■ R E V I E W

Nitric oxide and renal and cardiac dysfunction in cirrhosis

Joaquín GARCÍA-ESTAN*¹, M. Clara ORTIZ*¹ and Samuel S. LEE†²

*Departamento de Fisiología, Facultad de Medicina, Universidad de Murcia, Murcia 30100, Spain, and †Liver Unit, Gastroenterology Research Group, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1

A B S T R A C T

Nitric oxide (NO) has diverse physiological and pathophysiological effects. The roles of NO in the renal and cardiac dysfunction found in cirrhosis are reviewed. In the kidneys of experimental animals with cirrhosis, several lines of evidence speak in favour of an enhanced production of NO, through the activation of both endothelial constitutive and inducible isoforms of NO synthase. In contrast with the situation in normal animals, inhibition of NO synthesis in rats with cirrhosis improves sodium and water excretion via blood pressure-dependent and -independent mechanisms, which indicates that the renal sodium and water retention of cirrhosis is related to an excess of NO production. The deleterious effect of excessive NO on the kidney may be mediated by peroxynitrite, a potent oxidant that is readily formed whenever superoxide anions and the NO radical are produced together. The peroxidation of arachidonic acid by peroxynitrite leads to the formation of F_{2a} -isoprostanes, which are powerful renal vasoconstrictors. F_{2a} -isoprostane levels are correlated with the severity of liver injury during cirrhosis. However, whether peroxynitrite or F_{2a} -isoprostanes are the elusive mediator of the NO-induced renal alterations in cirrhosis remains to be firmly established. NO is also involved in cardiac contractility, probably in the normal heart as well as in disease conditions such as non-cirrhotic and cirrhotic cardiomyopathy. In the latter state, evidence suggests that inducible NO synthase attenuates ventricular contractility, mediated by cGMP. Another gas that transduces its signal through cGMP, carbon monoxide, is also likely to play a role in cirrhotic cardiomyopathy, but the nature of the interaction between NO and carbon monoxide in this syndrome remains unclear.

INTRODUCTION

“The animal spirit is *spiritus nitro-aerius*”

John Mayow, *Tractatus Quinque Medico-Physici*, 1674

In the 17th century the remarkably prescient English physiologist John Mayow described a ‘nitro-aerius’ gas, which he believed to be essential for life, and this a century before the discovery of oxygen and nitrogen. More than three centuries later, we know that a gas composed of oxygen and nitrogen, nitric oxide (NO), is

indeed critically important to maintain life. Over the past decade, a huge number of experimental studies have demonstrated the myriad functions and roles of NO in normal physiology, as well as in diverse disease states. It is currently known that NO is produced locally in many cells and tissues by the actions of NO synthase (NOS), which catalyses the conversion of L-arginine into L-citrulline with NO as the gaseous byproduct. NOS exists in three isoforms: neuronal (nNOS or NOS1), inducible (iNOS; NOS2) and endothelial constitutive (eNOS; ecNOS; NOS3). nNOS and eNOS are calcium-depen-

Key words: ascites, carbon monoxide, cGMP, cirrhotic cardiomyopathy, peroxynitrite, sodium retention.

Abbreviations: BDL rats, bile duct-ligated rats; HO, haem oxygenase; L-NAME, N^G -nitro-L-arginine methyl ester; L-NMMA, N^G -monomethyl-L-arginine; NOS, nitric oxide synthase; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; PDE, phosphodiesterase; PVS rats, portal vein stenosis rats; TBARS, thiobarbituric acid-reactive substances.

¹Present address: Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN, U.S.A.

Correspondence: Dr S. S. Lee (e-mail samlee@ucalgary.ca).

dent constitutively expressed isoforms, whereas iNOS is inducible, calcium-independent, and generates much greater concentrations of NO. Whatever the source, NO mediates its effects by rapid penetration of the target cell membrane and stimulation of soluble guanylate cyclase to produce its second messenger, cGMP. The interested reader is referred to recent reviews of general NO physiology [1,2].

The disease state of hepatic cirrhosis is associated with a host of haemodynamic and hydrodynamic abnormalities. These include the development of a hyperdynamic circulation (characterized by increased cardiac output and decreased peripheral vascular resistance), cirrhotic cardiomyopathy, hepatopulmonary syndrome and renal dysfunction with salt and water retention, leading to ascites and dilutional hyponatraemia. The pathogenesis of these phenomena remains incompletely understood, but a possible role for NO has been implicated in virtually all of these organs or vascular beds. Several recent publications have amply reviewed the role of NO in diverse aspects of the circulation in cirrhosis, including the hyperdynamic circulation [3], hepatosplanchnic vasculature [4], portal hypertensive gastropathy [5] and hepatopulmonary syndrome [6]. Because NO is so evanescent, with a lifespan of only seconds, it is likely to exert only paracrine effects in its local vascular bed. This explains the apparent paradox of an increased NO effect in most tissues but a decreased activity in certain organs such as the liver [4]. In the present review, we will summarize recent work concerning NO and the pathogenesis of renal and cardiac dysfunction in cirrhosis. Specifically, we will review the role of NO and related compounds in the salt and water retention of cirrhosis and in cirrhotic cardiomyopathy.

NO AND THE CIRRHOTIC KIDNEY

The peripheral vasodilatation associated with cirrhosis seems to be the origin of many clinical problems, such as sodium and water retention, ascites and renal failure. Although the origin of this haemodynamic alteration has not been completely elucidated, it is generally accepted that peripheral vasodilatation results from an imbalance between vasodilator and vasoconstrictor substances. There is now much evidence suggesting a role for NO in the peripheral vasodilatation of cirrhosis. Similarly, NO also seems to be an important mediator of the renal alterations. Human data, although scarce, generally support this idea. It has been demonstrated that patients with cirrhosis have increased plasma levels of nitrites. These plasma nitrite levels may correlate with plasma endotoxin levels [7] and with the severity of the liver disease [8], and thus might be used as a prognostic marker in cirrhosis [9]. Although advanced decompensated cirrhosis is associated with renal vasoconstriction,

patients with compensated cirrhosis have an increase in renal blood flow and glomerular filtration rate [10]. In this regard, Woitas et al. [11] have suggested that the hyperfiltration of patients with compensated cirrhosis is at least partly mediated by increased renal NO production. However, contradictory results in compensated patients have been reported by Sánchez-Rodríguez and colleagues [12], so this issue remains unresolved. Recently La Villa and colleagues [13] demonstrated that cirrhotic patients given the non-specific NOS inhibitor *N*^G-monomethyl-L-arginine (L-NMMA) at a dose sufficient to increase systemic vascular resistance by 26% also showed significant elevations of renal blood flow (12%), glomerular filtration rate (12%) and urinary sodium excretion (25%). To what degree these impressive renal effects were mediated by the increased systemic pressor effect remains unclear from these data, but the overall results certainly argue strongly in favour of a significant pathogenic role for NO in the renal dysfunction of cirrhosis.

