Reduced Natriuresis After Oral Sodium Load in Cholestatic Rats: Role of Compartment Volumes and ANP (44548)

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Abstract. **The purpose of this study was to assess the participation of the atrial natriuretic peptide (ANP)-cGMP system in electrolyte and volume handling of cholestatic rats submitted to an acute oral sodium load. Cholestasis was induced by ligation and section of the common bile duct (n = 51). Control rats were sham operated (n = 56). Three weeks after surgery, 24-hr urinary volume, sodium, potassium, cGMP and creatinine excretion were measured. Three days later, animals received 10 mmol/kg NaCl (1 *M*) by gavage, and urinary excretion was measured for 6 hr. In parallel groups of rats, plasma volume, electrolytes and ANP concentration, extracellular fluid volume (ECFV), and renal medullary ANP-induced cGMP production were determined in basal conditions or 1 hr after oral sodium overload. As compared with controls, cholestatic rats had a larger ECFV and higher plasma ANP (67.2 ± 5.2 vs 39.7 ± 3.5 pg/ml), but lower hematocrit and blood volume, and were hyponatremic. Cholestatic rats showed higher basal excretion of sodium, potassium, and volume than controls, but equal urinary cGMP. After the NaCl overload, cholestatic rats showed a reduced sodium excretion but equal urinary cGMP. One hr after sodium overload, both groups showed hypernatremia, but whereas in control rats ECFV and ANP increased (50.7 ± 4.1 pg/ml), in cholestatic rats ECFV was unchanged, and plasma volume and ANP were reduced (37.5 ± 5.8 pg/ml). ANP-induced cGMP production in renal medulla was similar in cholestatic and control nonloaded rats (14.2 ± 5.2 vs 13.4 ± 2.6 fmol/min/mg). One hr after the load, medullary cGMP production rose significantly in both groups, without difference between them (20.6 ± 3.1 vs 22.7 ± 1.7 fmol/min/mg). We conclude that the blunted excretion of an acute oral sodium load in cholestatic rats is associated with lower plasma ANP due to differences in body fluid distribution and cannot be explained by renal refractoriness to ANP.** [P.S.E.B.M. 2000, Vol 225:23–31]

Atrial natriuretic peptide (ANP), a hormone mainly produced in mammalian heart atria, is known to promote salt excretion and blood pressure reduction when exogenously administered. However, the participation of ANP in physiological sodium excretion is established only for acute volume expansion in humans and animal models, including ANP-knockout mice and rats autoimmunized against ANP (1–4). Although ANP's role in normal body fluid homeostasis is controversial, a resistance to the renal effects of ANP has been described in different states of pathological sodium retention (5).

Renal sodium and water retention is commonly observed in patients with chronic liver disease (6). The ethiology of this abnormality is multifactorial, and these patients do not seem to have a marked decrement in ANP synthesis or secretion (7, 8). Moreover, several investigators have reported increased (9–12) or normal (12, 13) ANP plasma levels in patients with hepatic cirrhosis and reduced natriuresis. Renal unresponsiveness to supra-physiologic doses of ANP has been demonstrated both in cirrhotic pa-

This work was supported by grants from the Chilean Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT) # 194/0628 and 1971222.

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Received January 4, 2000. [P.S.E.B.M. 2000, Vol 225]
Accepted April 17, 2000.

0037-9727/00/2251-0023$15.00/0

tients with severe sodium retention (14) and in precirrhotic chronic bile duct ligated (BDL) rats (15, 16).

A delayed natriuresis has been observed in BDL rats when submitted to an intravenous sodium load that triggers an important increase in plasma ANP levels (17). A reduced ability to excrete an acute oral sodium load, the normal route for sodium ingestion, has also been described in rats made cirrhotic with carbon tetrachloride (18); however, it is not known to what extent inappropriate ANP release or renal resistance to ANP may contribute to this phenomenon.

Thus, the first goal of this study was to evaluate the participation of ANP in the excretory response to acute oral sodium administration in normal and BDL rats. To meet this end, we measured sodium and cyclic guanosine 3′-5′ monophosphate (cGMP) urinary excretion, body fluid volumes, and plasma ANP levels before and after an acute oral NaCl load.

In previous studies, renal excretory resistance to ANP was demonstrated in isolated perfused kidneys from BDL rats (16), as well as from carbon tetrachloride–induced cirrhotic rats (18). However, BDL animals displayed moderate hypotension *in vivo*, and their isolated kidneys did not show an increase in perfusion pressure in response to ANP, as observed in control kidneys (16). Since ANP-induced natriuresis depends on renal perfusion pressure (19), it is possible that the observed blunted natriuresis was due to hemodynamic factors rather than to a defect in the renal mechanisms involved in the ANP response. The natriuretic response to ANP is mediated mostly by activation of medullary guanylate cyclase-ANP receptors and production of cGMP (20). A reduced cGMP accumulation in response to ANP, due to enhanced phosphodiesterase activity, has been found in renal medullary cells from BDL rats (17). Therefore, the second purpose of this study was to compare medullary generation of cGMP in response to ANP *in vitro*, in BDL and control rats.

