PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 764–770 (1974)

Effect of Renal Denervation and α-Adrenergic Blockade on Sodium Excretion in Dogs with Chronic Ligation of the Common Bile Duct[1] (38189)

CIDIO CHAIMOVITZ, SHAUL G. MASSRY, ROBERT M. FRIEDLER, AND JACK W. COBURN
(Introduced by Alvin Sellers)

From the Medical Research Institute and Division of Nephrology and Department of Medicine, Cedars-Sinai Medical Center, and the Research and Medical Services, Veterans Administration Wadsworth Hospital Center, and the Department of Medicine, UCLA School of Medicine

Evidence has accumulated showing that the sympathetic nervous system and circulating cathecholamines may play a role in the pathogenesis of sodium retaining states (1–4). Thus, Barger *et al.* reported that dibenzyline, an alpha-adrenergic blocking agent, infused into the renal artery of dogs with experimental congestive heart failure produced a significant increase in the excretion of sodium by the infused kidney (1). Furthermore, other studies have demonstrated that the renal sympathetic innervation and the circulating levels of catecholamines may be involved in the sodium retention observed in dogs with acute or chronic constriction of the thoracic inferior vena cava (2, 4).

The studies of Gliedman *et al.* (5, 6) as well as those reported from our laboratory (7) demonstrated that ascites may develop in dogs several weeks after division or ligation of the common bile duct. These observations suggest that a sodium retaining state may develop in such animals. Better and Massry (7) also demonstrated that chronic ligation of the common bile duct in dogs is accompanied by a blunted natriuresis following extracellular volume expansion (ECVE) produced by the infusion of hypotonic saline. The mechanisms underlying the altered renal handling of sodium in dogs with chronic bile duct ligation (CBDL) are not, as yet, elucidated. The present study was undertaken to investigate the effect of renal denervation and α-adrenergic blockade on the response to ECVE in dogs with CBDL.

Methods. Experiments were performed in female mongrel dogs weighing 18–25 kg. Twelve animals underwent a double ligation of the common bile duct 6 wk prior to the study and 6 normal animals were used as controls. Studies were done under light pentobarbital anesthesia, and the animals were ventilated with a Harvard respirator. The ureters were cannulated through bilateral flank incisions, for separate urine collections. A catheter was placed in the left renal vein with the tip directed toward the kidney; this was verified by the injection of a small amount of air. Another catheter was placed into the aorta through the femoral artery for blood pressure monitoring and arterial blood sampling. Blood was obtained simultaneously from the aorta and the left renal vein at the mid-point of each clearance period. Glomerular filtration rate was measured by exogenous creatinine clearance and renal plasma flow with para-aminohippurate clearance, utilizing the standard priming and sustaining infusion

[1] Work was supported by U.S.P.H.S. Grants Am 16093, AM 05630, RR05468, and a grant from the Kidney Foundation of Southern California. This work was presented before the American Physiological Society Meeting, April 15–20, 1973, Atlantic City, New Jersey.

techniques. Two experimental protocols were utilized:

(a) Renal denervation (6 normal dogs and 6 animals with CBDL): After the collection of three control clearance periods of 20–30 min duration, the left renal artery was stripped from all visible nerve fibers and coated with phenol. Two hr were allowed for recovery from the latter manipulation. Then, three urine collections of 20–30 min duration were obtained. Extracellular fluid expansion (ECVE) was produced with the intravenous infusion 0.45% NaCl solution given at progressively increasing rates of 0.5, 1.0, and 1.5 ml/kg body weight per min. The latter rate of infusion was maintained for 60 min after which 3 clearance periods of 10 min each were obtained. The total amount of hypotonic saline given in each study ranged between 130 and 135 ml/kg body weight.

