Drinking and blood pressure during sodium depletion or ANG II infusion in chronic cholestatic rats

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Fitts, Douglas A., Jeannine R. Lane, Elizabeth M. Starbuck, and Chi-Pei Li. Drinking and blood pressure during sodium depletion or ANG II infusion in chronic cholestatic rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R23–R31, 1999.—After a chronic ligation of the common bile duct (BDL), Long-Evans rats are hypotensive and have elevated saline intake during both sodium-depleted and nondepleted conditions. We tested whether BDL rats have exaggerated hypotension during sodium depletion or an elevated dipsogenic response to angiotensin II (ANG II) that might help to explain the saline intake. After 4 wk of BDL, rats were hypotensive at baseline and developed exaggerated hypotension during acute furosemide-induced diuresis. Without saline to drink, BDL rats increased water intake during depletion equal to sham-ligated rats. However, with saline solution available at 22 h after sodium depletion, the BDL rats drank more water and saline than did sham-ligated rats. This rapid intake temporarily increased their mean arterial pressure to equal that of sham-ligated rats. Intravenous infusion of ANG II induced equal blood pressure responses in the BDL rats relative to sham-ligated rats during both ad libitum and sodium-depleted conditions. Thus BDL rats have exaggerated hypotension during diuresis, and their hypotension is corrected by drinking an exaggerated volume of saline, but they do not have an increased drinking response to ANG II.

Methods

Subjects

Subjects were 115 male Long-Evans rats weighing between 300 and 500 g. They were housed individually in hanging wire mesh cages with Teklad laboratory chow and tap water continuously available except as noted. The rats received either water only or water in choice with 0.3 M NaCl as described under MAP and Drinking Experiment, beginning 2 days after the BDL or sham ligation surgeries. Saline was

Dilation of the common bile duct (BDL) in rats immediately stops the flow of bile from the liver to the duodenum and severely damages the liver over a period of a few weeks. Heart and kidney damage, reduced resting blood pressure, a blunted pressor response to angiotensin II (ANG II) and norepinephrine, and abnormalities of nitric oxide are present in BDL rats (2, 20) or in rats with hepatic cirrhosis resulting from carbon tetrachloride (CCl4) administration (21–23, 29, 30). Like jaundiced humans, BDL rats are more likely to suffer shock after a surgical hemorrhage because of a reduced ability to redistribute blood efficiently (14). These changes in pressure and distribution of blood volume in BDL rats have been noted as early as 3 days after the ligation (12–14) and may affect central mechanisms controlling ingestion of water and sodium.

BDL rats given access to water in choice with hypertonic saline double their intake of hypertonic saline on a daily basis about 3–4 wk after the ligation (16, 17). This effect occurs most reliably if a conditioned aversion to the taste of saline is avoided by introducing the saline to the rats after, rather than before, the ligation surgery (16, 18). BDL rats also drink more saline than sham-ligated rats after a loss of plasma volume induced by sodium depletion, and this effect becomes larger with multiple depletions (17). The mechanism of the increased salt intake under ad libitum conditions or sodium depletion is unclear, but the elevation in ad libitum saline intake appears in parallel with the development of hypotension (16). Intact rats also increase water or saline intakes during hypovolemia or hypotension (8).

There are at least three potential mechanisms by which hypotension may contribute to elevated daily saline intake in BDL rats (16): 1) a greater formation of, or dipsogenic sensitivity to, ANG II; 2) a direct neural stimulation of salt appetite via baroreceptors or volume receptors; or 3) a learned preference for saline based on a reversal of the unpleasant symptoms associated with hypotension.

We found no elevation of renin secretion in Long-Evans BDL rats expressing elevated daily saline intakes (19), so a greater formation of ANG II under ad libitum conditions is unlikely. An altered sensitivity to ANG II is possible because the pressor response is reduced after intravenous ANG II in BDL rats (1, 2). This reduced pressor response might indicate a general insensitivity to ANG II such that the rats drink less after a given dose than unligated animals. On the other hand, a reduced pressor response during ANG II formation may enhance the drinking response by reducing negative feedback from baroreceptors (5). In the sodium-depleted condition, BDL rats may experience exaggerated hypotension relative to the replete condition (14). This greater hypotensive response in BDL rats could increase peripheral ANG II synthesis or baroreceptor input that could enhance saline intake during depletion. Alternatively, the hypotension may cause symptoms of illness that are relieved by saline consumption.