Materials and Methods

Induction of Cholestasis. All experiments conformed to the *Guiding Principles in Care and Use of Animals* endorsed by the American Physiological Society. Adult female Sprague-Dawley rats, weighing 200–230 g, fed with a standard rodent chow, were prepared as described (16). Rats were randomized into controls and animals destined for induction of chronic cholestasis through bile duct ligation and section (BDL). Rats were anesthetized with sodium pentobarbital (40 mg/kg ip), and the common hepatic duct was sectioned between ligatures to produce cholestasis using standard surgical techniques. Sectioning was performed near the hepatic hilium to avoid recanalization and excessive bile accumulation in the remnant common duct. Control rats were sham operated (SO). During the third week after surgery, rats were placed for 6 hr in metabolic cages to reduce environmental stress at the moment of the experiments.

Four matched SO and BDL series were studied between 21 and 24 days after surgery. All rats were placed in metabolic cages without food, but with free access to water, and individual urine samples were collected overnight to check for sodium, potassium, and volume excretion. In a subgroup of 11 BDL and 11 SO rats, 24-hr urinary creatinine and cGMP were also measured. After this first urine collection period, the animals were returned to their normal conditions and 3 days later, they were subjected to different experimental protocols.

Three out of 54 BDL operated animals failed to develop cholestasis due to surgical problems and were excluded from the study. Structural changes of chronic cholestasis were confirmed by liver biopsy in 18 BDL animals.

Acute oral sodium load (AOSL). To study the ability of BDL and SO rats to handle ingested sodium, we submitted them to an acute oral sodium load maneuver, in which 10 ml/kg body wt of a 1 *M* NaCl solution, amounting to 10 mmol/kg body wt, was administered by gastric gavage (18).

Experimental Protocols. A first series of 10 BDL and 9 SO rats was used to study the time course of urinary sodium, potassium, volume, and cGMP excretion after AOSL. To this aim, rats were placed in metabolic cages at 1700 hr without food, but with free access to water. At 0900 hr of the next day, water supply was removed, rats received AOSL, and urine was collected every 2 hr for 6 hr. To get a better time resolution of the peak excretory response, in a subgroup of five BDL and five SO rats, the first period of urine collection was divided in two periods of 1 hr.

The maximal excretory rate was observed 1 hr after AOSL; therefore, this time interval was chosen for determination of body fluid volumes and ANP in the second, third, and fourth experimental series.

A second series of 10 BDL and 11 SO rats was used to assess glomerular function and determine plasma albumin. After 16-hr urine collection, half of the rats from each group were submitted to AOSL. One hour later, all animals were anesthetized, and an arterial blood sample was obtained to measure plasma concentration of creatinine, albumin, electrolytes, and hematocrit. Basal GFR was estimated by 16-hr creatinine clearance.

A third series of 13 BDL and 16 SO was used to assess plasma and extracellular fluid volume (ECFV) with standard indicator dilution techniques, and to measure electrolyte concentrations, both in normal conditions (seven BDL and seven SO) and 1 hr after AOSL (six BDL and nine SO).

A fourth series of rats was used to measure plasma ANP, and renal medullary cGMP production, in normal conditions (15 BDL and 17 SO), and 1 hr after AOSL (14 BDL and 13 SO). Rats were decapitated, and blood samples were collected in chilled tubes to measure plasma ANP in 10 animals of each experimental kind. In all rats, kidneys were rapidly excised, and the renal medullas were prepared for determination of cGMP content and accumulation in response to ANP *in vitro* as detailed below.

Analytical Techniques. Urinary volume was determined gravimetrically, sodium and potassium with an Ep-

pendorf flame photometer and creatinine with standard colorimetric technique in a Beckman analyzer. Plasma albumin concentration was determined by standard SMA12 techniques.

Plasma and extracellular fluid volumes were determined using Evans' Blue and ^{22}Na as intravascular and extracellular tracers, respectively. After overnight fasting, rats were anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg), and a catheter was placed in a femoral artery. Heparin (200 mU) was administered in 0.1 ml of isotonic saline and 3 min later, the same volume of blood was extracted to determine hematocrit and sodium and potassium plasma concentrations. Immediately, 0.2 ml of 0.5% Evans' Blue (w/v) solution containing 400,000 cpm of isotopic sodium (^{22}Na) was injected through the femoral vein. Five to eight min later, a 2-ml blood sample was obtained from the femoral artery for determinations of plasma volume and ECFV. This interval, that is about 10 times the rat mean circulatory time, was chosen to optimize plasma volume measurement, since it allows for proper dilution of the macromolecular tracer while preventing any significant extravasation into the interstitial space that would lead to an overestimation of plasma volume (21). ECFV may have been slightly underestimated since in rodents plasma concentration of ^{22}Na reaches its equilibrium within 10 min after intravenous injection (22). After centrifuging, the radioactivity content of a 1-ml plasma sample was determined in a Wallac gamma counter. After subtracting the background, typical readings between 7000 and 10,000 cpm/ml plasma were found. Blood volume was calculated as plasma volume/(1-hematocrit).