(b) Dibenzyline infusion into the renal artery (6 dogs with CBDL): A 23 gauge needle was placed into the left renal artery in the direction of blood flow. Patency of the renal arterial needle was maintained by a constant infusion of 0.45% at a rate of 0.5 ml per min. After three consecutive control clearance periods of 20–30 min duration, dibenzyline was added to the left renal arterial infusion in amounts sufficient to deliver 3.6 μg per min in 3 dogs and 15 μg per min in the remaining three animals. Three additional urine collections of 20–30 min duration were obtained 1 hr after starting the dibenzyline infusion. While the latter agent was still being infused, ECVE was produced as described above and three clearance periods of 10 min duration were obtained.

Determinations of creatinine, PAH and sodium as well as clearance calculations were performed according to methods previously described (7).

Results. The surgical procedure was well tolerated by the animals. The effects of the ligation of the bile duct on appetite, general well being of the animal and blood chemistries in the dogs of this study were similar to those reported previously (7).

The effects of renal denervation on the clearances of creatinine and PAH, extrac-

TABLE I. Effect of Denervation of the Left Renal Artery and Hypotonic Saline Infusion on the Clearance of Creatinine and Paraaminohippurate and on Sodium Excretion in Normal Dogs.[a]

	C_{Cr} ml/min		C_{PAH} ml/min		E_{PAH} %		$U_{Na}V$ μEq/min	
	L	R	L	R	L	R	L	R
Control	31.1 ± 4.8	28.6 ± 4.9	114.0 ± 18.7	102.2 ± 16.9	75.3 ± 3.2		16.0 ± 3.6	17.0 ± 4.1
Denervation	35.4 ± 7.1	31.2 ± 6.6	110.6 ± 11.2	96.0 ± 12.9	76.0 ± 3.1		21.7 ± 4.7	13.2 ± 4.9
Denervation + saline	38.2 ± 6.7	35.3 ± 7.3	138.3 ± 15.7	132.7 ± 21.2	62.5 ± 7.5		340.5 ± 43.8	324.6 ± 68.3

[a] C_{Cr} = exogenous creatinine clearance; C_{PAH} = clearance ot paraaminohippurate; E_{PAH} = extraction of paraaminohippurate; and $U_{Na}V$ = urinary sodium excretion; L = left kidney (experimental), and R = right (control) kidney. Each data point represents the mean ± SE of results obtained from six dogs.

TABLE II. Effect of Denervation of the Left Renal Artery and Hypotonic Saline Infusion on the Clearance of Creatinine and Paraaminohippurate and on Excretion of Sodium in Dogs with Chronic Bile Duct Ligation.[a]

Dog No.	C_{Cr} ml/min		C_{PAH} ml/min		E_{PAH} %	$U_{Na}V$ μEq/min	
	L	R	L	R	L	L	R
1. Control	21	19	93	97	69	1	1
Denervation	36	35	101	80	74	78	43
Denervation + saline	32	28	90	82	53	159	197
2. Control	27	29	86	95	84	4	4
Denervation	28	24	60	56	84	10	1
Denervation + saline	26	27	102	86	72	123	66
3. Control	48	45	233	209		2	1
Denervation	46	43	121	118		11	8
Denervation + saline	44	45	192	180		53	51
4. Control	33	37	140	158		2	1
Denervation	42	34	105	83		16	11
Denervation + saline	35	32	156	142		60	38
5. Control	43	39	116	108	83	7	7
Denervation	51	41	115	101	83	26	6
Denervation + saline	53	39	168	172	67	61	13
6. Control	24	28	102	109	57	18	34
Denervation	27	31	78	79	63	12	8
Denervation + saline	22	24	88	89	48	3	3
Mean ± SE Control	32.7 ± 4.4	32.8 ± 3.8	128.3 ± 22.3	129.3 ± 18.5	73.3 ± 6.4	5.7 ± 2.6	8.0 ± 5.3
Denervation	38.3 ± 4.0	35.7 ± 2.8	96.7 ± 9.5	86.2 ± 8.6	76.0 ± 4.9	25.5 ± 10.8	12.8 ± 6.2
Denervation + Saline	35.3 ± 3.4	32.5 ± 3.3	132.7 ± 18.3	125.2 ± 18.4	60.0 ± 5.7	76.5 ± 22.7	61.3 ± 28.8

[a] C_{Cr} = exogenous creatinine clearance; C_{PAH} = clearance of paraaminohippurate; E_{PAH} = extraction of paraaminohippurate; and $U_{Na}V$ = urinary sodium excretion; L = left kidney (experimental) and R = right kidney (control). Each data point represents the mean of 3 consecutive clearance periods.

