Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Heme oxygenase regulates renal arterial resistance and sodium excretion in cirrhotic rats

Marco Di Pascoli*, Francesca Zampieri, Santina Quarta, David Sacerdoti, Carlo Merkel, Angelo Gatta, Massimo Bolognesi

Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy

Background & Aims: Heme oxygenase (HO) catabolizes heme into biliverdin, carbon monoxide (CO), and free iron. CO generated in endothelial and smooth muscle layers of blood vessels modulates vascular tone by inducing relaxation of vascular smooth muscle cells. The aim of this study was to verify the role played by HO in regulating renal arterial resistance and Na^+ excretion in cirrhosis.

Methods: Twenty control rats and 20 rats with CCl_4^- induced cirrhosis, 10 of which were chronically treated with the HO inducer cobalt-protoporphyrin (CoPP), were studied. Pressurized renal interlobar arteries were challenged with increasing doses of phenylephrine (PE) and acetylcholine (ACh). Dose–response curves were evaluated under basal conditions and after inhibition of HO with chromium-mesoporphyrin (CrMP). HO-1 (inducible form) and HO-2 (constitutive form) expression was measured in the main and interlobar renal arteries. Serum and urinary levels of Na^+ and creatinine were also evaluated.

Results: In renal interlobar arteries from cirrhotic rats, the response to PE was increased, while that to ACh was blunted. After HO inhibition, the responsiveness to these vasoactive substances was comparable in the two groups. In cirrhotic rats, HO-1 expression was impaired in the main and the interlobar renal arteries. Chronic HO induction normalized the response to the vasoconstrictor, but not to the vasodilator. Cirrhotic rats treated with CoPP showed higher urinary Na^+ concentration and fractional Na^+ excretion, compared to both untreated cirrhotic and control rats.

Conclusions: In cirrhotic rats, an impaired HO-1 expression promotes vasoconstriction of renal interlobar arteries. Chronic HO induction normalizes the sensitivity to PE and promotes Na^+ excretion.

Keywords: Carbon monoxide; Cirrhosis; Heme oxygenase; Hepatorenal syndrome; Renal hemodynamics.

Received 26 February 2010; received in revised form 27 August 2010; accepted 28 August 2010; available online 18 September 2010

*Corresponding author.

E-mail address: m.dipascoli@tin.it (M. Di Pascoli).

Abbreviations: RBF, renal blood flow; GFR, glomerular filtration rate; CBDL, common bile duct ligation; NO, nitric oxide; HO, heme oxygenase; CO, carbon monoxide; K_{Ca}, calcium activated K^+ channel; CoPP, cobalt-protoporphyrin; MAP, mean arterial pressure; HR, heart rate; PE, phenylephrine; ACh, acetylcholine; CrMP, chromium-mesoporphyrin; FrE Na^+, fractional excretion of Na^+; EC_{50}, molar concentration of substance causing 50% of the maximal effect; C_{max}, maximal contraction; R_{max}, maximal relaxation.

Introduction

Patients with cirrhosis show renal functional alterations characterized by Na^+ and water retention and increased resistance to renal blood flow (RBF) [1,2]. These functional abnormalities are believed to relate to peripheral arterial vasodilation, which causes functional hypovolemia with activation of renin–angiotensin–aldosterone and the renal sympathetic nervous systems and increased secretion of catecholamines, anti-diuretic hormone, and endothelin [3]. The progressive vasoconstriction of the afferent renal artery causes pronounced hypoperfusion of the kidney, with reduced glomerular filtration rate (GFR) and increased tubular Na^+ and water reabsorption, with severe renal failure as an outcome [4,5]. Renal hemodynamic alterations begin early in the course of liver-related functional kidney failure, before changes in creatinine clearance are detected [6,7]. Indeed, in cirrhotic patients, changes in renal hemodynamics are already present before the onset of ascites [2,8]. An imbalance of vasoactive mediators produced directly in the kidney may also be implicated in the increase of resistance. A deficiency in the production of prostaglandin E2 and prostacyclin has been suggested in patients with hepatorenal syndrome [9–11]. In cirrhosis, also the renal production of 20-hydroxyeicosatetraenoic acid, epoxyeicosatrienoic acids and leukotriene E4 has been shown to regulate kidney perfusion [12–14]. Nitric oxide (NO) in the kidney has several important functions including the regulation of renal hemodynamics, maintenance of medullary perfusion, mediation of pressure-natriuresis, blunting of tubuloglomerular feedback, inhibition of tubular Na^+ reabsorption, and modulation of renal sympathetic neural activity. The net effect is to promote natriuresis and diuresis [15]. In cirrhotic rats with ascites an increased renal expression of constitutive endothelial NO synthase has been shown [16]. Local production of NO in the kidney may thus counterbalance the effect of endogenous vasoconstrictors on RBF and GFR. Heme oxygenase (HO) is a microsomal enzyme with two main distinct isoforms: inducible (HO-1) and constitutive (HO-2). It catalyses the rate limiting step in the degradation of heme into biliverdin, carbon monoxide (CO), and free iron. CO generated in the blood vessels induces relaxation of vascular