Urinary cGMP was determined by radioimmune assay (RIA) as described (23). Briefly, aliquots were diluted 1000–5000 times in RIA buffer (0.05 *M* sodium acetate, pH 6.2). Then 100 μl of appropriately diluted samples were acetylated by adding 10 μl triethylamine:acetic anhydride (2:1) and incubated overnight at 5°C in 500 μl RIA buffer containing anti-cGMP antibody (1:40,000) and 10,000 cpm of ^{125}I-2'-0-succinyl-cGMP-tyrosyl-methyl-ester. Similar tubes containing 10–1000 fmol acetylated cGMP were used for the standard curve. The antibody was precipitated with 50% ammonium sulfate and centrifuged. Bound counts were detected in a Wallac 1470 gamma counter equipped with an automatic RIA program.

Renal medullary cGMP content and production was assessed as follows (24). In every rat, each kidney was handled on ice, and the whole renal medulla was isolated with fine surgical scissors. Each medulla was blotted, weighed, minced with scissors, and preincubated at 37°C in 400 μl Hank's solution supplemented with 2.0 mg BSA, 5.5 m*M* glucose, 25 m*M* HEPES, pH 7.4 for 4 min. Then, one medulla received 100 μl ANP to a final concentration of 0.1 μ*M*, whereas the other received 100 μl Hank's solution. After three min of incubation with or without ANP, 500 μl of 10% trichloroacetic acid was added, and the tissue was homogenized with an Ultraturrax. After centrifugation, the supernatant was kept on ice and extracted three times with water-saturated ether (3:1). The aqueous phase was lyophilized and resuspended in 600 μl RIA buffer for cGMP determination. The 0.1 μ*M* ANP concentration was chosen since, in the conditions here described, it induces approximately a half-maximal cGMP production in renal medullas from control untreated rats, whereas maximal cGMP production is obtained with 0.5–1.0 μ*M* ANP (24).

Plasma ANP was measured using a commercial RIA Kit (Peninsula Lab., Belmont, CA). Briefly, blood samples were received in chilled tubes containing EDTA and aprotinin and centrifuged. Plasma was stored at –40°C until determination. One ml samples were acidified and extracted in C-18 reverse phase columns (Sep-Pak, Waters Corp., Milford, MA). The material eluted with 60% acetonitrile in 1% trifluoroacetic acid, was lyophilized and reconstituted in 250 μl RIA buffer according to the manufacturer's instructions.

All data are presented as mean ± SEM. The data were analyzed statistically using paired or unpaired *t* test, and by two-way ANOVA, as appropriate. A *P*-value of less than 0.05 was considered significant.

Results

Characterization of BDL and SO Rats. Three weeks after surgery, there was no difference in body weight between BDL and SO rats (247 ± 3.4 g, $n = 51$, vs 248 ± 2.2 $n = 56$ g). Liver biopsy of BDL rats uniformly showed a severe proliferation of bile ducts and advanced fibrosis of hepatic tissue. Control liver biopsy of two SO rats was within normal limits. There was no ascites and there was no diarrhea in the BDL rats.

BDL rats had a reduced hematocrit and hypoalbuminemia (Table I). In a previous study, we demonstrated that equally aged BDL rats showed mild hypotension, and elevated serum alkaline phosphatase and bilirubin (16).

BDL rats excreted significantly more sodium, urine, and potassium than SO controls (Fig. 1). In contrast, both groups excreted similar amounts of creatinine and cGMP. Plasma creatinine was moderately reduced in BDL as compared with SO (4.0 ± 0.46 μg/ml, $n = 8$ vs 5.11 ± 0.25 μg/ml, $n = 9$, $P < 0.05$); however, the calculated GFR was not significantly different between both groups (7.63 ± 1.21 in BDL vs 5.58 ± 0.31 ml/min/kg in SO rats).

Effects of AOSL. *Urinary excretion.* Confirming the results of 24 hr collection, BDL rats excreted significantly more sodium, urine, potassium, and equal cGMP as compared with SO during the preload period (Fig. 2). After AOSL, both groups showed transient, significant increases in urinary sodium, volume, and potassium and a nonsignificant increment in urinary cGMP (Fig. 2). In the first 2 hr post-AOSL, sodium excretion increased from 0.065 ± 0.008–3.13 ± 0.19 mmol/kg/hr in SO (60 ± 12 times) and from 0.203 ± 0.024–2.07 ± 0.30 mmol/kg/hr in BDL (only 12 ± 2 times). Volume excretion increased from 1.53 ± 0.32–11.36 ± 0.82 ml/kg/hr in SO (8.3 ± 1.3 times) and

Table I. Hematocrit and Plasma Electrolytes and Albumin

	Basal		AOSL	
	S.O.	B.D.L.	S.O.	B.D.L.
Hematocrit (%)	46.9 ± 0.8 (13)	39.4 ± 1.3 (11) $P < 0.00005$	45.0 ± 0.9 (14)[a]	43.9 ± 2.2 (10)[a] n.s.
Sodium (m*M*)	142.3 ± 1.4 (9)	137.1 ± 1.9 (9) $P < 0.005$	149.6 ± 1.6 (11)[a]	148.9 ± 2.5 (8)[a] n.s.
Potassium (m*M*)	4.02 ± 0.24 (9)	4.20 ± 0.23 (9) n.s.	3.29 ± 0.06 (10)[a]	4.49 ± 0.36 (8) $P < 0.001$
Albumin (mg/dl)	3.38 ± 0.12 (5)	2.60 ± 0.18 (5) $P < 0.05$	3.23 ± 0.14 (6)	2.48 ± 0.10 (5) $P < 0.01$

Note. *P* values underneath numbers denote differences between cholestatic (B.D.L.) and control (S.O.) rats.
[a] $P < 0.001$ versus respective Basal value.
Unpaired *t* test. The number of determinations appears in parentheses.