The main purposes of the present study were to narrow these possibilities by determining 1) if BDL rats suffer a greater loss of blood pressure during or after a furosemide diuresis than sham-ligated rats; 2) if BDL rats drink more water or saline than sham-ligated rats during sustained intravenous infusion of ANG II either in ad libitum or sodium-depleted conditions; and 3) if a rapid intake of saline solution repairs the hypotension of sodium-depleted BDL rats in the absence of an ANG II infusion.

Methods

Subjects

Subjects were 115 male Long-Evans rats weighing between 300 and 500 g. They were housed individually in hanging wire mesh cages with Teklad laboratory chow and tap water continuously available except as noted. The rats received either water only or water in choice with 0.3 M NaCl as described under MAP and Drinking Experiment, beginning 2 days after the BDL or sham ligation surgeries. Saline was
withheld during the periods of sodium depletion. Room temperature was held at 23°C, and lights were on for 12 h/day.

Surgies

Rats received either a BDL or a sham ligation surgery under halothane anesthesia. The common bile duct was ligated with 4–0 silk suture just above the duodenum anterior to the pancreas and posterior to the hilum of the liver, thereby eliminating the flow of bile from the liver in the duodenum. Another tie was made 2 mm anterior to the first, and the bile duct was then cut between the two ligatures. Sham ligation surgery consisted of locating the bile duct, manipulating it, and replacing it. Animals were given topical Betadine as antiseptic and 0.2 ml gentamicin intramuscularly to control post-surgical infection. Bupivacaine was used as a local anesthetic. All surgeries were performed using aseptic techniques.

Polyethylene catheters were inserted in the left femoral artery and vein of all rats under halothane anesthesia for later measurement of mean arterial pressure (MAP) and heart rate. Catheters were constructed of PE-10 tubing heat welded to a longer piece of PE-50 tubing; the latter tubing was tunneled subcutaneously to an exit wound between the scapulae. The catheters were filled with 100 U/ml heparin in sterile isotonic saline and obturated until the time of the experiment 2 days later.

Verification of BDL and Necropsy

At the end of the experiments, the presence of plasma or urinary bilirubin was determined to verify the completeness of the BDL surgery. For blood samples, rats were weighed and then rapidly anesthetized with halothane, and a sample of blood was drawn by cardiac puncture in syringes with heparinized needles. Plasma was frozen for later determination of total plasma bilirubin concentration by spectrophotometry (Sigma kit no. 552). Norms for plasma bilirubin in this laboratory range from 2 to 21 µmol/l in sham-ligated rats and from 70 to 150 µmol/l in 3- and 4-wk BDL rats. In other animals, a urine sample was obtained, and the presence of urinary bilirubin was tested qualitatively (Ames icotest reagent tablets). This test detects as little as 0.9 µmol/l bilirubin. After blood or urine collection, rats were given an overdose of pentobarbital sodium by intracardiac injection also under halothane anesthesia or by intraperitoneal injection. Ascites fluid accumulation and degree of bloating of the bile duct stump were noted qualitatively. In addition, the liver, kidneys, and heart were dissected, blotted dry, and weighed. Rats that had received a BDL treatment were rejected from the experiment if they did not have elevated plasma or urinary bilirubin.

MAP and Drinking Experiment

The purpose of this experiment was to study the blood pressure or drinking responses of sham-ligated and BDL rats during acute furosemide diuresis and after 22 h of sodium depletion either with or without intravenous infusion of ANG II. The experiment was conducted both at 3 and 4 wk postligation because the disease is progressive, and we have previously observed that elevated saline intake and hypotension develop in Long-Evans BDL rats sometime after 3 wk (16).

Catheters were implanted in the left femoral artery and vein of 105 rats either 3 or 4 wk after BDL or sham ligation. Each rat was used only one time. Two days after catheterization, the rats were weighed and transferred to cylindrical plastic cages for measurement of MAP. The arterial catheter was connected to a Cobe blood pressure transducer. The amplified pressure signal was digitized at 100 Hz with a Labmaster analog-to-digital board and stored on disk in a microcomputer. The pressure wave was later analyzed for MAP and heart rate (6). The data for sham-ligated rats at 3 and 4 wk postsurgery were nearly identical and were combined in a single sham-ligated group for each experimental condition.