smooth muscle cells by stimulating soluble guanylyl cyclase, opening large-conductance calcium activated K^+ (K_{Ca}) channels, and inhibiting the cytochrome P450 dependent monooxygenase system with a decrease in 20-HETE, which sustains contractile tone by inhibiting K_{Ca}s [17]. HO may mediate vasoregulation also through heme consumption: heme can bind to and inhibit large-conductance K_{Ca}s [18]. In renal interlobar arteries, CO reduces the sensitivity of vascular smooth muscle to constrictor agonists, apparently stimulating K_{Ca}s and interfering with the sensitizing influence of 20-HETE [19,20]. In renal interlobular arterioles and tubules from CBDL rats, the expression of HO-1 and HO-2 is decreased at 5 weeks. In CBDL rats and in sham rats treated with the selective HO inhibitor zinc-protoporphyrin, GFR and RBF are similarly reduced, suggesting that decreased renal HO-1 expression contributes to deteriorated renal function and hemodynamics in cirrhosis [21]. In the kidney, HO may also regulate the Na^+ excretion. Rodriguez et al. [22] showed that HO induction with hemin increases Na^+ and water excretion, and this effect is blocked by previous HO inhibition. Since CO uses similar signaling pathways as NO in the vasculature, it is possible that it activates similar pathways as NO in tubular epithelial cells. CO and biliverdin, whose antioxidant activity is known, may affect renal tubular function also limiting superoxide anion generation, which has been reported to stimulate Na^+ reabsorption [23,24]. The aim of this study was to verify the role played by HO in regulating renal arterial resistance and Na^+ excretion in experimental cirrhosis. Whereas in decompensated cirrhosis the mechanisms underlying sodium retention and renal vasoconstriction are quite well established, in pre-ascitic cirrhosis this issue remains elusive. Some authors have documented a reduction in renal plasma flow and an increase in renal vascular resistance at the pre-ascitic stage in humans [25] and in rats [26]. On the other hand, others have suggested that renal vascular resistance is reduced in compensated cirrhotic patients [27,28]. Therefore, we performed the experiments in rats with compensated cirrhosis, before the onset of ascites.

Materials and methods

Animals

The study was performed on 40 adult male Wistar rats (body weight, 200–225 g; Charles River Laboratories, Calco, Italy). The experiments were carried out in accordance with the legislation of Italian authorities (D.L. 27/01/1992 116), which complies with European Community guidelines (CEE Directive 86/609) for the care and use of experimental animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee. Cirrhosis was induced with the CCl_4 inhalation method in 20 rats as described previously [29]. Phenobarbital (0.30 g/L) was added in the rats drinking water. Treatment was followed for 16 weeks, and animals were free of treatment for the last week before the experiment. In 10 rats from this group, the HO-1 inducer cobalt-protoporphyrin (CoPP) (0.5 mg/100 g bw, s.c.) was injected once a week, starting the first day of CCl_4 inhalation, until three days before the rat sacrifice [30–32]. In nine control rats, CoPP was administered under the same conditions and for the same period of time. Under anesthesia with ketamine hydrochloride (100 mg/kg bw, i.m.), kidneys were excised and sectioned sagittally, and interlobar arteries were dissected out for immediate use. The absence of ascites was confirmed by visual examination at laparotomy. If ascites was present or its absence was not certain, the rat was not included in the study. Age-matched animals were used as controls.

Isolated microvessel preparation

The kidney was placed in a chilled oxygenated modified Krebs bicarbonate buffer [physiological salt solution (PSS)] containing 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.8 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM dextrose. Renal interlobar arteries were dissected into segments (2 mm in length) and mounted in a water-jacketed perfusion chamber (Living Systems Instrumentation, Burlington, VT) on a proximal micropipette connected to a pressure servo controller. Subsequently, the lumen of the vessel was flushed to remove residual blood, and the end of the vessel was mounted on a micropipette connected to a three-way stopcock. After the stopcock was closed, the intraluminal pressure was allowed to increase slowly until it reached 80 mm Hg. The vessel was superfused with PSS (4 ml/min) at 37 °C gassed with 95% O_2 and 5% CO_2 [29]. Intraluminal pressure was maintained at 80 mm Hg throughout the experiment.

Evaluation of the response to phenylephrine (PE) and acetylcholine (ACh) of renal interlobar arteries

After a 45-min period of equilibration, the vessels were challenged with PE, an α1-adrenoreceptor agonist (10^{-6} M). An artery was considered unacceptable for experimentation if it demonstrated leaks or failed to constrict by more than 20% to PE. The presence of a functional endothelium was determined on the basis of a prompt relaxation to ACh (10^{-6} M) in the vessel pre-contracted with PE (10^{-6} M). The effects of PE and ACh administration were evaluated as variations in the internal diameter of the vessels; the response to increasing doses of PE (10^{-8}–10^{-4} M) was reported as percent of contraction, and that to ACh (10^{-9}–10^{-4} M) as percent of inhibition of the contraction induced by PE (10^{-6} M). The HO inhibitor chromium-mesoporphyrin (CrMP) (15 μM) was then added to freshly prepared PSS, and 20-min drug-tissue contact time was allowed before re-testing the response to PE and ACh in the same vessel. PE and ACh were added to the bath (extraluminal application), and cumulative dose–response curves were generated, with 2–3-min intervals between doses. After each dose–response test, the tissues were washed with fresh PSS for at least 20 min. Vascular diameters were measured 1–3 min after the addition of PE and ACh with the use of a video system consisting of a microscope with a charge-coupled device television camera (Eclipse TS100-F; Nikon, Tokyo, Japan), a television monitor (Ultrak Inc., Lewisville, TX), and a video measuring system (Living Systems Instrumentation). Only one experiment was performed in each artery.

Chemicals

CoPP and CrMP were obtained from Porphyrin Products (Logan, UT). All other chemicals were obtained from Sigma Chemical (St. Louis, MO). CoPP injections were prepared by dissolving the compound in 0.2 mmol/L NaOH, adjusting its pH value to 7.4 with 1 mmol/L HCl, and diluting it with 0.9% NaCl to a final concentration of 1 mg/ml. CrMP was dissolved in a solution of 50 mM $NaCO_3$. PE and ACh were dissolved in de-ionized water and diluted with PSS.