TABLE III. Effect of Dibenzyline Infusion into the Left Renal Artery and Hypotonic Saline Infusion on the Clearance of Creatinine and Paraaminohippurate and on Sodium Excretion in Dogs with Chronic Bile Duct Ligation.[a]

Dog No.	C_{Cr} ml/min		C_{PAH} ml/min		E_{PAH} %	$U_{Na}V$ μEq/min	
	L	R	L	R	L	L	R
7. Control	36	37	67	126	84	10	7
Dibenzyline	38	38	71	115	82	17	4
Dibenzyline + saline	45	47	74	127	75	91	64
8. Control	10	15	37	37	66	4	3
Dibenzyline	15	17	33	38	66	18	7
Dibenzyline + saline	19	22	42	46	56	48	36
9. Control	22	20	75	80		18	14
Dibenzyline	26	24	82	78		34	14
Dibenzyline + saline	24	25	118	107		105	109
10. Control	15	22	60	77	77	1	2
Dibenzyline	21	27	83	94	73	5	2
Dibenzyline + saline	18	23	101	111	56	61	35
11. Control	23	27	79	82		3	2
Dibenzyline	31	28	87	69		20	5
Dibenzyline + saline	33	34	123	116		207	207
12. Control	20	19	71	70	67	10	6
Dibenzyline	23	25	76	74	65	25	5
Dibenzyline + saline	25	26	113	106	54	196	107
Mean ± SE Control	21.0 ± 3.6	23.3 ± 3.2	65.8 ± 6.2	78.6 ± 11.6	73.5 ± 4.3	7.7 ± 2.6	5.7 ± 1.9
Dibenzyline	25.8 ± 3.4	26.5 ± 2.8	72.0 ± 8.1	78.0 ± 10.5	71.5 ± 3.9	19.8 ± 3.9	6.2 ± 1.7
Dibenzyline + Saline	27.3 ± 4.1	29.5 ± 3.9	95.2 ± 12.8	102.2 ± 11.6	60.3 ± 4.9	117.8 ± 27.7	93.0 ± 26.3

[a] C_{Cr} = exogenous creatinine clearance; C_{PAH} = clearance of paraaminohippurate; E_{PAH} = extraction of paraaminohippurate; and $U_{Na}V$ = urinary sodium excretion; L = left kidney (experimental) and R = right kidney (control). Each data point represents the mean of 3 consecutive clearance periods.

tion of PAH and sodium excretion in normal dogs and in those with CBDL are presented in Tables I and II, respectively. The results of the studies with the infusion of dibenzyline into the renal artery are given in Table III. Renal denervation did not produce significant changes in creatinine and PAH clearances and in PAH extraction both in normal dogs and in those with CBDL. In the latter groups of animals, renal denervation was associated with an increase in sodium excretion; however, the mean difference between urinary sodium excretion from the denervated and the contralateral kidney was small, 12.7 ± 5.1 (SE) μEq/min, ($P < .05$). These findings were similar to those observed from the denervated kidney of the normal dog; urinary sodium excretion from the denervated kidney was 8.5 ± 2.9 μEq/min greater than that from the control kidney ($P < .05$).

The rates of sodium excretion observed after ECVE from both the denervated and the contralateral kidneys of dogs with CBDL were significantly lower ($P < .01$) than the values observed in normal animals tested similarly. Furthermore, during ECVE, sodium excretion from the denervated kidney (76.5 ± 22.7 μEq/min) was not different from that of the contralateral kidney (61.3 ± 28.8 μEq/min) in dogs with CBDL. Also, in the normal dogs, ECVE was not associated with a greater magnitude of natriuresis from the denervated kidney in comparison to the contralateral one.

