A Volume-Independent Component to Postdiuretic Sodium Retention in Humans\textsuperscript{1,2}

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ABSTRACT
Net sodium (Na) loss during diuretic administration is limited by postdiuretic renal salt retention. This could be a homeostatic response to extracellular fluid volume (ECV) depletion. However, rats infused with loop diuretics develop structural and functional adaptations in the distal nephron that enhance NaCl reabsorption. Therefore, the hypothesis that postdiuretic Na retention in humans contains a volume-independent component was tested. Normal volunteers were equilibrated to a 120 mmol/24 h Na intake. For the first protocol, subjects received, in random order, a placebo, bumetanide (B), or bumetanide accompanied by an infusion of an electrolyte solution at a rate adjusted to match urine flow and thereby to obviate Na losses (bumetanide plus volume replacement; B + VR). After the completion of B diuresis, there was a positive Na balance that restored 70% of the Na loss within 42 h. However, this positive Na balance was prevented by volume replacement (B + VR). For the second protocol, subjects received, in random order, a placebo injection and a 100-mmol NaCl load (P + NaCl) or a bumetanide injection plus volume replacement in addition to a 100-mmol NaCl load (B + VR + NaCl). Over the ensuing 42 h, 94% of the load was eliminated when it was infused alone (P + NaCl). In contrast, only 9% was eliminated when it was given with bumetanide and volume replacement (B + VR + NaCl). It was concluded that postdiuretic Na retention in normal human subjects is due both to ECV depletion and to volume-independent Na retention manifest as an inability to excrete a modest NaCl load. These findings emphasize the importance of salt restriction both during loop diuretic therapy and after its withdrawal to obviate these powerful Na-retaining mechanisms.

Key Words: Loop diuretics, sodium balance, sodium excretion, salt load

Diuretics and dietary salt restriction remain the cornerstone of treatment for edema and hypertension. Yet, little is known of the factors that regulate NaCl balance during diuretic therapy. Salt loss with loop diuretics is prevented in part by a decreased natriuretic response to repeated doses of the diuretic. However, this tolerance is prominent only during severe dietary NaCl restriction (1); in addition, during diuretic therapy at levels of Na intake equivalent to those used in clinical practice, diuretic-induced Na loss is limited primarily by postdiuretic Na retention (1,2). The administration of a loop diuretic leads to activation of the renin-angiotensin-aldosterone axis and the sympathetic nervous system. However, the period of postdiuretic Na retention is not dependent solely on these two well-defined pathways for ECV homeostasis because it is not prevented by the blockade of angiotensin II generation and aldosterone secretion with captopril or \( \alpha \)-adrenoceptors with prazosin, even when given together (3,4).

Rats infused with loop diuretics develop hypertrophy (5–7) and increased Na, K-ATPase activity (8,9) in the downstream nephron segments of the distal convoluted tubule and collecting ducts and enhanced Na absorption from perfused segments of the distal tubule via an aldosterone-independent mechanism (6,10). Therefore, we investigated the hypothesis that postdiuretic Na retention in humans contains ECV-dependent and ECV-independent components. The latter should be most evident during conditions in which distal NaCl delivery and reabsorption are augmented, such as during salt loading.

SUBJECTS AND METHODS
Twelve healthy human subjects were recruited. There were nine men and three women aged 25 to 65 yr and weighing 75 ± 6 kg. None were hypertensive or taking any medicines. For 3 days before the admission, they were requested to refrain from add-
ing salt to their food. Thereafter, they were admitted to the Clinical Research Center Metabolic Balance Ward for the duration of the study. One week before their admission, they were interviewed by a dietitian who prepared a fixed, palatable diet with sufficient calories to maintain their current weight and to provide 18 mmol/24 h of Na and 80 mmol/24 h of K. This diet was served as three meals taken at 6:00 to 7:00 a.m., 12:00 to 1:00 p.m., and 6:00 to 7:00 p.m. Each meal was supplemented with two 1-g NaCl tablets to give a 24-h Na intake of 120 mmol. This is equivalent to a salt-restricted diet for hypertension. Water was provided ad libitum, but alcohol consumption and smoking were not allowed. The study was passed by the Institutional Review Board of the University of Florida. All subjects gave informed consent.

