Effect of phosphodiesterase 5 inhibitor on alteration in vascular smooth muscle sensitivity and renal function in rats with liver cirrhosis

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CIRRHOSIS OF THE LIVER is associated with development of sodium retention by the kidney, renal vasoconstriction, and peripheral vasodilation especially in the splanchnic vasculature, leading to a hyperdynamic circulation (26). The pathogenesis of these findings has been the subject of numerous studies. Much evidence has been provided to support a role of NO in the genesis of splanchnic vasodilation (32). In addition, several studies have suggested that NO overproduction accounts for the decreased sensitivity of the mesenteric vasculature to vasoconstrictors (2, 3). However, some studies failed to support a role for NO in cirrhosis-induced vasodilation (12, 27), and it was recently shown that mice with a targeted deletion of endothelial nitric oxide synthase (eNOS) still developed the hyperdynamic circulation and peripheral vasodilation characteristic of portal hypertension (13), which suggested that other factors contributed to the observed vasodilation.

Among the other findings in cirrhosis is a resistance of the kidney to the natriuretic effects of atrial natriuretic peptide (ANP), an agent that, like NO, acts by raising intracellular cGMP levels (32). Recent studies in cirrhotic rats found a decrease in cGMP content in the kidney associated with an increase in phosphodiesterase (PDE)5 activity (1). In that study, administration of zaprinast, a selective inhibitor of PDE5 (17), increased the renal content of cGMP and resulted in an increase in renal plasma flow, glomerular filtration rate (GFR), and sodium excretion. Others had previously shown that the blunted renal response to ANP in cirrhotic rats was restored by treatment with zaprinast, which also enhanced the blunted natriuretic response to volume expansion (20). In that same study, the authors showed that after 7–10 days of bile duct ligation, there was an increase in cGMP PDE activity in rat renal cortex, which mediated renal resistance to ANP. These findings suggest that upregulation of PDE5 may explain the sodium retention, the renal vasoconstriction, and the resistance to the natriuretic effects of ANP encountered in cirrhotic animals. This may lead to development of newer treatment modalities targeting inhibition of PDE5 activity. It should be noted, however, that if a similar upregulation of PDE5 occurs in the splanchnic vascular bed, it would antagonize the effects of NO on the vasculature, which is the proposed mechanism of vasodilation there. Thus inhibition of PDE5 might further accentuate the mesenteric vasodilation and the general hemodynamic and humoral disturbance in cirrhosis. No studies have directly examined the effects of PDE5 inhibition or the role of PDE5 in vascular reactivity or in hemodynamic disturbances in cirrhosis. The aim of this study was to investigate this possible role, making use of 1,3-dimethyl-6-(2-propoxy-5-methanesulfonylamidophenyl)pyrazolo[3,4d]pyrimidin-4-(5H)-one (DMPPO), a more selective and potent inhibitor of the enzyme than zaprinast (7). More specifically, the study was aimed at 1) examining the sensitivity of aortic smooth muscle to NO donors [nitroglycerin (NG) and S-nitroso-N-acetyl- penticillamine (SNAP)] and phenylephrine in the absence and presence of DMPPO, and of DMPPO itself, in rats with liver cirrhosis and in normal rats and 2) assessing the effects of DMPPO on systemic hemo-

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and 25 NaHCO₃, gassed with 95% O₂-5% CO₂ and maintained at
length of the study.

Four weeks after BDL, rats were killed by cervical dislocation under
humane care according to the criteria outlined in the Guide for the
Care and Use of Laboratory Animals (National Research Council).

METHODS

The study was approved by the Institutional Animal Care and Use
Committee of the American University of Beirut. All animals received
Sprague-Dawley rats weighing 200 –250 g underwent either BDL or
Sham operation consisted of manipulating the common bile duct
without applying sutures or excision of the bile duct. The abdomen
was incised under anesthesia and the common bile duct was identified
and cut in between two sutures to achieve BDL. Sham operation
consisted of manipulating the common bile duct without applying sutures or excision of the bile duct. The abdomen
was double sutured, and rats were placed in separate cages for the
length of the study.

In Vivo Studies

Preparation of rat aortic rings and measurement of relaxation.