There were eight treatment groups in the experiment. Five groups were tested while depleted of sodium, and three groups were tested under ad libitum conditions. Three depleted groups (24 sham-ligated, 11 3-wk BDL, and 8 4-wk BDL rats) received a subcutaneous injection of 10 mg/kg furosemide followed by an intravenous infusion of ANG II after 22 h of sodium depletion without food available. Two depleted groups (10 4-wk sham-ligated and 8 4-wk BDL rats) received a furosemide injection followed by an intravenous infusion of the vehicle (isotonic dextrose 5%, D5W) instead of ANG II. The three nondepleted groups (21 sham-ligated, 12 3-wk BDL, and 11 4-wk BDL rats) received an intravenous infusion of the vehicle (isotonic dextrose 5%, D5W) instead of ANG II infusions without prior sodium depletion. These latter, nondepleted animals were part of a different study that measured fluid intakes, verification of completeness of BDL, or sham ligation surgery (16). About one-half of these nondepleted rats received access to 0.3 M NaCl solution in choice in water after surgery, as did all depleted rats. The other nondepleted rats received only water. Otherwise, the nondepleted rats were treated identically to the depleted groups. The prior fluid access condition (water or water and saline) did not affect the variables measured here, and the groups have been combined in the analysis. The daily fluid intakes, verification of completeness of BDL, and basal MAP data are reported elsewhere for the nondepleted rats (16). The pressor and drinking data from their ANG II infusions have not been reported previously.

The five depleted groups were tested for MAP before, during, and after an acute diuresis induced by a subcutaneous injection of furosemide. On the first day, the rats were connected to the pressure transducers, and baseline MAP was recorded for 60 min. Each rat was handled briefly for the subcutaneous injection, and pressure was recorded for another 60 min. The arterial catheters were then flushed and obturated, and the rats were weighed and left in this sodium-free environment with water but no food overnight. About 22 h after the depletion treatment, water intake was recorded, the rats were weighed, the arterial catheters were again connected to the blood pressure apparatus, and the venous catheters were connected to syringes on infusion pumps filled either with D5W vehicle or ANG II in D5W. MAP was recorded for 60 min, and 10 min of stable pressure data at the end of this period were used as a baseline value. The syringe pumps were then turned on to deliver ANG II intravenously at 30 ng/min in 0.6 ml/h for 90 min. After 30 min of infusion, all rats were offered both water and 0.3 M NaCl to drink in glass burettes, and intake was recorded to the nearest 0.1 ml at 15, 30, 45, and 60 min. Sixty minutes after the beginning of the drinking test, the pumps were turned off, and MAP was recorded for another 10 min.

For all rats tested under nondepleted conditions, the arterial and venous catheters were connected to the pressure transducers and Harvard Apparatus syringe pumps, respectively, and MAP was recorded for 60 min. Ten minutes of stable pressure data at the end of this period were used as a baseline value. The syringe pumps were then turned on to deliver ANG II intravenously as in the depleted groups. After 30 min of infusion, all rats were offered only water to drink in glass burettes, and intake was recorded as in the depleted...
groups. Saline was not offered because only half of the rats had been previously exposed to saline. Sixty minutes after the beginning of the drinking test, the pumps were turned off, and MAP was recorded for another 10 min.

Dose-Response Experiment

The purpose of this experiment was to determine whether a reduced pressor response to ANG II was a likely contributor to the exaggerated hypotensive response of BDL rats during a furosemide diuresis. Endogenous synthesis of ANG II was blocked with captopril before furosemide treatment, and the effects on MAP and heart rate of different intravenous doses of ANG II were determined beginning 1 h after furosemide.

