Carbon Monoxide-Mediated Activation of Large-Conductance Calcium-Activated Potassium Channels Contributes to Mesenteric Vasodilatation in Cirrhotic Rats

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ABSTRACT

Large-conductance calcium-activated potassium channels (BKCa) are important regulators of arterial tone and represent a mediator of the endogenous vasodilator carbon monoxide (CO). Because an up-regulation of the heme oxygenase (HO)/CO system has been associated with mesenteric vasodilatation of cirrhosis, we analyzed the interactions of BKCa and of HO/CO in the endothelium-dependent dilatation of mesenteric arteries in ascitic cirrhotic rats. In pressurized mesenteric arteries (diameter, 170–350 μm) of ascitic cirrhotic rats, we evaluated the effect of inhibition of BKCa, HO, and guanylyl-cyclase on dilatation induced by acetylcholine and by exogenous CO; and HO-1 and BKCa subunit protein expression. Inhibition of HO and of BKCa reduced acetylcholine-induced vasodilatation more in cirrhotic rats than in control rats, whereas inhibition of guanylyl-cyclase had a similar effect in the two groups. CO was more effective in cirrhotic rats than in control rats, and the effect was hindered by BKCa inhibition. The expression of HO-1 and of BKCaα-subunit was higher in mesenteric arteries of cirrhotic rats compared with that of control animals, whereas the expression of the BKCaβ1-subunit was lower. In conclusion, an overexpression of BKCaα-subunits, possibly due to HO up-regulation with increased CO production, participates in the endothelium-dependent alterations and mesenteric arterial vasodilatation of ascitic cirrhotic rats.

Mesenteric arterial vasodilation is a key mechanism in the pathophysiology of the hyperdynamic circulatory syndrome of cirrhosis. This syndrome is responsible for serious complications, such as ascites, hepatorenal syndrome, and gastrointestinal hemorrhage. The pathophysiological mechanism that supports the vasodilatation of mesenteric arteries in cirrhosis is a decrease in the response of the arteries to vasoconstricting agents (Sieber et al., 1993), caused by an increase in vasodilating substances of endothelial origin, such as nitric oxide (NO), prostacyclin, and, as recently demonstrated, carbon monoxide (CO) (Wiest and Groszmann, 1999; Fernandez et al., 2001; Gonzales-Abraldes et al., 2002).

We have recently shown that an increased action of the heme oxygenase (HO)/CO system plays a role in the hyporesponsiveness of small resistance mesenteric arteries to phenylephrine (PE) only in the advanced stage of experimental cirrhosis (Bolognesi et al., 2005). Therefore, the increased activity of the HO/CO system may participate in the evolution of cirrhosis from compensated to decompensated.

HO is a microsomal enzyme with two main distinct isoforms: the inducible isoenzyme HO-1 and the constitutive one HO-2 (Zhang et al., 2001; Johnson et al., 2003a). It is the rate-limiting enzyme in the degradation of heme to biliverdin, CO, and free iron (Motterlini et al., 1998). CO, generated by HO in endothelial and smooth muscle layers of arterial vessels, modulates vascular tone by inducing relaxation of vascular smooth muscle cells through stimulation on soluble guanylyl cyclase (sGC) and opening of large-conductance calcium-activated potassium channels (BKCa) (Zhang et al., 2001; Johnson et al., 2003a).
It also inhibits the formation of 20-hydroxyeicosatetraenoic acid (Zhang et al., 2001; Kaide et al., 2004), a vasoconstrictor that inhibits potassium channels.

BKCa-channel expression is by far the most abundant K+ channel expressed in vascular smooth muscle cells; their importance as physiological regulators of blood flow has long been recognized (Clapp and Jabr, 2003; Kotlikoff and Hall, 2003). The opening of these channels, in response to localized intracellular Ca2+ transients (Ca2+ sparks) (Jaggar et al., 2000), leads to hyperpolarization of smooth muscle cell and, thus, to relaxation. Their conductance is increased by membrane depolarization (Barriere et al., 2001), NO (Barriere et al., 2001; Wu et al., 2002), CO (Wu et al., 2002, Jaggar et al., 2005), and by other substances, such as epoxyeicosatrienoic acids (Archer et al., 2003).