Obviously, ethical considerations limit the experimental manipulations feasible in humans, so clarification of mechanisms underlying the effects of NO in the cirrhotic kidney requires the use of experimental animal models. The three most widely used rat models of liver disease are the carbon tetrachloride-induced cirrhosis model, the bile duct ligation model and the portal vein ligation model. The following discussion mostly focuses on studies in these animal models, with the caveat that, because of important differences between these models and human cirrhosis, extrapolation of these animal results to the human condition should be undertaken with caution.

In cirrhosis, sodium and water retention is due to increased renal tubular reabsorption [14]. The mechanisms mediating this elevated renal reabsorption are not completely clear. According to the peripheral arterial vasodilatation hypothesis, the main pathogenic factor for sodium and water retention is an arterial vasodilatation that induces a hyperdynamic circulatory state. This state would stimulate the anti-diuretic and anti-natriuretic systems that increase water and sodium reabsorption, and also impair the normal renal response to volume expansion [15,16]. During the past few years it has become increasingly clear that NO plays an important role in the pathogenesis of these altered mechanisms.

Many studies have suggested that excessive renal biosynthesis of NO may be involved in the local and systemic vasodilatation and associated abnormalities in cirrhosis. Elevated NO-dependent renal vasodilation has been described in cirrhotic rats [17]. Also, the renal excretion of end-products of NO metabolism, i.e. nitrates and nitrites, is increased in cirrhotic rats; inhibition of NO synthesis decreases excretion of these products [18,19]. Whether depressed renal metabolism of NO also contributes to the elevated nitrate/nitrite levels remains

unknown. Finally, acute inhibition of NO synthesis in experimental animals with cirrhosis, at doses that do not modify blood pressure, results in an improvement in renal excretory function [18–21]. This effect seems paradoxical, since in normal animals NO is a diuretic and natriuretic substance [22] and its inhibition results in a decrease in water and sodium excretion [21–23]. Similarly, chronic inhibition of NO in normal animals elevates arterial pressure and decreases sodium and water excretion, an effect associated with increased activity of renal and systemic angiotensin II [23–25].

In contrast, in cirrhotic rats, chronic inhibition of NOS improves renal salt and water excretion [26–28]. This beneficial effect seems to be mediated by increased blood pressure due to the inhibition of systemic vascular NO and the concomitant reduction in plasma levels of vasoactive substances such as vasopressin and aldosterone [27,28]. However, it has been shown that NOS antagonists at lower doses that do not elevate blood pressure can also induce natriuresis and diuresis in cirrhotic rats, which argues for an important intrarenal effect [21,29].

The precise role of each NOS isoform in the increased production of NO in cirrhosis has not yet been completely clarified. Thus activation of both iNOS and eNOS isoforms has been described in the renal tissue of cirrhotic rats [30,31]. While the results obtained with non-selective inhibitors, such as N^G -nitro-L-arginine methyl ester (L-NAME), and with inhibitors of nNOS suggest the involvement of the constitutive isoforms, other studies have demonstrated that the acute administration of aminoguanidine, a preferential inhibitor of iNOS, increases blood pressure and sodium and water excretion in two different rat models of cirrhosis [30,32]. However, chronic aminoguanidine administration did not produce any beneficial effect on the severely reduced excretion of sodium and water of conscious cirrhotic rats with ascites [32]. Clearly, further studies are needed using newer, more specific iNOS inhibitors, since the specificity of aminoguanidine has been questioned.

An important topic that has not yet been studied is the issue of the mechanisms responsible for the increased renal NO activity and/or production during the development of cirrhosis. Theoretically at least, there are two possibilities. First, elevated renal blood flows, which have been reported during the initial stages of cirrhosis, could stimulate NO release through a shear stress-related mechanism. However, it is not known if this renal vasodilation is the cause or the consequence of the increased NO production. A second possible mechanism for the stimulation of renal NO production is local, intrarenal elevation of vasoactive hormones. Thus elevated angiotensin II levels within the kidney, which occur early during the development of cirrhosis in bile duct-ligated (BDL) rats [33], are a very potent stimulus for intrarenal NO generation [34]. Other mechanisms

may also be at play, since we have observed that, as early as 24 h after ligation of the bile duct, urinary nitrites were increased and renal pressor responses to methoxamine were reduced, both of which could be blocked by NOS inhibitors (M. C. Ortiz and J. García-Esteban, unpublished work). Thus, at least in the BDL rat, another mechanism may exist that is activated very early, before the arterial vasodilation and the elevation in vasoactive substances are clearly established.

NO and oxidative stress

In addition to vasodilation, another consequence of the elevated levels of NO is its cellular toxicity. The direct toxicity of NO *per se* is modest, but is greatly increased on reaction with superoxide anions ($O_2^{\cdot-}$) to form peroxynitrite ($ONOO^-$). Although this reaction was viewed initially as a route for NO inactivation, the formation of peroxynitrite transforms two relatively unreactive free radicals, $\cdot NO$ and $O_2^{\cdot-}$, into much more bioreactive species. The formation of peroxynitrite depends on the concentration of both $\cdot NO$ and $O_2^{\cdot-}$ and, therefore, on the activities of both NOS and superoxide dismutase [35].

Oxygen-derived free radicals (among the more important of these is $O_2^{\cdot-}$), and consequently lipid peroxidation, are clearly enhanced in liver diseases. This may be due to decreased levels of endogenous antioxidants [36–42] or to enhanced generation stimulated by increased levels of angiotensin II [43], endothelin [44] or bile acids [45]. Thus, under these conditions, peroxynitrite is readily formed from the reaction between $O_2^{\cdot-}$ and $\cdot NO$. This reaction is at least three times faster than dismutation by superoxide dismutase [46].

Peroxynitrite is a highly reactive intermediate and one of the most potent oxidants known in biological systems. The mechanism of injury caused by peroxynitrite involves multiple factors, including initiation of lipid peroxidation and nitration of tyrosine-containing proteins [47]. Following the oxidative pathway through arachidonic acid, peroxynitrite induces the production of isoprostanes, which are prostaglandin-like compounds that have emerged as one of the most reliable markers of lipid peroxidation *in vivo* [48]. The most abundant isoprostane, F_{2a} -isoprostane, causes release of endothelin [49] and renal vasoconstriction, preferentially on afferent arterioles, through a thromboxane A_2 -like receptor-mediated process [50].