Five BDL and five sham-ligated rats with access to both water and saline for 4 wk after surgery received arterial and venous catheters 2 days before the experiment. The arterial and venous catheters were connected to pressure transducers and to syringes on an infusion pump, respectively, and baseline blood pressure was recorded for 30 min. The rats then received four 1-mg doses of the angiotensin-converting enzyme inhibitor captopril in 2.4-min intravenous infusions at 30, 60, 110, and 165 min after the beginning of the experiment, in addition to a continuous infusion of captopril at 2.5 mg/h in 0.6 ml/h of D5W and 10 U/ml heparin vehicle for the first 3 h. Thirty minutes after the beginning of the blockade, the rats were handled briefly for an injection of 10 mg/kg furosemide subcutaneously. About 2 h after the injection of furosemide and 10 min after the last intravenous bolus dose of captopril, the captopril syringes were disconnected from the catheters and replaced with new syringes loaded with ANG II in D5W vehicle. The dead space of the catheter was cleared by infusing the remaining captopril solution in the rat. ANG II was then infused at varying volumes in 10-s pulses with at least 10 min between doses. The doses were delivered in the order 0.22, 0.44, 0.85, 1.65, 0.001, and 3.26 µg, with the 0.001-µg dose serving as a control for our activity and the noise of the infusion pump. At the midpoint and end of the dose-response determinations, the rats were handled briefly for supplementary subcutaneous injections of 2.5 mg captopril, for a total of 16.5 mg of captopril by intravenous and subcutaneous routes distributed over 5 h. Another 10-s injection of 1.65 µg ANG II was delivered within 2 h after the end of the dose-response determinations, and 1.5 ml of blood were sampled from the arterial line beginning 30 s after the end of the infusion. Blood was transferred into chilled, EDTA-treated plastic tubes for immediate centrifugation in a freezing centrifuge and storage at −70°C for later determination of plasma ANG II by radioimmunoassay. The assay was performed by J. M. Hanesworth in the laboratory of Dr. J. W. Harding at Washington State University according to previously published methods (11). An additional sample of blood was drawn into a heparinized syringe and spun to obtain plasma for later assay of bilirubin.

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) and linear regression. Except for the body weight variable, between-subjects effects in the MAP and drinking experiment were analyzed with a one-way ANOVA followed by planned comparisons using Fisher’s least significant difference test or the Bonferroni test if the F was not significant. Within-subjects effects, when present, were crossed with the groups variable. In the MAP and drinking experiment, the nondepleted rats were randomized separately from the depleted rats. Consequently, potential differences between depleted and nondepleted rats are confounded with batches. For this reason, only within-batch comparisons were planned. A probability of less than 0.05 was required for significance. Results are presented as means ± SE.

RESULTS

MAP and Drinking Experiment

Body weights and verification of ligation. Initial body weights before any manipulations did not differ among the various ligation groups. Body weights were analyzed as the prefurosemide body weight for all sodium-depleted rats or as the necropsy weight for all nondepleted rats. The factors in the analysis were ligation treatment (sham vs. BDL) and weeks of treatment (3 or 4). Sham-ligated rats were significantly heavier than BDL rats at both weeks [F(1, 92) = 12.36, P = 0.001]. Sham-ligated rats weighed 423 ± 11 g at 3 wk and 446 ± 10 g at 4 wk. This was the only analysis for which the 3- and 4-wk sham-ligated groups were not pooled. BDL rats weighed 387 ± 14 g at 3 wk and 401 ± 11 g at 4 wk. For those rats receiving furosemide, the percent losses of body weight during the hour of baseline and 1 h of diuresis were similar for the sham-ligated (7.2 ± 0.2%), 3-wk BDL (7.3 ± 0.4%), and 4-wk BDL groups (6.7 ± 0.3%). A detailed study of sodium loss 1 h after this dose of furosemide in sham-ligated and BDL rats at various intervals after liglation determined that the effect was between 0.3 and 0.4 mmol/100 g body wt (17).

The effectiveness of the ligation was verified by the presence of hyperbilirubinemia or hyperbilirubinuria as well as the presence of significantly elevated liver and kidney weights at necropsy. Liver weights were 15 ± 1 g in sham-ligated rats and 24 ± 1 g in 3- and 4-wk BDL rats. Kidney weights were 3.3 ± 0.2 g in sham-ligated rats and 3.9 ± 0.2 g in BDL rats. Liver and kidney weights were not different between 3 and 4 wk in sham or BDL rats. No BDL rats in this study had a large amount of ascites. Small or moderate (<2 ml) ascites accumulation was noted in 17% of 3-wk and 26% of 4-wk BDL rats, and the rest of the BDL rats had no more free fluid in the peritoneum than sham-ligated rats. The data of BDL rats with a moderate amount of ascites did not appear exceptional and were included in the analyses. The reported sample sizes exclude 10 rats that were ligated but not used in the experiments because of low urinary bilirubin.

Mean arterial pressure. Analysis of baseline MAP for all 61 rats treated with furosemide in this experiment revealed that 4-wk BDL rats had significantly lower MAP (114 ± 2 mmHg) than 3-wk BDL rats (125 ± 3 mmHg) and that sham-ligated rats (119 ± 1 mmHg) had intermediate MAP [F(2, 58) = 4.71, P = 0.013]. Heart rate did not differ significantly among the groups. The percentage changes from these individual baseline values for all rats treated with furosemide are shown in Fig. 1 for the immediate 60 min after injection. The interaction of ligation treatment with time was significant [F(10, 290) = 2.64, P = 0.004], reflecting the progressive reduction in the MAP of BDL rats relative to sham-ligated rats. This drop in MAP was associated
with significant bradycardia and occasional arrhythmias (data not shown).