In this study, we tried to analyze whether the alteration of HO/CO system detected in ascitic cirrhosis is linked to alterations in the mechanisms transducing the NO signal in the smooth muscle cell. Therefore, the aim of the study was to analyze the role of BKCa, sGC, and HO/CO system in the response to acetylcholine (ACh) of small resistance mesenteric arteries of rats with CCl4-induced cirrhosis. In cirrhotic rats with ascites, we preliminarily analyzed the effect of HO, sGC, and BKCa, and the vasodilation. Finally, the hemodynamic effect of a CO donor, KCl (25, 50, and 100 nM) and then after a further 20 min of CrMP (15 μM) superfusion in arteries already evaluated after IbTx superfusion (10 μM) were determined in arteries superfused with PSS containing vehi-

Materials and Methods

Animals. The study was performed on 65 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 (CE 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 CCl4 inhalation method in 35 rats drinking phenobarbital (0.30 g/l in the drinking water) as described previously (Bolognesi et al., 2005). Treatment was followed for 16 weeks, and animals were free of treatment for the last week before the experiment. Under anesthesia with ketamine hydrochloride (100 mg/kg i.m.), a midventral laparotomy was performed, and a section of small intestine was removed. The presence of ascites was confirmed after preconstriction with PE by the absence of relaxation to ACh (1 μM). In these arteries, the absence of a functional endothelium was determined on the basis of a prompt relaxation to ACh (1 μM) in the vessel preconstricted with PE (1 μM). To remove the endothelium, 2 ml of air was flushed through the lumen (Sun et al., 1994). In these arteries, the absence of a functional endothelium was confirmed after preconstriction with PE by the absence of response to ACh with a normal response to sodium nitroprusside (SNP), an endothelium-independent vasodilator. The effects of ACh and CO administration were evaluated as variations in the vascular conductance in the internal diameter of the vessels preconstricted with 10 μM PE; all responses were reported as percent inhibition of the contraction induced by PE.

Evaluation of the Response to ACh of Small Mesenteric Arteries Preconstricted with Phenylephrine in CCl4 Cirrhotic Rats. Responses to increasing doses of ACh (10−9 to 10−4 M) were determined in arteries superfused with PSS-containing vehicles for the inhibitors tested. Inhibitors were added to freshly prepared PSS, and 20- to 30-min drug-tissue contact time was allowed before retesting the response to ACh in the same vessel. ACh was added to the bath (extraluminal application), and cumulative dose-response curves were generated, with 2- to 3-min intervals between doses. After each dose-response test, the tissues were washed with fresh PSS at least 20 min. Vascular diameters were measured 1 to 3 min after the addition of 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 (Ultran Inc., Lewisville, TX), and a video measuring system (Living Systems Instrumentation). In control rats and in ascitic cirrhotic rats, concentration-response curves to ACh were evaluated before and after 20-min superfusion with the HO inhibitor CrMP (15 μM); before and after 20-min superfusion with the sGC inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (10 μM) in arteries treated with indomethacin (Indo) (2.8 μM) and N5-nitro-L-arginine-methyl-ester (l-NAME) (1 mM) and then after a further 20 min of CrMP (15 μM) plus ODQ (10 μM) superfusion in arteries already evaluated after ODQ superfusion alone; and before and after 20-min superfusion with the BKCa inhibitor iberiotoxin (IbTx) (25 nM) in arteries treated with Indo (2.8 μM), l-NAME (1 mM), and ODQ (10 μM) and then after a further 20 min of CrMP (15 μM) plus IbTx (25 nM) superfusion in arteries already evaluated after IbTx superfusion alone. Concentration-response curves to ACh were also evaluated in small mesenteric arteries of control rats before and after 20-min superfusion with three different amounts of the BKCa inhibitor IbTx (25, 50, and 100 nM). Only one experiment was performed in each artery.

