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Effects of the neutral endopeptidase inhibitor thiorphan on cardiovascular and renal function in cirrhotic rats

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1 Cirrhosis is associated with cardiovascular and renal dysfunction including sodium retention. Many vasoactive peptides such as atrial natriuretic peptide (ANP) and endothelin-1 (ET-1) are degraded by neutral endopeptidase 24.11 (NEP). We investigated the hemodynamic and renal effects of thiorphan, a NEP inhibitor, in a rat cirrhosis model.

2 Cirrhosis was induced by chronic bile duct ligation, and controls had sham operation. Systemic and renal hemodynamics in conscious, restrained animals were determined using radiolabeled microspheres, and glomerular filtration rate (GFR) was measured by ^{3}H-inulin clearance. Plasma ANP and ET-1, and renal cGMP and $Na^+ - K^+$ ATPase activity were assayed. These variables were measured at baseline and after intravenous infusion of thiorphan (0.5 mg kg^{-1} loading dose followed by 0.1 mg kg^{-1} min^{-1} × 30 min).

3 Thiorphan significantly decreased cardiac output, and increased systemic vascular resistance in controls, whereas in cirrhotic rats these variables were unchanged.

4 Compared to the controls, cirrhotic rats showed a decreased baseline GFR and urine sodium excretion, and the latter was significantly increased by thiorphan.

5 Thiorphan increased plasma ET-1 levels in controls, but not cirrhotic rats. ANP levels were not significantly increased in either group by thiorphan.

6 Thiorphan significantly increased cGMP concentrations and decreased $Na^+ - K^+$ ATPase activity of renal medulla but not cortex in cirrhotic rats; no effect was observed in the control rats.

7 We conclude that thiorphan induces natriuresis in cirrhotic rats by a direct renal medullary mechanism via cGMP and $Na^+ - K^+$ ATPase, without affecting systemic hemodynamics. This may potentially be useful in patients with ascites.
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Abbreviations: ANP, atrial natriuretic peptide; BDL, bile duct ligation; cGMP, guanosine 3′,5′-cyclic monophosphate; ET-1, endothelin-1; GFR, glomerular filtration rate; $Na^+ - K^+$ ATPase, sodium – potassium adenosine triphosphatase; NEP, neutral endopeptidase.

Introduction

Neutral endopeptidase 24.11 (NEP) is a ubiquitous plasma membrane-bound zinc metalloprotease enzyme (Erdos & Skidgel, 1989). It catalyzes the degradation of a number of endogenous vasodilator peptides, including atrial natriuretic peptide (ANP) (Stephenson & Kenny, 1987), brain natriuretic peptide (Lang *et al*., 1992), C-type natriuretic peptide (Kenny *et al*., 1993), substance P (Skidgel *et al*., 1984), and bradykinin (Erdos & Skidgel, 1989), as well as vasoconstrictors including endothelin-1 (ET-1) (Abassi *et al*., 1992), angiotensin II (Erdos & Skidgel, 1989), and endogenous opioids (Erdos & Skidgel, 1989). In addition to degrading vasoactive peptides to inactive breakdown products, NEP can also convert big ET-1 to the active peptide ET-1 (Murphy *et al*., 1994). Therefore, the physiological actions of NEP *in vivo* will be the balance of its effects on the breakdown of vasodilators and vasoconstrictors and on the synthesis of ET-1 from big ET-1.

NEP is inhibited by several agents, including candoxatrilat (Danilewicz *et al*., 1989), thiorphan (Schwartz *et al*., 1990) and its prodrug, sinorphan (Gros *et al*., 1989), and phosphoramidon (Erdos & Skidgel, 1989). It was reported that thiorphan in noncirrhotic rats partially ameliorates the reduction in glomerular filtration rate (GFR) induced by cyclosporin A (Capasso *et al*., 2000), enhances the diuretic effects of ANP (Capasso *et al*., 2000), and increases urinary sodium excretion (Trapani *et al*., 1989). Previous work also showed that thiorphan induces a modest net vasoconstriction, which suggests accumulation of vasoconstrictors such as ET-1 (Haynes & Webb, 1994; Love *et al*., 1996). A recent study has demonstrated a diuretic and natriuretic effect of sinorphan in an experimental animal model of congestive heart failure (Solter *et al*., 2000).