Consistent with the prefurosemide data, analysis of the preinfusion baseline MAP immediately before the ANG II or D5W infusions in all 8 groups (Table 1) revealed that MAP of BDL rats was similar to like-treated sham-ligated rats after 3 wk of BDL but was reduced significantly after 4 wk of BDL \( F(7, 96) = 5.35, P < 0.001 \). Baseline heart rate did not differ significantly according to surgical treatment in either analysis.

The percentage changes in MAP from these individual preinfusion baseline values were averaged every 10 min for 90 min during the ANG II or D5W infusions and for 10 min after the infusions. The data are shown in Fig. 2 for the D5W-infused rats and in Fig. 3 for the ANG II-infused rats.

Thirty minutes of D5W infusion (Fig. 2) induced no further significant difference in MAP between sodium-depleted sham-ligated and BDL rats 4 wk after surgery. When fluids were offered after 30 min of infusion, all rats drank both water and saline rapidly, and MAP increased at this time largely as a consequence of the motor behavior of drinking. By the end of the infusion when all rats had finished drinking and were resting quietly, a difference between BDL and sham-ligated rats emerged, with BDL rats maintaining substantially higher gains in MAP than sham-ligated rats [group-by-time interaction \( F(63, 864) = 7.08, P < 0.001 \)]. The means and SE of MAP during the last 10 min of the experiment were 107 \( \pm \) 2 in sham-ligated rats and 109 \( \pm \) 5 in BDL rats.

Thirty minutes of ANG II infusion (Fig. 3) induced larger increases in MAP in sham-ligated than in BDL rats, and these differences generally persisted throughout the drinking period. Slightly greater increases in MAP were observed in depleted rats than in nondepleted rats when fluids were made available, and this correlated with a vigorous and persistent saline intake...
by the depleted rats. Heart rate did not differ significantly according to ligation group during the experiment (not shown). All animals reduced heart rate during ANG II infusion and increased heart rate at the onset of drinking. By the last half of the 90-min infusion, most rats were resting quietly, so the differences in MAP at this time were negligibly influenced by drinking behavior.

Drinking and salt appetite. Water intake during the 22-h period of sodium depletion after furosemide injection was not significantly different among the 3- or 4-wk BDL rats or the sham-ligated rats. The mean intakes in milliliters for these groups were 41 ± 5 for 3-wk BDL, 40 ± 4 for 4-wk BDL, and 36 ± 2 for all sham-ligated rats.

The acute 60-min drinking data for all rats in the experiment are shown in Table 1. Water intake did not differ significantly according to ligation treatment among the rats tested under ad libitum hydration conditions, and the average 60-min intake during ANG II infusion was ~3 ml. The mean cumulative 60-min water and saline intakes of the sodium-depleted rats were in the predicted direction of BDL groups greater than sham-ligated groups for both fluids, but these differences were not statistically significant. Total fluid intake (water plus saline) was significantly greater in the three BDL groups than in the two sham-ligated groups [Bonferroni t(56) = 2.72, P < 0.05]. Over half of this total fluid intake was consumed within the first 15 min of access, and the elevated intake of both water and saline by BDL rats was evident at that time. The mean sodium concentration of the total ingested fluid was slightly hypertonic in the sodium-depleted groups and ranged from 204 to 250 mmol/l. These did not differ significantly according to ligation group. The ANG II-infused groups tended to have slightly more diluted total fluid intake than the D5W-infused groups because of their slightly greater water intake, but this was also not significant.

Technical problems developed with the arterial or venous catheters of 12 rats, and these rats were removed from the above analysis. Nevertheless, they received a furosemide injection and completed the drinking experiment without any infusion or blood pressure measurement. By accident, these rats were equally divided between sham-ligated and BDL groups and 3- and 4-wk surgical treatments (n = 3 rats in each group). The total fluid intakes were 12.6 ± 2.2 ml for sham-ligated rats and 16.6 ± 2.3 for BDL rats, with the size and direction of difference being similar to those of the main study.

