Splanchnic hyposensitivity to glypressin in a haemorrhage/transfused rat model of portal hypertension: role of nitric oxide and bradykinin

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ABSTRACT

Hyposensitivity to vasopressin is a well documented phenomenon in animals with portal hypertension and patients with cirrhosis subject to haemorrhage. Haemorrhage is associated with the endogenous release of bradykinin, which may subsequently stimulate the formation of nitric oxide (NO). The present study investigated the relative contribution of NO synthase (NOS) isoforms and the role of bradykinin in the pathogenesis of splanchnic hyposensitivity to a long-acting vasopressin analogue, glypressin, in rats with portal hypertension induced by partial portal vein ligation (PVL). At 14 days after the operation, systemic and portal haemodynamics were measured in stable or bleeding PVL rats receiving an intravenous infusion of glypressin (0.07 mg/kg). In the treatment groups, Nω-nitro-L-arginine methyl ester (L-NAME; a non-selective NOS inhibitor), L-canavanine (a specific inhibitor of inducible NOS) or HOE 140 (a bradykinin B2 receptor antagonist) was administered 45 min before the infusion of glypressin. In rats with a hypotensive haemorrhage, 4.5 ml of blood was withdrawn and 50% of the withdrawn blood was re-infused before the administration of glypressin or various inhibitors. Splanchnic hyposensitivity to glypressin was demonstrated in the haemorrhage/transfused PVL rats. The infusion of L-NAME elevated the mean arterial pressure in the bleeding PVL rats without the modulation of portal pressure. The addition of L-NAME or HOE 140, but not L-canavanine, significantly and similarly potentiated the portal-hypotensive effects of glypressin. It is concluded that constitutive NOS and bradykinin are responsible, at least partly, for the splanchnic hyposensitivity to glypressin observed in the early stages of the haemorrhage/transfused rat model of portal hypertension.

INTRODUCTION

Bleeding from ruptured oesophageal varices is one of the leading causes of death in patients with liver cirrhosis. Vasopressin, an agent that decreases portal and collateral blood flow and portal pressure [1], has been used in cirrhotic patients for the management of oesophageal variceal bleeding [2]. Recent studies have demonstrated that vasopressin given during haemorrhage is less effective than when given during a stable state, both in experimental portal hypertension and in patients with cirrhosis (so-called hyposensitivity) [3–6]. Similar find-

Key words: bradykinin, glypressin, haemorrhage, hyposensitivity, nitric oxide, portal hypertension.

Abbreviations: L-NAME, Nω-nitro-L-arginine methyl ester; NOS, nitric oxide synthase; cNOS, constitutive NOS; iNOS, inducible NOS; PVL, portal vein ligation; TNF-α, tumour necrosis factor-α.

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ings were obtained when glypressin, a long-acting vaso-
pressin analogue [7], was administered in a haemorrhage/
transfused rat model of portal hypertension [8]. The exact
cause of this phenomenon is unknown. However, it may
be related to the excessive secretion of endogenous vasopressors [9] and the secretion of various mediators
during hypovolaemia [10].

Excessive formation of nitric oxide (NO) has been
recognized to play a key role in the pathogenesis of
vascular hyposensitiveness [11,12] and hyperdynamic
circulation [13,14] in portal-hypertensive states. In ad-
dition, the vascular hyporeactivity phenomenon ob-
served in haemorrhagic shock may also be mediated by
NO [15]. Furthermore, the addition of aminoguanidine,
a non-selective [16,17] or preferential [18,19] inhibitor of
inducible NO synthase (iNOS), could overcome the
splanchnic hyposensitivity to glypressin in portal-hy-
pertensive rats [8], suggesting an important role for NO
in the development of the hyposensitivity phenomenon.

NOS exists in two isoforms. The constitutive form
(cNOS), anchored on the internal surface of the en-
thelial membrane, may be activated by mechanical
factors, such as shear stress and pulsatile blood flow
[20,21], and endogenous vasodilators, such as bradykinin
[22–24]. It has been reported that haemorrhage is
associated with the endogenous release of bradykinin
[25,26]. On the other hand, the inducible form of NOS
represents newly synthesized enzyme. It is expressed in
macrophages and vascular smooth muscle following
activation by endotoxin and cytokines such as tumour
necrosis factor-α (TNF-α) and interleukin-6 [27]. Al-
though NO plays a role in the pathophysiology of
splanchnic hyposensitivity to glypressin, the contribu-
tion of NOS isoforms and bradykinin is unknown.