Hepatic cirrhosis is associated with abnormalities in cardiovascular and renal function. The circulation becomes

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hyperdynamic, manifested as peripheral vasodilatation and increased cardiac output, whereas renal excretory function diminishes, with reductions in GFR and renal sodium excretion. Numerous vasoactive substances have been hypothesized to play a pathogenic role in these circulatory and renal changes, including nitric oxide, ANP, ET-1, angiotensin, and endogenous opioids. Since many of these substances are degraded or inactivated by NEP, it would be reasonable to examine the effect of a NEP inhibitor on cardiovascular and renal function in cirrhosis. However, to our knowledge this has been done in only one previous study that investigated the effects of sinorphan in patients with cirrhosis and ascites (Dussaule *et al*., 1991). Dussaule *et al*. (1991) found that this drug induced natriuresis without changes in creatinine clearance or blood pressure. Since this was a human study, detailed invasive cardiovascular and renal parameters were not measured, nor was there a control group. We therefore aimed to elucidate the cardiovascular and renal effects of NEP inhibition and possible mechanisms of action in a commonly used rat model of cirrhosis.

Materials

The protocol was approved by the University of Calgary Animal Care Committee and the experimental procedures were carried out in accordance with guidelines established by the Canadian Council on Animal Care.

Animal preparation

Sprague – Dawley rats weighing between 250 and 350 g were used in the experiments. Cirrhosis was induced by the method of bile duct ligation (BDL) as previously described (Lee *et al*., 1986). Briefly, under halothane anesthesia, through a midline laparotomy, the common bile duct was doubly ligated with 4-0 silk thread and sectioned between the ligatures. Incisions were closed with silk, and the animals were given an intramuscular injection of benzathine penicillin G (30,000 IU) immediately after operation to prevent sepsis. Control rats (sham) underwent exactly the same surgical procedures, and the penicillin administration, but not ligation/section of the common bile duct. Animals were then kept for 3 – 4 weeks, by which time a body weight of 300 – 400 g had been attained and an obvious cirrhosis had developed. Before the hemodynamic experiment, the animals were fasted for 24 h in a metabolic cage with free access only to water. Water intake and urine output during 24 h were measured.

On the day of the study, animals were anesthetized with halothane, and PE-50 polyethylene catheters were inserted into the right carotid artery, right jugular vein, left femoral artery, and left femoral vein. The femoral artery catheter was connected to a pressure transducer (P23XL; Gould Instruments, Oxnard, CA, U.S.A.) connected to an RS 3400 recorder (Gould Instruments, Cleveland, OH, U.S.A.), and used to monitor blood pressure throughout the experiment. The carotid artery catheter was guided into the left ventricle, which was confirmed by the blood pressure tracing. The jugular vein cannula was used to infuse thiorphan. The femoral artery was used to obtain blood samples. The femoral vein was used to infuse saline or radioisotope-labeled markers. One PE-10 polyethylene catheter was also inserted into the right or left ureter to collect the urine sample. After the catheterizations were finished, the incisons were treated with lidocaine 5% gel and the animals were secured into a plastic restraint apparatus. To compensate for the estimated blood loss during the preparation and protocol, physiological saline in an amount equal to 1% of the total body weight was infused during the protocol, at a rate of $0.5\,ml\,h^{-1}\,100\,g^{-1}$ body wt. The studies were performed after the blood pressure and heart rate had been stable for at least 30 min, while animals were resting calmly.

Experimental procedures

The protocol was designed to compare the hemodynamics and kidney function changes before and after the administration of thiorphan. GFR was measured by ^{3}H-inulin (Amersham Life Science, Arlington Heights, IL, U.S.A.) clearance (Anderson *et al*., 1981). A solution of $2\,\mu Ci\ ^{3}H\text{-inulin}\,ml^{-1}$ was prepared with physiological saline and infused through the femoral vein at a rate of 0.375 ml bolus followed by $0.025\,ml\,min^{-1}$ continuous infusion until the end of the experiment. The urine sampling was started 1 h after the start of the ^{3}H-inulin infusion to assure a steady-state inulin level. Urine was collected continuously for 30 min-periods, while blood samples were obtained at the midpoint of each period at a rate of $0.8\,ml\,min^{-1}$ for 1 min. The radioactivity of the sample was determined by liquid scintillation beta counting (Wallack RackBeta, Turku, Finland). Urine volume was determined gravimetrically assuming a density of $1.0\,g\,ml^{-1}$. Urinary sodium concentration was determined by an Ion-Selective Electrode (Hitachi Instruments, Tokyo, Japan).