Evaluation of the Response to a CO Donor. Small mesenteric arteries of control rats and of rats with cirrhosis and ascites were prepared and mounted on glass micropipettes in a water-jacketed perfusion chamber as already described. After the equilibration pe-
Striated and after verifying their viability, the arteries were preconstricted with the addition of PE to the superfusion PSS. A CO donor (200 μM) (CO-releasing molecule (CORM-3) (Clark et al., 2003; Foresti et al., 2004) was then added to the superfusion PSS for 15 min, and the change in lumen diameter was recorded. The chemical composition of CORM-3 is: tricarbonylchloro(glycinato)ruthenium(II) ([Ru(CO)3Cl(glycinato)]). The concentration of CORM-3 was chosen after testing the hemodynamic effect of four different CORM-3 concentrations (25, 50, 100, and 200 μM) (Foresti et al., 2004) in a preliminary experiment on small mesenteric arteries of control rats. Only with the higher dose (200 μM) did we obtain a clear and measurable effect. Therefore, 200 μM was the minimal efficacious dose in our experimental system. In this preliminary experiment, we also verified that the hemodynamic effect of CORM-3 on small mesenteric arteries is slow because it could only be detected a approximately 3 to 4 min after administration and could be fully evaluated only after an exposure of 10 to 15 min. For this reason, to evaluate CORM-3 effect in our experimental set, the changes in lumen diameter were recorded for 15 min during a continuous exposure to CORM-3. Taking all this into account, we decided to test a single dose of CORM-3 using the minimal dose that was efficacious in our control animals (200 μM).

The effect of CORM-3 was also verified in arteries superfused with CrMP (15 μM) for 30 min, in arteries superfused with CrMP (15 μM) plus ODQ (10 μM) for 30 min, and in arteries superfused with CrMP (15 μM) plus l-NAME (25 nM) for 30 min. In a second series of experiments, the effect of CORM-3 was verified in arteries preconstricted with PE after removal of the endothelium. In these arteries, after verifying CO effect, CORM-3 was removed by the superfusing PSS, and when a stable baseline was obtained, the NO donor SNP (100 nM to produce a detectible dilation) was added. When a stable baseline was observed, CORM-3 was added again to the superfusing PSS, and the dilating effect was measured again.

**Western Blot Analysis of HO-1 and of Subunits α and β1 of BKCa Protein Expression in Mesenteric Arteries of CCl4 Cirrhotic Rats.** Standard techniques were used to evaluate protein expression. After removal of veins and adipose tissue, small mesenteric arteries (30–40 arteries with diameter <500 μm) were collected from each rat, snap-frozen in liquid N2, 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 protein expression was detected using a monoclonal murine antibody against HO-1 (StressGen Biotechnologies Corp., Victoria, BC, Canada). Only HO-1, and not HO-2, was tested in the present study because we had already demonstrated, in similar experimental condition, that among the two main HO isoforms (HO-1 and HO-2), only HO-1 is overexpressed in small mesenteric arteries of CCl4-induced cirrhotic rats (Bolognesi et al., 2005).

The protein expression of the two subunits of BKCa, β1- and α-subunits, were detected using polyclonal rabbit anti-slo β1 (KCNMB1) and anti-KCa1.1 (α subunit 1) (KCNMA1) antibody (Alomone Labs Ltd., Jerusalem, Israel). The secondary antibodies, anti-rabbit conjugated to horseradish peroxidase, were diluted 1:1000 in phosphate-buffered saline containing 2% nonfat dry milk. Antigenic detection was visualized by standard ECL-enhanced chemiluminescence (Amersham, Arlington Heights, IL) with exposure to X-ray film. Control antigens (Alomone Labs Ltd.) were used as positive controls. Protein expression was determined by densitometric analysis using the VersaDoc Imaging System (Bio-Rad Laboratories, Hercules, CA). After stripping, the blots were assayed for β-actin content as standardization of sample loading. The quantitative densitometric values of each protein of interest were normalized to β-actin and displayed in histograms.