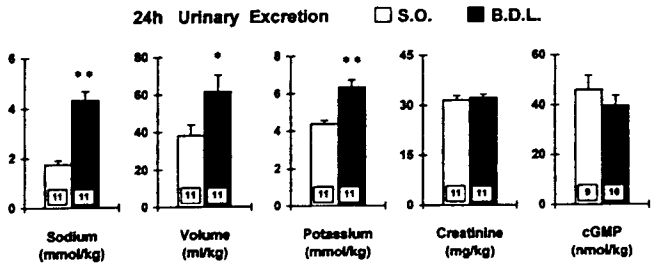

Figure 1. Baseline urinary excretion in 11 control, sham operated rats (S.O.) and 11 rats made cholestatic by ligature and section of the common bile duct (B.D.L.). Urine was collected individually in metabolic cages. cGMP could not be determined in two cholestatic, and one control urine sample. The number of valid determinations appears within the bars. $^*P < 0.05$, $^{**}P < 0.001$ vs S.O., unpaired *t* test.

from 2.77 ± 0.55–8.91 ± 1.24 ml/kg/hr in BDL (3.3 ± 0.7 times). Potassium excretion increased from 0.196 ± 0.008–0.703 ± 0.028 mmol/kg/hr in SO (3.7 ± 0.5 times) and from 0.343 ± 0.011–0.708 ± 0.144 in BDL (2.1 ± 0.4 times). The changes in cGMP excretion were from 6.51 ± 0.62–10.06 ± 2.00 nmol/kg/hr in SO (1.6 ± 0.3 times) and from 6.97 ± 1.19–12.05 ± 3.14 nmol/kg/hr in BDL (1.9 ± 0.4 times).

Sodium excretion remained significantly above baseline in both groups during the second (2–4-hr) and third (4–6-hr) collection periods post-load (Fig. 2). In contrast, during these periods, potassium excretion was substantially lower than baseline in both groups, whereas urinary volume and cGMP excretion was equal or moderately below baseline for both groups (Fig. 2). No diarrhea was observed in any animal.

As compared with SO rats, BDL rats excreted significantly less sodium in the first 2 hr post-load, a similar amount between 2 and 4 hr, and returned toward the baseline pattern excreting more sodium between 4 and 6 hr post-load (Fig. 2). Sodium excretion was significantly lower in BDL rats already at the first hour post-load (BDL: 1.42 ± 0.19 mmol/kg/hr, $n = 5$, vs SO: 2.15 ± 0.16 mmol/kg/hr, $n = 5$, $P < 0.01$). Diuresis and potassium excretion was not different between groups in the first 4 hr post-load. Water excretion was higher in BDL rats between 4 and 6 hr post-load, as in basal conditions. Interestingly, cGMP excretion was similar in both groups during the whole length of the experiment (Fig. 2).

Cumulative sodium excretion was lower in BDL than in SO rats, during every post-load period. In contrast, cumulative diuresis was not significantly different at any period. After 6 hr of collection, SO rats had excreted 82.2% ± 4.9% of the sodium load, whereas BDL rats had excreted only 64.9% ± 12.2% ($P < 0.05$ vs SO). Since AOSL was hypertonic (1 *M*), urine excretion exceeded the loaded volume (10 ml/kg) during the first 2 hr in both groups.

Body volumes, hematocrit, and electrolytes. Figure 3 shows the results of body volumes obtained in the third series, and Table I combines results of hematocrit and electrolyte determination from the second and third series.

Basal hematocrit was considerably lower in BDL than controls. After AOSL hematocrit showed a slight but statistically significant decrease in the SO group, whereas it frankly increased in the BDL rats, eliminating the difference between groups (Fig. 3, Table I). Neither group showed changes in plasma albumin after AOSL, and this variable remained about 30% lower in BDL rats (Table I).

Under basal conditions, there was no difference between plasma volumes in control and BDL rats. However, because of their reduced hematocrit, BDL rats had a lower blood volume as compared with SO (Fig. 3). One hour after the sodium load, plasma volume decreased significantly in the BDL group, becoming lower than in SO rats, in which this variable remained unchanged. AOSL did not modify blood volume in either group since this maneuver induced opposite changes in plasma volume and hematocrit, particularly marked in BDL. After AOSL, blood volume remained significantly lower in BDL than in SO (Fig. 3).

Under basal conditions, ECFV was significantly larger in BDL rats as compared with SO. After sodium load, this variable increased in control rats, whereas it did not change in BDL. Despite this, ECFV remained larger in BDL than in SO.

