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Research Article

Development of Acute Tolerance to Bumetanide: Constant-Rate Infusion Studies

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Bumetanide was administered intravenously to four mongrel dogs as a bolus of 8.7 μg/kg, immediately followed by a constant-rate infusion of 0.35 μg/min/kg at 0.036 ml/min. Treatment A consisted of a 90-min equilibration period and first hour (Phase I) of study in which animals were maintained under euvolemic conditions. During the subsequent 3 hr of Treatment A (Phase II), animals were maintained under hydropenic conditions. These experiments were then repeated 1 week later (Treatment B) with the temporal aspects of hydration reversed (Phase III, hydropenia; Phase IV, euvolemia). Serial plasma and urine samples were assayed for bumetanide by high-performance liquid chromatography (HPLC) and for sodium by flame photometry. The bumetanide excretion rate was not significantly different during the 4 hr of Treatment A, although minor differences were observed between Phase III and Phase IV of Treatment B. The sodium excretion rate showed significant differences between euvolemic and hydropenic conditions of both treatments. A two- to threefold difference in the sodium excretion rate persisted even when slight differences (<20%) in bumetanide excretion rates were taken into account. These results demonstrate that an acute tolerance does develop to constant-rate infusions of bumetanide when inadequate fluid and electrolyte replacement occurs and that this tolerance can be reversed by rehydration.

KEY WORDS: acute tolerance; bumetanide; kinetics; dynamics.

INTRODUCTION

Bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamoylbenzoic acid) is a potent diuretic that is similar to furosemide with respect to its pharmacologic action and clinical indications (1). Its major site of action is on the thick ascending limb of the loop of Henle, where it inhibits solute reabsorption, although inhibition of proximal tubular sodium transport also occurs (2–5). Bumetanide exerts its natriuretic and diuretic effects from the luminal surface of the nephron (4,6–8). Additionally, bumetanide has been shown to produce intrarenal hemodynamic changes (3,9–12).

An important factor in the dose–response relationship of a diuretic involves the regulation of normal salt and water homeostasis (13,14). It was reported previously that an acute diuretic tolerance can develop within a single intravenous dose of bumetanide when inadequate fluid and electrolyte replacement occurs (15). This tolerance effect was demonstrated by a parallel shift to the right of the dose–response curves with increasingly negative sodium balance. The data also demonstrated that the development of acute tolerance to bumetanide dosing was not the result of changes in the drug's pharmacokinetic parameters. Still, the exact mechanisms and how they affect the dose–response relationship of bumetanide remain unclear.

One possible explanation for the development of acute tolerance to bumetanide involves the renal feedback mechanisms brought into play in order to compensate for contractions in extracellular fluid volume. Seely and Dirks (16) have reported that a decrease in the glomerular filtration rate, caused either by tubuloglomerular feedback or by depletion of the extracellular fluid volume, can obscure the effect of diuretics on renal solute handling. Consistent with this hypothesis was the observation that both the creatinine clearance, a measure of the glomerular filtration rate, and the sodium excretion rate decreased by over 50% when going from euvolemic to hydropenic conditions in four mongrel dogs (15). Additionally, alterations in aldosterone secretion may modify the diuretic action of bumetanide. By increasing sodium reabsorption in the distal and collecting tubules, aldosterone can conserve or increase the blood volume and renal perfusion in the event of extracellular fluid volume depletion (17,18).

Thus, the purpose of this investigation was (i) to clarify further the mechanisms responsible for acute tolerance development to bumetanide and (ii) to characterize the temporal aspects of tolerance development.

To accomplish this end, constant-rate infusions of bumetanide were employed so that diuretic tolerance could be studied under relatively constant kinetic conditions.

MATERIALS AND METHODS

Materials

An aqueous solution of bumetanide (Lot 8193311811, Hoffmann–La Roche, Inc., Nutley, N.J.) in normal saline

