Pathogenesis of salt retention in dogs with chronic bile-duct ligation

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(Received 13 November 1980/18 May 1981; accepted 11 June 1981)

Summary

1. The present study investigates the role of mineralocorticoids in the pathogenesis of salt retention and ascites in dogs with chronic ligation of the common bile duct (CBDL).

2. After CBDL the natriuretic response to an intravenous sodium load [0.9% sodium chloride solution (150 mmol/l): saline; 10% of body weight] was markedly depressed. Urinary sodium excretion was 285 ± 62 vs 960 ± 58 μmol/min in the control period before CBDL (P < 0.001). This antinatriuresis was associated with a significant rise in plasma aldosterone concentration, from 52.5 ± 5.5 pg/ml before CBDL to 177 ± 50 pg/ml after CBDL (P < 0.02). Ascites was present in all salt-retaining CBDL dogs.

3. Bilateral adrenalectomy resulted in disappearance of ascites and in a rise in the natriuretic response to extracellular volume expansion. Urinary sodium excretion was 770 ± 124 μmol/min, a value significantly higher than in the CBDL dogs with intact adrenals (P < 0.001). Sodium balance studies in the adrenalectomized CBDL dogs during chronic deoxycorticosterone acetate (DOCA) treatment (25 mg/day) showed that in these animals there was failure to escape from the mineralocorticoid-induced sodium retention. Glomerular filtration rate and renal plasma flow did not change during the studies.

4. The present evidence supports the thesis that sodium retention in the CBDL dog results from a dual mechanism: (a) excess of circulating aldosterone and (b) an extra-adrenal factor which prevents escape from the salt-retaining effect of mineralocorticoids, in the CBDL dogs, thereby perpetuating the antinatriuresis in these animals.

Key words: aldosterone, liver, sodium excretion.

Abbreviations: ANG I, angiotensin I; CBDL, chronic bile-duct ligation; DOCA, deoxycorticosterone acetate; PAH, p-aminohippurate; PRA, plasma renin activity.

Introduction

Chronic bile-duct ligation (CBDL) in dogs is associated with avid sodium retention, formation of ascites and a blunted natriuretic response to acute extracellular volume expansion [1-3]. The derangements of renal function and of water and salt balance in CBDL dogs, therefore, resemble those observed in patients with cirrhosis of the liver [4, 5]. Previous studies have failed to elucidate the mechanisms responsible for the sodium and water retention in the CBDL dogs [3, 6-8]. Specifically, the antinatriuresis of these animals could not be explained by changes in renal haemodynamics since the glomerular filtration rate, renal plasma flow and intracortical distribution of renal blood have been shown to be preserved in the CBDL dogs [3, 8]. Nor can the antinatriuresis be attributed to increased renal sympathetic tone, since renal denervation did not correct the marked sodium retention of these animals [6]. It is important to determine therefore whether alterations in the plasma level of a humoral factor are the initiating step in the pathogenesis of salt retention in CBDL dogs.
Since hypersecretion of aldosterone is of primary importance in the pathogenesis of a number of sodium-retaining disorders, including thoracic caval constriction [9-11], the possibility that a similar mechanism operates in the CBDL dogs deserved exploration. The present studies were undertaken to define the possible role of increased adrenocortical activity in the renal sodium retention of the CBDL dogs.

Methods
Studies were conducted in five conscious trained female mongrel dogs weighing between 17 and 25 kg. The dogs were fed on a constant diet throughout the study, containing 70 mmol of sodium/day which they consumed entirely. Water was allowed ad libitum. The presence of ascites as a gross index of sodium retention was monitored throughout the study. In addition, the renal handling of sodium was assessed after 7 days of equilibration on the diet and in all subsequent stages of the experiment by the natriuretic response to an acute extracellular volume expansion. This is a well-established and useful tool for assessing renal sodium excretion in this experimental model [3, 6]. In the conscious animals two foreleg veins were catheterized, one for saline infusion and the other for inulin and p-aminohippurate (PAH) infusion. A 10% body weight volume expansion was accomplished with NaCl solution (0.9%; 150 mmol/l; saline) given intravenously at a rate of 15 ml/min. At the end of the saline load a 20 min clearance collection was obtained through a bladder catheter while the saline infusion was adjusted to match urine flow. Glomerular filtration rate and renal plasma flow were estimated from the clearance of inulin and PAH respectively.