Basal plasma sodium concentration was significantly lower in the BDL group. One hour after AOSL plasma sodium concentration increased in both groups, which

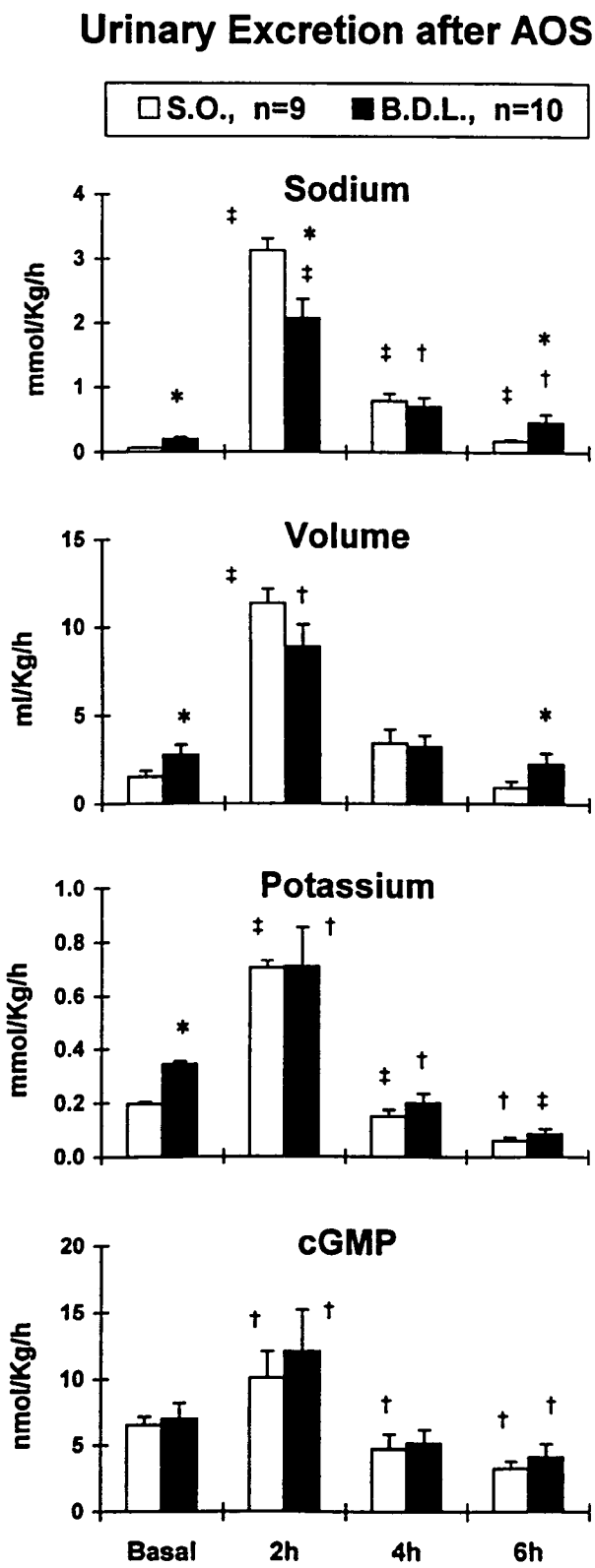

Figure 2. Time course of urinary excretion in control (S.O.) and cholestatic (B.D.L.) rats subjected to an acute oral sodium load (AOSL). Urine was collected in individual metabolic cages during 16 hr (Basal) without food but with free access to water. Then the water supply was removed, 10 mmol/kg NaCl in 10 ml/kg volume was administered by gavage, and urine was collected every 2 hr. *P < 0.05 vs S.O., unpaired t test. †P < 0.05; ‡P < 0.001 vs baseline, paired t test.