Radiolabeled microspheres and the reference sample method (Lee *et al*., 1986) were used to quantify the hemodynamic changes. Before and after thiorphan infusion, ^{113}Sn and ^{141}Ce (New England Nuclear, Boston, MA, U.S.A.)-labeled microspheres ($15 \pm 3\,\mu m$, sp. act $1.85\,mBq\,ml^{-1}$) were used, respectively (Lee *et al*., 1986). Immediately after the first urine collection was completed, the first microsphere (^{113}Sn, a precounted aliquot of $27\,\mu l$ containing approximately 20,000 microspheres) solution was injected and flushed with 1 ml saline over 45 s into the left ventricle. The reference blood sample from the femoral artery was obtained by a motorized withdrawal pump at $0.8\,ml\,min^{-1} \times 1\,min$. Then, thiorphan solution ($5\,mg\,ml^{-1}$ in 0.25 N sodium bicarbonate solution) was infused at a rate of $0.5\,mg\,kg^{-1}\,min^{-1}$ for 1 min and $0.1\,mg\,kg^{-1}\,min^{-1}$ for 30 min. This total dose of $3.5\,mg\,kg^{-1}$ was chosen because previous studies had indicated that this relatively low dose was sufficient to produce a natriuretic response (Trapani *et al*., 1994). At the 20th minute of thiorphan infusion, the second urine collection was started. At the midpoint of the second urine collection, 5 min after the end of the thiorphan infusion, the second blood sample was collected for determination of GFR. Within 5 min after this, the second microsphere (^{141}Ce) solution was injected, and the reference sample obtained, according to the same protocol for the first microsphere study. Immediately after the end of the second timed urine collection, the rats were killed with an overdose of pentobarbital sodium. Individual organs were dissected out and the radioactivity of each organ and the reference blood sample were counted

using a Gamma Counter (Wallack 1480 Wizard 3, Turku, Finland).

Calculations

Cardiac index (ml min^{-1} 100 g^{-1} body wt) was calculated as (total counts per minute (c.p.m.) injected (0.8 ml min^{-1} reference sample c.p.m.$^{-1}$))/100 g body wt. System vascular resistance (mmHg ml^{-1} min^{-1} 100 g^{-1} body wt) was calculated as mean arterial pressure/cardiac index. GFR (ml min^{-1} 100 g^{-1} body wt) was equated to the clearance of ^{3}H-inulin/100 g body wt. Renal plasma flow (ml min^{-1} 100 g^{-1} body wt) was calculated as renal blood flow × (1-Hct)/100 g body wt, where renal blood flow was calculated as kidneys c.p.m./0.8 ml min^{-1} × reference sample c.p.m.).

Determination of plasma ANP and ET-1 concentrations

Plasma ANP and ET-1 concentrations were measured by specific RIA, as reported previously (Trapani *et al*., 1989; Moller *et al*., 1995).

Measurement of cGMP level and $Na^+ - K^+$ ATPase activity in kidneys

In separate group of animals, BDL or sham-operated rats were infused with thiorphan using the same doses noted above. Control BDL and sham groups were infused with equivolumic vehicle solution. The rats were then killed and kidneys excised and frozen immediately in liquid nitrogen. The samples were stored at −70°C until the assays were carried out.

Renal cGMP assay Guanosine 3′, 5′-cyclic monophosphate (cGMP) concentrations in renal cortical and medullary homogenates were measured by ELISA using a commercially available kit (Amersham Life Science) according to the manufacturer's instructions. Renal cortex or medulla was dissected out and homogenized in cold 6% (w/v) trichloroacetic acid (TCA) at 4°C to give a 10% (w/v) homogenate. The homogenate was centrifuged at 2000 × g for 15 min at 4°C. The supernatant was washed with five volumes of water-saturated diethyl ether, and the upper ether layer was discarded after each wash. The extract was dried under a stream of nitrogen at 60°C and the dried extract was dissolved in 1 ml of assay buffer before analysis. The protein concentration was determined with Bio-Rad protein assay using bovine serum albumin as standard. We used the nonacetylation assay method.

Renal $Na^+ - K^+$ ATPase activity assay Microsomes of renal cortex or medulla were prepared according to methods described previously (Urayama & Nakao, 1979), with a few modifications. Briefly, the cortex or medulla was homogenized at a ratio of 1 g tissue to 9 ml of homogenizing medium consisting of 0.3 M sucrose, 5 mM Tris-HCl (pH 7.5), and 2 mM EDTA. The homogenate was centrifuged at 2450 × *g* for 15 min at 4°C, and after collecting the supernatant, the pellet was suspended in one-half of the original volume of homogenizing medium and centrifuged again as described above. The resulting supernatant was combined with the previous one and centrifuged at 32,800 × *g* for 35 min at 4°C. The pellet was suspended in 1 ml of homogenizing medium. The protein concentration was determined with Bio-Rad protein assay using bovine serum albumin as standard.