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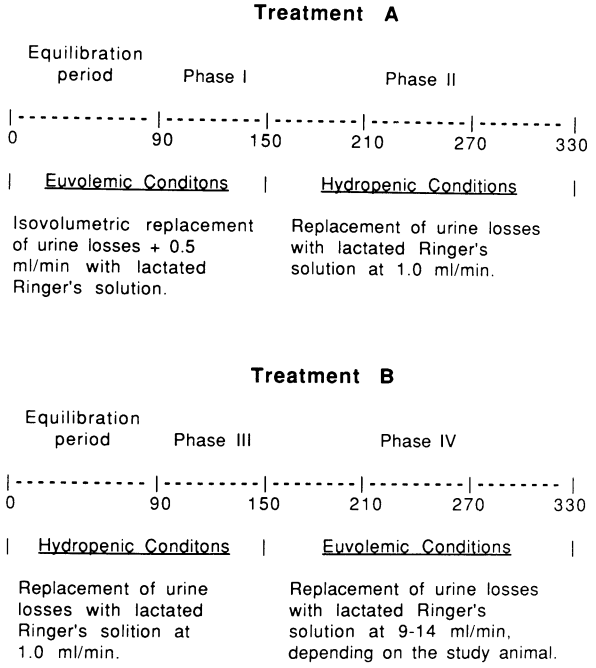
was prepared immediately prior to use with the aid of 0.4 *N* NaOH. All other chemicals and solvents were reagent grade or better, as previously reported (19).

Experimental Methods

Four male, mongrel, conditioned, unanesthetized dogs weighing from 24.1 to 27.3 kg were fasted the night before and throughout the entire experimental period. Heparinized catheters (Abbocath-T, 18 G × 2 in., Abbott Hospitals Inc., North Chicago) were placed in each foreleg and one hind leg of the dogs: one for the administration of bumetanide, one for the replacement fluids, and one for obtaining blood samples. Voided urine was collected via an indwelling bladder catheter (Swan-Ganz Flow-Directed Monitoring Catheter, Model-93-111-7F, American Edwards Laboratories, Santa Ana, Calif.). The bladder was flushed with 10 ml of air at the end of each urine collection period to ensure a complete catch. Plasma and urine samples were stored at −20°C until subsequent analysis.

Each animal received an intravenous loading dose of 8.7 μg/kg of bumetanide, immediately followed by a constant-rate infusion of 0.35 μg/min/kg at 0.036 ml/min. The animals were then equilibrated for 90 min to achieve steady-state plasma levels and urinary excretion rates of bumetanide. After equilibration, urine was collected every 15 min for 1 hr; blood samples were taken at the midpoint time of each urine collection. During the equilibration period and first hour of urine collections (Phase I), the urinary losses were replaced intravenously by equal volumes (plus 0.5 ml/min for insatiable water loss) of lactated Ringer's solution (euvolemic conditions). Urine samples were then taken every 15 min over the next 3 hr, with blood samples being taken at the midpoint time of each urine collection. However, during this 3-hr period, urinary losses were not rigorously replaced (hydropenic conditions; Phase II). Instead, lactated Ringer's solution was infused at 1 ml/min to obtain an adequate urine volume for analytical purposes and renal clearance calculations. These experiments were then repeated 1 week later, with a reversal of the temporal aspects of hydration. For example, during the equilibration period and first hour of sampling (Phase III), urinary losses were replaced by a 1-ml/min infusion of lactated Ringer's solution (hydropenic conditions). During the next 3 hr of sampling, urinary losses were replaced by lactated Ringer's at a flow rate equal to the urine volume (plus 0.5 ml/min for insatiable water loss) observed during the euvolemic conditions of the previous week's treatment (Phase IV). For clarification, Treatment A encompasses Phases I and II (euvolemia → hydropenia); Treatment B encompasses Phases III and IV (hydropenia → euvolemia). A schematic representation of the experimental design is shown in Fig. 1.



For Treatments A and B, urine collections were made over 15 minute intervals; plasma was sampled at the midpoint time of each urine collection period.

Fig. 1. Schematic representation of study design.

Analytical Methods

Plasma and urine samples containing bumetanide were analyzed using a sensitive and specific high-performance liquid chromatographic (HPLC) assay (19). Plasma and urine samples were assayed for sodium with a flame photometer (Model 455, Corning Medical and Scientific, Medfield, Mass.) and creatinine was determined colorimetrically using a commercial kit (Sigma Chemical Co., St. Louis, Mo.). Plasma aldosterone levels were measured with a commercially available solid-phase radioimmunoassay (Diagnostic Products Co., Los Angeles, Calif.).

