Role of vasopressin in impaired water excretion in conscious rats with experimental cirrhosis

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Role of vasopressin in impaired water excretion in conscious rats with experimental cirrhosis. The present study was undertaken to study the mechanism of impaired water excretion in experimental cirrhosis in the rat. Conscious rats in whom histologically proven cirrhosis was induced with carbon tetrachloride and phenobarbital were compared with control rats given phenobarbital alone. Impaired water excretion in experimental cirrhosis was verified by a basal hyponatremia (138 vs. 147 mEq/liter, $P < 0.005$) and an impaired excretion of water load (minimal urinary osmolality, 262 vs. 100 mOsm/kg; 58 vs. 102% of water load excreted, $P < 0.001$). To clarify the mechanism of this impaired water excretion, we measured glomerular filtration rate (GFR), renal blood flow (RBF), and vasopressin (VP) levels. In cirrhosis, GFR was normal but RBF was decreased (4.5 vs 6.8 ml/min/g, $P < 0.01$). VP levels were found to be higher in cirrhotic rats (1.61 vs. 0.71 pg/ml, $P < 0.025$). The significance of the impaired renal hemodynamics and the increase in VP was assessed by inducing cirrhosis in VP-free Brattle-boro (diabetes insipidus; DI) rats. Despite histologic, biochemical, and renal hemodynamic changes that were comparable to cirrhotic rats with an intact neurohypophysis, cirrhotic DI rats had no impairment in water excretion. To determine the cause of increased VP in experimental cirrhosis, we determined blood volume and systemic hemodynamics. Although plasma volume was greater in experimental cirrhosis (4.3 vs. 3.0 ml/100 g, $P < 0.05$), cirrhotic rats had a significantly lower peripheral resistance (0.37 vs. 0.42 mm Hg/ml/min/kg, $P < 0.05$) and mean arterial pressure (104 vs. 120 mm Hg, $P < 0.001$) than did control rats. These results document that experimental cirrhosis in the rat is associated with impaired renal water excretion in association with both abnormal renal hemodynamics and increased VP levels. The impaired water excretion, however, is solely VP mediated. The nonosmolar stimulus for VP release may be due to abnormal systemic hemodynamics.

Rôle de la vasopressine dans l'altération de l'excrétion de l'eau par le rat conscient atteint de cirrhose expérimentale. Cette étude a été entreprise pour élucider le mécanisme de l'altération de l'excrétion de l'eau au cours de la cirrhose du rat. Des rats conscients chez lesquels une cirrhose prouvée histologiquement a été induite par le tétrachlorure et le phénobarbital ont été comparés à des rats contrôles recevant seulement le phénobarbital. L'altération de l'excrétion de l'eau dans la cirrhose expérimentale a été vérifiée par l'hyponatrémie basale (138 vs. 147 mEq/litre, $P < 0,005$) et le défaut d'excrétion d'une charge en eau (osmolalité urinaire minimale 262 vs. 100 mOsm/kg; 58 vs. 102% de la charge d'eau sont excrétés, $P < 0,001$). Pour élucider le mécanisme de cette altération de l'excrétion de l'eau le débit de filtration glomérulaire (GFR), le débit sanguin rénal (RBF) et les concentrations de vasopressine (VP) ont été mesurés. Dans la cirrhose GFR est normal alors que RBF est diminué (4,5 vs. 6,8 ml/min/gm, $P < 0,01$). VP est plus élevée chez les rats cirrhotiques (1,61 vs. 0,71 pg/ml, $P < 0,025$). La signification des modifications de l'hémodynamique rénale et de l'augmentation de VP a été évaluée en créant des cirrhoses chez des rats sans VP de la souche Brattleboro (DI). Malgré des modifications histologiques, bio-chimiques et hémodynamiques rénales qui sont comparables à celles des rats dont la neurohypophyse est intacte, les rats DI cirrhotiques n'ont pas d'altération de l'excrétion de l'eau. Pour connaître la cause de l'augmentation de VP dans la cirrhose expérimentale le volume sanguin et l'hémodynamique systémique ont été étudiés. Quoique le volume plasmatique soit augmenté dans la cirrhose expérimentale (4,3 vs. 3,0 ml/100 g, $P < 0,05$) les rats cirrhotiques ont des résistances périphériques inférieures (0,37 vs. 0,42 mm Hg/ml/min/kg, $P < 0,05$) et une pression artérielle moyenne inférieure (104 vs. 120 mm Hg, $P < 0,001$) à celles des rats contrôles. Ces résultats indiquent que la cirrhose expérimentale du rat comporte une altération de l'excrétion de l'eau associée à une hémodynamique rénale anormale et à des concentrations de VP augmentées. L'altération de l'excrétion de l'eau, cependant, a la vasopressione comme seul médiateur. Le stimulus non osmolaire de la libération de VP pourrait être l'anomalie de l'hémodynamique systémique.

Derangements of renal function are frequently encountered in patients with cirrhosis [1–5]. Among these derangements, an impairment in renal water excretion that results in an inability to excrete a water load and hyponatremia occurs in a substantial number of individuals with liver disease [4, 6–10]. The potential mechanisms responsible for this defect in water excretion have been the subject of debate for many years.