The activity of $Na^+ - K^+$ ATPase in renal cortex and medulla was determined using the method of Khundmiri & Lederer (2002). In all, 50 μg protein of the extracted microsomes was incubated for 15 min at 37°C in medium containing 4.8 mM ATP, 120 mM NaCl, 24 mM KCl, 7.2 mM $MgSO_4$, and 48 mM Tris – HCl (pH 7.5), with or without 1.2 mM ouabain in a final volume of 1.5 ml. The reaction was stopped by adding 0.3 ml of 30% TCA. The difference in the ATPase activity assayed without or with 1.2 mM ouabain was taken as a measure of $Na^+ - K^+$ ATPase. Phosphorous (P_i) released as a result of the action of $Na^+ - K^+$ ATPase was determined using the method of Taussky & Shorr (1953). $Na^+ - K^+$ ATPase activity was expressed as nmol P_i released per mg protein.

Statistical analysis

Results are expressed as means ± s.e.m. To compare two independent groups, unpaired Student's *t*-test was used, and to compare responses in the same animal before and after thiorphan, paired *t*-tests were used. The significance level was set at $P<0.05$.

Results

Systemic hemodynamics

Control (basal) BDL rats exhibited the expected hyperdynamic circulation. This was manifested by a significant increase in cardiac index and a decrease in systemic vascular resistance in comparison with basal sham-operated rats (Figure 1). Thiorphan treatment in sham-operated rats decreased cardiac index and increased systemic vascular resistance, but lacked significant effect in the BDL (Figure 1). Mean arterial pressure (mmHg) was not affected by thiorphan in either group: sham baseline, 118.0 ± 4.6, sham thiorphan, 120.9 ± 3.3; BDL baseline, 105.6 ± 6.7, BDL thiorphan 107.8 ± 7.1.

Other regional vascular beds such as the liver (hepatic artery) and mesenteric viscera (stomach, spleen, intestine, colon, and mesentery-pancreas) were examined but no significant effect of thiorphan on these regional beds was found in either group (data not shown).

Renal function

In the baseline situation, BDL rats displayed lower GFR and urine sodium excretion compared to their corresponding sham-operated controls (Figures 2 and 3). Thiorphan treatment did not significantly change any of these variables in either group, with the exception of sodium excretion in the BDL rats. In this group, thiorphan significantly increased urinary sodium excretion (Figure 3). Thiorphan did not significantly affect urinary flow rates or renal plasma flow in either group. Urine flow rates (ml h^{-1} 100 g^{-1} body wt) were: sham baseline, 1.0 ± 0.1, sham thiorphan, 1.1 ± 0.2; BDL baseline, 0.8 ± 0.2, BDL thiorphan 1.2 ± 0.2. Renal plasma flows (ml min^{-1} 100 g^{-1} body wt) were: sham baseline, 8.7 ± 1.4, sham thiorphan, 6.9 ± 0.9; BDL baseline, 11.3 ± 1.7, BDL thiorphan, 9.4 ± 1.9.