There is evidence for *in vivo* peroxynitrite production in human chronic hepatitis and cirrhosis [51,52]. A marked overproduction of F_{2a} -isoprostanes in experimental animals and in humans with cirrhosis has also been reported [53–55], and these levels are correlated with the severity of liver injury [56]. Collectively, these data suggest that isoprostanes might contribute to the renal dysfunction of cirrhosis by an effect on the renal microcirculation. In early cirrhosis, there is a decrease in

Figure 1 Mechanisms by which oxidative stress could be involved in the renal alterations of liver cirrhosis

$O_2^{\cdot-}$, superoxide anion; $ONOO^-$, peroxynitrite.

the vascular volume of the cortex and an intrarenal redistribution of blood flow towards the juxtamedullary zones in the presence of relatively preserved total renal blood flow [57–60]. As the liver disease progresses, the extent of renal vasoconstriction intensifies, which can lead to oliguria and worsening sodium retention, and finally culminates in the hepatorenal syndrome [61,62], in which circulating isoprostane levels have been reported to be six times greater than in normal controls [63].

Whether F_{2a} -isoprostanes are the elusive mediators of these renal alterations that occur in cirrhosis remains to be established. However, some recent studies found that administration of antioxidants prevents the development of a hyperdynamic circulation in rats with portal hypertension [64] and in BDL rats [65], and ameliorates renal dysfunction and survival rate in patients with hepatorenal syndrome [66]. These improvements were associated with a striking inhibition of F_{2a} -isoprostane formation, suggesting a role for isoprostanes in both the systemic and renal alterations that occur during cirrhosis. Finally, we have recently reported, in preliminary form, that high-dose vitamin E confers protection against the systemic and renal functional alterations associated with chronic bile duct ligation in rats [55]. Specifically, this treatment normalized the arterial hypotension, renal blood flow, glomerular filtration rate and natriuresis in rats subjected to bile duct ligation. This recovery in renal function was accompanied by an enhancement in cortical and medullary vascular filling in the kidney, measured by

microcomputed tomography techniques [57]. Despite these improvements, vitamin E did not significantly lower the systemic isoprostane levels and caused only a small (but significant) decrease in circulating levels of thiobarbituric acid-reactive substances (TBARS). Renal tissue levels of TBARS or carbamylated protein were also unaltered, but the renal vein concentrations of both TBARS and isoprostanes decreased significantly. These results suggest that renovascular oxidative stress is decreased by vitamin E therapy, and that this may be related to the recovery of cortical vasoconstriction observed in these animals.

In addition to its pro-oxidant properties, peroxynitrite is itself a vasodilator, and induces tachyphylaxis at a concentration of $3 \mu\text{mol/l}$ [67], preventing a further response to its own vasodilator actions. It also causes long-lasting impairment of the vasoactive response to other vasodilators [68], and even catecholamines [69]. Therefore the indirect vasoconstrictive effects of peroxynitrite may be tempered by simultaneous biochemically mediated vasodilatory effects.

To summarize, of the many determinants of haemodynamic and renal alterations in cirrhosis, oxidative stress is strongly proposed as a contributory factor. In addition, excess NO production also plays a role in the pathogenesis of these abnormalities, as established during the past few years. The combination of the two factors, i.e. excessive synthesis of NO and free radical production, will facilitate the generation of other potent compounds,

such as peroxynitrite and isoprostanes, that amplify the individual actions of the primary substances (Figure 1). Consequently, lowering the concentration of either NO or $O_2^{\cdot -}$ [35,46] can reduce the biological effects of peroxynitrite. This protective effect in a given experimental disease model can be interpreted as compelling evidence for the involvement of peroxynitrite, when coupled with the direct demonstration of the ability of peroxynitrite to cause the particular type of injury [70,71]. Indeed, inhibition of NO synthesis has been used extensively as a powerful therapeutic tool in experimental cirrhosis. More recently, some studies have shown that antioxidant supplementation provides protection against the systemic and renal anomalies that characterize cirrhosis. Although the current evidence is preliminary or circumstantial, we speculate that the beneficial effects following inhibition of NO synthesis or antioxidant administration in cirrhosis are due, at least partly, to decreases in peroxynitrite and isoprostane levels.

CIRRHOTIC CARDIOMYOPATHY

Since the pioneering description half a century ago by Kowalski and Abelmann [72] that cardiac output is increased in cirrhosis, the heart has been the subject of much study. About three decades ago, studies of left ventricular function in patients with alcoholic cirrhosis showed that, despite the high baseline cardiac output in this group, when the ventricle was stressed by physiological or pharmacological stimuli, the contractile responsiveness was subnormal ([73,74]; reviewed in [75–78]). For many years this curious phenomenon was assumed to be the result of latent alcoholic cardio-

myopathy in these patients. Only in the mid- to late-1980s did the realization gradually dawn that this depressed ventricular responsiveness must be due to cirrhosis *per se*, and not alcohol, because the same syndrome was observed in animal models and in humans with non-alcoholic aetiologies of cirrhosis ([79–81]; reviewed in [75–78]). Thus this syndrome of increased basal cardiac output and depressed ventricular responsiveness to stimuli is now known as cirrhotic cardiomyopathy. The pathogenesis and management of cirrhotic cardiomyopathy have been extensively reviewed [76–78,82], and the interested reader is referred to those reviews. Herein we will limit discussion to the potential role of NO and related cGMP-active compounds in the pathogenesis of this condition. To understand fully a pathophysiological role for NO in cirrhotic cardiomyopathy, we should first briefly summarize what is currently known about physiological roles of NO in normal conditions and also in non-cirrhotic forms of heart disease.

NO and cardiac contractility

The physiological and possible pathophysiological roles of NO in cardiac function have been extensively reviewed recently [83,84]. In general, it appears that eNOS is expressed in different heart cell types, including myocytes and endothelial cells. NO produced in small amounts from eNOS in the heart may be physiologically active in several ways. As in several other tissues, the effects of NO often appear to be contradictory, depending on the site, concentration, local redox state and presence of other vasoactive substances. At low concentrations, NO exerts modest stimulatory effects on contractility, by

Figure 2 Schema of cellular events involved in cardiomyocyte contraction, and possible influences in the cirrhotic myocyte. + indicates a positive or stimulatory effect; — indicates a negative or inhibitory effect. Abbreviations: β AR, β -adrenergic receptor; AC, adenylate cyclase; PKA, protein kinase A; PKG, protein kinase G; SR, sarcoplasmic reticulum; iCa, intracellular calcium flux; HO, haem oxygenase.

improving diastolic relaxation and compliance [83]. On the other hand, higher concentrations of NO may depress contractility, predominantly by opposing or counterbalancing stimulatory effects of β -adrenergic influences. For example, some evidence suggests that the negative inotropic effect of cardiac muscarinic cholinergic stimulation is dependent on eNOS [84]. An exhaustive review of these myriad mechanisms is beyond the scope of this review, but the reader is referred to the aforementioned reviews of NO physiology in the heart [83,84]. However, a brief summary of ventricular contraction is necessary in order to understand possible pathophysiological effects in the cirrhotic myocardium.

