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Renal Response to Isotonic Saline Infusions into Portal and Jugular Vein in Sodium-Loaded, Conscious Rats (41160)

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Abstract. Evidence for the participation of the liver in the control of sodium excretion is controversial. In the present study, Sprague–Dawley rats were fed either a low sodium diet or regular chow plus saline drinking for at least 1 week. The day of the experiment, the unanesthetized, undisturbed animals received infusions of isotonic saline (2% of body weight; 0.2 ml/min) either in a branch of the portal vein or in the jugular vein via chronically implanted catheters. There were no differences in renal sodium handling between portal and systemic infusions in sodium-restricted animals. Both absolute and fractional sodium excretion in sodium-loaded animals receiving portal infusions exceeded those of systemically infused animals at 30 and 60 min. There were no significant differences in hematocrit, serum sodium or potassium concentration, filtered load of sodium, or inulin clearances between groups of animals receiving portal and jugular infusions on either diet. The results provide evidence for a role of the liver in the control of the sodium excretion.

Despite numerous investigations, the existence of a hepatic sodium receptor remains controversial (1–12). Although the initial studies in the dog suggested that infusions of hypertonic saline solutions into the portal vein produced far greater increases in sodium excretion than did similar infusions into a systemic vein (1, 2), more recent studies in nonanesthetized dogs did not confirm these observations (3–6). Studies in the cat (7), rat (8), and rabbit (9, 10), however, support the concept of a portal sodium monitor. Similarly, the data in man are inconclusive. Carey (11) recently demonstrated that normal subjects maintained on a low sodium diet had significantly greater natriuresis after oral than intravenous sodium administration. On the other hand, Gordon and Peart (12) were unable to confirm these results.

In the only studies performed in the rat, Perlmutt *et al.* (8) observed a greater sodium excretion when isotonic saline was infused via the portal as opposed to the caval route. In these studies, however, the sodium intake of the animals was not controlled and plasma values of solutes needed in the assessment of renal sodium handling were obtained in a separate group of animals.

The present studies were designed to evaluate renal function and renal sodium handling in both sodium-restricted and sodium-loaded, conscious rats after portal or systemic administration of isotonic saline. The present results provide evidence for a role of the liver in the control of sodium excretion and suggest that the enhanced sodium excretion after portal administration of saline results from inhibition of renal tubular sodium reabsorption.

Materials and Methods. *Animals.* Female Sprague–Dawley rats (200–250 g) were fed either low salt chow (sodium deficient diet (9 ppm)); ICN Nutritional Biochemicals, Cleveland, Ohio) and distilled water (sodium restricted) or regular chow plus normal saline (sodium loaded) for at least 1 week prior to studies. Twenty-four-hr urine collections were obtained in four animals receiving each diet the day prior to study in order to measure sodium excretion.

Five to 10 days prior to the experiments a nonocclusive plastic catheter (PE-10) was inserted into a jugular vein and/or into a portal vein tributary under ether anesthesia. Figure 1 shows the placement of the catheter in a small branch of the portal vein. The end of each catheter was

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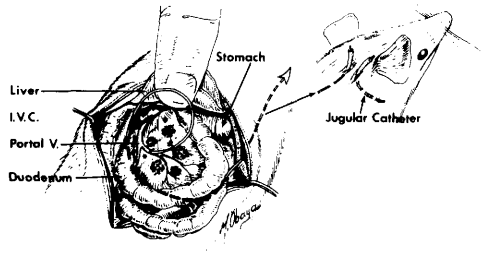

FIG. 1. Diagram indicating placement of catheters in a small branch of the portal vein and in the jugular vein. The catheters were exteriorized through a stab wound in the back of the animal. They remained patent from 1 to 8 weeks.

occluded with a metal rod and was exteriorized through a flank stab wound and secured to the back of the animal as shown in the diagram. The catheters were irrigated periodically with 0.5 ml of 5% dextrose in water. Prior to clearance studies, patency of the catheter was assessed by gentle aspiration while observing for reflux of blood. The correct location of the catheters was also confirmed by performing an autopsy at the end of the experiments.