Much of the controversy has revolved around the issue of whether the defect is due primarily to intrarenal factors such as abnormal delivery of fluid to the distal nephron or whether it is mediated by an extrarenal mechanism involving vasopressin release. The observation that maneuvers such as the infusion of mannitol [11], saline [11], saline plus albumin [12], ascitic fluid [13], and neck immersion [14] improve urinary dilution in patients with cirrhosis does not help clarify the above controversy because they presumably not only improve distal fluid delivery but also expand extracellular fluid volume, a process that would tend to suppress vasopressin secretion via the

baroreceptor pathways. Studies in which the administration of furosemide appear to improve free water excretion in cirrhotic patients [15] lend support to the intrarenal hypothesis because it involves no change or perhaps even a decrease in extracellular fluid volume. But because a similar improvement in free water generation with furosemide is seen in cirrhotic patients receiving exogenous vasopressin infusions, a role for persistent release of the hormone in the diluting defect could not be excluded. Experimental evidence supporting a role for vasopressin has in fact been indirect and has involved improvement of water excretion due to alcohol administration [16], an agent that suppresses antidiuretic hormone (ADH) release [17], and demeclocycline therapy [18], a drug that appears to antagonize the peripheral action of the hormone. Finally, measurements of hormone levels by bioassay in the urine and blood of patients with cirrhosis have yielded conflicting results [19–24].

The recent description of animal models of cirrhosis, the application of a sensitive radioimmunoassay for the measurement of ADH, and the use of rats with the hereditary absence of ADH have provided the opportunity to assess the role of ADH in the cirrhotic rat. In the present study, we demonstrate that cirrhotic rats have a defect in renal water excretion associated with elevated levels of vasopressin. Because cirrhotic rats with a congenital absence of vasopressin display no abnormality in renal water excretion, it is apparent that this hormone is the primary mediator of the impaired dilution of cirrhotic rats with an intact vasopressin secretory mechanism.

Methods

Studies were performed on male Sprague-Dawley (SD) rats and male rats of the Brattleboro strain with hereditary hypothalamic diabetes insipidus (DI). Each rat weighed between 200 and 250 g at the beginning of the study. In DI rats, the absence of vasopressin was supported by the finding of spontaneous urinary osmolality of less than 200 mOsm/kg and the finding that urinary osmolality (U_{Osm}) did not increase above 600 mOsm/kg after 14 hours of fluid deprivation.

Induction of experimental cirrhosis. Hepatic cirrhosis was induced in experimental SD rats by a modification of the methods described by McLean, McLean, and Sutton [25] and Lopez-Novoa et al [26–28]. In this model of cirrhosis, carbon tetrachloride is used as a hepatotoxin. The addition of phenobarbital to the drinking water during the induction of cirrhosis by carbon tetrachloride assures a high yield of cirrhotic animals and shortens the time required to induce cirrhosis [25–28]. The procedure consisted of adding phenobarbital 0.4 g/liter to the drinking water throughout the entire induction process. After 1 week of receiving phenobarbital, rats received carbon tetrachloride, 0.2 cc/kg i.p. After 7 days, inhalation of carbon tetrachloride was initiated. For gas inhalation, rats were placed in a plexiglass chamber (72 × 45 × 46 cm; that is, 150-liter capacity). Compressed air was passed via a flow meter (5 liters/min) bubbling through a flask containing carbon tetrachloride (Fisher Scientific Co., C-570) and into the box. At the time of gas exposure, the carbon tetrachloride vapor was delivered for a given number of minutes followed by an equal number of minutes of compressed air. The animals were dosed twice weekly for at least 6 weeks. Beginning with a 5-min exposure to the gas, the amount of time that animals remained exposed to it

was increased by 1 min every two sessions until by week 6 rats received 5 liters/min of carbon tetrachloride gas for 10 min followed by 5 liters/min of compressed air for 10 min. After this 6-week period of the gas inhalations, phenobarbital and carbon tetrachloride were discontinued, and the animals were allowed deionized water to drink. As has been previously noted [26], the induction process is associated with approximately a 30% mortality. Control animals drank phenobarbital throughout the study but were not exposed to carbon tetrachloride. All rats were allowed commercial rat chow ad lib. Following exposure to carbon tetrachloride and phenobarbital, animals were allowed to recover for at least 14 days before any protocol was initiated.

DI rats were treated in the same manner as the SD rats except that they received daily injections of 100 mU of Pitressin tannate in oil (Parke Davis, Detroit, Michigan) for 1 week prior to beginning phenobarbital and throughout the time of exposure to carbon tetrachloride. From each group of 6 to 10 SD rats, 2 to 4 were studied histologically. Cirrhosis was found in each gas-exposed rat whose liver was histologically examined. In addition, every SD rat that was exposed to the carbon tetrachloride had a defect in water excretion that was quantitated before the animal was used for further studies. Cirrhosis was verified histologically in every DI rat after the completion of the hemodynamic protocols.