Calculations

The following equations were used to calculate the pharmacokinetic and pharmacodynamic parameters for each urine collection period:

$$CL_p = R_o/C_{pss} \quad (1)$$

$$CL_r = (\Delta A_e/\Delta t)/C_{pss} \quad (2)$$

$$CL_{nr} = CL_p - CL_r \quad (3)$$

$$FE_{Na} = (\Delta Na/\Delta t)/Na_p/CL_{cr} \quad (4)$$

$$Eff = \Delta E/\Delta A_e \quad (5)$$

where CL_p is the total plasma clearance of bumetanide; R_o is the intravenous infusion rate; C_{pss} is the steady-state plasma concentration of bumetanide; CL_r is the renal clearance of bumetanide; $\Delta A_e/\Delta t$ is the bumetanide excretion rate; CL_{nr} is the nonrenal clearance of bumetanide; CL_{cr} is the creatinine clearance; FE_{Na} is the fractional sodium excretion; $\Delta Na/\Delta t$ is the sodium excretion rate; Na_p is the plasma sodium concentration at the midpoint of the urine collection interval; Eff is the efficiency of bumetanide as related to sodium excretion; and ΔE and ΔA_e are the amount of sodium and drug excreted in urine, respectively, over a 15-min collection period. CL_{cr} was calculated by dividing the urinary excretion rate of creatinine by its plasma concentration at the midpoint of the urine collection interval.

The data were grouped into 1-hr periods for analysis.

Statistical differences for the effect of hydration status on the kinetic and dynamic parameters were determined by a single-factor analysis of variance and a Newman–Keuls multiple range test. A P value of ≤ 0.05 was considered to be significant. Unless otherwise stated, data throughout the study are expressed as means ($\pm$SD).

RESULTS

Several significant differences were found in the pharmacokinetic parameters of bumetanide between the euvolemic and the hydropenic periods of Treatments A and B (Table I). During the first hour of urine collection in Treatment B (Phase III), the bumetanide excretion rate was significantly lower than in the following three periods. There were no significant differences in bumetanide excretion rate during the 4 hr of Treatment A. The plasma clearance of bumetanide was significantly higher in Phase I than in the last two collection periods of Phase II. There were no significant differences in the plasma clearance of bumetanide in Treatment B, although the same trend of higher plasma clearances during the more euvolemic periods was observed. Analysis of the renal clearances showed a significant difference in Treatment B, with the hydropenic first hour (Phase III) having a lower renal clearance than that for the last 2 hr of Phase IV. Analysis of variance testing of the renal clearances in Treatment A indicated a statistically significant difference ($P < 0.05$) between collection periods but the Newman–Keuls test revealed no significant differences between any of the groups studied ($P > 0.05$). However, the same trend of an increased renal clearance during euvolemia (Phase I) was present. Nonrenal clearances were statistically different between Phase I and hr 2 of Phase II only. In contrast, no significant differences were seen in Treatment B for the nonrenal clearances. Creatinine clearance was significantly different in both Treatment A and Treatment B, with lower CL_{cr} values for hydropenic vs euvolemic periods.

The differences observed in total plasma and renal clearances are relatively minor and change on the order of 20–30%. In contrast, creatinine clearances change from 80 to 100% during Treatments A and B, reflecting the kidney's attempt to compensate for depletion of the extracellular fluid volume. These small differences in bumetanide renal clearance are probably the result of hemodynamic changes rather than true alterations in the functional nephron mass. In a previous study by Lau *et al.* (20), a significant positive correlation was observed between bumetanide renal clearance and creatinine clearance in healthy subjects and chronic renal failure patients ($r = 0.995$; $P < 0.001$). This occurred even though the drug is highly bound to plasma proteins and over 95% is excreted through the kidneys by active secretion.

The pharmacodynamic parameters after bumetanide administration all showed significant differences in both treatments between euvolemic and hydropenic periods (Table II). Efficiency and sodium excretion rate decreased dramatically during hydropenic periods. Even when sodium excretion

Table I. Pharmacokinetic Parameters for Constant-Rate Infusions of Bumetanide[a]