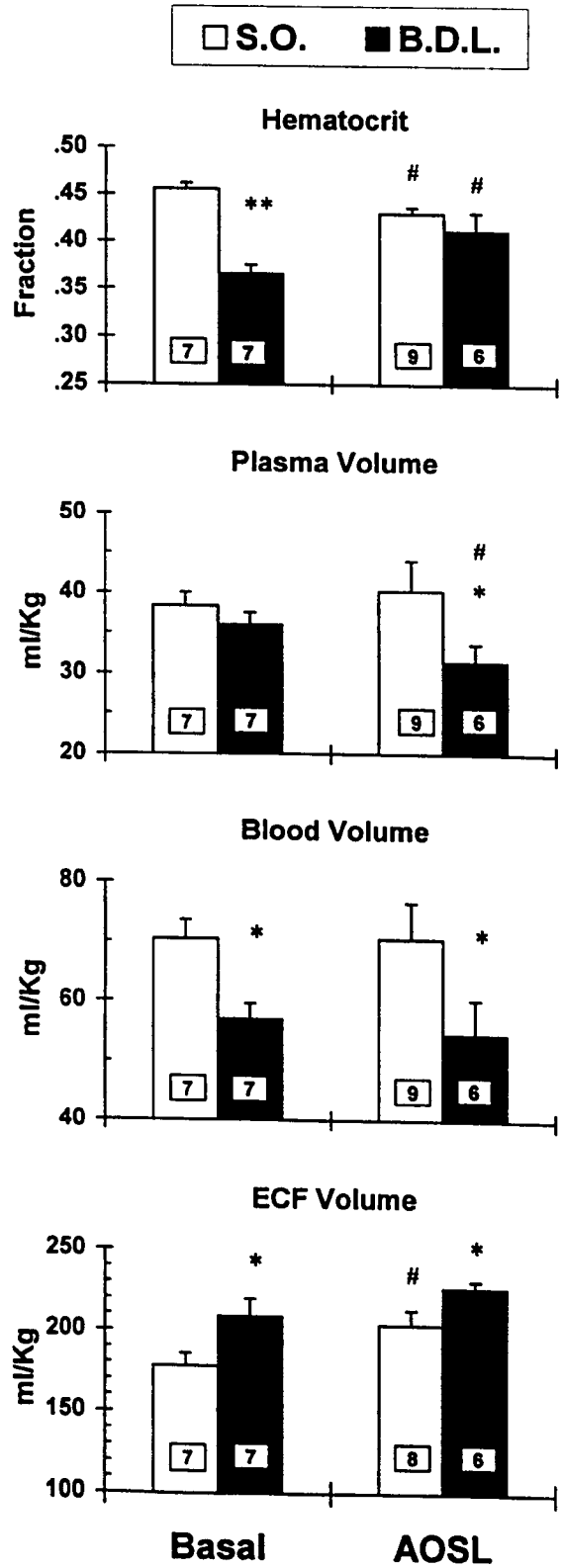

Figure 3. Hematocrit and body fluid volumes determined in control (S.O.) and cholestatic (B.D.L.) rats under control conditions (Basal) or 1 hr after receiving an acute oral sodium load (AOSL, 10 mmol/kg). The number of valid measurements appears within each bar. *P < 0.05 vs S.O.; #P < 0.05 vs its respective Basal value, unpaired t test.

reached a similar degree of hypernatremia (Table I). Basal plasma potassium concentration was similar between groups, but this variable decreased markedly in SO rats after AOSL, whereas it stayed unchanged in the BDL group (Table I).

The calculated extracellular sodium pool was not different between BDL and SO rats under control conditions (28.1 ± 1.5 vs 25.5 ± 1.3 mmol/kg body wt). This variable increased in both groups after AOSL, but it became significantly larger in BDL than in SO (35.1 ± 0.8 vs 31.4 ± 1.4 mmol/kg body wt).

Plasma ANP. Basal plasma ANP level was significantly higher in BDL rats as compared with SO (Fig. 4). In contrast, ANP levels measured 1 hr after the AOSL (i.e., at the moment of higher sodium excretion rate) were significantly lower in BDL than in SO rats. A significant increase in plasma ANP was found when comparing loaded versus nonstimulated SO rats (about +30%, $P < 0.05$ unpaired t test), whereas ANP levels in BDL rats were decreased by ≈ 44% after the NaCl load ($P < 0.001$, unpaired t test). When the data were subjected to a two-way ANOVA, a significant effect of NaCl load ($P < 0.05$) and a highly significant negative interaction between group and treatment ($P < 0.0001$) was found.

Renal medullary cGMP. Nonstimulated renal medullary cGMP content was not different between BDL and SO rats (Fig. 5). Incubation with 0.1 μ*M* ANP induced a significant increase in medullary cGMP content (6–10-fold the basal, $P < 0.001$, paired t test), and this increment was similar in both groups. Accordingly, the calculated ANP-induced cGMP medullary production was equal in BDL and SO (Fig. 5). The same pattern was found in medullas obtained 1 hr after AOSL, without difference between BDL and SO rats. Interestingly, in medullas from sodium-loaded rats, both baseline cGMP content and ANP-induced cGMP production were approximately twice the value obtained in medullas from nonstimulated rats ($P < 0.05$, unpaired t test) (Fig. 5).

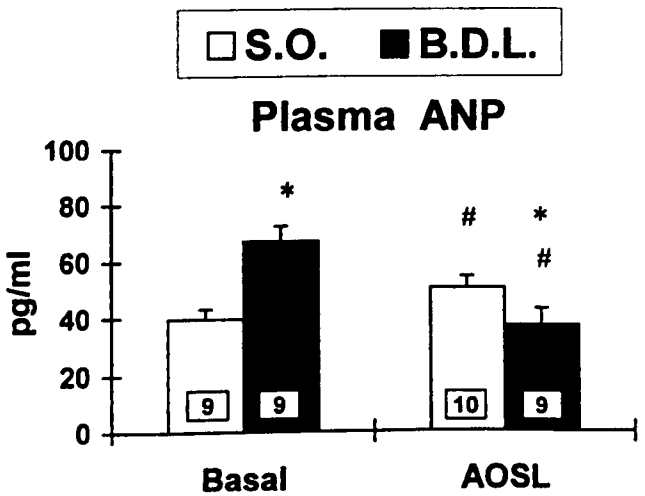

Figure 4. Plasma concentration of ANP as determined by RIA in control (S.O.) and cholestatic (B.D.L.) rats, either under control conditions (Basal) or 1 hr after the animals received an acute oral sodium load (AOSL) as explained in Figure 3. *$P < 0.05$ vs S.O., #$P < 0.05$ vs its respective Basal value, unpaired t test.

