Aminoguanidine ameliorates splanchnic hyposensitivity to glypressin in a haemorrhage-transfused rat model of portal hypertension

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ABSTRACT

1. Hyposensitivity to vasopressin is a well-documented phenomenon in animals with portal hypertension and patients with cirrhosis subjected to haemorrhage. Excessive formation of nitric oxide is at least partly responsible for the vascular hyporesponsiveness to vasoconstrictors observed in experimental portal hypertension or in rats with haemorrhagic shock. This study investigated whether addition of aminoguanidine, a preferential inducible nitric oxide synthase inhibitor, to glypressin (a long-acting vasopressin analogue) could enhance its portal hypotensive effect in portal-hypertensive rats with bleeding.

2. Portal hypertension was induced by partial portal vein ligation. Fourteen days after operation, systemic and portal haemodynamics were measured in stable or bleeding portal vein-ligated rats receiving intravenous glypressin (0.07 mg/kg) or aminoguanidine (70 mg/kg) followed by glypressin infusion. In rats with a hypotensive haemorrhage, 4.5 ml of blood was withdrawn and 50% of the withdrawn blood was reinfused before the administration of glypressin or aminoguanidine.

3. Glypressin resulted in a significantly greater decrease in portal pressure in portal vein-ligated rats without bleeding than in those with bleeding (P < 0.001). In contrast, glypressin induced similar changes in mean arterial pressure between the two groups (P > 0.05). The addition of aminoguanidine significantly potentiated the portal-hypotensive effect of glypressin in bleeding portal vein-ligated rats (P < 0.005) without an effect on the changes in mean arterial pressure induced by glypressin infusion (P > 0.05).

4. Splanchnic hyposensitivity to glypressin exists in a haemorrhage-transfused rat model of portal hypertension. This hyposensitivity can be ameliorated by the administration of aminoguanidine.

INTRODUCTION

Haemorrhage from oesophageal varices is a major complication of cirrhosis and results in a high mortality rate. Vasopressin and glypressin (a long-acting vasopressin analogue) are potent vasoconstrictors which greatly reduce portal venous inflow and have been used widely in the therapy of variceal haemorrhage [1]. The incidence of side effects and complications related to glypressin administration is less than that for vasopressin [1]. Recent
studies have demonstrated that vasopressin given during haemorrhage is less effective than when given during a stable state in experimental portal hypertension or patients with cirrhosis [2–5]. This desensitization phenomenon may be related to the excessive secretion of endogenous vasopressors [6] and the accumulation of toxic products during hypovolaemia [7]. However, there are no published results concerning the comparative therapeutic effect of glypressin administration in acute bleeding status.

Hyperdynamic circulation observed in portal hypertension is characterized by pronounced vasodilatation, increased systemic and regional blood flows, and augmented cardiac index [8,9]. Recent studies have shown that excessive formation of nitric oxide (NO) is responsible, at least in part, for the vascular hyporesponsiveness to vasoconstrictors [10,11] and hyperdynamic circulation [12,13] in experimental portal hypertension. Moreover, it has been claimed that the vascular hyporeactivity phenomenon observed in haemorrhagic shock may also be mediated by NO [14]. Therefore, it is reasonable to postulate that NO may be involved in the hyposensitivity to vasopressin or its analogues in portal hypertensive states during acute haemorrhage. Because the use of a non-selective NO synthase inhibitor may cause harmful complications [15–17], aminoguanidine, a preferential inducible NO synthase inhibitor [18,19], was selected in the present study for investigation.

This study was designed to clarify whether a hyporesponsiveness phenomenon to glypressin exists in haemorrhage-transfused portal vein-ligated rats. We also investigated whether the addition of aminoguanidine could enhance the portal-hypotensive effect of glypressin under these conditions.

METHODS

Animals
Male Sprague–Dawley rats weighing 300–350 g were used for this study. The rats were caged at 24 °C, with a 12-h light–dark cycle, and allowed free access to food and water until the time of experiments. Survival surgery and haemodynamic study were performed under ether anaesthesia followed by ketamine hydrochloride (100 mg/kg body weight intramuscularly). Portal hypertension was induced by partial portal vein ligation (PVL) as previously described [20]. In brief, portal vein stenosis was induced using a 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 produced. 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 fasted for 18 h before the experiments and had free access to water.