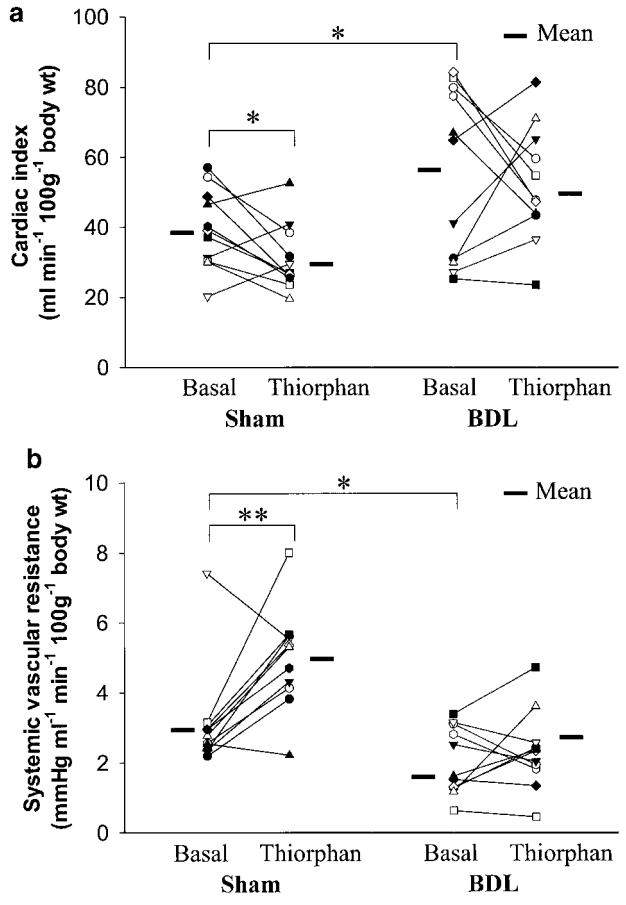

Figure 1 Cardiac index (a), systemic vascular resistance (b) in bile duct ligated (BDL) or sham-operated rats before (basal) and after thiorphan treatment. Data are expressed as mean ± s.e.m. of 11 animals. $*P<0.05$. $**P<0.01$ compared with basal shams.

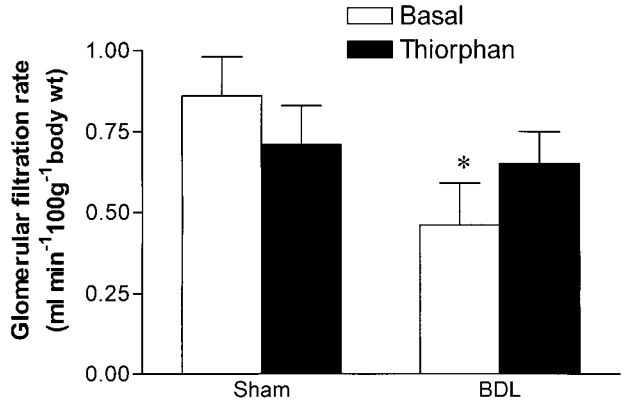

Figure 2 Glomerular filtration rate in bile duct ligated (BDL) or sham-operated rats before (basal) and after thiorphan treatment. Data are expressed as mean ± s.e.m. of eight animals. $*P<0.05$ compared with basal sham.

Plasma ANP and ET-1 concentrations

ANP concentration in BDL rats was 14.1 ± 3.4 fmol ml^{-1}, which was not significantly different from the sham group (21.2 ± 8.9 fmol ml^{-1}). Thiorphan treatment did not significantly change these concentrations in either BDL (23.9 ± 9.5) or sham (36.6 ± 15.5) rats.

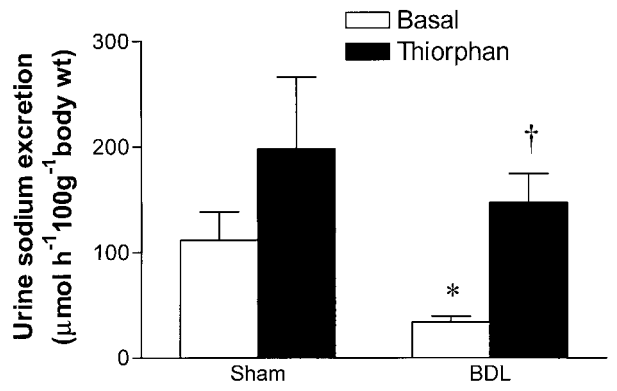

Figure 3 Urine sodium excretion in bile duct ligated (BDL) or sham-operated rats before (basal) and after thiorphan treatment. Data are expressed as mean ± s.e.m. of eight animals. $*P<0.05$ compared with basal sham, $\dagger P<0.05$ compared with basal BDL.

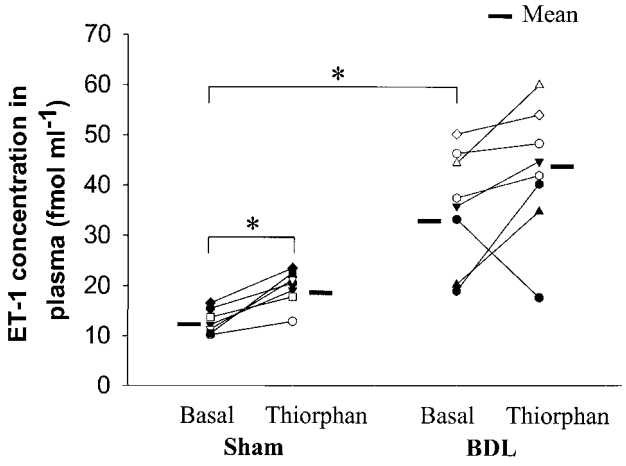

Figure 4 Plasma endothelin-1 (ET-1) concentrations in bile duct ligated (BDL) or sham-operated rats before (basal) and after thiorphan treatment. Data are expressed as mean ± s.e.m. of seven to eight animals. $*P<0.05$ compared with basal shams.