β -Adrenergic stimulation is mediated by a complex pathway that starts with receptor–ligand coupling in the myocyte sarcolemmal plasma membrane (Figure 2). The receptor–ligand complex activates the membrane-bound heterotrimeric G-proteins, and the $G_s\alpha$ subunit then stimulates the catalytic subunit of adenylate cyclase to produce the second messenger, cAMP. cAMP stimulates protein kinase A, which phosphorylates several proteins, leading ultimately to the influx of calcium via the membrane L-type calcium channel, and the release of calcium from intracellular sarcoplasmic reticulum calcium stores, the ryanodine receptors. The resultant calcium fluxes then activate actin–myosin cross-linking, and thus myocyte contraction. There are several regulatory and counterbalancing mechanisms. cAMP is metabolized by phosphodiesterases (PDEs), and three isoforms of PDE are found in the heart. The activity of the PDE II isoform is stimulated by cGMP, whereas PDE III is inhibited by cGMP. Thus cGMP may inhibit contractility in several ways: (a) by accelerating the breakdown of cAMP via its effect on PDE II, (b) activation of cGMP-dependent protein kinase, which inhibits the sarcolemmal L-type calcium channel [85], and (c) an inhibitory effect on the activity of the intracellular sarcoplasmic reticulum calcium-release channel (ryanodine receptor) [86,87].

These pathways, and their role in the pathogenesis of cirrhotic cardiomyopathy, have been the subject of several studies over the past decade (reviewed in [76–78]). Multiple defects have been described in the β -adrenergic signal transduction system, such as decreases in the density of β -adrenoceptors, $G_s\alpha$ levels and adenylate cyclase activity. There are also decreases in some of the inhibitory systems, such as the muscarinic m_2 receptors [88] and G_i protein levels [89], which probably represent a compensatory adjustment. Recently, decreased function of the ventricular L-type sarcolemmal calcium channel has been described in cirrhotic rats [90].

In several different non-cirrhotic models of cardiac dysfunction or failure, such as ischaemic, sepsis-induced and pacing-overdrive, iNOS is activated with significant negative inotropic effects. However, this general schema, with eNOS mediating some tonic physiological effects

and iNOS responsible for pathophysiological phenomena, has not been uniformly supported by all studies. For example, Stein and colleagues [91] found that expression of eNOS rather than iNOS was increased in human heart failure. However, the weight of evidence does support the idea that iNOS plays an important role in several forms of non-cirrhotic myocardial dysfunction. It was therefore not surprising that the possible role of NO in cirrhotic cardiomyopathy came to be examined.

NO in cirrhotic cardiomyopathy

In 1996, the first mention of a possible role for NO in the pathogenesis of cirrhotic cardiomyopathy was noted in two publications. While we briefly mentioned NO as an interesting topic for further study in a review [76], much more credit must be given to Van Obbergh and colleagues [92], who conducted an experimental study in the BDL cirrhotic rat. They administered the NOS inhibitor L-NMMA in an isolated working heart preparation, and observed improvement of blunted contractility [92]. L-NMMA had no effect in control hearts. This study was therefore the first clear demonstration of a possible role for NO in the blunted ventricular contractility found in cirrhosis.

More recently, Liu et al. [93] performed detailed studies in the BDL rat to elucidate further the role of the NO pathway in cirrhotic cardiomyopathy. Their hypothesis was that stimulation of iNOS by endotoxins or cytokines in the cirrhotic heart or circulation would lead to the overproduction of NO and thus to a net negative inotropic effect. First, it was observed that cardiac and serum levels of the cytokines tumour necrosis factor α and interleukin- 1β were elevated in the BDL rat, and that the negative inotropic effect of interleukin- 1β could be reversed by preincubation with L-NAME. Then it was demonstrated that L-NAME treatment restored the depressed isolated papillary muscle contractile responsiveness to isoprenaline in BDL rats, but had no effect in the muscles of control animals. Serum levels of nitrates/nitrites were elevated in BDL rats. Treatment with the NO donor *S*-nitroso-*N*-acetylpenicillamine decreased normal papillary muscle contractility. Finally, in BDL rat ventricles, increases were noted in soluble cGMP levels as well as in the content of iNOS mRNA and protein, without any change in the expression of eNOS. These experiments demonstrated conclusively the role of iNOS in the pathogenesis of cirrhotic cardiomyopathy. The authors believe [93] that the negative inotropic effects are mediated mainly by the increased cGMP levels, via the mechanisms noted above.

Battarbee and colleagues [94] have recently reported their investigations of NO activity in a rat model of prehepatic portal hypertension due to graded stenosis of the portal vein [portal vein stenosis (PVS) rats]. This rat model does not have any intrinsic liver disease, but mimics the human situation of portal vein obstruction

with a 'pure' presinusoidal portal hypertension. The PVS rat, like the human with this condition, suffers from many of the same cardiovascular derangements as in the cirrhotic condition, including a hyperdynamic circulation. However, whether this rat model also has myocardial contractile dysfunction remains controversial. Whereas Ma and colleagues [95] did not find evidence of cardiomyopathy, at least at the level of the myocyte membrane, Zavec and co-workers [96] documented decreases of 30–50% in systolic contractility in ventricular strips from PVS rats. These latter investigators also found that, in contrast with the decreased $G_s\alpha$ and $G_i\alpha$ content along with β -adrenoceptor and G-protein uncoupling described previously in the BDL rat [89], PVS rats show an entirely different pattern of cardiomyocyte intracellular and membrane changes. Specifically, these parameters were found to be unaltered in PVS rats, whereas membrane L-type Ca^{2+} channels and sarcoplasmic reticulum caffeine-sensitive calcium stores appeared to be dysfunctional [96]. Given the considerations described above, it is interesting to note that Battarbee and colleagues [94] found no evidence of iNOS or even NO activation in their PVS rats. Even serum levels of nitrites/nitrates were unaltered in their PVS rat model. At present, our opinion is that if cardiac dysfunction exists in prehepatic portal hypertension, it is clearly due to mechanisms different from those of cirrhotic cardiomyopathy.