Experimental procedures. The experimental protocol is shown in Fig. 2. On the day of an experiment, food was withdrawn 6 to 8 hr prior to study. The animals were then lightly anesthetized with ether for insertion of a plastic catheter into the bladder via the urethra. After recovery from anesthesia, rats were placed in a plastic (Lucite) restraining cage and left undisturbed for 30 min. At the beginning of the experiment, a priming dose (0.145 ml/100 g) of [^{14}C]inulin (sp act 1.3 μCi/mg; 2 μCi/ml; Amersham Co., Arlington Heights, Ill.) was administered intravenously. A sustaining infusion (0.010 ml/100 g) was infused into the jugular vein throughout the experiment

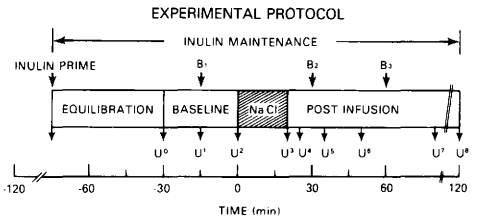

FIG. 2. Experimental protocol. B represents blood sampling and U, urinary collections. The isotonic NaCl infusion (2% of body wt) was begun at zero time.

with a Braun pump (B. Braun Apparatebau, Melsungen, W.G.). After 45 min of equilibration, two 15-min baseline clearance periods were obtained. The results of these two collection periods were averaged and the value will be referred to subsequently as baseline value.

The isotonic saline load (2% of body wt) was given at 0.2 ml/min either into the portal or the jugular vein by means of a Braun pump. The accuracy of the pump was checked intermittently using a graduated cylinder and a stopwatch. Urine collections were obtained at the end of infusion and subsequently at 5, 15, 30, 60, and 120 min for sodium and [^{14}C]inulin measurements. Approximately 120 μl of blood was obtained from the tail artery at the midpoint of the baseline period and at 15 and 45 min after completion of the saline infusions for hematocrit, sodium, potassium, and [^{14}C]-inulin determinations.

The following groups of animals were studied: (1) sodium restricted receiving portal infusions (n = 10), (2) sodium restricted receiving jugular infusions (n = 8), (3) sodium loaded receiving portal infusions (n = 6), and (4) sodium loaded receiving jugular infusions (n = 6).

Because of the difficulties in maintaining catheter patency, only three animals were studied twice (protocols 3 and 4) and one animal was evaluated with all four protocols.

Methods and calculations. Urine and blood samples were analyzed for sodium using a flame photometer (Model 143; Instrumentation Laboratory, Inc.). Hematocrits were measured in blood collected directly into microhematocrit tubes. Samples of plasma and urine (10 μl) were pipetted into plastic counting vials containing 10 ml of Aquasol (New England Nuclear Co.) and [^{14}C]inulin activity was counted using a liquid scintillation spectrometer (Packard Tricarb, Model 3380; Packard Instrument Co., Inc., Downes Grove, Ill.). Fractional excretion of sodium was calculated by the standard formula

$$\frac{\text{urine [sodium]} \times \text{plasma [inulin]}}{\text{plasma [sodium]} \times \text{urine [inulin]}} \times 100.$$

Statistical analysis was performed by either two-way analysis of variance or Student's *t* test where appropriate (13).

Results. There was no significant difference in the weight of the animals on the day of the study (sodium restricted, portal: 230 ± 10 (SE), jugular: 216 ± 5; sodium loaded, portal: 239 ± 9, jugular: 233 ± 15 g). Sodium-restricted animals excreted 8 ± 2 μeq of sodium and sodium-loaded animals excreted 9800 ± 1196 μeq on the day prior to the experiment.

Figure 3 shows urinary sodium excretion ($U_{Na}V$) over time for each of the four experimental groups. As expected, the baseline $U_{Na}V$ tended to be lower in sodium-restricted animals. In these animals there were slight and nonstatistically significant increases in $U_{Na}V$ after the NaCl infusion and the values in animals infused in the portal vein were not different from those in systemically infused animals. In contrast, $U_{Na}V$ increased significantly in both sodium-loaded groups and the excretion rates in animals receiving NaCl infusion in the portal vein exceeded those of rats infused in the jugular vein at 30 and 60 min.

Peak $U_{Na}V$ in the sodium-loaded groups (Fig. 5) was significantly higher after portal

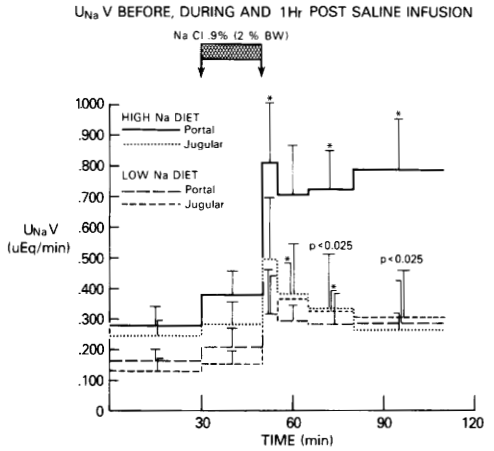

FIG. 3. Urinary sodium excretion in response to saline infusions into portal and jugular veins. Asterisks denote changes statistically different from baseline. *P* values indicate differences between portal and jugular infusions in sodium-loaded rats.

