac glycoside related to digoxin, samples of digoxigenin mono- and bisdigitoxoside[7], digoxigenin[7], and diginatin[7] were chromatographed twice, with 26 and 30% acetonitrile as mobile phases, and were compared by retention time to the impurity.

Concomitant Use of HPLC and UV Spectrophotometry—To obtain a full UV spectrum of chromatographically pure compound, the column effluent was passed first through a detector[8], fixed at 254 nm and connected to a recorder[9] to produce a chromatogram and then through a 10-mm flow cell positioned in a rapid-scanning spectrophotometer[10] to produce the spectrum. As the mobile phase passed through the flow cell, UV spectra were recorded every 2 sec until the intensity of the signal reached a maximum, at which time the solvent flow from the column was diverted, locking the sample in the flow cell. This permitted repetitive scanning of the sample and produced a smooth spectrum of the com-

[1] Model 204 liquid chromatograph; Waters Associates, Millipore Corp., Milford, MA 01757.
[2] Model 450 variable-wavelength detector; Waters Associates.
[3] Data Module; Waters Associates.
[4] WISP 710B; Waters Associates.
[5] μBondapak C-18, 10-μm particle size, 300 mm (length) × 3.9 mm (i.d.); Waters Associates.
[6] For the chromatographic column used in this work, 30% acetonitrile was preferred over 26% acetonitrile (the concentration recommended by the USP) because elution time was shortened without chromatographic interference from related cardiac glycosides. With 30% acetonitrile, the system suitability requirements of the USP (1) were met.
[7] Burroughs Wellcome Co., Inc., Research Triangle Park, NC 27709.
[8] Model 440 absorbance detector; Waters Associates.
[9] Model 3390A Reporting Integrator; Hewlett-Packard Co., Palo Alto, CA 94304.
[10] Model 8450A UV/visible spectrophotometer; Hewlett-Packard Co.

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