Two days after extracellular volume expansion, ligation of the common bile duct was performed in all five animals by a technique described previously [3]. Five weeks after this procedure the appearance of sodium retention was monitored weekly by the magnitude of the natriuretic response to extracellular volume expansion as described above.

After the appearance of salt retention, characterized by a blunted natriuretic response to extracellular volume expansion and the appearance of ascites, bilateral adrenalectomy was performed under anaesthesia through a midline abdominal incision. Since some ascitic fluid was lost during the procedure, intra-operative fluid replacement was undertaken to maintain systemic arterial pressure. A daily hormonal replacement with 7-5 mg of prednisone and 0.5 mg of deoxycorticosterone acetate (DOCA) was given to the animals [9]. For the first 2 postoperative days the animals also received 50 mg of hydrocortisone daily. Completeness of the adrenalectomy was assessed by measuring plasma cortisol concentration at the end of all the experiments after withdrawal of replacement treatment. On day 6 (postadrenalectomy) an intravenous saline load (extracellular volume expansion) was again performed.

Finally, renal escape from the sodium-retaining effects of a large dose of mineralocorticoid was studied in the five adrenalectomized CBDL dogs. For this purpose the animals were placed in metabolic balance cages and daily 24 h urinary collections and venous blood samples were analysed for sodium, potassium and creatinine. After 3 days of control studies the dose of DOCA was increased from 0-5 to 25 mg/day, which was given intramuscularly for 6 consecutive experimental days. It has been shown previously that in normal dogs on a similar sodium intake there is escape from the sodium-retaining effect of a chronic large dose of DOCA after 2 or 3 days [12]. On the last day extracellular volume expansion was performed by means of the saline load as described above.

Peripheral plasma renin activity (PRA) and aldosterone concentration were determined in the five dogs before CBDL and after sodium retention became evident in these animals. To establish the temporal relationship between the appearance of renal sodium retention and changes in the PRA and plasma aldosterone concentration these factors were also determined weekly after CBDL in three of the five dogs. Sodium, potassium, creatinine, inulin and PAH were determined by standard techniques as described previously [13]. Venous blood PRA and aldosterone concentration were determined by radioimmunoassay [13]. Values are reported as means ± SEM. Analysis of variance was used to determine statistical significance; P < 0.05 was considered the upper limit of significance.

Results
Effect of CBDL on the natriuretic response to extracellular volume expansion

All the animals tolerated CBDL well and became jaundiced. The effect of CBDL on liver function was as reported previously [3].

Sodium retention as monitored by the natriuretic response to extracellular volume expansion appeared 6–8 weeks after CBDL. After extracellular volume expansion in the salt-
Salt retention in experimental liver damage

Fig. 1. Rate of urinary sodium excretion, inulin (glomerular filtration rate, GFR) and PAH (renal plasma flow, RPF) clearance after extracellular volume expansion in all phases of the study: control, before CBDL; CBDL, after bile-duct ligation; Adrenx/CBDL, adrenalectomized bile-duct-ligated dogs under physiological DOCA replacement; Adrenx/CBDL + DOCA, adrenalectomized bile-duct-ligated dogs under chronic DOCA (25 mg/day).

Retaining CBDL dogs the rate of sodium excretion was 285 ± 72 μmol/min, a value significantly lower than that (960 ± 97 μmol/min) in the control period (P < 0.001) (Fig. 1). The clearance of inulin (82 ± 6 ml/min) and the clearance of PAH (378 ± 38 ml/min) were not significantly different from the original control values (87 ± 6 and 377 ± 31 ml/min respectively). Ascites was clearly present in all the salt-retaining animals. The filtered load of sodium of the CBDL dog of 11.5 ± 1.2 mmol/min was not statistically different from the control value of 11.8 ± 1.3 mmol/min.