Recent interest has focused on another evanescent gas with physiological and pathophysiological actions: carbon monoxide (CO). The only source of CO in mammals is via the metabolism of haem. The enzyme haem oxygenase (HO) catalyses the degradation of haem to iron, biliverdin and CO. The similarities between the HO/CO system and the NO pathway are striking. Analogous to the NOS isoforms, HO exists in inducible (HO-1) and constitutive (HO-2) isoforms. HO-1, also known as heat shock protein-32, is induced by more stimuli and factors than probably any other mammalian protein (reviewed in [97]). These include stimuli such as ischaemia, hyperthermia, hypothermia, sepsis, haemorrhage and altered redox states. CO, like NO, induces vasodilatation and negative inotropic effects via cGMP. Suematsu and colleagues have recently demonstrated an important physiological role for CO as a vasodilator in the hepatic microcirculation (reviewed in [98]). Moreover, Dillon and co-workers [99] have reported a correlation in cirrhotic patients between carboxyhaemoglobin, an indirect index of endogenous CO production, and plasma cGMP levels, suggesting that CO may contribute to the pathogenesis of the hyperdynamic circulation in cirrhosis.

In view of these considerations, Liu et al. [100] recently investigated the role of the HO/CO pathway in the pathogenesis of cirrhotic cardiomyopathy in BDL rats. They found that HO-1 mRNA transcription and protein

expression are significantly augmented in ventricles of cirrhotic animals compared with controls, whereas there is no difference in HO-2 mRNA or protein levels. In cirrhotic ventricles, treatment with the HO inhibitor zinc protoporphyrin significantly decreased the elevated cGMP content and reversed the decreased contractility of isolated papillary muscles. These results suggest that activation of the HO/CO pathway is involved in the pathogenesis of cirrhotic cardiomyopathy.

Not addressed in the above study were the issues of the relative contributions and possible interactions of the NOS/NO system and the HO/CO system. It is known that, in general, NO is a much more potent stimulator of soluble guanylate cyclase than CO [101,102], although the exact stoichiometry of cGMP production by these two systems in liver disease remains completely unstudied. Moreover, in certain circumstances the NO and CO pathways may potentiate or inhibit each other (reviewed in [97]). These questions, especially in the setting of the cirrhotic myocardium, remain to be clarified. Finally, in contrast with the few human studies of NO and the cirrhotic kidney, we are aware of no human studies of NO and cirrhotic cardiomyopathy. This deficit should be addressed in future studies.

ACKNOWLEDGMENTS

The research of M. C. O. and J. G.-E. was funded by grants from the Fundación Séneca de Murcia (PB/FS/99) and Comisión Interministerial de Investigación Científica y Técnica of Spain (SAF2000-0157). S. S. L. was funded by the Canadian Institutes of Health Research (FRN 12613), and salary support by a Senior Scholarship award from the Alberta Heritage Foundation for Medical Research.

REFERENCES

- Ignarro, L. J., Cirino, G., Casini, A. and Napoli, C. (1999) Nitric oxide as a signaling molecule in the vascular system: an overview. *J. Cardiovasc. Pharmacol.* **34**, 879–886
- Grisham, M. B., Jourdain, D. and Wink, A. M. (1999) Nitric oxide. I. Physiological chemistry of nitric oxide and its metabolites: implications in inflammation. *Am. J. Physiol.* **276**, G315–G321
- Hartleb, M., Michielsen, P. P. and Dziurkowska-Marek, A. (1997) The role of nitric oxide in portal hypertensive systemic and portal vascular pathophysiology. *Acta Gastroenterol. Belg.* **60**, 222–232
- Wiest, R. and Groszmann, R. J. (1999) Nitric oxide and portal hypertension: its role in the regulation of intrahepatic and splanchnic vascular resistance. *Semin. Liver Dis.* **19**, 411–426
- Ferraz, J. G. and Wallace, J. L. (1997) Underlying mechanisms of portal hypertensive gastropathy. *J. Clin. Gastroenterol.* **25** (Suppl. 1), S73–S78
- Fallon, M. B. (2000) Methylene blue and cirrhosis: pathophysiologic insights, therapeutic dilemmas. *Ann. Intern. Med.* **133**, 738–740