Balance studies. Balance studies were undertaken to determine the nutritional status of the cirrhotic animals at the time subsequent protocols were performed. Eight control SD and eight cirrhotic SD rats were placed in individual metabolism cages (Holtge Co., Cincinnati, Ohio) that allow for separation of food, urine, and feces. Animals were fed a diet containing 85 mEq of sodium and 248 mEq of potassium per kg of food. Both food and deionized water were allowed ad lib. After allowing 2 days for adaptation to the cages, the quantities of food ingested, the animal weights, and the urine volume over 24 hours was measured for 5 successive days as has previously been described [29]. After this study, and following the removal of water for 2 hours, the animals were anesthetized by the i.p. injection of phenobarbital (60 mg/kg). Blood was obtained by aortic puncture into heparinized tubes and was analyzed for sodium, potassium, osmolality, SGOT [30], bilirubin [31], alkaline phosphatase [32], albumin [33], and total protein [34]. Liver and kidney slices were obtained and fixed in 10% formalin for subsequent histologic evaluation.

Oral water loads in cirrhotic rats. To demonstrate the nature of the defect in water excretion caused by cirrhosis, we performed water loads in 12 phenobarbital-drinking and 12 cirrhotic SD rats. On each occasion, water loading was performed 2 hours after removing food and water. Animals were lightly anesthetized with ether and, following abdominal massage to assure emptying of their bladders, an oral water load (30 ml/kg body wt of tepid deionized water) was administered by gastric tube. The animals were then placed in individual metabolic cages where each spontaneously voided urine was collected. After 3 hours, following abdominal massage, a final urine sample was obtained. The total volume excreted was recorded, and the sample with the lowest osmolality was recorded as the minimal osmolality.

Water loads were performed on two occasions separated by at least 72 hours. In each rat, the results of both water loads

were comparable. Thus, the mean of the two studies is presented in the Results section. In earlier studies, the results of water loading were not found to be influenced by repeated water loads separated by 72 hours.

Intravenous sodium and water loads in cirrhotic animals. To demonstrate the effect of cirrhosis on renal sodium excretion, identical volumes of i.v. hypotonic solutions were administered to 10 cirrhotic SD and 7 phenobarbital-drinking SD rats. The animals were prepared for study with the use of ether, and jugular venous, carotid arterial, and bladder catheters were inserted. The animals were then allowed to awaken and recover in restraining cages for 30 to 60 min before any protocol was begun. Following recovery, 4% inulin in 0.9% sodium chloride was infused at a rate of 25 μl/min. During the subsequent 45 min, an i.v. water load equivalent to 5% body wt was administered as 2.5% dextrose. Three timed urine collections and a midpoint blood sample were obtained, and the blood was replaced with a equal volume of 0.9% sodium chloride. The resulting diuresis was then augmented by the infusion of 0.45% saline at rates of 0.2, 0.4, and 0.8 ml/min. At each infusion rate, the animal was allowed to equilibrate for 20 min, following which three urine samples and a midpoint blood sample were obtained. No animal had glycosuria or hemolysis. Glomerular filtration rate (GFR) was estimated from the clearance of inulin. Osmolar clearance, free water clearance (C_{H_2O}) and fractional sodium excretion (FE_{Na}) were calculated from standard formulae. Distal sodium delivery was approximated from the sum of $[C_{Na} + C_{H_2O}]$/GFR because this term reflects distal nephron fluid delivery in the absence of vasopressin [35]. All animals were vasopressin-free during the 0.8-ml/min saline infusion as evidenced by a U_{Osm} less than 150 mOsm/kg. Distal nephron sodium reabsorption was approximated by the formula $C_{H_2O}/[C_{Na} + C_{H_2O}] \times 100\%$.

Effect of cirrhosis on plasma arginine vasopressin levels. To correlate observed disturbances in water excretion with changes in plasma vasopressin levels, we obtained plasma for vasopressin from cirrhotic ($N = 8$) and control ($N = 8$) rats. In this study, animals were water loaded and 60 min later were sacrificed by guillotine. Free-flowing blood issuing from the trunk was collected in coded, chilled, heparinized tubes and centrifuged at 0° C. Aliquots of plasma were analyzed for sodium. The remaining plasma (approximately 2 ml) was stored at −20° C and subsequently assayed for arginine vasopressin in a blinded manner [36]. The lower limit of sensitivity of the assay is 0.5 pg/ml when 1 ml of plasma is extracted.

Effects of cirrhosis on water excretion in Brattleboro rats with congenital absence of vasopressin. To determine the contribution of vasopressin to the defect in water excretion, we performed serial water loads in 5 cirrhotic rats with congenital DI. These rats were treated identically to their SD controls with the exception that each animal was water loaded on two occasions prior to beginning phenobarbital and carbon tetrachloride administration. Following hemodynamic studies (vide infra), we obtained plasma samples for subsequent biochemical analysis. All DI rats were proven to have cirrhosis by histologic examination.

Effect of cirrhosis on blood volume, systemic and renal hemodynamics. Blood volume was determined in cirrhotic SD rats ($N = 6$) and control ($N = 7$) SD rats utilizing ^{125}I-albumin by methods described by Thiel et al [37] and Flamenbaum et al
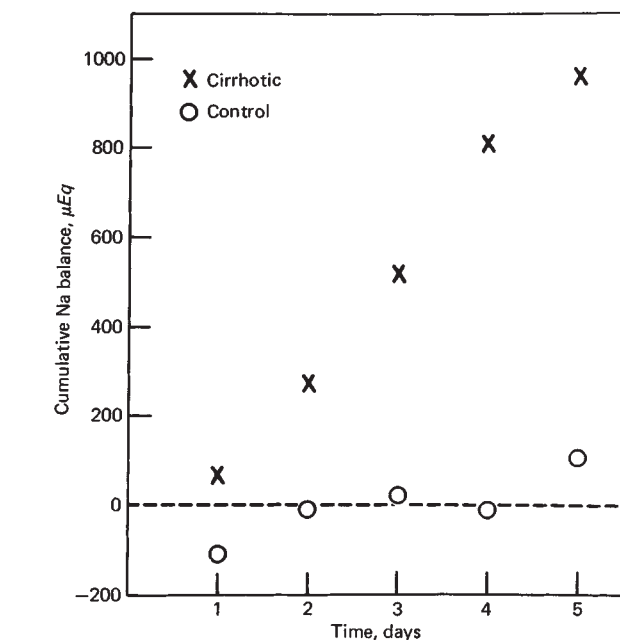