The study consisted of a 72-h equilibration period followed by a series of interventions at 48-h intervals. The order of these interventions was randomized between subjects. There were two protocols with eight normal subjects studied in each. Two subjects enlisted for both protocols. For them, 5 days for reequilibration were provided between the two sets of studies. The aim of Protocol 1 was to investigate the role of ECV depletion in postdiuretic Na retention. For this protocol, the interventions were placebo (P), bumetanide alone (B; 1 mg iv), or bumetanide with simultaneous volume replacement (B + VR) by infusion of fluid to match urinary losses. This replacement solution consisted of the following components (in millimoles per liter): Na, 67; Cl, 81; K, 14. Dextrose (20 g/L) was added to maintain isotonicity. Preliminary studies had shown that this electrolyte content corresponded to that of urine passed during a B diuresis. The diuresis was >95% complete within 3 h. The urinary volume during this 3-h period of the B + VR protocol was matched precisely by a 3-h iv infusion of the replacement solution. The average urine in the first 30 min after B was 600 mL. Therefore, 600 mL of replacement solution was given in the first 30 min and the rate of infusion was adjusted thereafter to match urine flow. In this way, subjects did not become volume depleted at any time in the 3 h after the diuretic. For the P and B study days, the subjects had an iv infusion of isotonic dextrose solution (50 g/L) at a rate equivalent to urine flow.

The aim of Protocol 2 was to investigate whether B would perturb the excretion of an NaCl load independent of major changes in ECV. For this protocol, there were two interventions given in random order. The first provided an NaCl load of about 100 mmol; subjects received a placebo injection followed by an iv infusion of 660 mL of 0.15 M NaCl solution over 3 h (P + NaCl). The second provided a B diuresis with volume replacement in addition to an equivalent NaCl load. Subjects received a B injection (1 mg iv) and a 3-h infusion delivered at a rate equal to urine flow, as in Protocol 1. However, this solution had an NaCl content above that of the urine passed and was calculated to provide a positive Na balance of approximately 100 mmol (B + VR + NaCl). This solution had the following composition (in millimoles per liter): Na, 100; K, 14; Cl, 114. Dextrose (10 g/L) was added to maintain isotonicity.

Subjects were seated throughout each intervention day. A vein was cannulated in each forearm for iv infusion and blood sampling. Sodium para-aminohippurate (PAH) was infused from 8:00 a.m. After 1 h for equilibration, there was a 2-h clearance period for creatinine and PAH clearances (11). Thereafter, B (1 mg) or P was given as an iv bolus and the infusions of replacement solutions or dextrose were started as described above for 3 h. A second 2-h clearance period was obtained 7 to 9 h after the administration of P or B.

Blood pressure (BP), measured by a sphygmomanometer, and heart rate (HR) were recorded 1 h before and 8 h after the administration of P or B. Mean BP was taken as diastolic BP (Kartotokf sound V) plus one third of pulse pressure. Urine samples for balance studies were collected every 6 h. On the experimental days, six 30-min collections were obtained after the administration of B or P to allow close monitoring of urinary losses. Blood for hematocrit (Hct) determination was obtained before and 8 h after the administration of B or P.

Routine chemical methods were used for the measurements of Na, creatinine, and PAH as described previously (2,11). The Hct was estimated as the mean of two values of microhematocrit with a hematocrit centrifuge (Clay Adams, Parsippany, N.J.). Cumulative Na balance was calculated at 6-h intervals as the difference between Na intake and renal excretion. Because this study was designed to investigate postdiuretic Na retention, the point at which diuresis was complete (3 h after the diuretic or P was administered) was selected as the starting point for calculating cumulative balances. This resulted in a total cumulative balance period of 42 h.

Results are reported as mean ± SE. The paired t test was used for within-subject statistical comparisons. Values were considered significant at P < 0.05.