Western blot analysis of HO-1 and HO-2 protein expression in main and interlobar renal arteries

Standard techniques were used to evaluate protein expression. Main and interlobar renal arteries were collected from each rat, snap-frozen in liquid N_2, and stored at −80 °C until analyzed. The vessels were homogenized in urea lysis buffer. Protein extracts were assayed for protein content using the BCA protein assay kit (Pierce, Rockford, IL). SDS–polyacrylamide gel electrophoresis and immunoblotting were performed on 50 μg of total protein extracts. HO-1 and HO-2 protein expression were detected using a monoclonal antibody against HO-1 (StressGen Biotechnologies Corp., Victoria, Canada) and polyclonal anti-HO-2 antibodies (StressGen Biotechnologies Corp.) diluted 1:1000 in phosphate buffered saline containing 2% non-fat dry milk. The secondary antibodies anti-mouse and -rabbit conjugated to horseradish peroxidase, respectively, were diluted 1:1000 in phosphate buffered saline containing 2% non-fat dry milk. After stripping, blots were assayed for β-actin content as a standardization of sample loading. Antigenic detection was visualized by standard ECL enhanced chemiluminescence (Amersham, Arlington Heights, Illinois, USA). Quantitative densitometric values of each protein were normalized to β-actin and displayed in histograms, using the VersaDoc Imaging System (Bio-Rad Laboratories, Hercules, California, USA).

Mean arterial pressure and heart rate measurement

To evaluate the effect of CoPP on systemic hemodynamics, the day before the sacrifice the mean blood pressure (MAP) and heart rate (HR) of conscious, warmed, and restrained control rats (treated and not treated with CoPP) were measured by the tail-cuff plethysmographic method. For each rat, five measurements were made at 10-min intervals, and the mean was calculated.

Renal function evaluation

Intracardiac blood samples (0.5 ml) were collected under anesthesia before the sacrifice of the animals. Serum Na^+ and creatinine values were determined using the appropriate analyzers (Aldrich/Sigma, St. Louis, MO, USA). Urine was obtained from a 24 h- collection before the sacrifice of the animals. Urinary Na^+ and creatinine values were determined using the appropriate analyzers (Aldrich/Sigma, St. Louis, MO, USA). Fractional excretion of Na^+ (FrE Na^+) was also calculated.

Data analysis

Data were expressed as mean ± SE. Concentration–response data derived from each vessel were fitted separately to a logistic function by nonlinear regression and EC_{50} (molar concentration of PE and ACh causing 50% of the maximal vasoconstrictor and vasorelaxant effect, respectively) was calculated and expressed as log [M]. From the same regression, the maximal contraction (C_{max}) and relaxation (R_{max}) of the artery were also calculated as percentage of contraction and relaxation (that is, reduction in vessel diameter relative to baseline diameter and increase in vessel diameter relative to the diameter after pre-contraction with PE, respectively). Data were analyzed by ANOVA or Student's *t* test for paired or unpaired observations when appropriate. Correlations were investigated by the least squares method. The *n* values quoted indicate the number of experiments. The null hypothesis was rejected at $p < 0.05$.

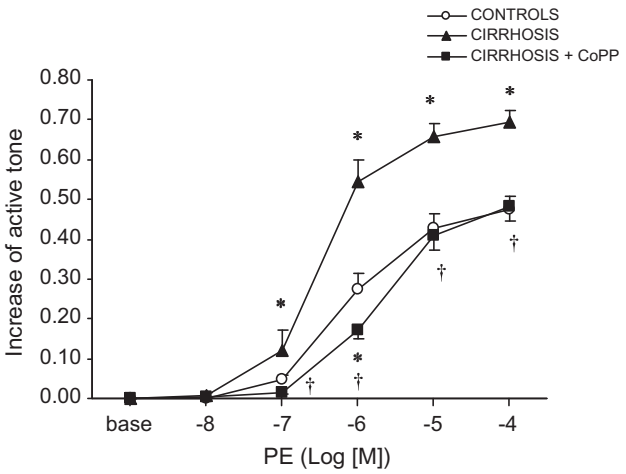

Fig. 1. Dose–response curves to phenylephrine (PE) in rat renal interlobar arteries. Results are mean ± SE. *p <0.05 vs. control rats. †p <0.05 vs. untreated cirrhotic rats.

Results

All rats treated with CCl_4 included in the study had macronodular or micronodular cirrhosis. Control rats had no appreciable alteration in liver appearance. At the time of the study no difference in body weight between control, cirrhotic untreated, and cirrhotic treated rats was observed.

Interlobar renal vascular response to PE

The response of interlobar arteries to PE was increased in untreated cirrhotic ($n = 9$) compared to control rats ($n = 18$): EC_{50}: −6.49 ± 0.14 log [M] vs. −6.0 ± 0.11 log [M] (p <0.01); C_{max}: −70 ± 3% *vs.* −49 ± 3% (p <0.01). In cirrhotic rats treated with CoPP ($n = 21$) the response to PE was blunted compared to untreated cirrhotic rats: EC_{50}: −5.70 ± 0.08 log [M] (p <0.01), C_{max}: −50 ± 4% (p <0.01). Comparing cirrhotic rats treated with CoPP and control rats, only EC_{50} was different ($p = 0.03$) (Fig. 1).

Interlobar renal vascular response to ACh

The maximal dilation of interlobar arteries to ACh was decreased in untreated cirrhotic rats ($n = 6$) compared to control rats ($n = 10$), (R_{max}: 39 ± 8% vs. 63 ± 5%, $p = 0.01$), but the sensitivity to ACh was not different between the two groups (EC_{50}: −7.01 ± 0.25 log [M] vs. −7.13 ± 0.16 log [M]). In cirrhotic rats treated with CoPP ($n = 5$) the response to ACh was decreased compared to control rats (R_{max}: 34 ± 6%, p <0.01; EC_{50}: −6.53 ± 0.22 log [M], $p = 0.05$), but similar to untreated cirrhotic rats (Fig. 2).