Fig. 1. *Daily cumulative sodium balance in control and cirrhotic Sprague-Dawley rats.*

[38]. A precise volume of ^{125}I-labeled human serum albumin (Mallinckrodt Inc., St. Louis, Missouri) diluted in isotonic saline was given i.v. with a micrometer syringe, and the catheter was flushed with 0.2 ml of saline. The total radioactivity of the administered dose was determined by delivering at least two identical volumes into counting vials. The range of individual samples was less than 3%. Twenty minutes after the injection, 0.4 ml of blood was withdrawn from a previously cannulated carotid artery using a heparinized syringe. Hemotocrits were determined in duplicate samples, and at least two 0.1-ml samples were obtained for isotope counting in a Beckman gamma spectrophotometer.

Cardiac index (CI), systemic vascular resistance (SVR), renal blood flow (RBF), and renal vascular resistance (RVR) were determined by a radioactive microsphere technique that was described originally for rats by Hsu, Kurtz, and Waldinger [39]. Seven conscious cirrhotic and 7 control SD rats were used for study. The microspheres used were 8.8 ± 0.9 μ in diameter and were labeled with strontium 85. In addition, hemodynamic studies were performed in 5 conscious cirrhotic DI and 6 control DI rats. In preliminary studies in conscious, intact SD rats, we determined that CI, SVR, RBF, and RVR were comparable using 8.8-μ and 15-μ microspheres. In further studies in 5 anesthetized SD rats, we obtained a continuous collection of renal venous blood for 2 min following left ventricular injection of 8.8-μ microspheres. Counts obtained on this renal venous blood were not different from background.

Sodium was determined by flame photometry and inulin by standard autoanalyzer methods [40]. Osmolality was determined by cryoscopy with an osmometer (Advanced Instrument 3L). Statistical analyses were performed using the unpaired and paired Student's t test. A P value less than 0.05 was considered significant. Data are expressed as the mean ± SEM.

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Table 1. Summary of plasma chemistries in cirrhotic Sprague-Dawley rats[a]

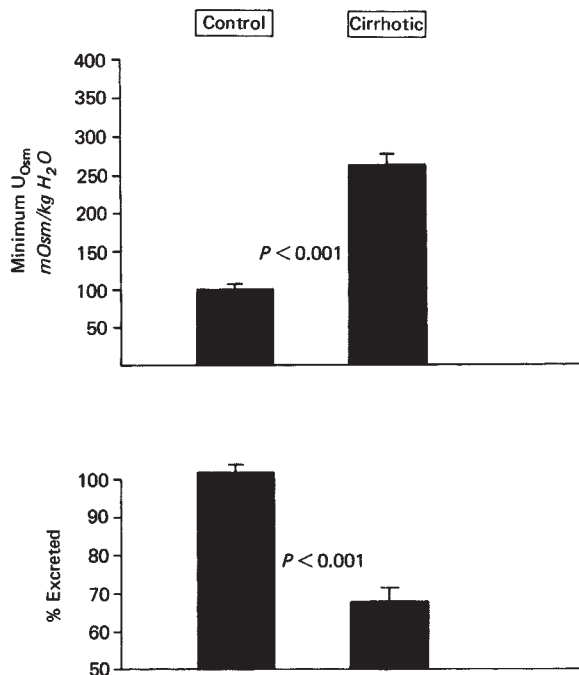|	Bilirubin *mg/dl*	GOT *IU*	Alkaline phosphatase *IU*	Total protein *g/dl*	Albumin *g/dl*	Sodium *mEq/liter*	Plasma osmolality *mOsm/kg H_2O*	Potassium *mEq/liter*
Control ($N = 8$)	0.18 ± 0.03	88 ± 11.5	193 ± 25	5.8 ± 0.08	3.84 ± 0.05	146.6 ± 1.0	292 ± 2.5	4.01 ± 0.13
Cirrhotic ($N = 8$)	0.50 ± 0.08	137 ± 19.0	376 ± 11	6.3 ± 0.10	3.69 ± 0.08	137.9 ± 2.0	273 ± 2.9	3.91 ± 0.10
P	< 0.005	< 0.05	< 0.001	< 0.005	NS	< 0.005	< 0.001	NS

[a] Values are the means $\pm$ SEM.