Protein expression of HO-1 was evaluated in four control rats, in six cirrhotic rats with ascites, and also in four cirrhotic rats without ascites. Protein expression of BKCa subunits was evaluated in five control rats, in four cirrhotic rats with ascites, and also in four cirrhotic rats without ascites.

**Data Analysis.** Data were expressed as mean ± S.E. Vasorelaxant responses were expressed as percent inhibition of the contraction induced by PE. Concentration-response data derived from each vessel were fitted separately to a logistic function by nonlinear regression and EC50 (molar concentration of ACh causing 50% of the maximal vasorelaxant effect) was calculated and expressed as log (molar) (pEC50). From the same regression, the maximal relaxation of the artery was also calculated. Two-way ANOVA was used to compare dose-response curves from controls and treated groups. Other data were analyzed by one-way ANOVA or Student’s t test for paired or unpaired observations when appropriate. The n values quoted indicate the number of experiments and animals used. The null hypothesis was rejected at p < 0.05.

**Results.** All rats treated with CCl4 included in the study had macronodular or micronodular cirrhosis. In 27 of 35 cirrhotic rats, the presence of ascites was confirmed by visual examination at laparotomy. The presence of ascites was dubious in two cirrhotic rats. These “intermediate” rats were classified as nonascitic, but they were not used for any experiment. Control rats had no appreciable alteration in liver appearance. At the time of the study, no difference in body weight between cirrhotic (nonascitic) rats, 570 ± 24 g; ascitic rats, 548 ± 11 g) and control rats (575 ± 20 g) was observed.

**Evaluation of the Response to ACh.** Comparing dose-response curves with ACh in baseline condition, ascitic cirrhotic rats (n = 6) showed a higher sensitivity to ACh with respect to control rats (p = 0.036) (Fig. 1).

**Effect of HO Inhibition.** Inhibition of HO with CrMP caused a slight shift of the concentration-response curve to ACh in control rats (F = 8.61, p < 0.001, two-way ANOVA), without a change in EC50 (Fig. 1). On the contrary, a marked rightward shift of the concentration-response curve to ACh was detected after CrMP in cirrhotic rats with ascites (F = 4.38, p = 0.0027, two-way ANOVA), with a significant increase in EC50 (Fig. 1). The increase in pEC50 after CrMP was significantly higher in cirrhotic rats with ascites (p = 0.03) (Fig. 1). Following CrMP, sensitivities of control and cirrhotic rats with ascites to ACh were the same (Fig. 1).

**Effect of sGC Inhibition and of HO Inhibition.** After treatment with Indo and l-NAME, mesenteric arteries of cirrhotic rats with ascites maintained a higher sensitivity to ACh with respect to control rats (p = 0.05) (Fig. 2). In arteries treated with Indo and l-NAME, the inhibition of sGC with ODQ provoked a shift of the dose-response curve to ACh, both in control rats (F = 3.58, p = 0.01, two-way ANOVA) and in cirrhotic rats with ascites (F = 8.09, p = 0.0005, two-way ANOVA) (Fig. 2). The addition of CrMP to ODQ provoked a further decrease in the response to ACh, both in control rats (F = 9.77, p < 0.001, two-way ANOVA) and in cirrhotic rats with ascites (F = 15.64, p < 0.001, two-way ANOVA) (Fig. 2). The effect of ODQ and of CrMP + ODQ on maximal relaxation of the artery was slightly but not significantly greater in control rats than in cirrhotic rats with ascites (Fig. 2).
Effect of BKCa Inhibition. Even after treatment with Indo, L-NAME, and ODQ, mesenteric arteries of cirrhotic rats with ascites maintained a higher sensitivity to ACh with respect to control rats (p = 0.024) (Fig. 3). The addition of IbTx provoked a decrease in the response to ACh both in control rats (F = 7.74, p = 0.007, two-way ANOVA) and in cirrhotic rats with ascites (F = 11.59, p = 0.001, two-way ANOVA) (Fig. 3). The effect of IbTx was more evident in ascitic cirrhotic rats than in control rats (the increase in pEC50 after IbTx was significantly higher in cirrhotic rats (p < 0.001, two-way ANOVA) and in ascites. The addition of CrMP (15 μM) to ODQ (10 μM) (closed square) decreased the effect of ACh both in control rats and in rats with cirrhosis and ascites. The addition of CrMP (15 μM) to ODQ (10 μM) (closed triangle) further reduced the effect of ACh in both groups. §, significantly different (p < 0.01) from the other two curves (two-way ANOVA). #, significantly different (p < 0.05) with respect to control rats; †, p = 0.05 with respect to control rats; *, significantly different (p < 0.05) from Indo + L-NAME; ‡, significantly different (p < 0.05) from Indo + L-NAME + ODQ.