Dose-Response Experiment

The baseline MAP and heart rate of 4-wk sham-ligated and BDL rats in the dose-response study are shown in Table 2. MAP was slightly but not significantly lower in the BDL rats relative to sham-ligated rats both after 30 min of baseline and after furosemide and captopril treatments. The values listed in Table 2 for the captopril-blocked, sodium-depleted condition represent the averages of all 2-min baseline periods before the ANG II injections. These baseline values were stable over the duration of the experiment, thus verifying the completeness of the captopril blockade. Percentage changes in MAP and heart rate after an injection were calculated relative to the immediately

### Table 2. Mean baseline MAP and HR and dose-response data

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<th>MAP</th>
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<td></td>
<td>n Pre-CAP</td>
<td>CAP Pre-CAP</td>
<td>Slope x1,000</td>
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<tr>
<td>Sham 5</td>
<td>124±7</td>
<td>92±5</td>
<td>393±14</td>
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<tr>
<td>BDL 5</td>
<td>117±4</td>
<td>90±5</td>
<td>379±13</td>
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Values are means ± SE; n, no. of rats. Units are µg/kg body wt. CAP, captopril. Slope and intercept were calculated by least-squares regression from percentage change at peak MAP after ANG II injection for each log µg/kg dose of ANG II. No differences were significant.
The difference was not significant between the BDL and sham-ligated groups in the peak increases in MAP or in the suppression of heart rate in response to these doses of ANG II during the captopril-induced blockade of angiotensin-converting enzyme and sodium depletion.

To compare the groups at doses that were comparable given the different body weights of the rats, the doses in micrograms per kilogram were calculated separately for each rat. The relationships between the percentage changes in MAP and heart rate plotted as a function of the six absolute microgram doses of ANG II are shown in Fig. 4. There were no differences between the BDL and sham-ligated groups in the peak increases in MAP or in the suppression of heart rate in response to these doses of ANG II during the captopril-induced blockade of angiotensin-converting enzyme and sodium depletion.

The plasma levels of ANG II after the 1.65-µg infusion of ANG II were 10.9 ± 2.7 ng/ml in the sham-ligated group and 4.9 ± 1.9 ng/ml in the BDL group. The difference was not significant [t(7) = 1.71, P = 0.13].

DISCUSSION

The major findings of this study include: 1) BDL rats of the Long-Evans strain were hypotensive at 4 wk under basal maintenance conditions; 2) furosemide-induced diuresis in BDL rats caused an exaggerated hypotensive response that lasted at least 1 h after injection and was accompanied by bradycardia and arrhythmias; 3) water intakes during the 22 h after furosemide were not different between BDL and sham-ligated rats but were about double that of nondepleted fasted rats of the same strain (20 ± 2 ml; see Ref. 28); 4) diuretic-induced hypotension was mostly corrected in BDL rats after 22 h of continued sodium depletion, with only mild hypotension remaining in 4-wk BDL rats; 5) rapid rehydration from sodium depletion by saline and water ingestion temporarily corrected the remaining hypotension in 4-wk BDL rats; 6) the increases of MAP in response to intravenous infusion of ANG II were blunted in 3- and 4-wk BDL rats relative to sham-ligated rats in both depleted and nondepleted conditions; 7) the water and saline drinking responses induced by intravenous infusion of ANG II were similar between BDL and sham-ligated rats in both depleted and nondepleted conditions; 8) total fluid intake after sodium depletion was significantly greater in BDL than in sham-ligated rats regardless of whether the rats received an intravenous infusion of ANG II or D5W vehicle; and 9) the exaggerated hypotensive response by 4-wk BDL rats during furosemide-induced diuresis did not correlate with a reduced pressor effectiveness of exogenous ANG II when endogenous synthesis of ANG II was controlled. The results will be discussed with respect to their implications for drinking behavior and pressor regulation.

Drinking Behavior

Hypotension and a loss of effective blood volume are associated with increased water or saline intake in normal rats as a result of the activation of the renin-angiotensin-aldosterone system of baroreceptors and volume receptors (4, 7, 8, 15, 26). Activities of these systems after sodium depletion were not measured in the present study, but they may also contribute to saline intake in BDL rats.