Fig. 2. *Effect of an acute water load on minimum urinary osmolality and percentage excreted in cirrhotic Sprague-Dawley rats.*

Results

Induction of experimental cirrhosis and balance studies. Histologic examination of livers was similar to that described by other [25–27] and showed moderate portal cirrhosis with fibrosis, periportal chronic inflammation, bile duct proliferation, and destruction of liver architecture with formation of regeneration nodules. Light microscopic evaluation of the kidneys from 12 cirrhotic rats was entirely normal. The histopathology of cirrhotic SD rats was indistinguishable from cirrhotic DI rats. Livers from rats drinking phenobarbital but not exposed to carbon tetrachloride and livers from rats exposed to carbon tetrachloride but not drinking phenobarbital appeared normal on histologic examination.

Although rats appeared ill and lost weight for the 3 to 5 days following i.p. carbon tetrachloride, they appeared well, ate, and gained weight during the 6 weeks of inhalation of carbon tetrachloride. Following exposure to carbon tetrachloride and prior to being subjected to any further experimental protocol, food intake (control, 20.5 ± 1.2 g/day; cirrhotic, 21.4 ± 0.7 g/day) and weight gain (control, 14 ± 1 g per 5 days; cirrhotic, 15 ± 1.5 g per 5 days) were comparable between control and cirrhotic rats. Figure 1 depicts cumulative daily sodium balance over a 5-day period for control and cirrhotic rats. As has been

noted in a recent study [28], cirrhotic rats were in positive sodium balance by 170 μEq/day more than control rats ($P < 0.05$). Thus, it is probable that the weight gain in cirrhotic rats during the balance period was partially due to the accumulation of sodium and water.

Table 1 describes some blood studies obtained from these animals. Cirrhotic rats had mild but significant increases in bilirubin, plasma GOT, alkaline phosphatase, and total protein when compared to control rats. Albumin concentrations were comparable between the two groups of rats. In addition, the spontaneous plasma sodium concentration (137.9 ± 2.0 vs. 146.6 ± 1.0 mEq/liter, $P < 0.005$) and plasma osmolality (273 ± 2.9 vs. 292 ± 2.5 mOsm/kg, $P < 0.001$) were significantly decreased in the cirrhotic rats.

Oral waterloads in cirrhotic rats with an intact neurohypophysis (Fig. 2). The spontaneous hyponatremia observed in the cirrhotic rats suggested a defect in water excretion in these rats. To better define this defect, we administered oral water loads. As is shown in Fig. 2, control rats had a minimum U_{Osm} of 100.4 ± 3.6 mOsm/kg and excreted $102.2 \pm 2.4\%$ of the water load. There was, however, a marked defect in water excretion in cirrhotic rats: minimum U_{Osm} was 262 ± 11.6 mOsm/kg; percentage excreted, $67.5 \pm 3.6\%$; both, $P < 0.001$ vs. control rats. Water excretion was also found to be normal in 5 rats exposed to carbon tetrachloride but not drinking phenobarbital (U_{Osm}, 98.6 ± 4.8 mOsm/kg; percentage excreted, $96 \pm 4.5\%$).

Intravenous sodium and water loads in cirrhotic rats (Table 2). Results obtained during infusion with dextrose and during the period of maximal absolute free water formation following the administration of 0.8 ml/min hypotonic saline are presented in Table 2. GFR was comparable between the two groups during both dextrose and hypotonic saline infusion. During the administration of dextrose, U_{Osm} was 220 ± 13 mOsm/kg in cirrhotic rats, a value significantly greater than the value found in control rats of 97 ± 2.1 mOsm/kg, $P < 0.001$. Mean arterial pressure was significantly decreased in cirrhotic rats (95.0 ± 1.0 vs. 120.4 ± 2.1 mm Hg, $P < 0.001$). Because the values obtained after intravenous administration were similar to those obtained after oral water loading, the possibility of impaired water excretion caused by abnormal water absorption by the intestine was excluded.

Following the infusion of hypotonic saline, cirrhotic rats were able to dilute their urine to the same degree as control rats (cirrhotic, 119 ± 6 mOsm/kg; control, 108 ± 6 mOsm/kg). As opposed to the decrease in mean arterial pressure in cirrhotic rats observed during dextrose infusion, mean arterial pressure was comparable in cirrhotic and control rats during hypotonic saline infusion. Estimated fractional distal nephron fluid delivery as estimated from these clearance studies was decreased in cirrhotic rats (12.2 ± 0.9 vs. $16.8 \pm 1.0\%$, $P < 0.01$), and distal

Table 2. Sodium excretion in cirrhotic Sprague-Dawley rats[a]

	2.5% Dextrose			0.45% Sodium chloride					
	GFR *ml/min/kg*	U_{Osm} *mOsm/kg*	MAP *mm Hg*	GFR *ml/min/kg*	U_{Osm} *mOsm/kg*	MAP *mmHg*	$[C_{Na} + C_{H_2O}]/GFR$ %	$C_{H_2O}/[C_{Na} + C_{H_2O}]$ %	FE_{Na} %
Control (n = 7)	7.4 ± 0.4	97 ± 2	120 ± 2	9.4 ± 0.3	108 ± 6	125 ± 3	16.8 ± 1.0	55 ± 3	7.7 ± 0.4
Cirrhotic (n = 10)	7.3 ± 0.4	220 ± 13	95 ± 1	9.6 ± 0.7	119 ± 6	124 ± 4	12.2 ± 0.9	68 ± 3	4.0 ± 0.4
P value	NS	< 0.001	< 0.001	NS	NS	NS	< 0.01	< 0.02	< 0.01