RESULTS

The purpose of the first protocol was to investigate the role of ECV depletion in postdiuretic Na retention. Three hours after receiving B, subjects lost a quantity of Na equivalent to approximately 1 L of ECV whereas Na balance was little changed after P. The volume replacement protocol (B + VR) successfully prevented Na loss over this 3-h period (Table 1). Thereafter, the pattern of cumulative Na balance after receiving B...
alone was quite different from that after P (Figure 1A). By 6 to 12 h, Na balance was more positive after B alone than after P. This difference became progressively more pronounced, and by 42 h, the cumulative Na balance of $+97 \pm 7$ mmol after B was significantly ($P < 0.001$) greater than the balance of $+23 \pm 6$ mmol after P. This positive Na balance restored 70% of the Na lost after B. During this 42-h period, the subjects had received a sodium intake of 200 mmol. Therefore, after the diuretic, subjects retained about half of the administered Na, whereas after P, they retained only 10%. As shown in Figure 1B, after the fluid and electrolytes were replaced in the 3 h after B was administered (B + VR), the cumulative Na balance of $+33 \pm 1$ mmol over the succeeding 42 h was almost identical to that after P. Thus, prevention of NaCl depletion during B diuresis effectively eliminated the postdiuretic Na retention.

The aim of the second protocol was to determine whether a diuretic could alter the elimination of a salt load independent of changes in ECV. Three hours after receiving the 100 mmol of Na with a P (P + NaCl), the subjects had retained $92 \pm 5$ mmol of Na (Table 1). It was hard to deliver a precise NaCl load during a B diuresis because of the rapid, and somewhat variable, rate of Na excretion. However, the positive Na balance of $76 \pm 15$ mmol was not significantly different from the value for Na balance over the 3 h after NaCl was given alone. Thereafter, when the NaCl load was delivered with a P (P + NaCl), the load was eliminated progressively. Yet, when it was given with B and volume replacement (B + VR + NaCl), there was no significant change in Na balance (Figure 1C). At the end of the 42-h period, 94% of the Na load had been eliminated when given with a P, yet only 9% was eliminated when given with B and volume replacement.

An examination of Figures 1A through C shows that, in each protocol, there was a period of positive Na balance 6 to 12 h after the intervention. The cause for this was not clearly identified. However, this period corresponded to the time from 8:00 p.m. to 2:00 a.m. in the evening after the completion of the clearance studies when the subjects were in bed. During this time period, this is normally a time of reduced Na excretion. This positive Na balance, which averaged 23 mmol in the P protocol, was retained throughout the remainder of the balance study.

The BP, HR, Hct, and renal hemodynamics before and after the interventions are shown in Table 2. There was a modest increase in HR and Hct and a fall in PAH clearance after B alone in Protocol 1 and a fall in Hct after saline infusion in Protocol 2. Otherwise, none of these parameters were changed 7 to 9 h after the diuretic, P, or NaCl load.

**DISCUSSION**

This study was undertaken at an Na intake of 120 mmol/24 h, which is equivalent to a no-added-salt diet. B led to Na loss, followed by progressive renal Na retention sufficient to restore two thirds of the Na lost within 42 h. Because this period of postdiuretic Na retention was effectively abolished by the simultaneous infusion of an electrolyte solution equivalent to urinary losses, we conclude that ECV depletion is an important trigger for postdiuretic renal Na retention. The mediator(s) of this Na retention was not apparent from these studies. However, there was a small reduction in PAH clearance 7 to 9 h after B, accompanied by a rise in the calculated filtration fraction that was prevented by volume repletion. Therefore, renal vasoconstriction and a rise in the filtration fraction may have contributed to Na retention, at least during the early phase of the response (12). Our previous studies at a higher level of dietary salt intake indicate that neither the renin-angiotensin-aldosterone system nor α-adrenoceptors individually or in combination are essential for this response (3,4).

In contrast to the results of the first protocol, which clearly pointed to the importance of overt ECV depletion in conditioning the renal response to the diuretic, the results of the second protocol demonstrated that the administration of a single dose of a diuretic, together with volume replacement, prevented the elimination of a modest NaCl load. This indicates that the diuretic had activated a subtle, ECV-independent, Na-retaining process that persisted for at least 42 h. Two possible explanations for the sodium-retaining state should be considered.