Effect of CrMP on the response to PE

CrMP increased C_{max} in control ($n = 11$, from −55 ± 3% to −65 ± 2%, p <0.01) and cirrhotic rats treated with CoPP ($n = 7$, from −54 ± 4% to −62 ± 5%, $p = 0.01$). On the other hand, in untreated cirrhotic rats ($n = 9$) CrMP did not modify C_{max} (−70 ± 3%). CrMP increased the sensitivity to PE in control rats (EC_{50} from −6.09 ± 0.14 log [M] to −6.21 ± 0.14 log [M], $p = 0.05$) and in cirrhotic rats treated with CoPP (EC_{50} from −5.62 ± 0.16 log [M] to −6.09 ± 0.18 log [M], $p = 0.01$), but not in untreated cirrhotic rats (EC_{50} from −6.49 ± 0.14 log [M] to −6.53 ± 0.11 log [M]). After CrMP administration, the response to PE, evaluated both as C_{max} and EC_{50}, was similar in the three groups (Fig. 3).

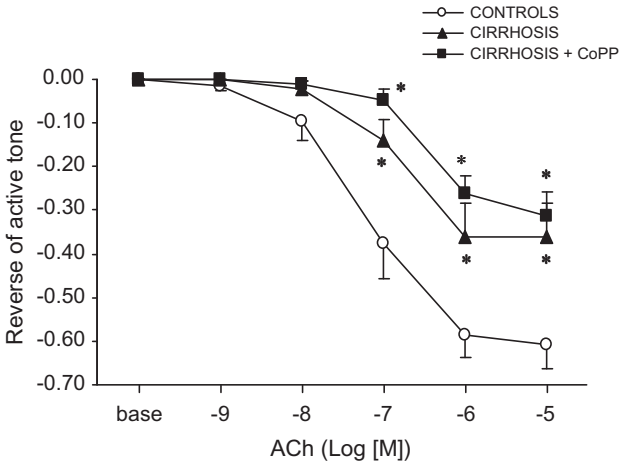

Fig. 2. Dose–response curves to acetylcholine (ACh) in rat renal interlobar arteries. Results are mean ± SE. *p <0.05 vs. control rats.

Effect of CrMP on the response to ACh

CrMP reduced the maximal dilation (R_{max}) to ACh: in control rats ($n = 11$) from 60 ± 7% to 38 ± 6% (p <0.01), in untreated cirrhotic rats ($n = 5$) from 45 ± 5% to 31 ± 6% ($p = 0.03$), and in cirrhotic rats treated with CoPP ($n = 5$) from 32 ± 6% to 16 ± 5% ($p = 0.01$). EC_{50} did not change after administration of CrMP: in control rats from −7.23 ± 0.17 log [M] to −6.95 ± 0.24 log [M], in untreated cirrhotic rats from −6.78 ± 0.12 log [M] to −6.75 ± 0.16 log [M], and in cirrhotic rats treated with CoPP from −6.56 ± 0.20 log [M] to −6.77 ± 0.40 log [M]. After CrMP administration, the response to ACh considered as EC_{50} was similar in the three groups. R_{max}

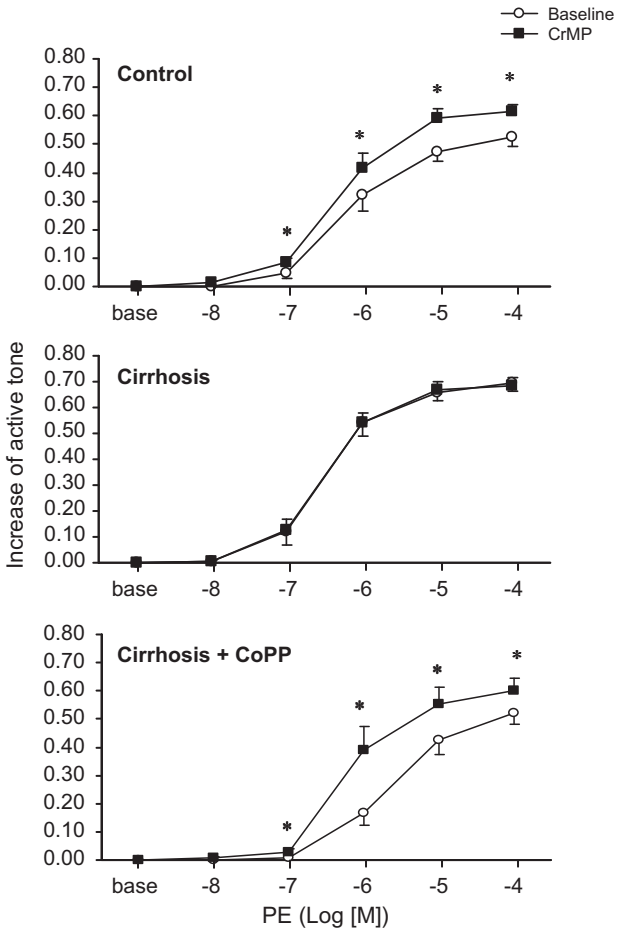

Fig. 3. Effect of HO inhibition with chromium-mesoporphyrin (CrMP) on interlobar renal arteries response to phenylephrine (PE). Results are mean ± SE. *p <0.05 vs. baseline.