Evaluation of the Response to CORM-3 of Small Mesenteric Arteries Preconstricted with Phenylinephrine in CCl4 Cirrhotic Rats. The addition of CORM-3 to the superfusion caused a slight but not significant dilatation (5 ± 3%) in mesenteric arteries of control rats (p = N.S.), whereas it caused a significant vasorelaxation (25 ± 6%) in cirrhotic rats with ascites (p = 0.05) (Fig. 5). Vasorelaxation was more evident in ascitic cirrhotic rats than in controls (p = 0.012) (Fig. 5). In arteries treated with CrMP and preconstricted with PE, CORM-3 provoked a significantly greater vasorelaxation both in controls and in ascitic cirrhosis (p = 0.016 and p = 0.022, respectively), but in the latter group, the vasorelaxation was more evident (p = 0.036) (Fig. 5). In arteries pretreated with CrMP and ODQ and preconstricted with PE, CORM-3 did not provoke any significant dilatation, both in control and in cirrhotic rats with ascites (Fig. 5). In arteries pretreated with CrMP and IbTx and preconstricted
with PE, CORM-3 caused a significant dilatation in controls rats (14 ± 3%, p = 0.004), which was slightly but not significantly lower than the dilatation obtained in the arteries pretreated only with CrMP (14 ± 3% versus 20 ± 3%, respectively, p = N.S.) (Fig. 5); on the contrary, in cirrhotic rats with ascites, pretreatment with CrMP and IbTx markedly reduced the dilator effect of CORM-3 with respect to the effect obtained after pretreatment with only CrMP (4 ± 1 versus 46 ± 11%, respectively, p = 0.003) (Fig. 5).

After endothelium removal, CORM-3 provoked a significant but slight dilatation both in controls (p = 0.002) and in cirrhotic rats (p = 0.056) but more evident in controls (p = 0.015) (Fig. 6). After the addition of a low concentration of SNP, which caused per se a modest dilatation (25 ± 8% versus 25 ± 5%, p = N.S., in controls and cirrhotic rats, respectively), the vasodilating effect of CORM-3 was more evident, both in controls (p = 0.0003) and in cirrhotic rats (p = 0.037), but remained more evident in control rats (p = 0.0008) (Fig. 6).

Western Blot Analysis. In small mesenteric resistance arteries, HO-1 and BKCa α-subunit protein expression was increased in rats with cirrhosis, particularly in those with ascites (Figs. 7 and 8). On the contrary, BKCa β1-subunit protein expression was significantly decreased both in cirrhotic rats with and without ascites (Fig. 8).
Because CO is the vasoactive molecule produced by HO (Zhang et al., 2001; Jaggar et al., 2002; Wu et al., 2002), inhibition of HO did not lead to any modification in the sensitivity to ACh only in cirrhotic rats with ascites, suggesting a role of BKCa in the altered vasoactive response that occurs in this pathological condition. Further inhibition of HO did not lead to any modification in the response to ACh, both in controls and in cirrhotic rats with ascites, excluding other mediators of HO.

We evaluated the effect of inhibition of BKCa in mesenteric arteries pretreated with the inhibitors of COX, NOS, and sGC. Sensitivity to ACh was increased in ascitic cirrhotic rats also after the inhibition of these three systems. The inhibition of BKCa caused a decrease in the maximal relaxation to ACh, particularly in control rats, but it provoked a marked decrease in the sensitivity to ACh only in cirrhotic rats with ascites, suggesting a role of BKCa in the altered vasoactive response that occurs in this pathological condition. Further inhibition of HO did not lead to any modification in the response to ACh, both in controls and in cirrhotic rats with ascites, excluding other mediators of HO.