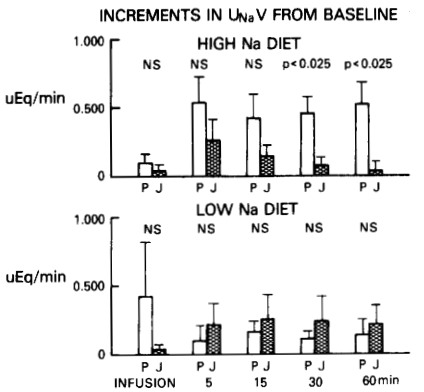

FIG. 4. Increments in $U_{Na}V$ following infusion of isotonic NaCl into portal (P) or jugular (J) veins of sodium-loaded and -restricted rats. Bars represent mean ± 1 SEM.

(1.1 ± 0.2 μeq/min) than after jugular infusions (0.5 ± 0.1 μeq/min; $P < 0.025$). In the sodium-restricted groups, no differences in peak $U_{Na}V$ between portal and jugular infusions were detected. When the data were expressed as increments in $U_{Na}V$ above baseline, the values for portal infusions of sodium-loaded rats exceeded those of systemically infused animals at 30 and 60 min (Fig. 4). No differences between the two groups given a low sodium diet were discerned.

Figure 5 shows total urinary sodium excretion in 60 min expressed as a percentage of the administered dose:

$$\left(\frac{\text{experimental} - \text{baseline value}}{\text{administered dose}} \times 100\right).$$

Sodium-loaded animals infused in the portal vein excreted 5 ± 0.1% of the administered

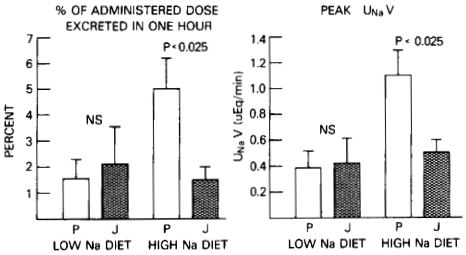

FIG. 5. Percentage of administered dose excreted in 1 hr and peak $U_{Na}V$ following NaCl infusion into portal (P) or jugular (J) vein of sodium-loaded or sodium-restricted animals. Bars represent mean ± 1 SEM.

dose in 1 hr while animals receiving jugular infusions excreted 1.5 ± 0.4% of the load ($P < 0.025$). The differences between portal and jugular infusions in the sodium-restricted groups (portal 1.6 ± 0.7; jugular 2.1 ± 1.5%) were not statistically significant. In three animals in each group in which $U_{Na}V$ was measured during the second hour postsaline infusion, the $U_{Na}V$ values remained similar to those observed between 30 and 60 min (data not shown).

The changes in serum sodium, hematocrit, inulin clearances (C_{IN}) and fractional excretion of sodium (FE_{Na}) during baseline, 0- to 30-, and 30- to 60-min periods are shown in Fig. 6. Hematocrit and serum sodium concentrations did not change significantly in any of the groups (except for a slight drop in hematocrit in the sodium-loaded animals) and the values in animals infused in the portal vein were not different from those in animals infused in the jugular vein. C_{IN} tended to increase in all groups between 0 and 30 min and returned to baseline between 30 and 60 min. There were no differences in C_{IN} between portal and jugular infusions. FE_{Na} increased significantly in both groups of sodium-loaded animals and the values at 0–30 and 30–60 min were significantly higher in animals infused by the portal route than in those infused in the jugular vein. Similar results were obtained when the increments in FE_{Na} were compared (0–30 min, portal: 0.293 ± 0.06%, jugular: 0.133 ± 0.03; 30–60 min, portal: 0.237 ± 0.04, jugular: 0.01 ± 0.01%; ($P < 0.025$ and $P < 0.001$, respectively)). The FE_{Na} increases at 30 and 60 min in the sodium-restricted animals were not statistically significant.