Four weeks after BDL, rats were killed by cervical dislocation under
CO₂ narcosis. The thoracic aorta was quickly dissected, cleaned of fat
and connective tissues, and cut into four rings 3 – 4 mm in length. The rings were mounted under 2 g of resting tension in 15-ml organ baths
containing Krebs solution of the following composition (mM): 118
NaCl, 4.7 KCl, 1.25 CaCl₂, 1.14 KH₂PO₄, 1.19 MgSO₄, 10 glucose,
and 25 NaHCO₃, gassed with 95% O₂-5% CO₂ and maintained at
37°C. Preparations were allowed to equilibrate for 60 min with
periodic washing before the experiments started. Tension was mea-
sured with a force displacement transducer, which was connected to a
Myobath system for recording (World Precision Instruments, Sara-
sota, FL). In some experiments, the endothelium of aortic rings was
removed by gently rubbing the intimal surface; in others, care was
taken to maintain the integrity of the endothelium. Nonfunctional
endothelium was tested by the inability of acetylcholine (10 μM) to
induce relaxation of aortic rings precontracted with phenylephrine (1
μM). Aortic rings with functional endothelium exhibited at least 50%
relaxation under identical conditions.

Measurement of aortic sensitivity to NG, SNAP, and DMPPO.
The effects of NG, SNAP, and DMPPO were tested in aortic rings
precontracted with phenylephrine (1 μM). Increasing concentrations
of NG or SNAP were added to the bath to obtain a dose-response
curve for relaxation of the aortic rings. Each experiment consisted of
copies or duplicates, the average of which was used to represent the result of
one particular experiment. Similar dose-response curves were ob-
tained in separate aortic rings from BDL and sham-operated rats that
were preincubated with DMPPO (0.1 μM), a selective inhibitor of
PDE5 (7), to examine the role of PDE5 in these responses. In this
study, 0.1 μM DMPPO was used to specifically and maximally inhibit
PDE5 without acting on other PDE isoenzymes such as PDE3 and PDE4
implicated in vasorelaxation (9, 18). In separate studies, the relaxant
effect of DMPPO itself and of the non-endothelium-dependent vaso-
dilator diazoxide was investigated in aortic rings with intact endo-
thelium, and a concentration-response curve was obtained.

Measurement of aortic sensitivity to phenylephrine.

In this exper-
iments, aortic rings were mounted as described above. Rings from
BDL and sham-operated rats with intact endothelium or denuded
endothelium were exposed to increasing concentrations of phenyleph-
rine to achieve maximal contraction. In two other groups, endothelium-
denuded rings were preincubated with SNAP (0.1 μM) or with SNAP
(0.1 μM) and DMPPO (0.1 μM) and then exposed to increasing
concentrations of phenylephrine.

In Vivo Studies: Effect of DMPPO on Systemic Hemodynamics
and Renal Function

This protocol assessed the cardiovascular response to DMPPO in
sham-operated and BDL rats. Twenty-eight days after BDL or sham
operation, rats were anesthetized with intraperitoneal injections of
pentobarbital (25–50 mg/kg). The trachea was cannulated with PE-
205 tubing to maintain a patent airway. The right jugular vein was
catheterized with PE-50 tubing for administration of fluids. The right
carotid artery was cannulated with PE-50 tubing and connected to a
blood pressure transducer (TXD-310) attached to a BPA-100 blood
pressure analyzer (Micro-Med, Louisville, KY); this was used for
continuous monitoring of mean arterial pressure (MAP). A thermistor
probe was introduced in the left carotid artery and advanced to the
aortic arch to measure cardiac output by the thermodilution technique
after administration of 200 μl of ice-cold 0.9% NaCl solution. The
probe was connected to a cardiac output computer (Columbus Instru-
mants, Columbus, OH). A spring-loaded syringe was used to ensure a
constant injection rate and volume. In addition, the urinary bladder
was catheterized with PE-190 tubing through the urethra for collection
of urine samples. A 0.9% NaCl solution containing 1 μCl/ml of
[^3]H]Inulin was administered intravenously as a bolus of 0.75 ml,
followed by an infusion of 50 μl/ml, which continued until the end of
the experiment. The body temperature was maintained between 37
and 38°C with a heating lamp.