Mean plasma aldosterone concentration was increased to 177 ± 50 pg/ml in the salt-retaining CBDL dogs from a control value of 52.5 ± 5.5 pg/ml (P < 0.02). As in a previous study [14], the mean PRA in the salt-retaining animals (2.5 ± 0.16 ng of ANG I/ml) was not significantly different from the control value (2.8 ± 0.7 ng of ANG I/ml) (Fig. 2). Sequential measurements of plasma aldosterone concentrations after CBDL revealed a close temporal relation between the increase in plasma aldosterone levels and the appearance of salt retention and ascites. PRA remained similar to the values before CBDL.

Effect of bilateral adrenalectomy on the natriuretic response to extracellular volume expansion in the CBDL dogs

The animals recovered from the adrenalectomy uneventfully and ate their regular diet. Signs of adrenal insufficiency such as apathy, weakness
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and hyperkalaemia were not observed. After bilateral adrenalectomy, ascites disappeared and after extracellular volume expansion there was a significant increase in the rate of sodium excretion in all the dogs (770 ± 124 μmol/min) compared with the values in the CBDL dogs with intact adrenals (P < 0.001) (Fig. 1). This natriuresis occurred in the face of a decrease in filtered load of sodium, which was not, however, statistically significant (10.9 ± 2.7 mmol/min in CBDL adrenalectomized vs 11.5 ± 1.2 mmol/min in CBDL animals). Although this natriuresis was somewhat smaller than that observed in the pre-CBDL control period, the difference was not significant by either analysis of variance or Student's paired t-test. The inulin and PAH clearances were not significantly different from the control study, averaging 78 ± 1.9 and 392 ± 34 ml/min respectively.

Effect of a pharmacological dose of DOCA on sodium balance of the adrenalectomized dogs

When the adrenalectomized CBDL dogs were given 25 mg of DOCA/day, a progressively positive sodium balance was observed throughout the 6 experimental days (Fig. 3). The dogs failed to show escape from the sodium-retaining effect of mineralocorticoid administration and ascites reaccumulated in all the animals. The large-dose DOCA therapy resulted in a marked antinatriuresis, urinary sodium excretion averaging 296 ± 69 μmol/min, when the adrenalectomized CBDL dogs were studied with an acute saline load (Fig. 1). This blunted natriuretic response to extracellular volume expansion did not differ significantly from the value of 285 ± 72 μmol/min recorded in the CBDL dogs with intact adrenals. In these DOCA-treated animals the filtered load of sodium was 11.6 ± 0.9 mmol/min, a value similar to 11.5 ± 1.2 mmol/min in the CBDL animals before adrenalectomy. The clearances of inulin and PAH (84 ± 5.8 and 378 ± 43 ml/min respectively) did not change significantly when compared with the original control values before CBDL. The discontinuance of DOCA administration was associated with disappearance of ascites in the ensuing 7–10 days. Plasma sodium, potassium and albumin concentrations remained in the normal range in all the animals throughout the experiments.

Discussion

The CBDL model has been utilized to examine the mechanisms of renal sodium retention in chronic liver disease [1–3]. The antinatriuresis in these animals is not attributable to major renal haemodynamic alterations, as glomerular filtration rate and renal plasma flow are preserved both before and after an acute saline load [2, 3, 6]. Likewise, intrarenal blood flow distribution is not altered in the CBDL dogs [8]. We have shown previously that an augmented renal sympathetic tone does not mediate the sodium retention in these animals, since this phenomenon was not corrected by renal denervation or α-adrenoceptor blockade [6]. Furthermore, the normal plasma albumin concentration, normal renal plasma flow, normal renal perfusion pressure and filtration fraction [3, 6, 7] found in the CBDL dogs suggest that factors other than peritubular physical forces may mediate the antinatriuresis in this model.

Our attention must therefore be focused on the importance of circulating humoral factors in the pathogenesis of the salt-retaining state associated with CBDL in dogs. As the use of aldosterone antagonists in nonazozaemic cirrhotic patients has resulted in the loss of oedema and ascites [15, 16], our first step was to study the role of mineralocorticoids in the CBDL model.