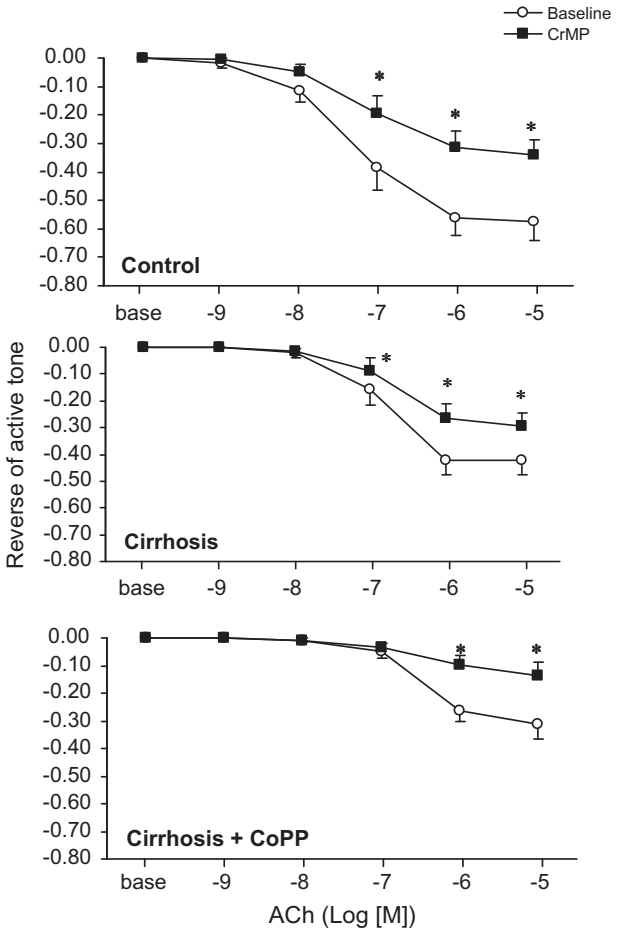

Fig. 4. Effect of HO inhibition with chromium-mesoporphyrin (CrMP) on interlobar renal artery response to acetylcholine (ACh). Results are mean ± SE. *p <0.05 vs. baseline.

was different only between control and cirrhotic rats treated with CoPP (p = 0.02) (Fig. 4).

Western blot analysis of HO-1 and HO-2 protein expression

In the main and interlobar renal arteries from untreated cirrhotic rats (n = 3), HO-1 expression was impaired compared to control rats (n = 3) (p = 0.04). In cirrhotic rats treated with CoPP (n = 3) the enzyme expression was increased compared to both untreated cirrhotic (p <0.01) and control rats (p = 0.03). HO-2 protein expression was not modified in untreated cirrhotic rats and cirrhotic rats treated with CoPP (Fig. 5).

Systemic hemodynamics and renal function

In control rats CoPP administration (n = 9) did not have any effect on MAP and HR, but increased FrE Na^+ compared to untreated rats (n = 11) (p <0.01). Serum creatinine concentration was similar in control, untreated cirrhotic (n = 6), and cirrhotic rats treated with CoPP (n = 6). Urinary Na^+ concentration was higher in cirrhotic rats treated with CoPP compared to untreated cirrhotic rats (p = 0.04). FrE Na^+ was higher in cirrhotic rats treated with CoPP compared to both untreated cirrhotic (p <0.01) and control rats (p <0.01). A direct inverse correlation was found between the interlobar arteries sensitivity to the α-adrenergic stimulus of PE, analyzed as EC_{50}, and FrE Na^+ (r = 0.71, p <0.01) (Tables 1, 2 and Fig. 6).

Discussion

This study shows that in renal interlobar arteries from rats with compensated cirrhosis, vasoconstrictor response to PE is increased while vasodilator response to ACh is blunted. In these rats, renal function was normal, which emphasizes that changes in renal vascular response occur even before Na^+ retention. These data are consistent with the evidence that renal arterial resistance indexes are increased in patients with cirrhosis before the onset of ascites [2,8]. Therefore, in cirrhotic patients, an early increased response of interlobar renal arteries to adrenergic stimuli may occur. HO, through CO production and heme consumption, plays an important role in regulating renal vascular tone [18,19]: its deregulation may be critical in altering the renal response to vasoactive factors in cirrhosis. In control rats, HO inhibition reduces GFR and RBF to values similar to the ones observed in cirrhotic rats [21]. Accordingly, we demonstrated that CrMP administration increases the sensitivity of renal interlobar arteries to PE in control but not in cirrhotic rats; after HO inhibition, the response to

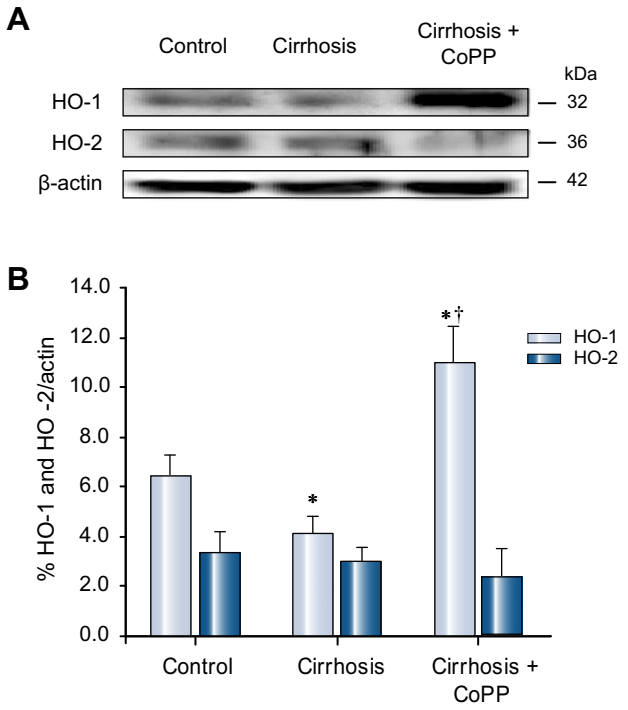

Fig. 5. HO-2 protein expression not modified in untreated cirrhotic rats and cirrhotic rats treated with CoPP. (A) Western blot analysis of inducible heme oxygenase (HO-1) and constitutive heme oxygenase (HO-2) in the main and interlobar renal arteries of control, cirrhotic, and chronically treated with cobalt-protoporphyrin (CoPP) cirrhotic rats. The reported blots are representative of three experiments per each group. Examples of β-actin expression, analyzed as an index of the adequacy of sample loading, are also displayed. (B) Quantitative densitometric evaluation, normalized to β-actin, of inducible heme oxygenase (HO-1) and constitutive heme oxygenase (HO-2) in the main and interlobar renal arteries of control, cirrhotic, and chronically treated with cobalt-protoporphyrin (CoPP) cirrhotic rats. Results are from three rats per each group (mean ± SE). *p <0.05 vs. control rats. †p <0.05 vs. untreated cirrhotic rats.