The infusion of dibenzyline into the renal artery of dogs with CBDL did not produce a significant change in creatinine and PAH clearance and in PAH extraction. Sodium excretion increased from 7.7 ± 2.6–19.8 ± 3.9 μEq/min ($P < .01$). Also, the mean difference in sodium excretion between the infused and the contralateral kidneys was significant (13.7 ± 2.6 μEq/min, $P < .01$). The infusion of dibenzyline, however, did not cause a significant improvement in the natriuretic response to ECVE. The excretion of sodium from the infused kidney was not significantly different from that of the contralateral one.

During ECVE, the fractions of filtered sodium excreted from the denervated kidney and that from the kidney infused with dibenzyline in dogs with CBDL were each significantly less ($P < .01$) than the fractions of filtered sodium excreted from either intact or denervated kidneys of the normal dogs (Fig. 1).

Discussion. The results of the present study indicate that renal denervation was associated with a slight but significant increase in the excretion of sodium in both normal dogs and animals with CBDL. A similar change in sodium excretion was also noted after α-adrenergic blockade with dibenzyline in dogs with CBDL. However, both renal denervation or the infusion of dibenzyline into the renal artery did not correct the blunted natriuresis following ECVE in dogs with CBDL. Urinary excretion of sodium and its fractional excretion from the experimental kidney were not different from values observed from the control kidney, and these values were significantly lower than those noted in normal dogs undergoing similar degrees of ECVE. Also urinary excretion of sodium and its fractional excretion from the experimental and the control kidneys in dogs with CBDL of the present study were not different from those observed previously in such animals in our laboratory (7).

Several observations indicate that increased sympatho-adrenal activity may play an important role in various sodium retaining states. Thus, Barger *et al.* (1) were able to produce a substantial increase in sodium excretion by the infusion of dibenzyline

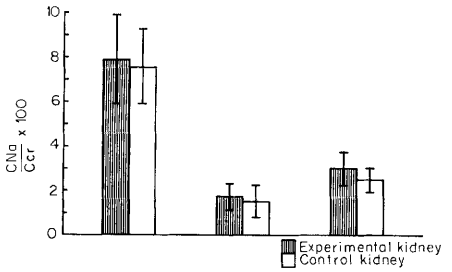

FIG. 1. Fractional excretion of sodium ($C_{Na}/C_{cr} \times 100$) from the experimental and control kidney of normal and CBDL dogs. Denervation or infusion of dibenzyline into the renal artery was performed in the experimental kidney.

into one renal artery of dogs with congestive heart failure. The difference in sodium excretion between the experimental and control kidneys in their study was 7–10 times greater than that observed in our dogs with CBDL receiving a similar intrarenal infusion of dibenzyline. Gill *et al.* (2) reported that the intravenous administration of pentolinium, an autonomic ganglion blocker, to dogs with constriction of the thoracic inferior vena cava significantly increased sodium excretion, from a mean of 5 ± 2–56 ± 23 μEq/min. Pentolinium also significantly improved the response to saline infusion in these dogs; sodium excretion was 32 ± 8 μEq/min without pentolinium and 165 ± 47 during the administration of the agent. Recently, Azer, Gannon and Kaloyanides reported data indicating that renal denervation produced marked improvement in the natriuretic response to saline infusion in dogs with acute constriction of the thoracic inferior vena cava (4).

Although the results of the present study demonstrate that renal denervation or α-adrenergic blockade in dogs with CBDL produced a slight but significant increment in urinary sodium excretion, these changes were not different from those observed after renal denervation in normal animals. Also these procedures did not improve the response to saline infusion in our animals. These data indicate that the sympatho-adrenal activity does not play a paramount role in the salt retaining state that follows CBDL.