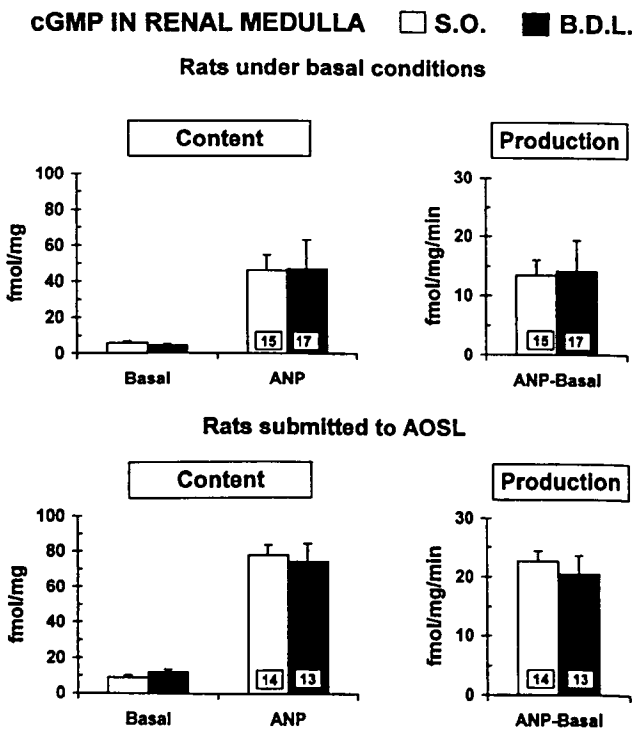

Figure 5. Cyclic guanosine 3′-5′monophosphate (cGMP) content in isolated whole renal medullas incubated without (Basal) or with 0.1 μ*M* ANP (ANP) during 3 min at 37°C, as measured by RIA. cGMP average minute production in response to ANP was determined as the difference between cGMP content in stimulated and nonstimulated medulla of each rat divided by the time of stimulus. AOSL: acute oral sodium load.

Discussion

The major findings of this study are: first, BDL rats, without ascites or macroscopic edema, present a diminished ability to excrete an oral sodium load (Fig. 2), resembling the previously reported blunted natriuretic response after an intravenous sodium load (17). Second, changes in body volume distribution following AOSL differed markedly between both groups: BDL rats showed a contraction of plasma volume and reduced ECFV expansion (Fig. 3). Third, contrasting to what has been reported after an intravenous sodium load (17), plasma ANP concentration did not increase after AOSL in BDL rats, as it did in SO rats, but actually decreased (Fig. 4). Fourth, urinary cGMP excretion was similar in BDL and control rats, in basal conditions and after the load (Fig. 2). Fifth, *in vitro* renal medullary cGMP production in response to ANP was similar in both groups of rats (Fig. 5). We have previously shown that BDL rats and their isolated perfused kidneys present a reduced diuretic natriuretic response to exogenous ANP (16). Renal refractoriness to ANP in BDL rats is similarly suggested by the elevated plasma ANP and normal urinary cGMP levels observed under basal conditions in the present study. However, our study failed to provide any evidence

that renal refractoriness to ANP or a higher cGMP turnover rate actually contribute to the delayed sodium excretion observed in BDL rats after AOSL. This study rather indicates that delayed natriuresis can be ascribed to inadequate ECFV expansion in BDL rats.

Cholestatic rats showed elevated baseline urinary salt and water excretion, as compared with controls. Similar findings have been described in BDL dogs (25) and rats (16), and may be attributed to a saluretic effect of negatively charged biliary salts (26). Glomerular function, as assessed by clearance of creatinine, was similar between BDL and SO rats, confirming previous determinations of GFR in this model using inulin clearance (16). Consistent with their elevated saluresis, BDL rats have lower sodium plasma concentrations. BDL rats have a similar plasma volume as compared with SO rats, but present a larger ECFV, which may be explained by a reduced colloid osmotic pressure caused by their hypoalbuminemia. Because of hyponatremia and expanded ECFV, BDL rats have a similar extracellular sodium pool as compared with their controls.

Nevertheless, as it would be expected in a pathological salt-retaining state, the natriuretic response after AOSL in cholestatic rats was significantly slower than in control rats, amounting to only 65% of the total sodium load after 6 hr. This result resembles that obtained in rats made cirrhotic by carbon tetrachloride (18), and extends previous reports showing that BDL rats present a blunted natriuretic response after an intravenous isotonic sodium load (17). Therefore, despite their elevated baseline urinary sodium excretion, BDL rats show an impaired capacity to handle an additional sodium load, either by gastric or parenteral route.

ECF sodium content increased in both groups following the sodium load. According to the estimated change in this variable, 1 hr after AOSL, about 85% of ingested sodium was accounted for as expansion plus excretion in each group. This finding rules out a significant impairment in intestinal sodium absorption to explain the delayed sodium excretion in BDL rats. The sodium load was accompanied by a +15% increase in ECFV in control rats but only by a +9% (nonsignificant) change in the already expanded ECFV of BDL.

As expected from the increase in plasma sodium concentration (that may likely lead to an increase in plasma osmolarity), hematocrit was slightly reduced in SO after AOSL. In contrast, hematocrit was dramatically increased in BDL, despite a proportionally greater increase in plasma sodium concentration 1 hr after AOSL in these rats, which showed an increase of 11.8 m*M* (+8.6%) vs an increase of 7.2 m*M* (+5.1%) in SO. This observation may indicate a defect in the mechanisms of cell volume regulation in BDL, leading to an abnormal erythrocyte enlargement after AOSL. Cholestasis is associated with changes in membrane lipid composition that result in a reduced Na^+/K^+ ATPase activity of the erythrocyte membrane, as shown *in vitro* (27) and *in vivo* (28). Furthermore, isotonic volume expansion has been reported to cause an increase in cell sodium content and a reduced Rb influx in erythrocytes of cirrhotic rats as compared with controls (29). Consistent with this interpretation, BDL rats showed no difference in plasma potassium after AOSL, whereas SO rats presented hypokalemia, likely reflecting enhanced cellular potassium uptake due to a higher activity of membrane transporters, triggered by hypertonicity and/or the increased cellular sodium influx. Initial sodium sequestration in the intracellular space after AOSL may contribute to the delayed natriuretic response.