Experimental design (Figure 1)
PVL rats were divided into three groups: without bleeding group (n = 13), with bleeding group (n = 13), and with bleeding plus aminoguanidine group (n = 14). Blood was withdrawn for 15 min at a constant rate of 0.3 ml/min from the rats subjected to haemorrhage after the measurements of baseline mean arterial pressure, heart rate and portal blood pressure. After a 20-min stabilization, 50% of the withdrawn blood was reinfused at the same rate as used for haemorrhage [2]. The infusion and withdrawal of blood were performed using an infusion/withdrawal pump (model SP 210 iw, World Precision Instruments, Sarasota, 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 reinfused in these two periods. Aminoguanidine (70 mg/kg in 0.35 ml of normal saline) or normal saline (0.35 ml) were infused intravenously for 2 min after the second record of the haemodynamic parameters. The dose of aminoguanidine was selected from preliminary experiments in PVL rats to induce an increase in mean arterial pressure of at least 10%. Forty-five minutes later, the third haemodynamic measurements were taken, and then glypressin (0.07 mg/kg) was infused intravenously for 2 min. Mean arterial pressure, heart rate and portal pressure were determined again at 10 min after the glypressin administration. Both glypressin (Ferring, Kiel, Germany) and aminoguanidine (Sigma Chemical Co., St. Louis, MO, U.S.A.) were infused by an infusion pump (model SP 210 iw, World Precision Instruments) via a PE-50 catheter connected to the right jugular vein.

Measurements of systemic and portal haemodynamics
The right femoral artery of PVL rats was cannulated with a PE-50 catheter 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 analysis were performed using one-way analysis of
variance with Scheffe test and unpaired Student’s t-test accordingly. Results were considered statistically significant at $P < 0.05$.

RESULTS

Haemodynamic assessments at baseline

The body weights were similar in the three groups (without bleeding, 322 ± 7 g; with bleeding, 326 ± 6 g; with bleeding plus aminoguanidine, 342 ± 6 g; $P > 0.05$). There was no significant difference in the mean arterial pressure, portal pressure and heart rate among the three groups at baseline (Table 1).

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

Glypressin infusion resulted in a significantly greater reduction 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 after glypressin infusion were similar in without bleeding and with bleeding groups ($3.5 ± 3.8\%$ and $4.4 ± 3.8\%$ respectively, $P > 0.05$).

Systemic and portal haemodynamic effects of aminoguanidine in PVL rats with bleeding

In PVL rats with bleeding, aminoguanidine infusion resulted in a significant elevation of mean arterial pressure compared with saline infusion (Figure 3). There was no significant change in portal pressure (Figure 3) or heart rate (aminoguanidine, $10.2 ± 2.6\%$; saline, $9.8 ± 3.2\%$; $P > 0.05$) after aminoguanidine infusion.

Haemodynamic effects of glypressin alone or glypressin plus aminoguanidine in PVL rats with bleeding

Compared with bleeding PVL rats receiving glypressin alone, the addition of aminoguanidine significantly potentiated the portal-hypotensive effect of glypressin in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Systemic and portal haemodynamics at baseline</th>
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</thead>
<tbody>
<tr>
<td>Haemodynamic parameter</td>
<td>Without bleeding ($n = 13$)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>$85.6 ± 4.8$</td>
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<tr>
<td>Portal pressure (mmHg)</td>
<td>$11.3 ± 0.5$</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>$318 ± 11$</td>
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</table>

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bleeding PVL rats (Figure 4). However, no difference was observed in the change of mean arterial pressure (Figure 4) or heart rate (glypressin, 4.4 ± 3.8%; glypressin plus aminoguanidine, 1.7 ± 2.8%; P > 0.05) between the two groups.

**DISCUSSION**

Our study demonstrates that during acute bleeding, glypressin induced a significant elevation in the mean arterial pressure of PVL rats but had no noticeable effect on portal pressure. In PVL rats without bleeding, however, glypressin administration achieved similar effects on the mean arterial pressure but effectively reduced portal pressure. These results suggest the existence of hyposensitivity to glypressin in bleeding PVL rats, similar to the hyposensitivity phenomenon with vasopressin infusion [2,3]. In addition, the above findings disclose that the vascular reactivity to glypressin may be selectively blunted over splanchnic circulation in PVL rats with acute bleeding (i.e. splanchnic hyposensitivity). It has been suggested that only a dose of vasopressin 10 times larger than the standard dose could achieve the same portal-hypotensive effect in the haemorrhage-transfused animals [2]. Since only one dose of glypressin was used in our study, it is unknown whether the lack of effect after glypressin infusion can be overcome with a larger dose. However, caution must be advised since a larger dose of glypressin may cause harmful complications.