The mechanisms underlying the salt retention and the blunted natriuresis after saline infusion in dogs with CBDL are not yet delineated. Histological studies of liver of these animals, 6–8 wk after bile duct ligation, revealed cholestasis, centrilobular degeneration of liver cells, septal fibrosis and inflammatory cell infiltration (unpublished data). These changes are similar to those seen in the precirrhotic stage of liver disease. Patients with cirrhosis of the liver may display abnormalities in renal sodium handling similar to those observed in dogs with CBDL (8, 9). It appears that a normal liver with intact circulation and biliary drainage is important for normal sodium homeostasis. Certain observations support this postulate. First, the magnitude of natriuresis following a saline load to the dog is greater when the infusion is administered into the portal vein than into a systemic vein (10, 11). Second, the exclusion of the liver from the circulation in dogs diminishes or abolishes the natriuretic response to saline infusion (12). Recently Melman and Massry (13) reported preliminary data indicating that saline infusion does not produce renal vasodilatation in dogs with CBDL, although the renal arteries of these animals do respond to a renal vasodilator such as acetylcholine. These authors suggested that, at least, part of the blunted natriuretic response to saline infusion is due to inability of the latter to cause renal vasodilatation in animals with CBDL. They postulated that a circulating substance normally produced by the intact liver during saline infusion is responsible for renal vasodilatation and is not elaborated by the diseased liver in these animals.

Summary. Chronic ligation of the common bile duct (CBDL) is dogs causes sodium retention and a blunted natriuretic response to extracellular volume expansion (ECVE). Since increased sympatho-adrenal activity plays an important role in other sodium retaining states, the present study was undertaken to evaluate the role of renal denervation and α-adrenergic blockade on the renal handling of sodium in dogs with CBDL. Both renal denervation and the infusion of dibenzyline into the renal artery produced a slight but significant increment in urinary sodium excretion, but these changes were not different from those observed after renal denervation in normal animals. Also, these procedures did not improve the response to saline infusion in dogs with CBDL. These data indicate that the sympatho-adrenal activity does not play an important role in the salt retaining state that follows chronic ligation of the common bile duct.

Anne Harrington and Miriam Bick provided technical help and Mrs. Catherine Hoyt provided secretarial assistance.

1. Barger, A. C., Muldowney, F. P., and Liebowitz, M. R., Circulation **20**, 273 (1959).

2. Gill, J. R., Jr., Carr, A. A., Fleischmann, L. E., Casper, A. G. T., and Bartter, F. C., Amer. J. Physiol. **212**, 191 (1967).

3. Gill, J. R., Jr., and Casper, A. G. T., J. Clin. Invest. **48**, 915 (1969).

4. Azer, M., Gannon, R., and Kaloyanides, G. G., Amer. J. Physiol. **222**, 611 (1972).

5. Gliedman, M. L., Carroll, H. J., Popovitz, L., and Mullane, J. F., Surg. Gynecol. Obstet. **131**, 34 (1970).

6. Mullane, J. F., and Gliedman, M. L., Surg. Res. **10**, 11 (1970).

7. Better, O. S., and Massry, S. G., J. Clin. Invest. **51**, 402 (1972).

8. Schedl, H. P., and Bartter, F. C., J. Clin. Invest. **39**, 248 (1960).

9. Earley, L. E., and Martino, J. A., Circulation **42**, 323 (1970).

10. Daly, J. J., Roe, J. W., and Harrocks, P., Clin. Sci. (London) **33**, 481 (1967).

11. Strandhoy, J. W., and Williamson, H. E., Proc. Soc. Exp. Biol. Med. **133**, 419 (1970).

12. Mullane, J. F., Hollman, T., McNamara, J. J., and Yhap, E. O., Clin. Res. **29**, 541 (1971).

13. Melman, A., and Massry, S. G., Clin. Res. **21**, 284 (1973).

Received Feb. 25, 1974. P.S.E.B.M., 1974, Vol. 146.