We showed that inhibition of HO was more effective on the endothelium-dependent vasodilatation in cirrhotic rats with ascites with respect to controls rats (Fig. 1). To analyze the mechanisms that could link HO to the ACh-induced mesenteric vasorelaxation, we studied the relationship between HO and the effectors of CO on smooth muscle cells, i.e., sGC and nitric oxide synthase (NOS), respectively, both the inhibition of sGC and, afterward, of HO had a similar effect on ACh-induced vasorelaxation in control and in ascitic cirrhotic rats. Hence, the action of HO on smooth muscle cells is not exclusively due to the activation of sGC; moreover, the action on sGC is not what differentiates the HO action in ascitic cirrhotic rats.

Fig. 6. Effect of CORM-3 (200 μM) on the internal diameter of small mesenteric arteries after endothelium removal. The effect was evaluated before and after the administration of the NO donor SNP (100 nM). After endothelium removal, CORM-3 provoked a significant but slight dilatation both in controls (n = 4) (p = 0.002) (closed diamond) and in cirrhotic rats with ascites (n = 5) (p = 0.056) (open triangle); the dilatation was more evident in controls (p = 0.015). After the addition of a low concentration of SNP, which caused per se a modest dilation, the vasodilating effect of CORM-3 was more evident, both in controls (p = 0.0003) and in cirrhotic rats (p = 0.037), but remained more evident in control rats (p = 0.0008). The effect of CORM after SNP was measured when a new stable baseline was obtained after the administration of SNP. #, p < 0.05 with respect to control rats; *, p < 0.05 with respect to values obtained before SNP administration.

Discussion

This study demonstrates that in small mesenteric arteries of rats with CCl4-induced cirrhosis, the response to ACh is increased, and it is normalized by the inhibition of HO and BKCa. CO was more effective in cirrhotic than in control rats, and the effect was hindered by BKCa inhibition. In mesenteric arteries of cirrhotic rats, there is an overexpression of the α-subunit of BKCa, which together with the increased expression of HO-1 and an increased production of CO may cause the increased response to ACh.

BKCa channels are composed of the pore-forming α-subunit and of the auxiliary regulatory β-subunits that modulate channel gating (Tanaka et al., 2004). A change in the expression of BKCa subunits has been reported in experimental arterial hypertension. Indeed, a decrease in β1-subunit expression has been reported in genetic (Amberg and Santana, 2003a) and in acquired angiotensin II-induced hypertension (Amberg et al., 2003b), suggesting that it may contribute to the development of hypertension. Bratz et al. (2005) reported a decrease in the expression of BKCa α-subunit in superior mesenteric arteries from rats made hypertensive with Nω-nitro-L-arginine. Liu et al. (1997, 1998) reported an increase in the expression of the pore-forming α-subunit in aortas (Liu et al., 1997) and in cerebral arteries (Liu et al., 1998) of spontaneously hypertensive rats, and they suggested that such an increase may be a compensatory vasodilatory reaction in systemic hypertension (Liu et al., 1998). Our data demonstrate that an altered expression of BKCa may participate in the exaggerated mesenteric vasodilatation of cirrhosis. BKCaS are stimulated by the CO locally produced by endothelial HO (Naik et al., 2003a), which is also overexpressed in experimental cirrhosis (Bolognesi et al., 2005).

We showed that inhibition of HO was more effective on the endothelium-dependent vasodilatation in cirrhotic rats with ascites with respect to controls rats (Fig. 1). To analyze the mechanisms that could link HO to the ACh-induced mesenteric vasorelaxation, we studied the relationship between HO and the effectors of CO on smooth muscle cells, i.e., sGC and BKCa (Zhang et al., 2001; Jaggar et al., 2002; Wu et al., 2002).