Phase	Hour	Condition	$\Delta A_e/\Delta t$ (μg/min)	C_{pss} (μg/ml)	CL_p (ml/min/kg)	CL_r (ml/min/kg)	CL_{nr} (ml/min/kg)	CL_{cr} (ml/min/kg)
				Treatment A				
I	1	Euvolemia	4.43	35.9	9.91	4.99	4.94	1.98
			(0.80)	(5.2)	(1.71)	(0.92)	(1.12)	(0.61)
II	1	↓[b]	4.23	41.4	8.84	4.18	4.67	1.50
			(0.70)	(0.97)	(1.96)	(1.26)	(1.17)	(0.45)
			NS	NS	NS	NS	NS	$P < 0.01$
II	2	↓	4.50	47.3	7.78	3.88	3.90	1.11
			(0.74)	(10.7)	(1.89)	(1.17)	(1.10)	(0.26)
			NS	$P < 0.01$	$P < 0.025$	NS	$P < 0.05$	$P < 0.001$
II	3	Hydropenia	4.48	46.1	7.89	3.99	3.91	1.01
			(1.00)	(9.7)	(1.75)	(1.42)	(0.98)	(0.28)
			NS	$P < 0.01$	$P < 0.025$	NS	NS	$P < 0.001$
				Treatment B				
III	1	Hydropenia	4.00	45.8	8.05	3.61	4.43	1.00
			(0.73)	(7.3)	(1.46)	(1.15)	(0.76)	(0.23)
IV	1	↓	4.85	46.3	8.17	4.31	3.86	1.52
			(1.21)	(10.8)	(2.00)	(1.40)	(1.47)	(0.53)
			$P < 0.025$	NS	NS	NS	NS	$P < 0.01$
IV	2	↓	4.98	41.8	8.91	4.88	4.04	1.83
			(0.76)	(8.1)	(1.88)	(1.06)	(1.23)	(0.59)
			$P < 0.01$	NS	NS	$P < 0.025$	NS	$P < 0.001$
IV	3	Euvolemia	4.67	41.0	9.07	4.69	4.39	1.75
			(0.56)	(7.7)	(1.86)	(1.07)	(1.10)	(0.63)
			$P < 0.05$	NS	NS	$P < 0.025$	NS	$P < 0.001$

[a] P values compare the subsequent 3 hr of Phase II with Phase I (Treatment A) and the subsequent 3 hr of Phase IV with Phase III (Treatment B).

[b] Arrows indicate the trend from euvolemia toward hydropenia in Treatment A and the trend from hydropenia toward euvolemia in Treatment B.

Table II. Pharmacodynamic Parameters for Constant-Rate Infusions of Bumetanide[a]

Phase	Hour	Condition	ΔNa/Δt (mEq/min)	Eff (mEq/μg)	Fe_{Na}
			Treatment A		
I	1	Euvolemia	1.27	0.287	0.199
			(0.19)	(0.052)	(0.064)
II	1	↓[b]	0.864	0.200	0.169
			(0.207)	(0.045)	(0.029)
			$P < 0.001$	$P < 0.001$	NS
II	2	↓	0.589	0.127	0.157
			(0.107)	(0.022)	(0.033)
			$P < 0.001$	$P < 0.001$	$P < 0.05$
II	3	Hydropenia	0.459	0.098	0.136
			(0.094)	(0.022)	(0.033)
			$P < 0.001$	$P < 0.001$	$P < 0.001$
			Treatment B		
III	1	Hydropenia	0.472	0.115	0.143
			(0.092)	(0.017)	(0.026)
IV	1	↓	0.848	0.179	0.167
			(0.240)	(0.068)	(0.029)
			$P < 0.01$	$P < 0.005$	$P < 0.05$
IV	2	↓	1.17	0.233	0.194
			(0.24)	(0.064)	(0.034)
			$P < 0.001$	$P < 0.001$	$P < 0.001$
IV	3	Euvolemia	1.17	0.243	0.202
			(0.28)	(0.054)	(0.040)
			$P < 0.001$	$P < 0.001$	$P < 0.001$

[a] *P* values compare the subsequent 3 hr of Phase II with Phase I (Treatment A) and the subsequent 3 hr of Phase IV with Phase III (Treatment B).

[b] Arrows indicate the trend from euvolemia toward hydropenia in Treatment A and the trend from hydropenia toward euvolemia in Treatment B.

was corrected for differences in creatinine clearance (FE_{Na}), substantial differences were still found between euvolemic and hydropenic conditions in Treatments A and B.

DISCUSSION

Acute tolerance was observed under the experimental conditions of the present study. As observed in Figs. 2 and 3 and in Table II, there was a 64% decrease in the sodium excretion rate between Phase I and hr 3 of Phase II (Treatment A). When the temporal aspects of hydration status were reversed, an increase of 146% occurred for sodium excretion rate in hr 3 of phase IV as compared to Phase III (Treatment B). Changes in glomerular filtration rate can explain some of these differences, as the fractional sodium excretion drops only 30% between the extremes of euvolemia and hydropenia. Although the mechanisms are unclear, this compensatory adjustment in glomerular filtration may be the result of acute extracellular volume contraction and/or tubuloglomerular feedback control (16,21–23).