The results of the present study demonstrate that bilateral adrenalectomy abolishes the
sodium-retaining state of the CBDL dogs. Thus, after adrenalectomy, a normal natriuretic response to acute saline load was demonstrated in these dogs and ascitic fluid did not reaccumulate. This correction of the antinatriuresis by adrenalectomy occurred in the face of a slight though not statistically significant decrease in the filtered load of sodium. Furthermore, our data show that the adrenal hormone responsible for the sodium retention in the CBDL dogs is a mineralocorticoid, since chronic treatment with pharmacological doses of DOCA reproduced both a blunted natriuresis during volume expansion and ascites formation. Therefore, the present findings support the view that the hyperaldosteronism found in the CBDL dogs is a key factor in the pathogenesis of renal sodium retention in these animals.

Since plasma aldosterone concentration is dependent on both the rate of secretion by the adrenal gland and the rate of inactivation by the liver, the extent to which either of these two mechanisms is responsible for the hyperaldosteronism in the CBDL dogs remains unclear. Several lines of evidence from this study, however, argue against the importance of low hepatic clearance in the aetiology of the hyperaldosteronism in the CBDL dogs. Although marked impairment in liver function could be demonstrated as early as 2 weeks after CBDL, the increase in circulating aldosterone concentration was detected much later concomitantly with signs of sodium retention. Moreover, a low metabolic clearance as a single explanation for the hyperaldosteronism in CBDL dogs is unlikely, since the resulting hypokalaemia and suppressed PRA would interrupt aldosterone secretion via the homeostatic feedback-control mechanism. Furthermore, features such as salt retention leading to ascites and oedema, a non-suppressed PRA and normal arterial blood pressure found in the CBDL dogs are characteristic of secondary hyperaldosteronism.

The hyperaldosteronism cannot, therefore, fully be explained by a decreased metabolic clearance and factors which may enhance aldosterone biosynthesis must be invoked. Since neither PRA nor serum potassium was elevated in our animals, the mechanism of this enhanced synthesis remains unclear. CBDL dogs have a low systemic peripheral vascular resistance [7] and it is likely that their effective blood volume is decreased. It is attractive, therefore, to speculate that, as in hypovolaemic subjects, the sensitivity of the adrenal cortex to angiotensin II is enhanced [17]. Such a mechanism would explain the high aldosterone levels of our animals in the presence of normal PRA as well as a similar dissociation between aldosterone and PRA noted by Wilkinson et al. [18] in cirrhotic patients receiving a β-adrenoceptor antagonist.

Regardless of the mechanism of hyperaldosteronism, its importance in the genesis of salt retention and ascites rests on the demonstration of an absence of the renal escape to a chronic mineralocorticoid effect. Thus we have demonstrated that adrenalectomized CBDL dogs, unlike control animals, sustain persistent sodium retention with DOCA, leading to the reaccumulation of ascites. The lack of a mineralocorticoid escape in these experimental animals implicates extra-adrenal mechanisms, which, in addition to hyperaldosteronism, contribute to salt retention in the CBDL dogs. This finding in our dogs supports previous data showing that patients with cirrhosis fail to manifest mineralocorticoid escape [19, 20].

The mechanisms whereby in the CBDL dogs there is failure to escape from the chronic mineralocorticoid-induced sodium retention remain obscure. The results of our studies are in accordance with those employing other experimental models of oedema formation. All such models, including dogs with thoracic inferior vena cava constriction [9], dogs with congestive heart failure due to pulmonary artery constriction [10], animals with high-output failure due to aortic-caval fistula [11] and rats with aminonucleoside nephrotic syndrome [21], have in common the potential for correction of the renal salt retention with adrenalectomy and the absence of mineralocorticoid escape.

In conclusion, the present study demonstrated two factors which are essential to the renal sodium retention of the CBDL dogs, namely (1) an excess of circulating aldosterone and (2) extrarenal factors which, by preventing the escape phenomenon, perpetuate the salt-retaining effect of mineralocorticoids. The nature of these extra-adrenal sodium-retaining factors remains to be elucidated.

Acknowledgments

We thank Mr Dubois and Mr Asaria for their technical assistance and Mrs Bilha Savell for her secretarial help.

References