A period of 45 min was allowed for stabilization after surgery, after
which the experiment was started. The experiment was divided into
two consecutive periods of 30 min each. During the first period
baseline measurements of the cardiac output and MAP were obtained
at 5-min intervals. Urine was collected for 30 min in preweighed
microtubes, and a blood sample was obtained at 15 min. After this
period a single intravenous dose of DMPPO (5 mg/kg) was adminis-
tered over 5 min, and the same measurements were obtained over the
next two periods. Plasma and urine samples were used to measure
radioactivity with a beta-scintillation counter, and urine samples
were used to determine urinary (UNaV) and fractional (FE Na) sodium
excretion. Cardiac index (CI) was calculated by normalizing the
cardiac output to body weight and was expressed as milliliters per
minute 100 g of body weight. Total peripheral resistance (TPR) was
estimated with the formula TPR = MAP/CI GFR was estimated from
the clearance of inulin.

Statistical Analysis

The results are expressed as means ± SE. Comparison of responses
between BDL and sham-operated rats was performed with the
Student’s unpaired one-tailed t-test. When more than two groups were
involved, one-way ANOVA (with repeated measures for the in vivo
studies) was initially performed, followed by either the Newman-
Keuls test for individual comparisons or, for the in vivo studies, the
Dunnett’s test for comparison of values to the control value (i.e.,
comparison of hemodynamic values in periods 1 and 2 with those
during the baseline period). A P value of ≤0.05 was considered
significant.

RESULTS

Vascular Sensitivity to NG, SNAP, diazoxide, and DMPPO

In endothelium-denuded aortic rings from sham-operated and BDL rats precontracted with phenylephrine, NG induced a
concentration-dependent relaxation (Fig. 1). Aortic rings from
BDL rats had a reduced sensitivity to NG, with a shift of the
dose-response curve to the right. Aortic rings that were incu-
bated with DMPPO (0.1 μM) had an enhancement of the relaxant responses to NG in both sham-operated and BDL rats, such that the difference between the two curves was eliminated except at one concentration (10 nM), at which aortic rings from cirrhotic rats remained less sensitive to NG than those from sham-operated rats. The relaxant responses to SNAP in the BDL group were, like those to NG, attenuated compared with the responses in the corresponding sham-operated group. This difference in sensitivity was eliminated by treatment with DMPPO (0.1 μM) (Fig. 2). The relaxant response to diazoxide was not different in aortic rings with intact endothelium from cirrhotic or control rats (Fig. 3). In endothelium-denuded aortic rings, however, the responses to diazoxide were significantly attenuated relative to intact rings. Nevertheless, the effect of diazoxide remained similar in rings from BDL and sham-operated rats. The relaxant response to DMPPO in aortic rings with intact endothelium precontracted with phenylephrine (1 μM) was attenuated in the cirrhotic rats compared with the sham-operated rats (Fig. 4). In endothelium-denuded aortic rings, there was no discernible relaxant effect of DMPPO in any of the groups at the concentrations used in this study (data not shown).

Vascular Sensitivity to Phenylephrine

In aortic rings with endothelium, the phenylephrine dose-response curve was right-shifted in BDL rats, showing a significantly reduced response to phenylephrine in cirrhotic rats compared with sham-operated rats (Fig. 5). Aortic rings denuded of endothelium had an enhanced response to phenylephrine in both sham-operated and cirrhotic rats, and the difference between the two groups was no longer observed (Fig. 5). Preincubation of aortic rings with DMPPO attenuated the vasoconstriction in both groups: it shifted the dose-re-
response curve to the right and decreased the maximal response to phenylephrine in both the sham-operated and cirrhotic groups, and it eliminated the differences between them that were observed in aortic rings with endothelium (Fig. 6). When endothelium-denuded rings were incubated with SNAP or with SNAP and DMPPO and then exposed to phenylephrine, there was no difference in response between cirrhotic rats and sham-operated rats, contrary to the response observed in aortic rings with intact endothelium, nor were there changes in the responses observed within either the sham-operated or the cirrhotic group after these pretreatments (Figs. 7 and 8).