Aldosterone plasma concentrations were also measured in an attempt to explain the development of acute tolerance to bumetanide. However, only two of the four dogs studied had levels above the limit of the assay sensitivity (25 pg/ml). In the dogs with measurable levels, plasma aldosterone did not show any marked changes until 1 to 2 hr after the change

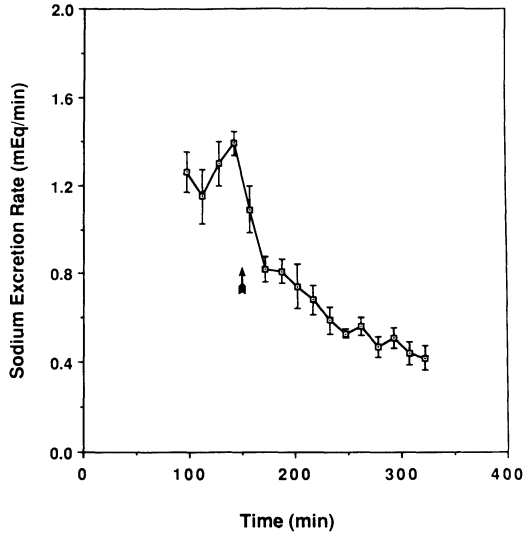


Fig. 2. Sodium excretion rate vs midpoint time plot for Treatment A. The arrow marks the change from euvolemic toward hydropenic conditions. Data are expressed as the mean ± SE ($N = 4$).

from euvolemic toward hydropenic conditions (Fig. 4), or visa versa. In contrast, sodium excretion rates changed rapidly and within the first 30 min of an altered hydration status (Figs. 2 and 3). This suggests that aldosterone, at least initially, is not responsible for changes in sodium retention. This finding is consistent with previous investigations in which changes in aldosterone plasma levels to salt and water imbalance occurred more slowly than that necessary to account for the development of acute tolerance to diuretics (24–28). In addition, it was reported (29) that during furosemide infusion in volume-depleted conscious rats, both proximal and distal fractional sodium reabsorptions were enhanced as compared to those in volume-replaced rats. This difference was evident when furosemide was infused for only 15–30 min without volume replacement and indicates

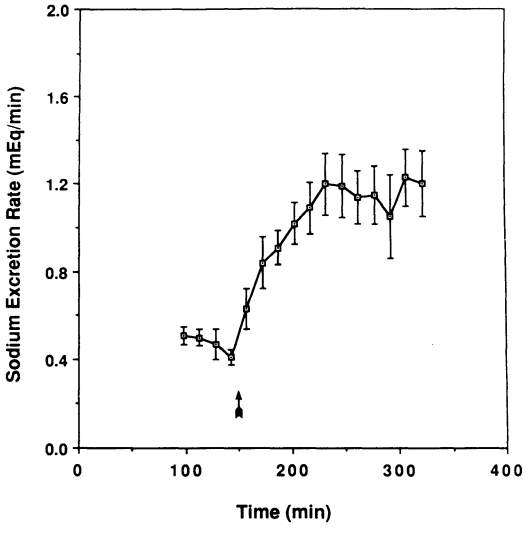


Fig. 3. Sodium excretion rate vs midpoint time plot for Treatment B. The arrow marks the change from hydropenic toward euvolemic conditions. Data are expressed as the mean ± SE ($N = 4$).

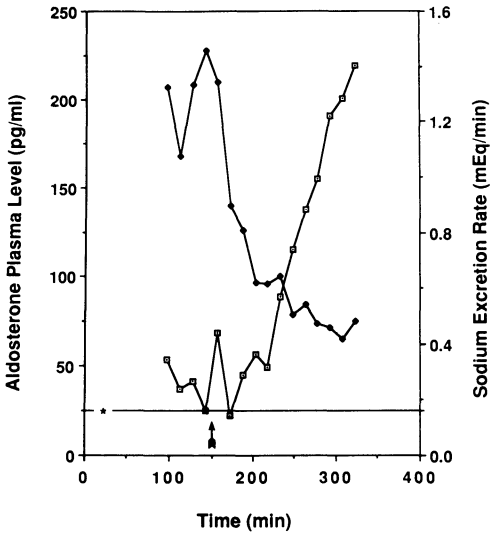


Fig. 4. Aldosterone plasma concentration (□) and sodium excretion rate (◆) vs midpoint time plots for dog 2, Treatment A. This relationship was observed in two of the four dogs studied (see text). The arrow marks the change from euvolemic toward hydropenic conditions. The asterisk denotes the aldosterone assay limit of 25 pg/ml.