The present findings replicate an increase in the intake of hypertonic total fluid in BDL rats 22 h after a single depletion of sodium (17). One possible explanation for this increased fluid intake is that BDL rats may have a greater stimulus for drinking after sodium depletion both because of a greater excretion of sodium or a greater loss of effective blood volume and blood pressure. This condition in BDL rats may create greater plasma levels of ANG II or aldosterone or may enhance the sensitivity to the dipsogenic effects of ANG II, hypovolemia, or hypotension. If true, we should expect to see greater water intake by BDL rats either during sodium depletion when no saline is available or during ANG II infusion into ad libitum-hydrated or sodium-depleted rats. We did observe exaggerated hypotension during a furosemide diuresis in BDL rats at

Fig. 4. Mean peak pressor and heart rate (HR) responses by sham-ligated (4 wk, n = 5) and BDL (4 wk, n = 5) rats after varied doses of ANG II delivered iv in a 10-s injection.
either 3 or 4 wk, and we previously observed a greater excretion of sodium during the furosemide diuresis in BDL rats at 4 wk but not at 3 wk (17). In neither this nor our prior experiments (17), however, did we observe a significant elevation of water intake during the depletion, and an infusion of ANG II in the present study did not produce a greater drinking response in BDL rats either during ad libitum hydration or sodium depletion. Furthermore, the exaggerated hypotension during the furosemide diuresis did not continue to the time of the salt appetite test 22 h later, suggesting that changes in hormonal concentrations or neural activity related to the hypotension may not have persisted until the test.

Elevated renin activity has sometimes been observed in BDL rats under basal maintenance conditions (24, 25). We examined plasma renin activity and aldosterone concentrations in 3-wk Long-Evans BDL and sham-ligated rats either under basal maintenance conditions or when stimulated with a 0.1 mg/ml concentration of captopril delivered either in the drinking water or by gavage (19). Both groups had an appropriate increase in renin activity with captopril administration, but renin activity was never significantly higher in BDL rats than in sham-ligated rats, and aldosterone levels were reduced in BDL rats. Nevertheless, the BDL rats drank more saline solution than sham-ligated rats under both control and captopril-stimulated conditions. The increase in daily intake of saline by control, nonstimulated BDL rats has previously been reported (3, 16, 17). Thus there is a precedent for elevated saline intake in BDL rats without a greater activation of the peripheral renin-angiotensin system. Whether the same applies to BDL rats after the exaggerated hypotension induced by acute sodium depletion is unknown.

Even if plasma levels of ANG II are not ordinarily elevated in BDL rats, it could be argued that the rats’ sensitivity to the dipsogenic effects of ANG II might be increased, thus producing greater fluid intakes with a similar dose. The plasma ANG II levels after exogenous injection of ANG II during captopril blockade in the dose-response experiment were not significantly different between BDL and sham-ligated rats, suggesting that the volumes of distribution were not greatly different. That is, the smaller body size of the BDL rats may have been offset by a slightly larger relative plasma volume. If the plasma ANG II levels during sustained infusions of ANG II were also similar between BDL and sham-ligated rats, indicating that the volume of distribution, metabolism, and suppression of endogenous renin secretion were similar, then the drinking data would suggest that the BDL rats do not have a greater sensitivity to ANG II.

The additional water and saline intakes attributable to the intravenous infusion of ANG II during sodium depletion were small in all groups. This probably results from a potent inhibition of water and saline intake by hypertension (5). Fitts and Thunhorst (7) infused the same dose of ANG II in intact sodium-depleted rats that had been treated with captopril by intravenous infusion all night to block ANG II synthesis. During ANG II infusion the next day, the rats increased MAP from low (e.g., Table 2) to normal levels and drank both water and saline (7). This thus dose of ANG II is dipsogenic in our stock of rats if the pressor effects are avoided.

The findings to date provide no evidence that a greater hormonal concentration or sensitivity of BDL rats during sodium depletion explains their greater saline intake during ad libitum or stimulated conditions. Learning may contribute to the link between hypotension and increased salt intake in BDL rats without invoking different hormonal responses. Conditioned aversions play a major role in the reduced intake of solutes in BDL rats (16, 18). Such effects were avoided here by initially exposing the rats to the saline solution 2 days after the surgery (16, 17, 19). A conditioned preference for saline may arise when the post-estive effects of saline counteract the unpleasant sequelae of hypotension.