Baseline plasma ET-1 levels were significantly higher in BDL rats compared to sham-control rats. In sham-operated rats, thiorphan significantly increased ET-1 levels, whereas in cirrhotic rats, it had no significant effect (Figure 4).

Renal cGMP concentrations

The BDL cirrhotic rats had decreased baseline renal medullary cGMP concentrations (Figure 5). Thiorphan treatment reversed the decreased cGMP concentrations in the renal medulla of BDL rats in a significant fashion when compared to the baseline. Thiorphan treatment exerted no significant change in cGMP concentrations in the medulla of sham-operated rats (Figure 5). The cGMP concentrations in renal cortex were not significantly different in thiorphan-infused vs vehicle-infused BDL or sham-operated rats (Figure 5).

Renal $Na^+ - K^+$ ATPase activity

The vehicle-treated BDL rats showed increased medullary $Na^+ - K^+$ ATPase activity compared to vehicle-treated sham controls, and thiorphan treatment significantly decreased this elevated $Na^+ - K^+$ ATPase activity (Figure 6). In sham-

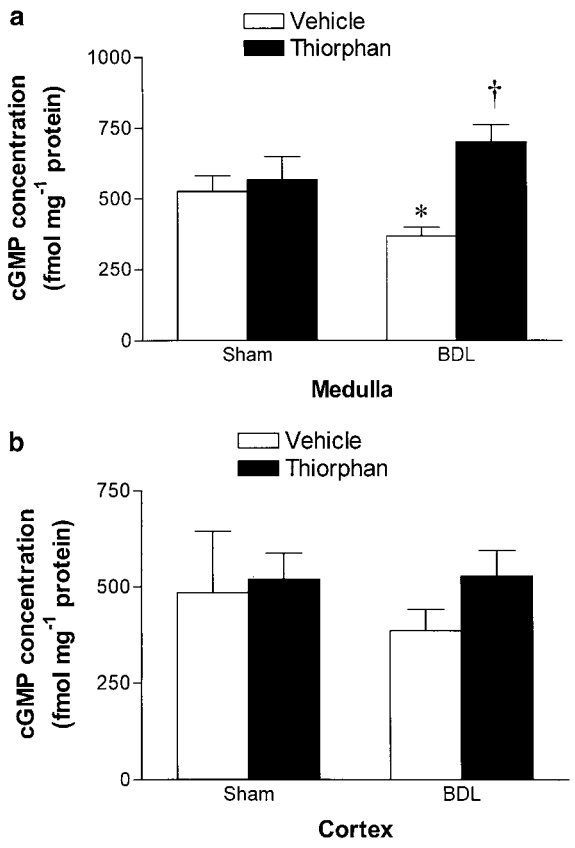

Figure 5 cGMP concentration in renal medulla (a) and cortex (b) in bile duct ligated (BDL) or sham-operated rats treated with vehicle or thiorphan. Data are expressed as mean ± s.e.m. of six animals. $*P<0.05$ compared with sham-vehicle group. $\dagger P<0.05$ compared with BDL-vehicle rats.

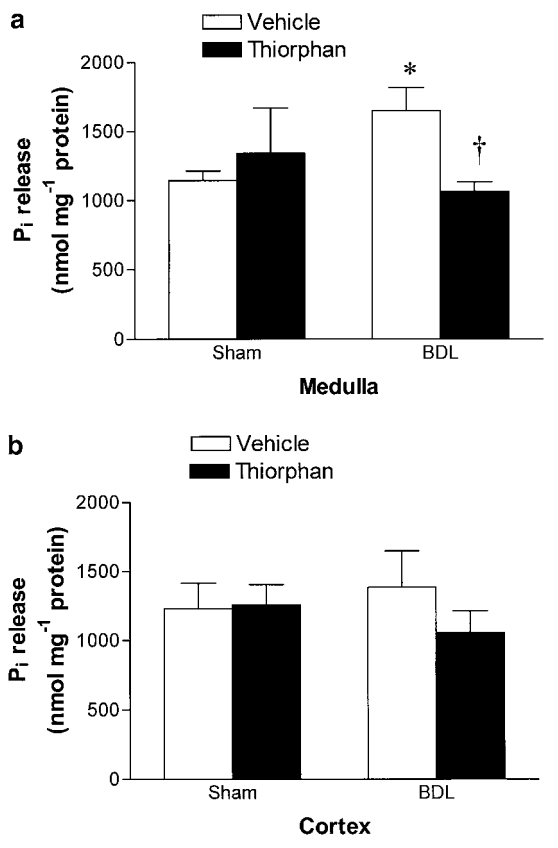

Figure 6 $Na^+ - K^+$ ATPase activity (phosphorus, P_i, release) in renal medulla (a) and cortex (b) in bile duct ligated (BDL) or sham-operated rats treated with vehicle or thiorphan. Data are expressed as mean ± s.e.m. of six animals. $*P<0.05$ compared with sham-vehicle group. $\dagger P<0.05$ compared with BDL-vehicle rats.

operated control rats, medullary $Na^+ - K^+$ ATPase activity was unchanged after thiorphan treatment. In neither BDL nor sham rats did thiorphan significantly affect $Na^+ - K^+$ ATPase activity in renal cortex.