In the present study, Nω-nitro-L-arginine methyl ester
(l-NAME; a non-selective inhibitor of NOS [28]) and L-
canavanine (a specific inhibitor of iNOS [29,30]) were
used to clarify which isoform of NOS is involved in the
pathogenesis of glypressin hyposensitivity. In addition,
we also investigated whether the addition of HOE 140 (a
bradykinin B₂ receptor antagonist [31,32]) may enhance
the portal-hypotensive effect of glypressin in haemor-
hage/transfused portal-hypertensive rats.

**MATERIALS AND METHODS**

**Animals**

Male Sprague–Dawley rats weighing 300–350 g were used.
The rats were caged at 24 °C, with a 12-h light/dark
cycle, and were allowed free access to food and water
until the time of the experiments. Portal hypertension
was induced by partial portal vein ligation (PVL), as
described previously [33]. In brief, portal vein stenosis
was induced using 3-0 silk to ligate both the portal vein
and a 20-gauge needle. The needle was then removed and

a calibrated stenosis of the portal vein was induced.
Survival surgery and the haemodynamic study were
performed under ether anaesthesia followed by ketamine
hydrochloride (100 mg/kg body weight; intramuscular).
Haemodynamic studies were performed 14 days after
ligation. The experiments reported here were conducted
according to the American Physiological Society guiding
principles for the care and use of laboratory animals. All
rats were deprived of food for 18 h before the experi-
ments, and had free access to water.

**Experimental design (Figure 1)**

The PVL rats were divided into five groups: without-
bleeding group (n = 18), with-bleeding group (n = 18),
with-bleeding plus l-NAME group (n = 17), with-bleed-
ing plus L-canavanine group (n = 18), and with-bleeding
plus HOE 140 group (n = 18). Blood was withdrawn
for 15 min at a constant rate of 0.3 ml/min from those
rats subject to haemorrhage, following the measurement
of baseline mean arterial pressure, heart rate and portal
pressure. After a 20-min stabilization period, 50% of the
withdrawn blood was re-infused at the same rate as in
haemorrhage [3]. The infusion and withdrawal of blood
were performed using an infusion/withdrawal pump
(model SP 210 iw; World Precision Instruments, Sara-
sota, FL, U.S.A.) via a PE-50 catheter connected to the
right carotid artery. In the without-bleeding group, no
blood was withdrawn or re-infused in these two periods.
l-NAME (15 mg/kg in 0.35 ml of normal saline), L-
canavanine (100 mg/kg in 0.35 ml of normal saline),
HOE 140 (1.5 μg/kg in 0.35 ml of normal saline) or
normal saline (0.35 ml) was infused intravenously for
2 min after the second recording of the haemodynamic
parameters. After a further 45 min [31,34–36], the third
haemodynamic measurement was carried out, and then
glypressin (0.07 mg/kg) was infused intravenously for
2 min. Mean arterial pressure, heart rate and portal
pressure were determined again 10 min after glypressin
administration. Various inhibitors were infused using an
infusion pump (model SP 210 iw; World Precision
Instruments) via a PE-50 catheter connected to the right
jugular vein.

**Reagents**

Glypressin was purchased from Ferring (Kiel, Germany).
l-NAME and L-canavanine were purchased from Sigma
Chemical Co. (St. Louis, MO, U.S.A.), and HOE 140
was acquired from Research Biochemicals International
(Natick, MA, U.S.A.).

**Measurement of systemic and portal
haemodynamics**

The right femoral artery of the PVL rats was canulated
with a PE-50 cather that was connected to a Spectramed
DTX transducer (Spectramed Inc., Oxnard, CA, U.S.A.),
and continuous recordings of mean arterial pressure were made on a multi-channel recorder (model RS 3400; Gould Inc., Cupertino, CA, U.S.A.). The external zero reference limit was placed at the mid-portion of the rat. Heart rate was determined from the recording. The abdomen was then opened with a mid-line incision, and a mesenteric vein was cannulated with a PE-50 catheter connected to a Spectramed DTX transducer. The abdominal cavity was closed and the portal pressure was recorded on a Gould model RS 3400 recorder.