Conditioned preferences have been demonstrated previously (e.g., see Ref. 27), although the reinforcement is usually a caloric load delivered to the gut instead of sodium. The possibility that sodium intake is reinforced in BDL rats by a reversal of hypotension is illustrated in Figs. 1 and 2 as follows. During the diuresis, BDL rats experienced exaggerated hypotension, bradycardia, and arrhythmias. During rehydration the next day, the BDL rats drank more fluid and had considerably more positive changes in MAP than sham-ligated rats. This temporary benefit of drinking saline may enhance future bouts of saline ingestion, such as during repeated depletions of sodium when BDL rats have progressively exaggerated salt appetite (17). Similarly, the gradual doubling of saline intake under ad libitum hydration conditions in BDL rats (16, 17, 19) may result from many trials of temporary relief of minor symptoms of hypotension through small bouts of saline intake. This proposed mechanism for elevated saline intake makes no assumptions about the endocrine state and is therefore consistent with the present data as well as our captopril and ad libitum data (19).

Pressor Regulation

A typical response of MAP during a rapid oral rehydration of extracellular volume, as demonstrated by the sham-ligated rats receiving infusions of D5W vehicle in Fig. 2, is a decrease in MAP of –5–10% after 1 h. Presumably, vasodilation during a rapid intravascular rehydration reduces MAP slightly and prevents massive natriuresis while the saline is absorbed and distributed. The 4-wk BDL rats did not experience this decrease of MAP (Fig. 2), and their ending MAP was about equal to that of the sham-ligated rats. The failure to decrease MAP by 5–10% does not imply that the BDL rats enter a natriuresis, however, because sodium balances of chronic BDL rats are even more positive than those of sham-ligated rats after 2 h of rehydration (17). Thus the BDL rats seem to have recovered, temporarily at least, from their hypotension and reduced effective plasma volume at the end of the rehydra-
tion period, and this temporary recovery may serve to reinforce the saline drinking response.

Others have found that 3-day BDL rats are hypotensive (1, 12–14). We found that BDL rats were not hypotensive until 4 wk after ligation. The difference in the time of onset of hypotension probably results from a difference in the strain of rats used, i.e., Long-Evans versus albino (usually Sprague-Dawley). Clear differences in the regulation of hydration have been noted between Long-Evans and Sprague-Dawley rats (9, 10).

BDL rats are more sensitive than sham-ligated rats to the hypertensive effects of a rapid loss of blood volume (14), and, similarly, the BDL rats in the present study had a marked hypertensive response to a rapid furosemide diuresis. Acute intravenous injections of ANG II produce blunted pressor responses during ad libitum hydration in BDL rats or in CCl₄ cirrhotic rats (1, 2, 21, 22), and, similarly, BDL rats in our study had a reduced pressor response to sustained intravenous infusion of ANG II at both 3 and 4 wk. However, intravenous injections of ANG II did not produce blunted pressor responses in sodium-depleted, captopril-blocked BDL rats. Those data, as well as the normal drinking responses during ANG II infusions, suggest that a defect of ANG II receptors does not contribute to the exaggerated hypotension during the diuresis in 4-wk BDL rats. The difference between the pressor effects of ANG II in captopril-blocked and unblocked BDL rats is unexplained.

An uncontrolled factor that is not considered in many in vitro studies of BDL in rats is the possible difference in the volume of distribution of injected hormone and the actual plasma hormone concentrations after the infusions or injections. Even when BDL rats do not have large amounts of ascites fluid, which was the case with all rats used in this study, the rats may nevertheless have a considerable amount of bile sequestered in the ligated stump of the ligated common bile duct. This fluid accumulation is highly variable between rats and may account for a large proportion of body weight, e.g., 10% in extreme cases. The degree to which injected substances mix with this fluid is unknown. More work needs to be done to understand cardiovascular and behavioral regulation in the chronic BDL rat.

Cholestatic human patients often have hypotension, and a reduced pressor response to physiological stimuli, and an increased susceptibility to hemorrhagic shock during surgery. The BDL rat has been widely used as a model of human cholestatic disease. The experiments reported here demonstrate that hypotension, bradycardia, and arrhythmias can readily be detected during acute natriuresis in chronic BDL rats, thus confirming this aspect of the 4-wk BDL rat cardiovascular system as a potential human model. In addition, the finding that avid saline ingestion by BDL rats temporarily corrects their hypotension supports a possible role for learning in the development of the high-salt preference in BDL rats.

We thank J. W. Harding and J. M. Hanesworth of Washington State University for generously providing the radioimmunoassays for ANG II and Yong Ran Kim, Sherman Louie, Peter Lee, Liz Snyder, and Ed Evans for technical assistance.

This research was supported by National Institutes of Health Research Grant NS-22274 and by National Research Service Award AA-05390. Captopril was a gift from Bristol-Myers Squibb, Princeton, N. J.

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Received 17 November 1997; accepted in final form 3 September 1998.

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