**Effects of DMPPO on Systemic Hemodynamics**

At the time of the experiment there was no difference in body weight between cirrhotic (265.5 ± 8.4 g) and sham-operated (255 ± 3.9 g) rats. The values of MAP, CI, and TPR in the two groups of rats before and after administration of DMPPO are shown in Table 1. Baseline values in BDL compared with sham-operated rats showed significantly higher CI and lower TPR, but there was no significant difference in MAP. Administration of DMPPO resulted in a decrease in MAP and TPR in both groups compared with the basal values, but no changes occurred in CI, such that the significant differences in both CI and TPR between sham-operated and cirrhotic rats that were observed before DMPPO administration remained valid after administration.

**Effects of DMPPO on Renal Function**

GFR, \( U_{NaV} \), and \( FE_{Na} \) are shown in Table 1. No significant difference was observed in the basal value of GFR between BDL rats and sham-operated rats, and administration of DMPPO had no effect on GFR in either group. In contrast, BDL rats tended to have lower basal values of \( U_{NaV} \) and \( FE_{Na} \) compared with the sham-operated controls, but this was not statistically significant. After DMPPO administration, there were significant, almost threefold, increases in \( U_{NaV} \) and \( FE_{Na} \) values in BDL rats. In contrast, in sham-operated rats, although there tended to be similar responses in \( U_{NaV} \), this did not reach statistical significance by repeated-measures ANOVA \((P = 0.07)\). As for \( FE_{Na} \) in the sham-operated group, repeated-measures ANOVA revealed a significant difference from baseline \((P = 0.03)\), which was evident during the first experimental period only. Comparison of values in the two groups during corresponding experimental periods with the Student’s unpaired \( t \)-test did not reveal a significant difference.

**DISCUSSION**

The results of this study reveal that in this model of cirrhosis in rats, there is a decreased sensitivity to the vasodilator effects of NG, an NO donor, compared with control rats. It is well established that the relaxant effect of NO is due to the action of cGMP produced as a result of stimulation of guanylate cyclase. PDE5 is the primary phosphodiesterase responsible for hydrolysis of cGMP in vascular smooth muscle. Theoretically, this
The difference in response may be due to one of several factors: 1) decreased release of NO from the NO donor, 2) decreased formation of cGMP due to decreased activation of guanylyl cyclase by NO, possibly due to downregulation of soluble guanylate cyclase as a result of increased NO release in vessels from cirrhotic rats (14), and 3) increased hydrolysis of cGMP due to increased activity of PDE5 in cirrhotic rats. To examine whether this decreased responsiveness was limited to NG or extended to other NO donors, we conducted experiments with SNAP. These revealed essentially the same pattern of response, with decreased response in the BDL group compared with the sham-operated group, a difference that was eliminated by pretreatment of endothelium-denuded aortic rings with DMPPO. These results are consistent with a recent study that showed decreased response to acetylcholine in mesenteric vessels from BDL rats (6) but contrary to other studies that found no difference between vessels from cirrhotic and control animals in their response to bethanecol or NG (16). Colle et al. (6) attributed this reduced response to impaired endothelium-dependent relaxation or dysfunction at the level of vascular smooth muscle. However, two findings in the present study argue against this. 1) This decreased response was observed in endothelium-denuded rings; hence it was independent of the endothelium. 2) The similar response to diazoxide in vessels from BDL and control rats suggested that there was a specific decrease in sensitivity to the NO-cGMP pathway and not a nonspecific decline in vascular smooth muscle reactivity. The fact that DMPPO corrected the decreased sensitivity to NG and SNAP suggests that of the three mechanisms proposed above to explain this difference, the last, i.e., an increase in PDE5 activity, is the most plausible. Interestingly, the response to diazoxide was attenuated in endothelium-denuded vessels, suggesting that a small part of the response may be endothelium dependent. This was reported previously for low concentrations of diazoxide (30 μM) and was linked to NO production; higher concentrations of diazoxide were not affected by endothelium removal or by treatment with a NOS inhibitor (11). Others have reported that removal of the endothelium enhances the vasodilating effects of potassium channel openers like pinacidil and levocromakalim (19). On the other hand, Deka et al. (8), using these same compounds, reported differential modulation by NO of their effects, such that removal of the endothelium led to enhancement of the effects of pinacidil and inhibition of the effects of levocromakalim. It is obvious that this requires further investigation, but the fact remains that the major component of vasodilation by diazoxide is endothelium independent, and there was no difference between BDL and sham-operated rats in response to diazoxide.