- 7 Guarner, C., Soriano, G., Tomás, A. et al. (1993) Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology* **18**, 1139–1143
- 8 Hori, N., Okanou, T., Kashima, K. et al. (1996) Endogenous nitric oxide production is augmented as the severity advances in patients with liver cirrhosis. *Clin. Exp. Pharmacol. Physiol.* **23**, 30–35
- 9 Heller, J., Kristeleit, H., Brensing, K. A., Woitas, R. P., Spengler, U. and Sauerbruch, T. (1999) Nitrite and nitrate levels in patients with cirrhosis of the liver: influence of kidney function and fasting state. *Scand. J. Gastroenterol.* **34**, 297–302
- 10 Wong, F., Massie, D., Colman, J. and Dudley, F. (1993) Glomerular hyperfiltration in patients with well-compensated alcoholic cirrhosis. *Gastroenterology* **104**, 884–889
- 11 Woitas, R. P., Heller, J., Stoffel-Wagner, B., Spengler, U. and Sauerbruch, T. (1997) Renal functional reserve and nitric oxide in patients with compensated liver cirrhosis. *Hepatology* **26**, 858–864
- 12 Sánchez-Rodríguez, A., Oliveira, A., Martín-Oterino, J. A. et al. (1999) Renal function in compensated hepatic cirrhosis: effects of an amino acid infusion and relationship with nitric oxide. *Dig. Dis.* **17**, 235–240
- 13 La Villa, G., Barletta, G., Pantaleo, P. et al. (2001) Hemodynamic, renal and endocrine effects of acute inhibition of nitric oxide synthase in compensated cirrhosis. *Hepatology* **34**, 19–27
- 14 López-Novoa, J. M., Rengel, M. A., Rodicio, J. L. and Hernando, L. (1977) A micropuncture study of salt and water retention in chronic experimental cirrhosis. *Am. J. Physiol.* **232**, F315–F318
- 15 López-Novoa, J. M. and Martínez-Maldonado, M. (1982) Impaired response to splanchnic infusion of hypertonic saline in conscious cirrhotic rats. *Am. J. Physiol.* **242**, F390–F394
- 16 Atucha, N. M., Quesada, T. and García-Estañ, J. (1993) Reduced papillary plasma flow in non-ascitic cirrhotic rats. *Clin. Sci.* **85**, 139–145
- 17 García-Estañ, J., Atucha, N. M., Sabio, J. M., Vargas, F., Quesada, T. and Romero, J. C. (1994) Increased endothelium-dependent renal vasodilation in cirrhotic rats. *Am. J. Physiol.* **267**, R549–R553
- 18 Ramírez, A., Atucha, N. M., Pérez, M. C. et al. (1994) Efectos de la inhibición de la síntesis de óxido nítrico en la hemodinámica y excreción renal de ratas con cirrosis biliar primaria. *Nefrología* **14**, 656–662
- 19 Atucha, N. M., Ramírez, A., Pérez, M. C. et al. (1994) Papel del óxido nítrico en las alteraciones renales de la cirrosis hepática. *Nefrología* **14**, 651–655
- 20 Clària, J., Jiménez, W., Ros, J. et al. (1992) Pathogenesis of arterial hypotension in cirrhotic rats with ascites: role of endogenous nitric oxide. *Hepatology* **15**, 343–349
- 21 Atucha, N. M., García-Estañ, J., Ramírez, A., Pérez, M. C., Quesada, T. and Romero, J. C. (1994) Renal effects of nitric oxide synthesis inhibition in cirrhotic rats. *Am. J. Physiol.* **267**, R1454–R1460
- 22 Romero, J. C., García-Estañ, J. and Atucha, N. M. (1996) The role of nitric oxide on renal function: the control of blood pressure under normal conditions and during cirrhosis. In *The Kidney in Liver Disease* (Epstein, M., ed.), pp. 373–386. Hanley & Belfus Inc., Philadelphia
- 23 Lahera, V., Navarro, J., Cachofeiro, V., García-Estañ, J. and Ruilope, L. M. (1997) Nitric oxide, the kidney and hypertension. *Am. J. Hypertens.* **10**, 129–140
- 24 García-Estañ, J., Navarro, J., Atucha, N. M. et al. (1996) Chronic effect of nitric oxide and prostaglandin inhibition on pressure diuresis and natriuresis in rats. *Kidney Int.* **49** (Suppl. 55), S141–S143
- 25 Fortepiani, L. A., Rodrigo, E., Ortiz, M. C. et al. (1999) Pressure natriuresis in nitric oxide-deficient hypertensive rats: effect of antihypertensive treatments. *J. Am. Soc. Nephrol.* **10**, 21–27
- 26 Martín, P. Y., Ohara, M., Ginès, P. et al. (1998) Nitric oxide synthase inhibition for one week improves renal sodium and water excretion in cirrhotic rats with ascites. *J. Clin. Invest.* **101**, 235–242
- 27 Ortiz, M. C., Fortepiani, L. A., Martínez-Salgado, C. et al. (2002) Renal effects of chronic L-NAME and aminoguanidine in cirrhotic rats with ascites. *Nefrología*, in the press
- 28 Xu, L., Carter, E. P., Ohara, M. et al. (2000) Neuronal nitric oxide synthase and systemic vasodilation in rats with cirrhosis. *Am. J. Physiol.* **279**, F1110–F1115
- 29 Atucha, N. M. and García-Estañ, J. (1996) Intrarenal alterations in experimental liver cirrhosis. *News Physiol. Sci.* **11**, 48–52
- 30 Criado, M., Flores, O., Ortiz, M. C. et al. (1997) Elevated glomerular and blood mononuclear lymphocyte nitric oxide production in rats with chronic bile duct ligation: Role of inducible nitric oxide synthase activation. *Hepatology* **26**, 268–276
- 31 Bosch-Marcé, M., Morales-Ruiz, M., Jiménez, W. et al. (1998) Increased renal expression of nitric oxide synthase type III in cirrhotic rats with ascites. *Hepatology* **27**, 1191–1199
- 32 Ortiz, M. C., Fortepiani, L. A., Martínez, C., Atucha, N. M. and García-Estañ, J. (1996) Renal and pressor effects of aminoguanidine in cirrhotic rats with ascites. *J. Am. Soc. Nephrol.* **7**, 2694–2699
- 33 Ubeda, M., Matzilevich, M., Atucha, N. M. et al. (1994) Renin and angiotensinogen mRNA expression in the kidneys of rats subjected to long-term bile duct ligation. *Hepatology* **19**, 1431–1436
- 34 Ortiz, M. C., Fortepiani, L. A., Ruiz, F. M., Atucha, N. M. and García-Estañ, J. (1998) Role of AT₁ receptors in the acute and chronic effects of nitric oxide inhibition. *Am. J. Physiol.* **274**, R760–R766
- 35 Squadrito, G. L. and Pryor, W. A. (1998) Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite and carbon dioxide. *Free Radical Biol. Med.* **25**, 392–403
- 36 Panozzo, M. P., Basso, L., Balint, L. et al. (1995) Renal functional alterations in extrahepatic cholestasis: Can oxidative stress be involved? *Eur. Surg. Res.* **27**, 332–339
- 37 Bianchi, G., Marchesini, G., Fabbri, A., Ronchi, M., Chianese, R. and Grossi, G. (1997) Lipoperoxide plasma levels in patients with liver cirrhosis. *Hepatol. Gastroenterol.* **44**, 784–788
- 38 Krahenbuhl, S., Talos, C., Lauterburg, B. H. and Reichen, J. (1995) Reduced antioxidative capacity in liver mitochondria from bile duct ligated rats. *Hepatology* **22**, 607–612
- 39 Tsai, L. Y., Lee, K. T., Tsai, S. M., Lee, S. C. and Yu, H. S. (1993) Changes of lipid peroxide levels in blood and liver tissue of patients with obstructive jaundice. *Clin. Chem. Acta* **215**, 41–50
- 40 Tsai, L. Y., Lee, K. T. and Lu, F. J. (1997) Biochemical events associated with ligation of the common bile duct in Wistar rats. *J. Formosan Med. Assoc.* **96**, 17–22
- 41 Halim, A. B., el-Ahmady, O., Hassab-Allah, S., Abdel-Galil, F., Hafez, Y. and Darwish, A. (1997) Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. *Ann. Clin. Biochem.* **34**, 656–663
- 42 Parola, M., Leonarduzzi, G., Robino, G., Albano, E., Poli, G. and Dianzani, M. U. (1996) On the role of lipid peroxidation in the pathogenesis of liver damage induced by long-standing cholestasis. *Free Radical Biol. Med.* **20**, 351–359
- 43 Pagano, P. J., Clark, J. K., Cifuentes-Pagano, M. E., Clark, S. M., Callis, G. M. and Quinn, M. T. (1997) Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 14483–14488
- 44 Barton, M., Shaw, S., d'Uscio, L. V., Moreau, P. and Luscher, T. F. (1997) Angiotensin II increases vascular and renal endothelin-1 and functional endothelin converting enzyme activity *in vivo*: role of ET_A receptors for endothelin regulation. *Biochem. Biophys. Res. Commun.* **238**, 861–865
- 45 Bomzon, A., Holt, S. and Moore, K. (1997) Bile acids, oxidative stress and renal function in biliary obstruction. *Semin. Nephrol.* **17**, 549–562