**Statistical analysis**

All results are expressed as means ± S. E. M. Statistical analyses were performed using paired and unpaired Student’s t tests or by one-way ANOVA with Scheffe’s test, as appropriate. Results were considered statistically significant at \( P < 0.05 \).

**RESULTS**

**Haemodynamic assessments at baseline**

Body weight was similar among the five groups (Table 1). There were no significant differences in mean arterial pressure, portal pressure or heart rate among these groups at baseline (Table 1).

**Effects of blood withdrawal/re-infusion on haemodynamics**

The procedure of blood withdrawal/re-infusion produced no obvious changes in mean arterial pressure (before, 103.7 ± 4.6 mmHg; after, 105.1 ± 6.3 mmHg; \( P > 0.05 \)) or portal pressure (before, 11.6 ± 0.4 mmHg; after, 11.6 ± 0.5 mmHg; \( P > 0.05 \)) in PVL rats with bleeding (n = 18).

**Systemic and portal haemodynamic effects of glypressin in PVL rats with or without bleeding**

Both groups of PVL rats (with and without bleeding) showed significant increases in mean arterial pressure after the infusion of glypressin (with-bleeding group: before, 104.1 ± 6.8 mmHg; after, 148.0 ± 4.4 mmHg; \( P < 0.001 \); without-bleeding group: before, 106.6 ± 12.2 mmHg; after, 148.0 ± 4.4 mmHg; \( P < 0.001 \)).
Table 1  Body weights and baseline haemodynamic parameters of the different experimental groups

No significant differences existed between groups for any of the parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without bleeding (n = 18)</th>
<th>With bleeding (n = 18)</th>
<th>With bleeding plus L-NAME (n = 17)</th>
<th>With bleeding plus L-canavanine (n = 18)</th>
<th>With bleeding plus HOE 140 (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>333 ± 8</td>
<td>337 ± 7</td>
<td>341 ± 8</td>
<td>358 ± 7</td>
<td>343 ± 8</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>104.1 ± 3.9</td>
<td>103.7 ± 4.6</td>
<td>103.4 ± 4.0</td>
<td>110.5 ± 3.4</td>
<td>108.4 ± 4.1</td>
</tr>
<tr>
<td>Portal pressure (mmHg)</td>
<td>11.4 ± 0.4</td>
<td>11.6 ± 0.4</td>
<td>11.2 ± 0.4</td>
<td>11.1 ± 0.3</td>
<td>12.3 ± 0.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>315 ± 11</td>
<td>314 ± 14</td>
<td>304 ± 12</td>
<td>329 ± 13</td>
<td>297 ± 10</td>
</tr>
</tbody>
</table>

Figure 2  Effects of glypressin on mean arterial pressure (MAP) and portal pressure (PP) in PVL rats with or without bleeding

NS, not significant.

4.5 mmHg; after, 154.5 ± 4.4 mmHg; P < 0.001). The portal pressure was significantly decreased after glypressin infusion in the without-bleeding group (before, 11.6 ± 0.3 mmHg; after, 9.5 ± 0.5 mmHg; P < 0.001), but not in the with-bleeding group (before, 11.4 ± 0.6 mmHg; after, 11.1 ± 0.6 mmHg; P > 0.05). Glypressin infusion resulted in a significantly greater decrease in portal pressure in PVL rats without bleeding than in those with bleeding (Figure 2). However, the changes in mean arterial pressure (Figure 2) and heart rate (without-bleeding, −1.9 ± 3.7%; with-bleeding, −3.3 ± 3.2%; P > 0.05) after glypressin infusion were similar in the two groups.

Systemic and portal haemodynamic effects of L-NAME, L-canavanine and HOE 140 in PVL rats with bleeding

After the infusion of L-NAME, a significant elevation of the mean arterial pressure was noted (P < 0.001; Figure 3). Administration of L-canavanine or HOE 140 had no obvious effect on mean arterial pressure (P > 0.05; Figure 3). The changes in portal pressure were comparable among these groups (Figure 3). As compared with bleeding PVL rats receiving normal saline, alleviation of tachycardia was observed in the L-NAME-treated group (normal saline, 11.7 ± 2.4%; L-NAME, −0.1 ± 2.6%; P < 0.05). Infusion of L-canavanine or HOE 140 did not modify heart rate (P > 0.05).

