Nitric oxide and renal and cardiac dysfunction in cirrhosis

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ABSTRACT

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 F2α-isoprostanes, which are powerful renal vasoconstrictors. F2α-isoprostane levels are correlated with the severity of liver injury during cirrhosis. However, whether peroxynitrite or F2α-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-aerien”
John Mayow, Tractatus Quinque Medico-Physici, 1674

In the 17th century the remarkably prescient English physiologist John Mayow described a ’nitro-aeriens’ 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-
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-Rodriguez 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\textsuperscript{\textsubscript{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 vasodilatation 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 unclear.
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\(^\bullet\)-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-Estañ, unpublished work). Thus, at least in the BDL rat, another mechanism may exist that is activated very early, before the arterial vasodilatation and the elevation in vasoactive substances are clearly established.

**NO and oxidative stress**

In addition to vasodilatation, 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\(_2^-\)) 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, ‘NO and O\(_2^-\)’, into much more bioreactive species. The formation of peroxynitrite depends on the concentration of both ‘NO and O\(_2^-\)’ and, therefore, on the activities of both NOS and superoxide dismutase [35].

Oxygen-derived free radicals (among the more important of these is O\(_2^-\)), 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\(_2^-\) and ‘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\(_{2\alpha}\)-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\(_{2\alpha}\)-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

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$_{\alpha}$-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$_{\alpha}$-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 μmol/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 \( \text{O}_2^\cdot \) \([35,46]\) 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 \([73,74]\;\text{reviewed in}\;[75–78]\). 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 \([79–81]\;\text{reviewed in}\;[75–78]\). 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](image-url)  
**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: \( \beta \text{AR} \), \( \beta \)-adrenergic receptor; AC, adenylate cyclase; PKA, protein kinase A; PKG, protein kinase G; SR, sarcoplasmic reticulum; \( i \text{Ca} \), 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$_\alpha$ subunit then stimulates the catalytic subunit of adenylyl 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 intraacellular 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$_\alpha$ levels and adenylate cyclase activity. There are also decreases in some of the inhibitory systems, such as the muscarinic m$_3$ receptors [88] and G 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 \( G_\alpha \) and \( G_\beta \) content along with \( \beta \)-adrenoceptor and G-protein uncoupling described previously in the BDL rat [89], PVS rats show an entirely different pattern of cardio-myocyte 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 nitrates/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 recently 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.

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**REFERENCES**