Table 1. Hemodynamic parameters and renal function markers in control rats treated with CoPP.

Group	MAP (mmHg)	HR (beats/min)	S.Creat. (μmol/L)	FrE Na^+ (%)
Control	107.6 ± 3.2	400.7 ± 11.8	37.64 ± 1.87	0.73 ± 0.28
CoPP	112.6 ± 2.9	380.2 ± 7.5	36.60 ± 1.57	2.29 ± 0.28*

n = 11 control, 9 controls treated with CoPP. *p <0.05 vs. control rats.

Table 2. Renal function markers: serum creatinine concentration, urinary Na^+ concentration, urinary 24 h Na^+, fractional excretion of Na^+.

Group	S.Creat. (μmol/L)	U. Na^+ (mmol/L)	U. Na^+/24h (mmol)	FrE Na^+ (%)
Control	37.64 ± 1.87	42,1 ± 6,7	1.17 ± 0.19	0.73 ± 0.28
Cirrhosis	41.33 ± 3.22	35.2 ± 5.8	1.13 ± 0.24	0.66 ± 0.18
Cirrhosis + CoPP	40.83 ± 1.19	68.7 ± 14.3†	1.68 ± 0.64	2.19 ± 0.41*†

n = 11 control, 6 cirrhosis, 6 cirrhosis + CoPP. *p <0.05 vs. control rats. †p <0.05 vs. cirrhosis.

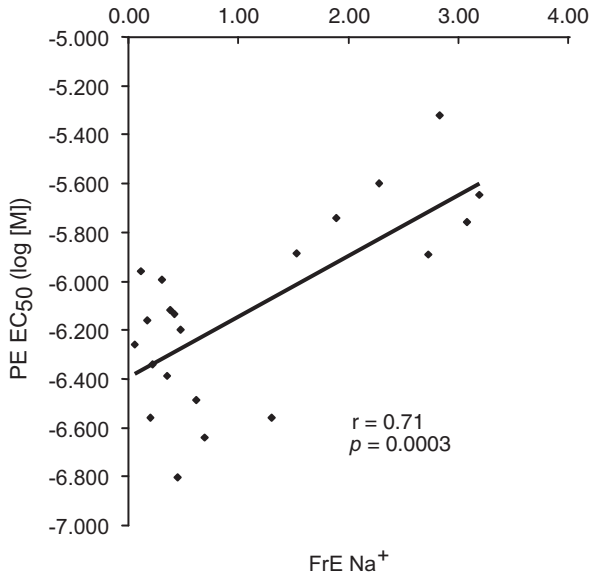

Fig. 6. Relationship between the interlobar artery sensitivity to the α-adrenergic stimulus evaluated as EC_{50} (molar concentration of phenylephrine (PE) causing 50% contraction) and the fractional excretion of Na^+ (FrE Na^+).

the vasoconstrictor in the two groups was comparable. These data suggest that a lack of vascular HO activity/expression contributes to the increased renal sensitivity to PE in cirrhosis. Miyazono et al. [21] observed a decrease in HO-1 expression in renal interlobar arteries 5 weeks after CBDL. In our study, HO-1 expression was reduced in renal arterial vessels from cirrhotic rats. To verify the role of HO in controlling the renal vascular response to PE in cirrhosis, we also performed the experiment in a group of cirrhotic rats chronically treated with the HO inducer CoPP. In the arteries of these animals, the response to PE was normal. Also, unlike the untreated cirrhotic rats and similarly to control rats, the artery's sensitivity to CrMP was retrieved. After HO inhibition, the contractile response was comparable to that of the other two groups under the same conditions. Analyzing the dose–response curves to ACh, which causes an endothelium-dependent vasodilation, we found that in cirrhotic rats renal interlobar arteries are less responsive than in control rats. After CrMP was administered, the response to ACh was markedly reduced in control rats while it was only slightly reduced in cirrhotic rats. HO inhibition made the response to the vasodilator in these two groups comparable suggesting that the decreased expression of HO-1 may have a role in the blunted response of renal interlobar arteries to ACh in cirrhosis. In contrast, and unexpectedly, chronic HO induction with CoPP did not improve the artery's response to ACh in cirrhotic rats. To explain this lack of effect, we can hypothesize that the overexpression of HO-1 induced by CoPP (see Fig. 5) caused an increase in CO beyond its physiological levels. High levels of CO, despite its direct vasodilator effect, inhibit the production of the potent vasodilator NO, that is essential to maintain a normal renal perfusion [33]. Previous studies have shown that low levels of CO (0.001–0.1 μmol/L) stimulate NO release, while higher levels ($\geqslant$1 μmol/L) inhibit NO synthase [34]. In the renal circulation, CO buffers the excessive vasoconstriction observed after blockade of NO, suggesting a major role for CO protecting the renal vasculature from excessive vasoconstriction when the renal NO system is deficient [35,36]. In contrast, overexpression of HO-1 in the vascular