Haemodynamic changes after glypressin in various groups of bleeding PVL rats

The addition of L-NAME or HOE 140, but not L-canavanine, significantly potentiated the portal-hypotensive effect of glypressin in bleeding PVL rats (Figure 4). The increase in mean arterial pressure following glypressin infusion was less prominent in the L-NAME-treated group as compared with the other groups (Figure 4). Although the differences were not statistically signifi-
Nitric oxide and bradykinin in glypressin hyposensitivity

DISCUSSION

Our study disclosed that vascular reactivity to glypressin was selectively blunted over the splanchic circulation in PVL rats with acute bleeding (i.e. splanchic hyposensitivity). As glypressin had a significant effect on the systemic circulation, splanchic hyposensitivity cannot be explained by an inadequate dose of glypressin. The exact pathogenetic factors responsible for this hyposensitivity phenomenon have not been clarified; however, the possible roles of NO and bradykinin were investigated in the present study.

NO has been suggested to contribute to the mesenteric hyperaemia and the splanchic hyposensitivity to vasoconstrictors associated with portal hypertension [11–14]. With regard to the discriminatory roles of NOS isoforms in the development of a hyperdynamic circulation, the enhanced expression of cNOS stimulated by the consequences of a hyperdynamic circulation, such as shear stress and pulsatile blood flow, has been well documented in PVL rats [37–39]. However, the involvement of iNOS in PVL rats is still controversial [39–41]. Previous studies have demonstrated that the inhibition of NO synthesis in unbled cirrhotic patients and portal-hypertensive animals could correct the observed vasodilatation, without modulation of portal pressure [13,42–44].

The lack of a change in portal pressure after the addition of L-NAME could enhance the vasoconstrictive effects of glypressin and subsequently overcome the phenomenon of glypressin hyposensitivity. Due to the prior vasoconstrictive action created by NO inhibition, a modest elevation of the mean arterial pressure after glypressin was observed in bleeding PVL rats treated with L-NAME. However, restored vascular activity was hinted at by the observation that L-NAME-treated PVL rats showed the highest elevation of mean arterial pressure when the mean arterial pressure before the administration of different inhibitors was used as the baseline. In addition, the elevation of mean arterial pressure following L-NAME administration may suppress the secretion of other neurohormonal vasopressors, such as endogenous vasopressin, catecholamines and the renin/angiotensin/aldosterone system [45–47].

Haemorrhage has been reported to induce an increase in serum TNF-α and interleukin-6 levels [48], which may subsequently stimulate iNOS to generate NO [27]. However, our present study failed to demonstrate a significant role for iNOS activation in the pathogenesis of splanchic hyposensitivity. It has been reported that the release of NO from endothelial tissue after exposure to TNF-α is first evident at 8 h, and becomes maximal at 16–24 h [49]. Therefore we can postulate that, during acute bleeding, the amount of NO contributed by iNOS activation is relatively low compared with that produced

![Figure 4 Effects of glypressin alone, or glypressin plus L-NAME, l-canavanine or HOE 140, on mean arterial pressure (MAP) and portal pressure (PP) in PVL rats with bleeding](image-url)

The letters from a to h indicate comparisons between the two groups indicated; aP < 0.05, bP < 0.01, b′P < 0.05, cP < 0.001 and c′P < 0.001 indicate a significant difference between the compared groups.

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by cNOS. This may be the reason why L-canavanine infusion exerted no significant effects on splanchnic hyposensitivity to glypressin in our present study. The lack of an effect on glypressin-induced hyposensitivity was not due to inadequate inhibition of iNOS, as the half-life of L-canavanine (1.56 h [34]) is long enough to ensure adequate iNOS inhibition during the period of haemodynamic assessment.

In our previous study, the addition of aminoguanidine, a non-selective [16,17] or preferential [18,19] inhibitor of iNOS, could overcome the splanchnic hyposensitivity to glypressin in portal-hypertensive rats [8]. However, in the present study, activation of cNOS rather than iNOS was proved to mediate the hyposensitivity to glypressin observed in PVL rats with bleeding. Therefore the ability of aminoguanidine to ameliorate the splanchnic hyposensitivity to glypressin in PVL rats was probably due to its ability to inhibit cNOS.