[a] Data noted is at times of maximal urinary dilution. Values are the means ± SEM. Abbreviations are GFR, glomerular filtration rate; U_{Osm}, urinary osmolality; MAP, mean arterial pressure; C_{Na}, sodium clearance; C_{H_2O}, free water clearance; FE_{Na}, fractional excretion of sodium.
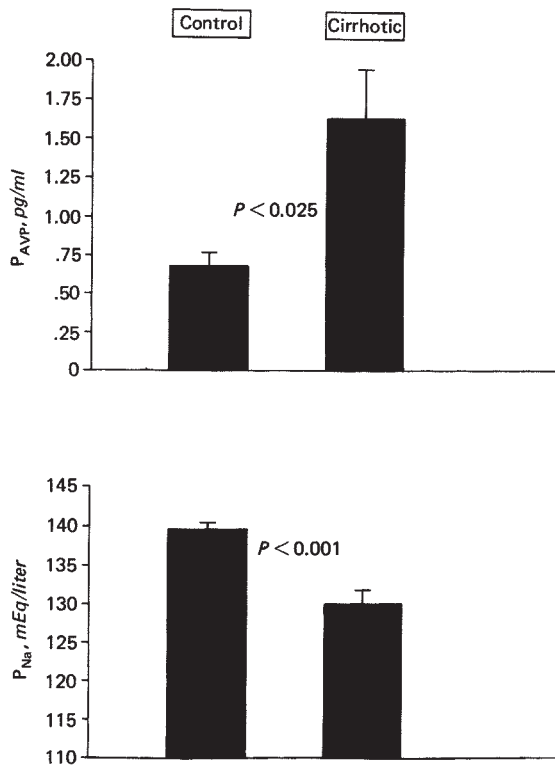

Fig. 3. *Relationship between plasma arginine vasopressin levels and plasma sodium concentration following an oral water load in Sprague-Dawley rats.*

Fig. 4. *Effect of an acute water load on minimum urinary osmolality and percentage excreted in cirrhotic diabetes insipidus rats.*

nephron sodium reabsorption was increased in cirrhotic rats (68 ± 3 vs. 55 ± 3%, *P* < 0.02). The net result of these estimated changes in segmental sodium transport was that fractional sodium excretion was decreased in cirrhotic rats (4.0 ± 0.4 vs. 7.7 ± 0.4%, *P* < 0.01). These observations are thus in agreement with the more direct micropuncture results seen in this experimental model [27]. Thus, when cirrhotic rats were challenged with a sodium load, there was an increase in sodium reabsorption throughout the nephron and a marked decrease in urinary sodium excretion.

Plasma arginine vasopressin levels in cirrhotic rats (Fig. 3). To determine if increased vasopressin levels could account for the observed abnormalities in water excretion, we measured plasma vasopressin levels following an oral water load in cirrhotic rats. Plasma arginine vasopressin levels were significantly elevated in cirrhotic rats when compared to control rats (1.61 ± 0.33 vs. 0.71 ± 0.11 pg/ml, *P* < 0.025). Because the sensitivity of the assay is 0.5 pg/ml, this value was used in
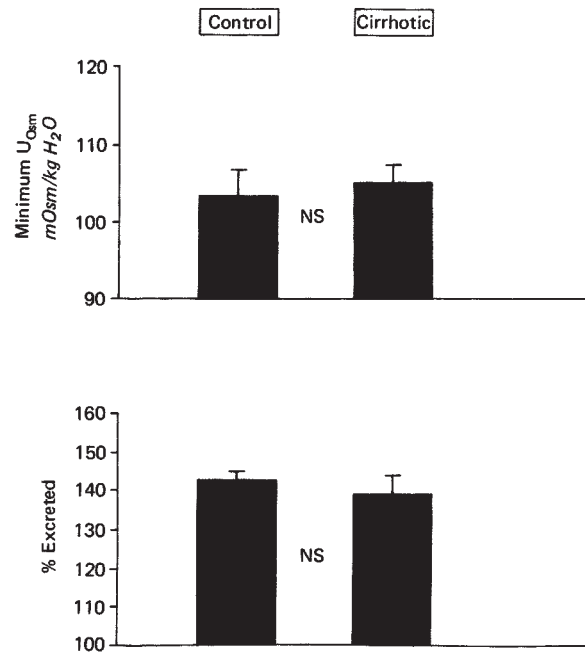
samples reported as undetectable. Such was the case in 4 of 8 controls but not in any cirrhotic rats. The noted means therefore underestimate the difference in plasma arginine vasopressin levels between the two groups. In addition, whereas the mean U_{Osm} of the control rats was 120 ± 0.3 mOsm/kg, only 3 of the cirrhotic rats had spontaneously voided at the time of sacrifice (U_{Osm}, greater than 250 mOsm/kg).