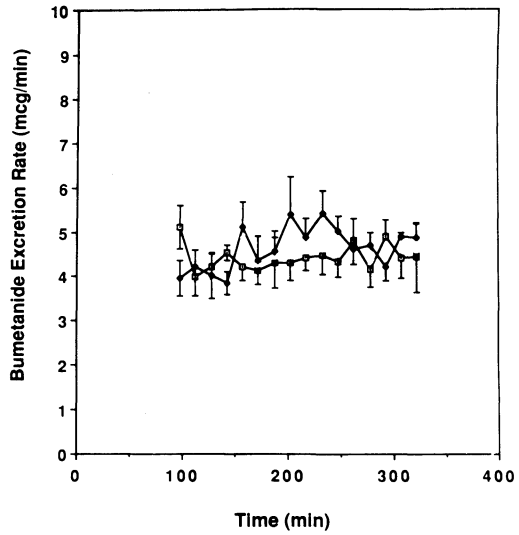


Fig. 6. Bumetanide excretion rate vs midpoint time plots for Treatments A (□) and B (◆). Data are expressed as the mean ± SE (N = 4).

that the mechanisms operating on either nephron segment had a fast onset of action.

The changes in pharmacokinetic parameters are most likely due to decreases in the extracellular fluid volume. As observed in Fig. 5 and Table I, plasma levels are slightly higher during hydropenic conditions in Treatment A. Bumetanide excretion rate (Fig. 6 and Table I), the critical determinant with respect to bumetanide-induced diuresis and natriuresis (4,6–8) showed a modest difference only during Treatment B, in which the urinary drug excretion of Phase III was 80% of that in the subsequent hours of Phase IV. Even when differences in sodium excretion rate were corrected for small differences in urinary excretion of bumetanide, there was still a two- to threefold difference in the efficiency of bumetanide between the first and the last study hours of both treatments. Therefore, due to the marginal differences in drug disposition with altered hydration status, changes in the pharmacokinetics of bumetanide cannot satisfactorily explain the development of acute diuretic tolerance to this drug.

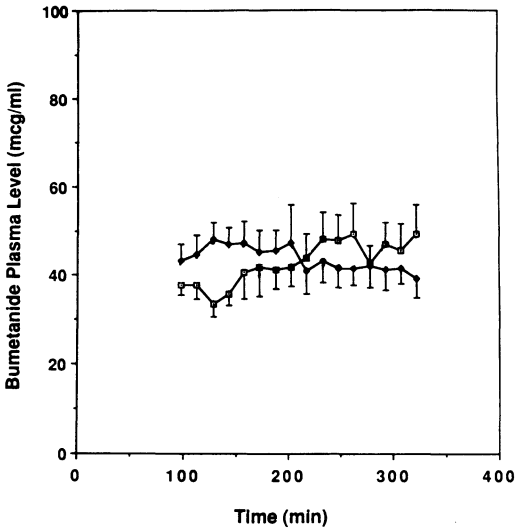


Fig. 5. Bumetanide plasma concentration vs midpoint time plots for Treatments A (□) and B (◆). Data are expressed as the mean ± SE (N = 4).

In summary, an acute diuretic tolerance can develop rapidly to constant-rate infusions of bumetanide when inadequate fluid and electrolyte replacement occurs. Furthermore, this tolerance effect can be reversed by rehydration. Although the exact mechanisms are not totally clear, changes in glomerular filtration rate can account in part for this phenomenon; aldosterone levels can increase sodium retention in the latter part of hydropenia but do not precipitate the development of acute diuretic tolerance. It is clear that pharmacokinetic changes in bumetanide are not responsible for the tolerance observed. In addition, this study has demonstrated that steady-state conditions with constant-rate infusions of drug are a valuable tool for studying the dose–response relationship of bumetanide and its potential for acute tolerance development.

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