First, the diuretic might have engendered false signals of ECV depletion. Although external balances for Na were maintained by the schedule of volume replacement, there could have been undetected shifts between body fluid compartments. However, such shifts should be minimized because the fluid loss
Figure 1. Mean ± SE values for cumulative Na balance in the period 3 to 45 h after the administration of placebo or bumetanide. Panels A and B depict data from Protocol 1. Panel A contrasts the changes after the completion of B diuresis with P. Panel B contrasts changes after diuresis with B + VR with P. Panel C depicts data from Protocol 2 contrasting Na balance 3 to 45 h after the administration of P and an NaCl load (P + NaCl) compared with a similar load administered with B and volume replacement (B + VR + NaCl). *P < 0.05.

was directly from the plasma compartment into the urine whereas the volume replacement solution was infused simultaneously into the plasma. Even without plasma volume depletion, loop diuretics can stimulate renin release via a macula densa-mediated mechanism (13). Moreover, increases in angiotensin II after iv loop diuretics can cause sufficient prostaglandin-dependent venodilation to reduce cardiac filling pressures, which might thereby stimulate volume-conserving mechanisms despite the maintenance of the plasma volume (14,15). However, these effects are short lived (14,15) and renal diuretic elimination is more than 90% complete within 3 h of iv administration (1). Therefore, these effects of the drug are unlikely to explain actions that persisted for 42 h. Moreover, after B + VR + NaCl, there were no detectable changes in mean blood pressure, HR, or the clearance of creatinine or PAH to suggest effective ECV depletion (Table 2).

The second explanation that could account for
postdiuretic Na retention is an adaptive change in tubular transport capacity. Rats infused with loop diuretics for several days have an enhanced ability to reabsorb Na from perfused nephron segments of the distal convoluted and connecting tubules via an aldosterone-independent mechanism (6,10). Hypertrophic changes in the collecting ducts can develop within hours of diuretic administration in the rat (7).

In a previous study, we provided indirect evidence for enhanced Na reabsorption in the distal convoluted tubule of hypertensive subjects receiving loop diuretics for 1 month. These subjects were found to have an enhanced natriuretic response to a thiazide, which predominantly acts on the distal convoluted tubule despite ECV depletion induced by the month of loop diuretic administration (2). Moreover, we noted an increase in the Na, K-ATPase activity of the distal convoluted tubule of rats that had received a single dose of loop diuretic 8 to 12 h previously (L.C. Garg and C.S. Wilcox, unpublished observation). Regardless, the findings presented here indicate that a single dose of a loop diuretic can prevent the elimination of an NaCl load by a mechanism that appears independent of changes in ECV.

DeWardener and MacGregor and colleagues (16, 17) have proposed that some patients with idiopathic edema suffer from diuretic-induced fluid retention. Withdrawal of diuretics from these subjects was followed by a sharp increase in their body weight in the following days. However, most of these subjects could eliminate this excess fluid and clear their edema without the reintroduction of diuretics over the next 2 to 4 wk. Those authors proposed that the diuretic had induced sodium retention that required ongoing diuretic administration for its control. These findings in normal healthy subjects demonstrate that even a single dose of a loop diuretic can prevent the excretion of a modest salt load. Thus, these results support the hypothesis proposed by DeWardner and MacGregor et al.

If these findings may be extrapolated to the care of patients receiving diuretic therapy, they would emphasize the importance of the restriction of dietary salt, both during diuretic therapy and for some time after its withdrawal, to obviate the period of salt retention. Otherwise, the physician may reach an erroneous conclusion that the patient has a primary salt-retaining condition requiring ongoing diuretic treatment for its control. In fact, the reappearance of edema or signs of ECV expansion after the abrupt discontinuation of diuretic therapy may represent a normal, adaptive response of the kidney to diuretics that persists for some time after the drug has been withdrawn. However, it is important to emphasize that these studies were conducted on normal individuals. Additional studies will be required to determine whether these findings apply to patients receiving diuretic therapy.

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