Water excretion in cirrhotic rats with the congenital absence of vasopressin (Fig. 4). To determine the physiologic significance of the elevated vasopressin levels in cirrhotic rats, we performed studies in DI rats. Cirrhotic DI rats had histologic and liver functional anormalities that were comparable to cirrhotic SD rats: bilirubin, 0.54 ± 0.1 mg/dl; GOT, 144 ± 20 IU; alkaline phosphatase, 367 ± 43 IU. Plasma sodium concentration, however, was comparable to untreated DI rats in our laboratory: cirrhotic DI, 145.6 ± 1.03 (*N* = 5); control DI rats, 147.4 ± 1.1 mEq/liter (*N* = 6). Following a water load, control DI rats with the hereditary absence of vasopressin had a minimum U_{Osm} of 103.4 ± 2.9 mOsm/kg and excreted 142.6 ± 2.2% of the water load. In cirrhotic DI rats, water excretion was identical to control DI rats (minimum U_{Osm}, 104.6 ± 2.4

Table 3. Blood volume in cirrhotic Sprague-Dawley rats[a]

	Weight *g*	Blood volume *ml/100 g*	Hematocrit *vol %*	Plasma volume *ml/100 g*
Control (N = 7)	383 ± 8	5.35 ± 0.5	43.8 ± 1.4	3.03 ± 0.25
Cirrhotic (N = 6)	386 ± 18	7.32 ± 0.5	41.6 ± 2.6	4.33 ± 0.45
P	NS	< 0.01	NS	< 0.05

[a] Values are the means ± SEM.

Table 4. Hemodynamic studies in cirrhotic Sprague-Dawley and Brattleboro diabetes insipidus rats[a]

	Mean arterial pressure *mm Hg*	Cardiac index *ml/min/kg*	Systemic vascular resistance *mm Hg/ml/min/kg*	Renal blood flow *ml/min/g*	Renal vascular resistance *mm Hg/ml/min/g*
Sprague-Dawley rats (intact neurohypophysis)					
Control (N = 7)	120 ± 1	283 ± 6	0.42 ± 0.01	6.77 ± 0.13	17.72 ± 0.28
Cirrhotic (N = 7)	104 ± 3	286 ± 15	0.37 ± 0.02	4.46 ± 0.35	24.34 ± 2.22
P	< 0.001	NS	< 0.05	< 0.001	< 0.02
Brattleboro rats (central diabetes insipidus)					
Control (N = 7)	122 ± 2	292 ± 8	0.41 ± 0.01	6.22 ± 0.21	19.6 ± 0.6
Cirrhotic (N = 7)	100 ± 3	288 ± 12	0.35 ± 0.02	4.11 ± 0.32	24.3 ± 1.8
P	< 0.001	NS	< 0.025	< 0.001	< 0.05

[a] Values are the means ± SEM.

mOsm/kg; 139.4 ± 4.8% excreted). Thus, in the absence of vasopressin, water excretion was normal in cirrhotic rats. These results provide strong evidence that the mechanism of the defect in water excretion in cirrhotic rats with an intact neurohypophysis is mediated by vasopressin rather than by a decrease in distal nephron fluid delivery.

Blood volume and hemodynamics in cirrhotic rats (Tables 3 and 4). As is shown in Table 3, both whole blood and plasma volumes were increased in cirrhotic rats. It is possible, however, that although total plasma volume was increased in cirrhotic rats, the albumin distribution space may have differed between the two groups of rats. Despite the probable increase in blood volume, there were marked hemodynamic changes in cirrhotic rats. Mean arterial pressure was decreased in cirrhotic rats (104 ± 3.2 vs. 120 ± 1.2 mm Hg, P < 0.001). This decrease in mean arterial pressure was caused by a decrease in SVR in cirrhotic rats (0.37 ± 0.02 vs. 0.42 ± 0.01 mm Hg/ml/min/kg, P < 0.05) because the CI was comparable to control rats.

Although GFR was comparable to control rats (Table 2), cirrhotic rats had a significant decrease in RBF (4.46 ± 0.35 vs. 6.77 ± 0.13 ml/min/g, P < 0.001), and a significant increase in RVR (24.34 ± 2.2 vs. 17.72 ± 0.28 mm Hg/ml/min/g, P < 0.02).

As compared with control DI rats, cirrhotic DI rats also had significant decreases in mean arterial pressure, SVR, and RBF, whereas there was a significant increase in RVR, thus completely mimicking the hemodynamic changes noted in SD cirrhotic rats.

Discussion

In the present study we systematically investigated the mechanism of the abnormal water excretion in experimental cirrhosis by using a model of the disease in rats. The model used in this study for induction of chronic liver disease results in the development of many of the features observed in patients with early chronic alcoholic portal cirrhosis. Rats exposed to pheno-

barbital and carbon tetrachloride developed mildly abnormal liver function abnormalities and histologic abnormalities consistent with cirrhosis. As in some patients with compensated cirrhosis [41], cirrhotic rats were free of ascites but when challenged with a sodium load were unable to excrete sodium normally (Table 2). The sodium-retaining characteristics of this experimental model of cirrhosis have also been documented by others using both balance and micropuncture techniques [27, 28, 42]. There were also hemodynamic and renal functional abnormalities that are often seen in patients with cirrhosis [1–10, 43–47]. In this regard, blood volume was increased whereas systemic vascular resistance and arterial pressure were significantly decreased. As in early human cirrhosis, GFR was well maintained, RBF was decreased, and RVR was increased. There are differences between this experimental model of liver disease and cirrhosis in patients. In particular, although impaired water excretion has been described in the compensated cirrhotic patient [41], it is uncommon. Moreover, hyponatremia is uncommon in the absence of ascites. Despite these differences, we feel that this is an acceptable experimental model for the study of water excretion. We chose to study rats prior to the development of ascites because at that time the rats appeared well, ate and gained weight at the same rate as control rats. Because, in the absence of ascites, cirrhotic rats developed a reproducible defect in water metabolism without decrements in glomerular filtration, they appeared ideally suited for a study of the mechanism of the defect in water excretion in the absence of a failing kidney.