- 46 Pryor, W. A. and Squadrito, G. L. (1995) The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* **268**, L699–L722
- 47 Beckman, J. S. and Koppenol, W. H. (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol.* **271**, C1424–C1437
- 48 Morrow, J. D. and Roberts, II, L. J. (1997) The isoprostanes: unique bioactive products of lipid peroxidation. *Prog. Lipid Res.* **36**, 1–21
- 49 Fukunaga, M., Takafumi, Y. and Badr, K. (1995) Stimulatory effect of 8-epi-PGF_{2α} and F₂-isoprostane, on endothelin-1 release. *J. Cardiovasc. Pharmacol.* **26** (Suppl. 3), S51–S52
- 50 Takahashi, K., Nammour, T. M., Fukunaga, M. et al. (1992) Glomerular actions of a free radical-generated novel prostaglandin, 8-epi-prostaglandin F_{2α}, in the rat. Evidence for interaction with thromboxane A₂ receptors. *J. Clin. Invest.* **90**, 136–141
- 51 Cuzzocrea, S., Zingarelli, B., Villari, D., Caputi, A. P. and Longo, G. (1998) Evidence for in vivo peroxynitrite production in human chronic hepatitis. *Life Sci.* **63**, 25–30
- 52 Banan, A., Fields, J. Z., Decker, H., Zhang, Y. and Keshavarzian, A. (2000) Nitric oxide and its metabolites mediate ethanol-induced microtubule disruption and intestinal barrier dysfunction. *J. Pharmacol. Exp. Ther.* **294**, 997–1008
- 53 Pratico, D., Iuliano, L., Basili, S. et al. (1998) Enhanced lipid peroxidation in hepatic cirrhosis. *J. Investig. Med.* **46**, 51–57
- 54 Awad, J. A., Roberts, L. J., Burk, R. F. and Morrow, J. D. (1996) Isoprostanes–prostaglandin-like compounds formed in vivo independently of cyclooxygenase: use as clinical indicators of oxidant damage. *Gastroenterol. Clin.* **25**, 409–427
- 55 Ortiz, M. C., Romero, J. C., Nath, K. A. and Juncos, L. A. (2000) Vitamin E improves systemic and renal hemodynamic and renal excretory function in bile duct ligated rats. *J. Am. Soc. Nephrol.* **11**, 606A (abstract)
- 56 Nanji, A. A., Khwaja, S., Tahan, S. R. and Sadrzadeh, S. M. (1994) Plasma levels of a novel noncyclooxygenase-derived prostanoid (8-isoprostane) correlate with severity of liver injury in experimental alcoholic liver disease. *J. Pharmacol. Exp. Ther.* **269**, 1280–1285
- 57 Ortiz, M. C., García-Sanz, A., Bentley, M. D. et al. (2000) Micro-computed tomography of kidneys following chronic bile duct ligation. *Kidney Int.* **58**, 1632–1640
- 58 Sacerdoti, D., Bolognesi, M., Merkel, C., Angeli, P. and Gatta, A. (1993) Renal vasoconstriction in cirrhosis evaluated by duplex Doppler ultrasonography. *Hepatology* **17**, 219–224
- 59 Sacerdoti, D., Merlo, A., Merkel, C., Zuin, R. and Gatta, A. (1986) Redistribution of renal blood flow in patients with liver cirrhosis. *J. Hepatol.* **2**, 253–261
- 60 Yarger, W. E., Schrader, N. W. and Boyd, M. A. (1976) Intrarenal mechanism of salt retention after bile duct ligation in rats. *J. Clin. Invest.* **57**, 408–418
- 61 Epstein, M. (1994) Hepatorenal syndrome. Emerging perspectives of pathophysiology and therapy. *J. Am. Soc. Nephrol.* **4**, 1735–1753
- 62 Arroyo, V., Ginès, P., Gerbes, A. L. et al. (1996) Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. *Hepatology* **23**, 164–176
- 63 Morrow, J., Moore, K., Awad, J. et al. (1993) Marked overproduction of non-cyclooxygenase derived prostanoids (F₂-isoprostanes) in the hepatorenal syndrome. *J. Lipid Med.* **6**, 417–420
- 64 Bimbi, F., Marley, R., Holt, S. et al. (1998) N-Acetylcysteine prevents development of the hyperdynamic circulation in the portal hypertensive rat. *Hepatology* **28**, 689–694
- 65 Marley, R., Holt, S., Bimbi, F. et al. (1999) Lipoid acid prevents development of the hyperdynamic circulation in anesthetized rats with biliary cirrhosis. *Hepatology* **29**, 1358–1363
- 66 Holt, S., Goodier, D., Marley, R. et al. (1999) Improvement in renal function in hepatorenal syndrome with N-acetylcysteine. *Lancet* **353**, 294–295
- 67 Villa, L. M., Salas, E., Darley-Usmar, V. M., Radomski, M. W. and Moncada, S. (1994) Peroxynitrite induces both vasodilatation and impaired vascular relaxation in the isolated perfused rat heart. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12383–12387
- 68 Benkusky, N. A., Lewis, S. J. and Kooy, N. W. (1998) Attenuation of vascular relaxation after development of tachyphylaxis to peroxynitrite in vivo. *Am. J. Physiol.* **275**, H501–H507
- 69 Benkusky, N. A., Lewis, S. J. and Kooy, N. W. (1999) Peroxynitrite-mediated attenuation of α - and β -adrenoceptor agonist-induced vascular responses in vivo. *Eur. J. Pharmacol.* **8**, 151–158
- 70 Crow, J. P. and Beckman, J. S. (1995) The role of peroxynitrite in nitric oxide-mediated toxicity. *Curr. Top. Microbiol. Immunol.* **196**, 57–73
- 71 Szabo, C. (1996) The pathophysiological role of peroxynitrite in shock, inflammation and ischemia-reperfusion injury. *Shock* **6**, 79–88
- 72 Kowalski, H. J. and Abelmann, W. H. (1953) Cardiac output at rest in Laennec's cirrhosis. *J. Clin. Invest.* **32**, 1025–1033
- 73 Gould, L., Sharif, M., Zahir, M. and Di Lieto, M. (1969) Cardiac hemodynamics in alcoholic patients with chronic liver disease and a presystolic gallop. *J. Clin. Invest.* **48**, 860–868
- 74 Limas, C. J., Guiha, N. H. and Lekagui, O. (1974) Impaired left ventricular function in alcoholic cirrhosis. *Circulation* **49**, 755–760
- 75 Lee, S. S. (1989) Cardiac abnormalities in liver cirrhosis. *West. J. Med.* **151**, 530–535
- 76 Ma, Z. and Lee, S. S. (1996) Cirrhotic cardiomyopathy: getting to the heart of the matter. *Hepatology* **24**, 451–459
- 77 Liu, H. and Lee, S. S. (1999) Cardiopulmonary dysfunction in cirrhosis. *J. Gastroenterol. Hepatol.* **14**, 600–608
- 78 Myers, R. P. and Lee, S. S. (2000) Cirrhotic cardiomyopathy and liver transplantation. *Liver Transpl.* **6** (Suppl. 1), S44–S52
- 79 Caramelo, C., Fernandes-Munoz, D., Santos, J. C. et al. (1986) Effect of volume expansion on hemodynamics, capillary permeability, and renal function in conscious, cirrhotic rats. *Hepatology* **6**, 129–134
- 80 Lee, S. S., Marty, J., Mantz, J., Samain, E., Brailion, A. and Lebrec, D. (1990) Desensitization of myocardial β -adrenergic receptors in cirrhotic rats. *Hepatology* **12**, 481–485
- 81 Ingles, A. C., Hernandez, I., Garcia-Estan, J., Quesada, T. and Carbonell, L. F. (1991) Limited cardiac preload reserve in conscious cirrhotic rats. *Am. J. Physiol.* **260**, H1912–H1917
- 82 Ma, Z. and Lee, S. S. (1999) Management of cirrhotic cardiomyopathy. In *Medical Management of Liver Disease* (Krawitt, E. J., ed.), pp. 583–589, Marcel Dekker, New York
- 83 Paulus, W. J. and Shah, A. M. (1999) NO and cardiac diastolic function. *Cardiovasc. Res.* **43**, 595–606
- 84 Balligand, J. L. (1999) Regulation of cardiac β -adrenergic response by nitric oxide. *Cardiovasc. Res.* **43**, 607–620
- 85 Mery, P. F., Pavoin, C., Belhassen, L., Pecker, F. and Fischmeister, R. (1993) Nitric oxide regulates cardiac Ca²⁺ current. Involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. *J. Biol. Chem.* **268**, 26286–26295
- 86 Zahradnikova, A., Minarovic, I., Venema, R. C. and Meszaros, L. G. (1997) Inactivation of the cardiac ryanodine receptor calcium release channel by nitric oxide. *Cell Calcium* **22**, 447–454
- 87 Eu, J. P., Xu, L., Stamler, J. S. and Meissner, G. (1999) Regulation of ryanodine receptors by reactive nitrogen species. *Biochem. Pharmacol.* **57**, 1079–1084
- 88 Jaue, D. N., Ma, Z. and Lee, S. S. (1997) Cardiac muscarinic receptor function in rats with cirrhotic cardiomyopathy. *Hepatology* **25**, 1361–1365
- 89 Ma, Z., Miyamoto, A. and Lee, S. S. (1996) Role of altered β -adrenergic receptor signal transduction in the pathogenesis of cirrhotic cardiomyopathy in rats. *Gastroenterology* **110**, 1191–1198