In animals suffering from acute haemorrhage, compensation of hypotension may induce an early and sustained vasoconstriction over the non-hepatic splanchnic organs but this compensatory response is insufficient to raise total systemic vascular resistance [21–24]. A delayed rise in the systemic vascular resistance then takes place, which may be due to the vasoconstriction reaction over adipose tissue, muscles and skin. It has been suggested that a rapid release of various vasoconstrictors (e.g. catecholamines) may be responsible for a selective initial splanchnic vasoconstriction. In addition, the
delayed release of endogenous vasopressin may explain the later increase in the non-splanchnic vascular resistance [25–27]. If this is true, the hyposensitivity phenomenon to vasopressin and its analogues during haemorrhage may be explained, at least in part, by the competition of catecholamines and endogenous vasopressin. In addition, autoregulatory escape of various vascular beds during haemorrhage may alter the responsiveness to exogenous vasopressin and blunt its therapeutic effect [28].

Recent studies have demonstrated that excessive formation of NO may be responsible for the vascular hyporeactivity to vasoconstrictors [10,11] and hyperdynamic circulation [12,13] observed in experimental portal hypertension. In unbled PVL rats, blockade of NO synthesis by chronic aminoguanidine administration significantly modulated the hyperdynamic status without changes in portal pressure [29,30]. However, the haemodynamic situation during acute haemorrhage may be quite different since many toxic products are accumulated during acidosis, hypoxaemia and hypovolaemia, which may interfere with or down-regulate the response of various vasoactive mediators. In addition, it has been suggested that NO formation may also be stimulated during this situation and mediate the vascular hyporeactivity to vasoconstrictors in haemorrhagic shock [14].

In our study, acute aminoguanidine infusion significantly increased mean arterial pressure without changes in portal pressure in bleeding PVL rats; similar to the results obtained with chronic aminoguanidine administration [29,30]. This suggests that excessive formation of NO may exist in PVL rats with bleeding due to endogenous NO overproduction mixed with the compensatory response to hypotension. The lack of changes in portal pressure after aminoguanidine administration may be explained by a concomitant increase in portal-collateral resistance [30].

In the present study, the splanchnic hyposensitivity to glypressin in haemorrhage-transfused PVL rats could be ameliorated by aminoguanidine infusion, indicating NO may be involved in the vascular hyporeactivity phenomenon observed in portal hypertensive models with
bleeding. The NO synthase exists in two isoforms. The constitutive form, anchored on the internal surface of the endothelial membrane, may be activated by endogenous vasodilators such as bradykinin [31], and mechanical factors such as shear stress and pulsatile blood flow [32,33]. The inducible form represents newly synthesized enzyme, which is expressed in macrophages and vascular smooth muscles after activation by endotoxin and cytokines such as tumour necrosis factor-α and interleukin-6 [34]. It has been reported that haemorrhage is associated with endogenous release of various substances including bradykinin, 5-hydroxytryptamine, adenosine triphosphate and tumour necrosis factor-α [35–37]. These mediators may subsequently stimulate NO formation through the action on both constitutive and inducible NO synthases. In addition, we cannot neglect the possibility that hyperdynamic circulation itself may also stimulate constitutive NO synthase, although the influence seems less prominent during acute haemorrhage. However, the discriminative role of NO synthase isoforms in the pathogenesis of glypressin hyporesponsivity observed in bleeding PVL rats still awaits further investigations.

In the current study, apparent regional selectivity to glypressin infusion in bleeding PVL rats was demonstrated. Although the actual reasons are unknown, the possibility of uneven distribution of NO synthase isoforms over systemic and splanchnic circulation may partly explain this phenomenon. Niederberger et al. [38] have disclosed that the mesenteric constitutive NO synthase activities were increased in PVL rats, whereas aortic constitutive NO synthase activities did not differ from those of controls. In addition, a similar distribution of endogenous vasopressors and NO synthase stimulators may also develop during acute bleeding status, and subsequently result in the regional selectivity to the administration of glypressin. However, more research in this area is required for further elucidation.

In summary, our present study suggests the existence of splanchnic hyporesponsivity to glypressin in

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**Figure 4** Effects of glypressin alone or in combination with aminoguanidine on mean arterial pressure (MAP) and portal pressure (PP) in PVL rats with bleeding

NS, not significant.
haemorrhage-transfused PVL rats. This hyposensitivity could be ameliorated by the administration of amino-guanidine, suggesting excessive formation of NO may play a role in the pathophysiology of glypressin hyposensitivity.

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