Although carbon tetrachloride has been reported to cause renal functional abnormalities [48], there was no evidence to suggest that it was a nephrotoxin in this study. As opposed to the renal functional changes that might have been expected to occur in rats with toxic nephropathy, cirrhotic rats had a normal GFR and have enhanced rather than decreased renal sodium reabsorption. Moreover, light microscopic evaluation of kid-

neys obtained from cirrhotic rats was entirely normal. It thus appears that the observed diluting defect is not a consequence of carbon tetrachloride-induced nephrotoxicity. These findings also suggested that in this model of cirrhosis, carbon tetrachloride increases renal vascular resistance indirectly by causing hepatic dysfunction.

The pathogenesis of the impairment in water excretion in cirrhosis has been attributed to either a decrease in the delivery of filtrate to the diluting segment of the nephron or to an increase in vasopressin activity [11–14, 16, 19–24]. Both of these mechanisms could have occurred in cirrhotic SD rats. Distal nephron fluid delivery as estimated by clearance techniques (Table 2) and by micropuncture studies was found to be decreased in cirrhotic rats [27]. In addition to the decrease in distal nephron delivery, plasma arginine vasopressin levels were significantly elevated in cirrhotic rats. To our knowledge, this is the first demonstration of an increase in radioimmunoassayable arginine vasopressin levels in cirrhotic rats or humans following a water load. But, the presence of arginine vasopressin in the circulation does not in itself define the relative roles of the hormone and of the observed intrarenal factors in the pathogenesis of the abnormal excretion of water.

The availability of Brattleboro rats with the hereditary absence of vasopressin allowed for distinguishing which of these two possibilities was the pathogenic mechanism of impaired water excretion in cirrhosis. Cirrhotic DI rats, who had disturbances in systemic and renal hemodynamics that were indistinguishable from cirrhotic rats with an intact neurohypophysis, did not have a defect in water excretion. It is of interest that similar findings have been reported in a patient with cirrhosis and central DI [49]. This patient had a normal response to water loading during severe decompensation. Thus, the defect in water excretion in cirrhotic rats with an intact neurohypophysis was related to vasopressin. Furthermore, the results obtained in cirrhotic DI rats exclude the possibility that the defect in water excretion is due to a vasopressin independent increase in water permeability of the collecting duct in the cirrhotic rats or some other nonspecific consequence of carbon tetrachloride.

The present study also provides some insights as to the mechanism of enhanced vasopressin activity associated with experimental cirrhosis in the rat. Cirrhotic rats had a defect in water excretion associated with elevated levels of plasma vasopressin as measured by radioimmunoassay. This defect in water excretion was accompanied by a diminished plasma sodium concentration, an osmotic effect that alone should have suppressed vasopressin release. Moreover, the elevated levels of vasopressin were associated with an increase in plasma volume, a nonosmotic effect that should also have suppressed vasopressin release [50]. There was, however, a significant decrease in systemic vascular resistance and in arterial pressure, both of which are known nonosmotic stimuli for the release of vasopressin [50]. It would appear likely that the nonosmotic stimulus for vasopressin release in cirrhotic rats was mediated by these decreases in systemic hemodynamics. As in human cirrhosis, the pathophysiologic cause of the decrease in systemic resistance remains to be elucidated.

Another possible mechanism for the increase in vasopressin activity in the cirrhotic rats is that there may have been abnormalities in the metabolic clearance of vasopressin. Although earlier studies suggested that there was no decreased

capacity to inactivate either endogenous or exogenous ADH in cirrhosis [51, 53], recent preliminary studies by Skowsky, et al [54] have demonstrated a decreased metabolic clearance rate of arginine vasopressin in six patients with alcoholic cirrhosis. Although the present study was not designed to assess arginine vasopressin kinetics in rats with cirrhosis, the ability of cirrhotic rats to rapidly and maximally dilute their urine following hypotonic volume expansion with 0.45 M saline would suggest that there is no defect in the metabolic clearance of vasopressin in these rats. Further kinetic studies using the radioimmunoassay will be necessary to determine the contributions of abnormalities in production and clearance to the enhanced vasopressin activity in cirrhotic rats.

In summary, the present results confirm an earlier demonstration that it is possible to produce an experimental model of chronic liver disease in the rat resembling cirrhosis. Cirrhotic rats have impaired water excretion that is associated with impaired renal hemodynamics and increased plasma vasopressin levels as measured by radioimmunoassay. In cirrhotic Brattleboro rats with hereditary hypothalamic DI, there was no defect in water excretion, thus demonstrating that the mechanism of impaired water excretion in cirrhotic rats with an intact neurohypophysis was primarily vasopressin mediated. The findings of this study do not exclude the possibility that changes in renal hemodynamics contribute to impaired water excretion under other circumstances; for example, in the severely decompensated edematous, ascitic patient with cirrhosis. The pathophysiologic mechanism for enhanced vasopressin activity is likely to be mediated by known nonosmotic stimuli, namely, a decrease in peripheral resistance and in arterial pressure.

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