- 90 Ward, C. A., Liu, H. and Lee, S. S. (2001) Altered cellular calcium regulatory systems in a rat model of cirrhotic cardiomyopathy. *Gastroenterology* **121**, 1209–1218
- 91 Stein, B., Eschenhagen, T., Rudiger, J., Scholz, H., Forstermann, U. and Gath, I. (1998) Increased expression of constitutive nitric oxide synthase III, but not inducible nitric oxide synthase II in human heart failure. *J. Am. Coll. Cardiol.* **32**, 1179–1186
- 92 Van Obbergh, L., Vallieres, Y. and Blaise, G. (1996) Cardiac modifications occurring in the ascitic rat with biliary cirrhosis are nitric oxide related. *J. Hepatol.* **24**, 747–752
- 93 Liu, H., Ma, Z. and Lee, S. S. (2000) Contribution of nitric oxide to the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. *Gastroenterology* **118**, 937–944
- 94 Battarbee, H. D., Zavec, J. H., Grisham, M. B. et al. (1999) Cardiac impairment and nitric oxide synthase activity in the chronic portal vein-stenosed rat. *Am. J. Physiol.* **276**, G363–G372
- 95 Ma, Z., Meddings, J. B. and Lee, S. S. (1995) Cardiac plasma membrane physical properties and beta-adrenergic receptor function are unaltered in portal hypertensive rats. *Hepatology* **22**, 188–193
- 96 Zavec, J. H., Bueno, O., Maloney, R. E., O'Donnell, J. M., Roerig, S. C. and Battarbee, H. D. (2000) Cardiac excitation-contraction coupling in the portal hypertensive rat. *Am. J. Physiol.* **279**, G28–G39
- 97 Maines, M. D. (1997) The heme oxygenase system: a regulator of second messenger gases. *Annu. Rev. Pharmacol. Toxicol.* **37**, 517–554
- 98 Suematsu, M. and Ishimura, Y. (2000) The heme oxygenase-carbon monoxide system: a regulator of hepatobiliary function. *Hepatology* **31**, 3–6
- 99 Dillon, J. F., Selby, C., Hadoke, P. W. F. et al. (1998) Hypothesis and preliminary data to suggest carbon monoxide as the mediator of the hyperdynamic circulation of cirrhosis? *Med. Biochem.* **1**, 93–96
- 100 Liu, H., Song, D. and Lee, S. S. (2001) Role of heme oxygenase pathway in pathogenesis of cirrhotic cardiomyopathy in the rat. *Am. J. Physiol.* **280**, G68–G74
- 101 Sharma, V. S. and Magde, D. (1999) Activation of soluble guanylate cyclase by carbon monoxide and nitric oxide: a mechanistic model. *Methods* **19**, 494–505
- 102 Hoenicke, M., Becker, E. M., Apeler, H. et al. (1999) Purified soluble guanylyl cyclase expressed in a baculovirus/Sf9 system: stimulation by YC-1, nitric oxide, and carbon monoxide. *J. Mol. Med.* **77**, 14–23