Basal plasma ANP concentration was found significantly elevated in BDL animals, in agreement with previous studies (17), despite the fact that BDL rats have similar plasma volume and reduced blood volume as compared with SO rats. Since BDL rats excreted normal amounts of cGMP, it can be argued that the elevated ANP levels compensate for the reduced responsiveness found in these animals to exogenous ANP (16). It has been shown that plasma ANP levels rise significantly more in BDL than in control rats after an intravenous isotonic NaCl expansion (17). In contrast, our study showed that AOSL produced a significant reduction in plasma ANP levels in BDL rats, as opposed to the increase observed in SO. In our study, these inverted changes in ANP levels appeared to follow the changes in plasma volume (i.e., whereas plasma volume increased by 5% after AOSL in SO animals, it was reduced by 13% in BDL rats). Since blood volume did not change significantly in either group, we can only speculate that other factors, related to central venous tone or the increase in plasma sodium concentration and the consequent changes in neurohumoral regulatory systems, may have differently affected atrial ANP release after AOSL.

Renal refractoriness to ANP in models of experimental cirrhosis has been explained by hemodynamic differences, such as systemic hypotension (30), and reduced glomerular vasoactive responses (16, 31). Atuchá *et al.* found reduced natriuresis and a lower papillary plasma flow under equal renal perfusion pressure both in carbon tetrachloride–treated (32) and BDL rats (33). Enhanced efferent renal sympathetic nerve activity has also been proposed as a major factor leading to sodium retention in BDL rats, both after an intravenous sodium load (34) and after exogenous ANP administration (15). In addition, a blunted hepatorenal chemosensitive reflex, that normally decreases efferent renal sympathetic nerve activity during ingestion of high-NaCl food, has been described in carbon tetrachloride-induced cirrhotic rats after portal vein injections or oral administration of a hypertonic NaCl solution (35).

An intrinsic renal tubular resistance to ANP is supported by cellular studies. Ni *et al.* (17), showed that inner medullary collecting duct (IMCD) cells freshly isolated from male BDL rats present a reduced cGMP accumulation in response to ANP, as compared with controls. This effect partially depended on the augmented efferent renal sympathetic nerve activity. Moreover, the lower ANP-induced cGMP accumulation in IMCD cells from BDL rats appears to be entirely due to an enhanced cGMP phosphodiesterase

activity, rather than to a smaller cGMP generation (5, 17). Therefore, it was important to evaluate whether similar mechanisms were relevant in the excretory response of BDL rats to AOSL. We used a previously characterized model of whole renal medullas incubated *in vitro* (24). In the present report, we did not detect any difference in cGMP accumulation in response to ANP between female BDL and SO rats, either under basal conditions or after AOSL, in the absence of phosphodiesterase inhibitors. The discrepancy with previous reports (17) may be accounted for by differences in the experimental approach. Besides the change in rat gender, it is possible that enzymatic activities provided by other cell types present in our preparation might mask differences in cGMP accumulation that would be detectable in isolated IMCD cells. The ANP concentration used (0.1 μM) provides a good estimate of the tissue response, since it induces submaximal cGMP stimulation (17, 24). Interestingly, cGMP accumulation in response to ANP was increased in medullas obtained from rats submitted to AOSL from either group. This enhanced response may be related to the above mentioned hepatorenal reflex–mediated reduction in medullary phosphodiesterase activity. The lack of difference between groups does not support the possibility that an impairment in this mechanism plays a role in the delayed excretory response to AOSL in female BDL rats. A reduction in efferent renal nerve activity in BDL rats may also explain why *in vivo*, these rats excreted similar amounts of cGMP after AOSL, despite their reduced plasma ANP at that time.

In conclusion, the present results extend our previous report showing that BDL rats have a blunted excretory response to exogenous ANP both *in vivo*, and in their isolated perfused kidney (16). Our findings support the importance of hemodynamic factors over an intrinsic renal ANP resistance as cause of the altered sodium handling observed in cholestatic rats. Lack of significant impairment in ANP transduction mechanisms is evidenced because, as compared with controls, cholestatic rats presented similar renal cGMP excretion/production after AOSL, both *in vivo* and *in vitro*. We speculate that the reduced plasma volume and plasma ANP concentration after AOSL, and eventually defects in cell volume regulation, contributed to the delayed sodium excretion in BDL rats. Other factors, such as metabolic parameters or neural activity, which were not studied, also can be involved in mediating this delayed natriuretic response.

We thank Drs. Ignacio Duarte and Helmar Rosenberg for interpreting the liver biopsies and Dr. Mireya Ortíz and Mr. José Cornejo for their technical assistance and handling of experimental animals.

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