It was noticeable that the magnitude of the difference in the response to SNAP between sham-operated and BDL rats was less than that observed for NG. These results support recent reports of decreased vascular responsiveness to NG and SNAP in cirrhotic livers (10). Those authors suggested that this decreased sensitivity was due to both decreased metabolism of NG to NO and the inability of the hepatic vasculature to respond to NO. Our results suggest that the observed inability to respond to NO may be due to increased PDE5 activity in the...
and that this activated form of PDE5 has a lower sensitivity to superior mesenteric artery, thoracic aorta, and portal vein of rats. Cahill et al. (4) also showed increase in eNOS activity in that tissue. Alternatively, the decreased response to DMPPO in cirrhotic rats may thus be explained by the increased activity of PDE5, which manifested functionally as a reduced responsiveness to NO donors. This is consistent with previous studies that demonstrated increased activity and expression of PDE5 in the vascular bed. Taking the thoracic aorta as a model, we have observed differences in response to phenylephrine, as previously suggested (22). These changes in PDE5 will tend to counteract NO production (2, 3, 23, 32). This conclusion was based in studies have provided evidence to support a role for NO in mechanisms of vascular dysfunction in liver cirrhosis and other diseases (4, 16, 22, 23). The peripheral vasodilation hypothesis of ascites formation in cirrhosis suggests that splanchnic vasodilation is the primary event that leads to activation of compensatory mechanisms, including the renin-angiotensin and sympathetic nervous systems, to result in sodium retention by the kidney and eventually renal vasoconstriction, culminating in the hepatorenal syndrome (26). Many studies have provided evidence to support a role for NO in splanchnic vasodilation (32). This conclusion was based in many instances on correction of the hemodynamic abnormalities by NOS inhibitors, with some studies demonstrating increased levels of NO metabolites or increased NOS activity in that vascular bed. Taking the thoracic aorta as a model, we have provided evidence to suggest increased activity of PDE5, which manifested functionally as a reduced responsiveness to NO donors. This is consistent with previous studies that demonstrated increased activity and expression of PDE5 in the kidney (20, 21). These changes in PDE5 will tend to counteract the effect of increased NO generation or of any process that is mediated by cGMP, such as the effect of ANP in the kidney. It is possible therefore, that in cirrhotic animals appear to have both an increase in NO synthesis and an increase in PDE5 activity that antagonizes the effects of NO; however, the increased NO synthesis predominates over the increased cGMP hydrolysis and leads to a reduced response to phenylephrine. In support of this explanation is the finding that in endothelium-deficient rings responses to phenylephrine were similar in the two groups. Interestingly, when endothelium-denuded rings were incubated with SNAP or with SNAP + DMPPO, thus eliminating the contribution of the differences in NO production and providing a constant and similar NO tone, the responses to phenylephrine remained similar to those in the sham-operated group, indicating that restoring a basal NO tone is not sufficient to reveal the difference in response based on a difference in PDE5 activity. This suggests that phenylephrine induces novel NO synthesis in the endothelium that partially antagonizes its direct vasoconstrictor effect and that differences in NOS activities in the two groups account for the observed differences in response to phenylephrine, as previously suggested (22).