Discussion

Hepatic cirrhosis is associated with a unique pattern of circulatory and functional derangements in many organs and vascular beds. Abnormalities in the pulmonary (Fallon & Abrams, 2000), hepatosplanchnic (Hartleb *et al.*, 1997; Wiest & Groszmann, 1999), renal (Gerbes, 1993; Moller *et al.*, 2001), central nervous (Butterworth, 2000) and cardiac (Liu & Lee, 1999) systems have been recently reviewed. Despite the hyperdynamic circulation with increased baseline cardiac output, the ventricular contractile response to stress is blunted, a condition termed cirrhotic cardiomyopathy (Lee, 1989). Moreover, the cirrhotic kidney functions as if the renal circulation is inadequate, with reductions in both GFR and renal sodium excretion.

The BDL rat model that we studied is commonly used to investigate the cardiovascular and renal derangements of cirrhosis (Martinez-Prieto *et al.*, 2000; Garcia-Estan *et al.*, 2002). Many studies over the past seven decades have conclusively established that this rat model is afflicted by hyperdynamic circulation, portal hypertension, cirrhotic cardiomyopathy, hepatopulmonary syndrome, splanchnic hyperemia, sodium retention, and ascites. It thus mirrors all the salient pathophysiological features of human cirrhosis. Comparing the vehicle-treated BDL rats to the vehicle-treated sham-operated group, we were able to reconfirm that this model exhibits hyperdynamic circulation, depressed GFR, and sodium retention.

In view of the myriad cardiovascular and renal changes of cirrhosis, it is surprising that there has not been more interest in exploring a possible therapeutic role of NEP inhibitors to correct some of these derangements. The net vasoconstrictive and natriuretic properties of this class of drugs could, at least theoretically, counterbalance the vasodilatation and sodium retention of cirrhosis. An intriguing study a decade ago by Dussaule *et al.* (1991) remains the only previous examination of a NEP inhibitor, in that case sinorphan, in cirrhotic patients. In that study, GFR was estimated by creatinine clearance, and arterial pressure was the only hemodynamic variable measured. The problem of using creatinine clearance to estimate GFR is that it tends to overestimate the true GFR under conditions of low GFR, such as in advanced cirrhosis. Despite these methodological limitations, those investigators found a significant natriuretic effect in the absence of changes in creatinine clearance and arterial pressure. However, it could not be determined from their results whether the natriuresis was mediated by an intrarenal effect or by a systemic/renal hemodynamic change such as increased renal perfusion.

The present results in our cirrhotic rats agree very well with these human data. Specifically, we also found a significant natriuretic effect of thiorphan in cirrhotic rats, in the absence of changes in systemic hemodynamics and inulin clearance. In the cirrhotic rats, our results clearly demonstrate that the effect of thiorphan is mediated by a direct intrarenal mechanism rather than through systemic or renal perfusion changes. Whether the natriuresis is mediated by ANP, as suggested by Dussaule *et al.* (1991), or other humoral substances remains unclear from our results. The augmentations of both ET-1 and ANP induced by thiorphan in the cirrhotic rats did not reach statistical significance, so a firm role for these substances remains unclear. However, it is becoming increasingly clear that the notion that NEP inhibitors act merely by increasing the blood levels of ANP, ET-1, and other vasoactive substances is an oversimplification. Rather, recent studies using sinorphan or thiorphan have convincingly demonstrated intrarenal effects, predominantly in the tubules where NEP activity is high, independent of the degree of plasma ANP augmentation which is inconsistent (Trapani *et al.*, 1989; Willenbrock *et al.*, 1996; Solter *et al.*, 2000). Therefore, we remain unconcerned about the insignificant elevations of ET-1 and ANP in our cirrhotic rats; the significant increase in natriuresis in this group confirms, in our view, the efficacy of the thiorphan dose despite the inability to raise these vasoactive substance levels in plasma. Finally, we cannot rule out a possible role of other vasoactive substances, including NO, angiotensin, glucagon, bradykinins, prostaglandins, bile acids, enkephalins, and catecholamines, as many of these are degraded by NEP. However, it was beyond the scope of the present study to measure this vast array of substances.