smooth muscle cells attenuates NO-mediated vasodilatation [37]. Moreover, elevated levels of endogenous CO contribute to arteriolar NO dysfunction in Dahl salt-sensitive rats, and responses to ACh are restored upon inhibition of HO [38]. In isolated carotid arteries from angiotensin II hypertensive mice, HO-1 induction does not improve ACh dependent vascular relaxation [39]. Therefore, we assume that in conditions of excessive renal vasoconstriction, increases in vascular CO may be beneficial, but need to be tempered so they do not interfere with endogenous NO production, which can lead to impaired vascular relaxation. This study investigated the role of HO in regulating both the vascular tone and renal Na^+ excretion in experimental cirrhosis. In this condition, daily Na^+ balance is highly variable in the pre-ascitic stage, before becoming openly and steadily positive at the time of ascites formation [40,41]. Accordingly, we found that Na^+ excretion was not significantly decreased in untreated cirrhotic rats compared to control rats. In cirrhotic rats, HO induction did not modify serum creatinine concentration but increased renal Na^+ excretion as measured by urinary Na^+ concentration and FrE Na^+. We assume that this was not secondary to an effect on systemic hemodynamics, as under physiological conditions CoPP administration significantly increases FrE Na^+ without affecting MAP and HR (see Table 1). Moreover, it has been shown that stannous chloride, another HO-1 inducer, promotes natriuresis in spontaneously hypertensive rats, despite a reduction in blood pressure [42]. We found an inverse correlation between the interlobar artery's sensitivity to the α-adrenergic stimulus and the FrE Na^+, thus HO, by reducing preglomerular resistance, may inhibit Na^+ retention in cirrhosis. In cirrhotic rats treated with CoPP, FrE Na^+ was also higher compared to control rats, suggesting that CO overproduction in the renal tubules directly induces the secretion or reduces the reabsorption of Na^+. Along the same lines, Rodriguez et al. [22] showed that HO induction with hemin enhances renal Na^+ excretion, without interfering with GFR. HO may also have a natriuretic effect limiting the production of superoxide anion, which has been shown to promote Na^+ reabsorption [24]. In conclusion, our study shows that in cirrhotic rats chronic HO induction normalizes the sensitivity of renal interlobar arteries to PE and promotes Na^+ excretion.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgments

We thank Cristian Turato for scientific support and Antonietta Sticca and Roberta Venturini for expert technical assistance.

References

[1] Schrier RW, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. Hepatology 1989;9:797–798.
[2] Sacerdoti D, Bolognesi M, Merkel C, Angeli P, Gatta A. Renal vasoconstriction in cirrhosis evaluated by duplex Doppler ultrasonography. Hepatology 1993;17:219–224.
[3] Møller S, Henriksen JH. Circulatory abnormalities in cirrhosis with focus on neurohumoral aspects. Semin Nephrol 1997;17:505–519.
[4] Ring-Larsen H. Renal blood flow in cirrhosis: relation to systemic and portal haemodynamics and liver function. Scand J Clin Lab Invest 1977;37:635–642.
[5] La Villa G, Barletta G, Pantaleo P, Del Bene R, Vizzutti F, Vecchiarino S, et al. Hemodynamic, renal, and endocrine effects of acute inhibition of nitric oxide synthase in compensated cirrhosis. Hepatology 2001;34:19–27.
[6] Epstein M, Berk DP, Hollenberg NK, Adams DF, Chalmers TC, Abrams HL, et al. Renal failure in the patient with cirrhosis. The role of active vasoconstriction. Am J Med 1970;49:175–185.
[7] Maroto A, Ginès A, Saló J, Clària J, Ginès P, Anibarro L, et al. Diagnosis of functional kidney failure of cirrhosis with Doppler sonography: prognostic value of resistive index. Hepatology 1994;20:839–844.
[8] Rivolta R, Maggi A, Cazzaniga M, Castagnone D, Panzeri A, Solenghi D, et al. Reduction of renal cortical blood flow assessed by Doppler in cirrhotic patients with refractory ascites. Hepatology 1998;28:1235–1240.
[9] Zipser RD, Radvan GH, Kronborg IJ, Duke R, Little TE. Urinary thromboxane B2 and prostaglandin E2 in the hepatorenal syndrome: evidence for increased vasoconstrictor and decreased vasodilator factors. Gastroenterology 1983;84:697–703.
[10] Rimola A, Ginés P, Arroyo V, Camps J, Pérez-Ayuso RM, Quintero E, et al. Urinary excretion of 6-keto-prostaglandin F1 alpha, thromboxane B2 and prostaglandin E2 in cirrhosis with ascites. Relationship to functional renal failure (hepatorenal syndrome). J Hepatol 1986;3:111–117.
[11] Govindarajan S, Nast CC, Smith WL, Koyle MA, Daskalopoulos G, Zipser RD. Immunohistochemical distribution of renal prostaglandin endoperoxide synthase and prostacyclin synthase: diminished endoperoxide synthase in the hepatorenal syndrome. Hepatology 1987;7:654–659.
[12] Sacerdoti D, Balazy M, Angeli P, Gatta A, McGiff J. Eicosanoid excretion in hepatic cirrhosis. Predominance of 20-HETE. J Clin Invest 1997;100:1264–1270.
[13] Miyazono M, Zhu D, Nemenoff R, Jacobs ER, Carter EP. Increased epoxyeicosatrienoic acid formation in the rat kidney during liver cirrhosis. J Am Soc Nephrol 2003;14:1766–1775.
[14] Moore KP, Taylor GW, Maltby NH, Siegers D, Fuller RW, Dollery CT, et al. Increased production of cysteinyl leukotrienes in hepatorenal syndrome. J Hepatol 1990;11:263–271.
[15] Mount PF, Power DA. Nitric oxide in the kidney: functions and regulation of synthesis. Acta Physiol 2006;187:433–446.
[16] Bosch-Marcé M, Morales-Ruiz M, Jiménez W, Bordas N, Solé M, Ros J, et al. Increased renal expression of nitric oxide synthase type III in cirrhotic rats with ascites. Hepatology 1998;27:1191–1199.
[17] Zhang F, Kaide JI, Rodriguez-Mulero F, Abraham NG, Nasjletti A. Vasoregulatory function of the heme–heme oxygenase–carbon monoxide system. Am J Hypertens 2001;14:62S–67S.
[18] Tang XD, Xu R, Reynolds MF, Garcia ML, Heinemann SH, Hoshi T. Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels. Nature 2003;425:531–535.
[19] Kaide JI, Zhang F, Wei Y, Jiang H, Yu C, Wang WH, et al. Carbon monoxide of vascular origin attenuates the sensitivity of renal arterial vessels to vasoconstrictors. J Clin Invest 2001;107:1163–1171.
[20] Kaide J, Zhang F, Wei Y, Wang W, Gopal VR, Falck JR, et al. Vascular CO counterbalances the sensitizing influence of 20-HETE on agonist-induced vasoconstriction. Hypertension 2004;44:210–216.
[21] Miyazono M, Garat C, Morris Jr KG, Carter EP. Decreased renal heme oxygenase-1 expression contributes to decreased renal function during cirrhosis. Am J Physiol Renal Physiol 2002;283:F1123–F1131.
[22] Rodriguez F, Kemp R, Balazy M, Nasjletti A. Effects of exogenous heme on renal function: role of heme oxygenase and cyclooxygenase. Hypertension 2003;42:680–684.
[23] Matsumoto H, Ishikawa K, Itabe H, Maruyama Y. Carbon monoxide and bilirubin from heme oxygenase-1 suppresses reactive oxygen species generation and plasminogen activator inhibitor-1 induction. Mol Cell Biochem 2006;291:21–28.
[24] Ortiz PA, Garvin JL. Superoxide stimulates NaCl absorption by the thick ascending limb. Am J Physiol Renal Physiol 2002;283:F957–F962.
[25] Woitas RP, Heller J, Stoffel-Wagner B, Spengler U, Sauerbruch T. Renal functional reserve and nitric oxide in patients with compensated liver cirrhosis. Hepatology 1997;26:858–864.
[26] Sansoè G, Aragno M, Smedile A, Rizzetto M, Rosina F. Solute-free water retention in preascitic cirrhotic rats following intravenous water loading. J Physiol Pharmacol 2009;60:111–117.
[27] Wong F, Massie D, Colman J, Dudley F. Glomerular hyperfiltration in patients with well-compensated alcoholic cirrhosis. Gastroenterology 1993;104:884–889.