In arteries treated with Indo and L-NAME, to inhibit any possible interfering action from cyclooxygenase (COX) and nitric oxide synthase (NOS), respectively, both the inhibition of sGC and, afterward, of HO had a similar effect on ACh-induced vasorelaxation in control and in ascitic cirrhotic rats. Hence, the action of HO on smooth muscle cells is not exclusively due to the activation of sGC; moreover, the action on sGC is not what differentiates the HO action in ascitic cirrhotic rats.

Fig. 7. Western blot analysis of HO-1 in the small mesenteric arteries of control rats and of cirrhotic rats, with and without ascites. The reported blots are representative of four to six experiments. Lower, densitometric analysis of HO-1. *, p < 0.05 with respect to control rats. In cirrhotic rats, the expression of HO-1 was increased.
arteries of control rats, the vasodilating response to CORM-3 was insignificant, whereas it was evident after elimination of endogenous CO by pretreatment with the HO inhibitor (Sacerdoti et al., 2006) (Fig. 5). A similar trivial effect of exogenous CO in baseline conditions has been reported by Kozma et al. (1999), who reported that in first order gracilis muscle arterioles, CO does not produce arteriolar dilatation unless the preparation is exposed previously to CrMP. One possible explanation for the ineffectiveness of exogenous CO as a vasodilator in preparations not exposed to an inhibitor to HO is that, in such a setting, the vasodilatory mechanism mediated by endogenous CO is maximally active (Kozma et al., 1999; Zhang et al., 2001). Another explanation may be that inhibition of endogenous CO results in increased NOS activity (Johnson and Johnson, 2003b), which, in turn, permits the exogenous CO to cause dilatation (Barkoudah et al., 2004). At any rate, in control rats, the vasodilatory effect of exogenous CO after HO inhibition was completely abolished after inhibition of sGC but only partially and not significantly reduced after inhibition of BKCa (the final response was significantly lower with respect to control rats, $p < 0.015$), underlining the pivotal role of these channels for the action of CO in this condition. The increased vasodilating effect of CO in ascitic cirrhotic rats was reversed in mesenteric arteries without endothelium, a condition in which the CO effect was lower with respect to control rats, even after SNP pretreatment. These results, taken together, not only support the hypothesis that the HO/CO system plays a role in the vasodilatation of mesenteric arteries in ascitic cirrhosis but also suggest that in ascitic cirrhotic rats, the effect of CO is mainly through BKCa channels.

Western blot data confirmed and supported the results of the
hemodynamic experiments. There was an increase in protein expression of HO-1 and of BKCa α-subunit in mesenteric arteries of cirrhotic rats, particularly in those with ascites, associated with a decrease in the expression of β1-subunit. This finding supports the hypothesis that CO could play a key role in the regulation of mesenteric vasorelaxation in cirrhosis because the α-subunit is the target of CO on BKCa. CO action on BKCa has been identified in the capacity of enhancing the coupling of Ca^{2+} sparks to BKCa. (Jaggar et al., 2002). More recently, Jaggar et al. (2005) have demonstrated that CO activates BKCa by binding to heme and modifying its interaction with an α-subunit-heme-binding domain. The effect of CO on BKCa is independent from the regulatory β1-subunit (Wu et al., 2002; Jaggar et al., 2005). Indeed, the presence of BKCa β-subunit is not necessary for the effect of CO, whereas on the contrary, it is essential for the stimulating effect of NO (Wu et al., 2002). The decreased expression of β1-subunit may be due to activation of the Renin-Angiotensin-Aldosterone system present in ascitic cirrhosis (Wilkinson and Williams, 1980; Schrier et al., 1988) because β1-subunit synthesis is inhibited by high levels of angiotensin II (Amberg et al., 2003b). However, the decreased presence of β1-subunit seems not sufficient to inhibit the action of BKCa because of the increased expression of the α-subunits and of the increased expression of HO, which through CO stimulation of heme oxygenase (HO), is necessary for the effect of CO, whereas on the contrary, it is required for the stimulating effect of NO (Wu et al., 2002). The BK channel: protective or detrimental in genetic hypertension. Circ Res 194 Bolognesi et al.