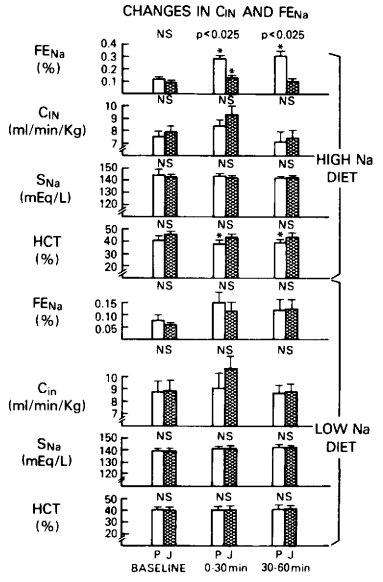

FIG. 6. Changes in renal sodium handling, inulin clearances, serum sodium concentration, and hematocrit at 0–30 min and 30–60 min after NaCl infusions into portal (P) or jugular (J) veins. Asterisks denote values statistically different from baseline. Bars represent mean ± 1 SEM.

There were no differences between the portal and jugular infusions in urine flow rate, filtered load of sodium, or serum potassium concentration. Urine flow rate increased significantly from baseline (two- to fourfold) only in animals on a high sodium intake (data not shown).

Discussion. The present studies were designed to evaluate the effect of varying sodium intake on the renal response to portal and jugular infusions of isotonic saline. We reasoned that prolonged ingestion of a high sodium diet might stimulate the activity of any hepatic factor involved in the regulation of renal sodium excretion, thus, magnifying any differences between portal and systemic infusions of saline. The studies were performed in conscious, undisturbed rats, thus, permitting us to evaluate concomitant changes in renal function without the complicating effects of anesthesia and surgical manipulations.

In animals drinking NaCl, intraportal administration of a saline load produced a twofold greater natriuresis compared to animals receiving the sodium load via the jugular vein. This differing natriuresis occurred in the absence of associated changes in C_{IN}, filtered sodium load, hematocrit, serum sodium, and potassium concentrations. A significantly higher FE_{Na} resulted from the portal infusions indicating that they promoted greater inhibition of tubular reabsorption of sodium than did jugular infusions. In contrast to the sodium-loaded rats, sodium-restricted animals did not manifest a differing natriuresis and exhibited smaller, nonsignificant increases in urinary sodium excretion. We can not

exclude the possibility, however, that the sodium depletion masked any differences in $U_{Na}V$ between portal and jugular infusions.

Our studies confirm and extend the observations of Perlmutt *et al.* (8). These authors found an exaggerated natriuresis within 1 hr after portal infusions of isotonic saline in the rat and no differences between portal and caval infusions of isosmotic glucose suggesting that the responses to saline were not related to the osmotic load. In addition, the changes were not abolished following vagotomy and it was suggested that some humoral factor might mediate the response to isotonic saline.

Previous studies evaluating the role of the liver in the control of sodium excretion have resulted in conflicting results (1–12). These discrepancies might have been related, at least in part, to species differences, use of anesthetized animals in some studies, route of administration and concentration (hypertonic versus isotonic) of the sodium load, and the use of a variable endpoint to quantify sodium excretion. For instance, portal infusions of hypertonic saline in the dog were reported to produce greater natriuresis than similar infusions in a peripheral vein (1, 2). In more recent studies, however, in unanesthetized animals, investigators have been unable to confirm the presence of a hepatic sodium monitor (3–6). Moreover, Hanson *et al.* (6) evaluated renal function in dogs both on low and high sodium intake and found no evidence for a differential natriuresis following a sodium load given by stomach tube. Despite these observations, a large body of evidence in other species is consistent with the interpretation that specific sodium receptors are present in the liver (7–12). The results of the present studies as well as a previous observation (8) suggest that in the rat the liver may play a role in the control of sodium excretion. In addition, it has been shown that partially hepatectomized rats exhibit decreased sodium excretion after saline infusions (14).

The mechanism responsible for changes in renal sodium excretion after portal infusion of salt-containing solutions has not been elucidated. The studies of Milies (15) and the above-mentioned experiments of Perlmutt *et al.* (8) suggest that a humoral factor might mediate the changes. That this factor is not aldosterone is suggested by the time course of events and by the prior findings of Carey (11). Other studies, however, have shown that neural mechanisms may mediate the natriuresis of hepatic origin (7). In addition, electrophysiologic studies support the presence of sodium receptors in the liver (16). Recordings from afferent nerves in rabbit liver showed responsiveness to increases or decreases in sodium concentration. In contrast, no discharge was observed in response to glucose or mannitol suggesting that the receptors were not sensitive to osmotic changes.

In conclusion, we have demonstrated that rats exhibit an augmented natriuresis when infused with isotonic saline in the portal vein as compared with similar infusions via the systemic route. This natriuresis which was manifest in sodium-loaded, but not in sodium-restricted rats, appears to be mediated by inhibition of renal tubular sodium reabsorption. The results provide further evidence, in the rat, for the participation of the liver in the control of sodium excretion.

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