In the sham-operated control rats, because of the significant individual variability in thiorphan responses, the augmentation of natriuresis induced by the drug was not statistically significant. This, in addition to the relatively small sample size, raises the possibility of a type II error in the normal control group. We chose a relatively low thiorphan dose that would provide a pathophysiologically relevant degree of endopeptidase inhibition to avoid supraphysiological pharmacological effects. The drawback of this approach is the possibility that the degree of endopeptidase inhibition with this dose was insufficient in the control animals and even some of the cirrhotic rats. Moreover, it is possible that some of the observed differences between the two groups were because of decreased metabolism/clearance of thiorphan in the cirrhotic group. However, if this were the case, the other hemodynamic effects such as the changes in cardiac output and systemic vascular resistance should also have been more pronounced in the cirrhotic animals, and the opposite result was observed. Nevertheless, in the absence of direct thiorphan measurements in plasma and urine, these possibilities cannot be firmly ruled out.

The prodrug of thiorphan, sinorphan (also known as ecadotril), has recently been extensively studied in another salt-retaining state, congestive heart failure. In both humans and animal models of heart failure, sinorphan has been shown to increase significantly urine output and sodium excretion. Detailed renal and hemodynamic studies in these animal models have shown a pattern of effect very similar to our results: natriuresis in the absence of hemodynamic changes (Wilkins *et al.*, 1990; Solter *et al.*, 2000). Solter *et al.* (2000) showed that the intrarenal effect of sinorphan in canine congestive heart failure is because of decreased distal tubular sodium reabsorption. NEP activity in the kidney is highest in the proximal convoluted tubule, whereas ANP receptors are found throughout the nephron, but especially in the distal tubule and collecting ducts. Thus, these authors proposed that sinorphan mediates its effect by allowing access of ANP to distal tubular binding sites that it normally does not reach (Solter *et al.*, 2000).

A similar mechanism may be at work in our cirrhotic rats treated with thiorphan. The effects on cGMP concentration and $Na^+ - K^+$ ATPase activity in the medulla, but not the cortex of BDL rats, suggest the involvement of ANP. It is known that both cGMP and $Na^+ - K^+$ ATPase are involved in the natriuretic effect of ANP (Zeidel *et al.*, 1987; Syren, 1997). ANP has been shown to increase cGMP synthesis in the rabbit and rat inner médullary collecting ducts (Nonoguchi *et al.*, 1987; Zeidel *et al.*, 1987; Gunning *et al.*, 1989), and inhibit $Na^+ - K^+$ ATPase activity in the renal medulla (Gorny *et al.*, 1994), rat medullary slices (Scavone *et al.*, 1995), and the medullary thick ascending limb (Syren *et al.*, 1996; Syren, 1997). The studies of Scavone *et al.* (1995) and Beltowski *et al.* (1998) indicate that the regulation of the $Na^+ - K^+$ ATPase activity is mediated by cGMP, because the effect of ANP is mimicked by 8-bromo-cGMP, the membrane-permeable analogue of cGMP. Therefore, we speculate that the thiorphan effect in the cirrhotic kidney is similar to the sinorphan mechanism hypothesized by Solter and colleagues above: in the proximal nephron, thiorphan may increase ANP levels enough to eventually access natriuretic ANP receptors in the inner medullary collecting ducts that normally would not see ANP.

Thiorphan in the sham-operated controls exerted a net vasoconstriction with decreased cardiac output and systemic vascular resistance. This may have been mediated, at least in part, by the increased ET-1 levels. However, this is speculative because many other vasoactive substances were not measured as noted above. It is interesting that apart from the natriuresis, the cirrhotic rats were completely unaffected by the thiorphan infusion. Similar desensitization of cardiovascular responses has been noted in cirrhosis to a whole host of substances including catecholamines, ANP, endothelins, glucagons, and prostaglandins (Asbert *et al.*, 1993; Ohsuga *et al.*, 1994; Pak & Lee, 1994, Moller *et al.*, 1995; Tsai *et al.*, 1997; Helmy *et al.*, 2001).

As sodium retention is a major clinical problem in patients with cirrhosis, the current results, along with the earlier Dussaule study (Dussaule *et al.*, 1991), would suggest thiorphan or sinorphan as a potentially useful treatment in these patients. In particular, natriuresis in the absence of significant hemodynamic changes would be very desirable in patients with cirrhosis and ascites.

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