How do these results suggesting increased PDE5 activity in the aorta and kidneys of cirrhotic animals relate to the hemodynamic disturbances in cirrhosis? Although the aorta may not be the ideal vessel to study, being a conduit rather than a resistance vessel, it has been used frequently as a model to examine the mechanisms of vascular dysfunction in liver cirrhosis and other diseases (4, 16, 22, 23). The peripheral vasodilation hypothesis of ascites formation in cirrhosis suggests that splanchnic vasodilation is the primary event that leads to activation of compensatory mechanisms, including the renin-angiotensin and sympathetic nervous systems, to result in sodium retention by the kidney and eventually renal vasoconstriction, culminating in the hepatorenal syndrome (26). Many studies have provided evidence to support a role for NO in splanchnic vasodilation (32). This conclusion was based in many instances on correction of the hemodynamic abnormalities by NOS inhibitors, with some studies demonstrating increased levels of NO metabolites or increased NOS activity in that vascular bed. Taking the thoracic aorta as a model, we have provided evidence to suggest increased activity of PDE5, which manifested functionally as a reduced responsiveness to NO donors. This is consistent with previous studies that demonstrated increased activity and expression of PDE5 in the kidney (20, 21). These changes in PDE5 will tend to counteract the effect of increased NO generation or of any process that is mediated by cGMP, such as the effect of ANP in the kidney. It is possible therefore, that the increased PDE5 activity may be an adaptive mechanism to the increased NO generation. This is

Table 1. Effect of DMPPO on systemic hemodynamic and renal parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Period 1</th>
<th>Period 2</th>
<th>RM-ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>118 ± 4</td>
<td>97 ± 4*</td>
<td>99 ± 5*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cardiac index (ml·min⁻¹·100 g⁻¹)</td>
<td>121 ± 5</td>
<td>98 ± 2*</td>
<td>96 ± 4*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg·min⁻¹·100 g⁻¹)</td>
<td>26.6 ± 1.1</td>
<td>27.4 ± 1.1</td>
<td>27.0 ± 1.1</td>
<td>0.21</td>
</tr>
<tr>
<td>Glomular filtration rate (ml·min⁻¹·100 g⁻¹)</td>
<td>37.8 ± 1.9</td>
<td>39.3 ± 2.4†</td>
<td>40.1 ± 1.9†</td>
<td>0.24</td>
</tr>
<tr>
<td>Urinary Na excretion (% of body weight per day)</td>
<td>4.4 ± 0.2</td>
<td>3.5 ± 0.1†</td>
<td>3.6 ± 0.2*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fractional Na excretion (%)</td>
<td>3.3 ± 0.2†</td>
<td>2.6 ± 0.2*†</td>
<td>2.5 ± 0.2*†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sham</td>
<td>0.61 ± 0.06</td>
<td>0.56 ± 0.05</td>
<td>0.68 ± 0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>BDL</td>
<td>0.68 ± 0.09</td>
<td>0.90 ± 0.21†</td>
<td>0.72 ± 0.09</td>
<td>0.30</td>
</tr>
<tr>
<td>Sham</td>
<td>1.43 ± 0.28</td>
<td>2.75 ± 0.76</td>
<td>1.88 ± 0.54</td>
<td>0.06</td>
</tr>
<tr>
<td>BDL</td>
<td>1.25 ± 0.26</td>
<td>4.03 ± 0.85†</td>
<td>3.16 ± 0.56*</td>
<td>0.01</td>
</tr>
<tr>
<td>Sham</td>
<td>0.54 ± 0.09</td>
<td>1.14 ± 0.32</td>
<td>0.80 ± 0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>BDL</td>
<td>0.46 ± 0.07</td>
<td>1.24 ± 0.26*</td>
<td>1.13 ± 0.15*</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE of the effects of 1.3-dimethyl-6-(2-proproxy-5-methanesulfonylamidophenyl)pyrazolo[3,4-d]pyridinimid-4(SH)-one (DMPPO) on systemic hemodynamic and renal parameters in bile duct ligation (BDL; n = 9) sham-operated (Sham; n = 16) rats during a 30-min baseline period and 2 consecutive 30-min periods after administration of DMPPO (5 mg/kg iv). Comparisons among the 3 periods within the same group were conducted by repeated-measures ANOVA (RM-ANOVA). *P < 0.05 comparing baseline values with periods 1 and 2 (after DMPPO administration); †P < 0.05 comparing Sham and BDL rats.
reminiscent of the suggestion that upregulation of PDE1 and/or PDE5 activity may explain the tolerance to the chronic administration of NG (15, 25); in liver cirrhosis, however, it is increased endogenous NO production, rather than exogenous administration of NO donors, that triggers the increase in PDE5 activity. Alternatively, this increase in PDE5 activity may be a primary event of liver cirrhosis that is independent of enhanced NO production and results directly from the disease process itself.