[28] Magri P, Auletta M, Andreucci M, Somma G, Musone D, Fiorillo M, et al. Sodium retention in preascitic stage of cirrhosis. Semin Nephrol 2001;21:317–322.
[29] Bolognesi M, Sacerdoti D, Di Pascoli M, Angeli P, Quarta S, Sticca A, et al. Haeme oxygenase mediates hyporeactivity to phenylephrine in the mesenteric vessels of cirrhotic rats with ascites. Gut 2005;54:1630–1636.
[30] Ma LJ, You Y, Bai BX, Li YZ. Therapeutic effects of heme oxygenase-1 on psoriasiform skin lesions in guinea pigs. Arch Dermatol Res 2009;301:459–466.
[31] Botros FT, Schwartzman ML, Stier Jr CT, Goodman AI, Abraham NG. Increase in heme oxygenase-1 levels ameliorates renovascular hypertension. Kidney Int 2005;68:2745–2755.
[32] Turkseven S, Kruger A, Mingone CJ, Kaminski P, Inaba M, Rodella LF, et al. Antioxidant mechanism of heme oxygenase-1 involves an increase in superoxide dismutase and catalase in experimental diabetes. Am J Physiol Heart Circ Physiol 2005;289:H701–H707.
[33] Reyes AA, Purkerson ML, Karl I, Klahr S. Dietary supplementation with L-arginine ameliorates the progression of renal disease in rats with subtotal nephrectomy. Am J Kidney Dis 1992;20:168–176.
[34] Thorup C, Jones CL, Gross SS, Moore LC, Goligorsky MS. Carbon monoxide induces vasodilation and nitric oxide release but suppresses endothelial NOS. Am J Physiol 1999;277:F882–F889.
[35] Rodriguez F, Lamon BD, Gong W, Kemp R, Nasjletti A. Nitric oxide synthesis inhibition promotes renal production of carbon monoxide. Hypertension 2004;43:347–351.
[36] Botros FT, Navar LG. Interaction between endogenously produced carbon monoxide and nitric oxide in regulation of renal afferent arterioles. Am J Physiol Heart Circ Physiol 2006;291:H2772–H2778.
[37] Imai T, Morita T, Shindo T, Nagai R, Yazaki Y, Kurihara H, et al. Vascular smooth muscle cell-directed overexpression of heme oxygenase-1 elevates blood pressure through attenuation of nitric oxide-induced vasodilation in mice. Circ Res 2001;89:55–62.
[38] Johnson FK, Durante W, Peyton KJ, Johnson RA. Heme oxygenase inhibitor restores arteriolar nitric oxide function in Dahl rats. Hypertension 2003;41:149–155.
[39] Stec DE, Vera T, McLemore GR, Kelsen Jr S, Rimoldi JM, Gadepalli RS, et al. Heme oxygenase-1 induction does not improve vascular relaxation in angiotensin II hypertensive mice. Am J Hypertens 2008;21:189–193.
[40] Jiménez W, Martinez-Pardo A, Arroyo V, Bruix J, Rimola A, Gaya J, et al. Temporal relationship between hyperaldosteronism, sodium retention and ascites formation in rats with experimental cirrhosis. Hepatology 1985;5:245–250.
[41] López C, Jiménez W, Arroyo V, Clària J, La Villa G, Asbert M, et al. Temporal relationship between the decrease in arterial pressure and sodium retention in conscious spontaneously hypertensive rats with carbon tetrachloride-induced cirrhosis. Hepatology 1991;13:585–589.
[42] Sacerdoti D, Escalante B, Abraham NG, McGiff JC, Levere RD, Schwartzman ML. Treatment with tin prevents the development of hypertension in spontaneously hypertensive rats. Science 1989;243:388–390.