Since cNOS activity is already enhanced in PVL rats with a stable haemodynamic status, one may question why it plays such an important role in the splanchnic hyposensitivity to glypressin observed in haemorrhage/transfused PVL rats. Two possible mechanisms may be involved. One is the alteration of vascular bed reactivity following haemorrhage, including the desensitization of vasopressin receptors [50], a post-receptor defect [10], and an autoregulatory escape phenomenon [51]. These events may aggravate the vascular hyperresponsiveness to vasoconstrictors and attenuate the therapeutic effect of glypressin. In stable PVL rats, glypressin administration could still accomplish the portal-hypotensive effect in the presence of the blunted vascular reactivity produced by cNOS. However, after the occurrence of vascular bed alterations created by haemorrhage, glypressin failed to achieve the desired therapeutic effect in the haemorrhage/transfused condition.

Another possible mechanism is the stimulation of cNOS by endogenously released substances associated with haemorrhage, including bradykinin. Bradykinin is a peptide that occurs naturally in the gut wall and acts as a physiological mesenteric vasoregulator [52]. Infusion of bradykinin increases portal vein calibre and pressure, probably as a result of mesenteric vasodilatation and augmented intestinal blood flow [53,54]. In addition, bradykinin can antagonize noradrenaline-induced constrictor responses in isolated perfused rat gut preparations [55]. Physiologically, bradykinin influences cellular function by binding to the receptor subtypes located on the cell surface, and the bradykinin B₂ receptor mediates the fundamental vascular actions of bradykinin [56]. It is generally accepted that the endothelial B₂ receptor is linked to G-proteins, and receptor binding results in the activation of cNOS to generate NO with the cooperation of calmodulin [23,24].

In the present study, a long-acting bradykinin B₂ receptor antagonist, HOE 140, was used to block the physiological action of endogenous bradykinin. It has been shown that, at a concentration of 1 μg/kg, HOE 140 completely prevented the bradykinin-induced fall in blood pressure [31]. In addition, a much lower dose of HOE 140 (0.001 μg/kg) efficiently inhibited NO generation derived from bradykinin in an animal model of acute pancreatitis [57]. Although our present study did not measure the plasma levels of NO following HOE 140 administration, it is reasonable to assume that, at the administered dose of 1.5 μg/kg, HOE 140 would sufficiently antagonize the actions of endogenous bradykinin. Our results disclosed that splanchnic hyposensitivity to glypressin could be ameliorated by the addition of HOE 140, suggesting that the endogenous release of bradykinin during haemorrhage may participate in this phenomenon. Unlike L-NAME, infusion of HOE 140 in bleeding PVL rats did not elicit obvious haemodynamic changes. Previous studies have demonstrated that even higher doses of HOE 140 (10–100 μg/kg) did not induce changes in the mean arterial pressure of rats [31,58,59]. This may be due to the fact that inhibition of endogenous bradykinin alone is insufficient to induce haemodynamic changes. During acute bleeding, vasopressors secreted in response to haemorrhage [9,60] may add further to the difficulty of interpretation. However, we believe that blockade of bradykinin potentiated the portal-hypotensive effect of glypressin through the modulation of vascular reactivity.

Although the portal-hypotensive effects after glypressin infusion were similar in the HOE 140- and L-NAME-treated groups, we cannot solely attribute the actions of HOE 140 on the mesenteric vasculature to the inhibition of NO. In addition to cNOS activation, other mediators, such as prostacyclin and endothelium-derived hyperpolarizing factor, have been reported to be involved in the vasodilatory action of bradykinin [61,62]. In addition, blockade of prostacyclin activity by indomethacin may attenuate the vascular hyporeactivity to vasoconstrictors associated with portal hypertension [63,64]. Whether prostacyclin and/or endothelium-derived hyperpolarizing factor are also involved in the splanchnic hyposensitivity to glypressin in bleeding PVL rats awaits further elucidation.

In summary, our present study indicates that activation of cNOS, rather than iNOS, may be responsible for the observed splanchnic hyposensitivity to glypressin that occurs in the early stages of the haemorrhage/transfused rat model of portal hypertension. The addition of HOE 140 significantly potentiated the portal-hypotensive effects of glypressin, suggesting that the endogenous release of bradykinin may also participate in the pathogenesis of glypressin-induced hyposensitivity. However, the lack of an effect on mean arterial pressure after the infusion of HOE 140 indicates that HOE 140 is probably acting independently of its effects on cNOS.
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