It has already been shown that acute administration of a PDE5 inhibitor improves renal sodium excretion (20, 21). However, this same intervention can potentially worsen the hemodynamic disturbance by enhancing the splanchic and peripheral vasodilation in cirrhosis, which may then worsen renal function and sodium retention, as proposed by the peripheral vasodilation hypothesis. We therefore examined the effects of DMPPO on hemodynamic and renal parameters in cirrhotic and control rats. The results demonstrated that DMPPO enhanced the hemodynamic disturbance by causing worsening of vasodilation and hypotension; however, this response occurred in both sham-operated and BDL rats and was of equal magnitude. Sildenafil was previously shown to decrease MAP in cirrhotic rats but to a lesser extent than in controls. In that study, however, sildenafil was given as bolus injections and not as an infusion, and no measurements of other systemic hemodynamic parameters were undertaken (5). The similar decrease in arterial pressure and vascular resistance in control rats and in those with BDL does not appear to be consistent with the results from our experiments on isolated aortas, which showed that DMPPO had a lower effect on relaxation in cirrhotic rats. It should be noted, however, that the in vivo situation differs from the in vitro situation in that production of NO by other vascular beds, notably the splanchic bed, will influence the extent of vasodilation in response to DMPPO in vivo. Thus the increased NO production should lead to a greater reduction in TPR in response to DMPPO in cirrhotic relative to control animals, whereas the proposed increase in PDE5 activity should attenuate the effects of DMPPO. The net result of these opposing influences may be a similar effect of DMPPO on TPR in the cirrhotic and control animals in vivo. Furthermore, the in vivo situation is characterized by presence of baroreflexes and other vasoactive mediators that may influence the effects of DMPPO on hemodynamics (e.g., angiotensin II, the sympathetic system, ANP).

In the present study, despite the decrease in arterial pressure induced by DMPPO, there was an increase in sodium excretion in both the cirrhotic and control groups. Although unpaired analysis did not show a difference between the sham-operated and cirrhotic groups at any time point, likely because of the wide variability in values, repeated-measures analysis within each group revealed that the increase in sodium excretion was more consistent and reproducible in the cirrhotic group, resulting in a significant increase from baseline sustained during the two periods after DMPPO administration. These results emphasize the role of PDE5 activity locally in the kidney and the influence of PDE 5 inhibition on sodium handling by the kidney, both under control and, more prominently, under cirrhotic conditions. They are in agreement with previous findings that also showed a more consistent and more significant increase in sodium excretion after PDE5 inhibition with zaprinast in cirrhotic rats, although control rats also sustained an increase in sodium excretion (1). It should be noted, however, that this was an acute intervention, and that the chronic effect of administration of PDE5 inhibitors in cirrhosis may be different and may actually worsen sodium retention because it will potentiate vasodilation, which may further stimulate the compensatory sodium-retaining mechanisms. This is especially relevant considering recent findings in which a single dose of sildenafil administered to patients with liver cirrhosis and ascites reduced sodium excretion and arterial pressure and was associated with increases in plasma renin activity and angiotensin II and aldosterone levels (28). The reasons for these contradictory results are not clear, but it should be noted that all these patients had advanced stages of cirrhosis (with ascites), indicating severe sodium retention and activation of compensatory mechanisms. Any further vasodilation and reduction in arterial pressure could cause a marked worsening of their renal function. Furthermore, the dose of the PDE5 inhibitor may be an important consideration because a relatively high dose may lead to a marked reduction in arterial pressure that will counteract the direct, intrarenal natriuretic effect of the drug. Specific studies are needed to address these issues in the future.

In conclusion, this study revealed novel findings suggesting a role for PDE5 in regulating vascular reactivity, hemodynamics, and renal function in cirrhotic rats. In at least two tissues (the aorta and kidney), there is evidence to suggest an increase in activity of PDE5 that has important functional consequences. Further studies are warranted to examine the usefulness of chronic administration of PDE5 inhibitors in ameliorating the renal complications of cirrhosis, especially sodium retention.

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