Chronic administration of octreotide increases vascular responsiveness in rats with portal hypertension

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1. It has been reported that octreotide partially corrects the hyperdynamic state in patients and animals with portal hypertension. The aim of the present study was to investigate whether chronic administration of octreotide can increase vascular responsiveness in rats with portal hypertension.

2. Portal hypertension was induced by partial portal vein ligation. Octreotide was given for 9 days subcutaneously (100 μg/kg every 12 h) starting 1 day before ligation. The aorta and mesenteric artery were then removed to study contraction after pressure recording.

3. Octreotide treatment significantly reduced portal pressure and plasma glucagon concentrations compared with the vehicle-treated group. Both phenylephrine and vasopressin induced concentration-dependent contractile responses in the aorta and mesenteric artery from both groups. The maximum contractile responses to phenylephrine and vasopressin in aorta and mesenteric artery were significantly greater in the octreotide-treated group than in the vehicle-treated group. The EC50 values for phenylephrine and vasopressin were significantly different in the aorta, but not in the mesenteric artery, between the two groups. In contrast, octreotide treatment did not alter the contractile responsiveness of arteries from sham-operated rats.

4. These results show that, in rats with portal vein stenosis, octreotide increases arterial contractile responsiveness and reduces portal pressure.

INTRODUCTION

The hyperdynamic circulation in portal hypertension is characterized by a decrease in arterial blood pressure and peripheral vascular resistance and an increase in cardiac output and splanchnic blood flow [1]. In addition, portal hypertension is associated with both decreased vascular responsiveness to endogenous vasoconstrictors and increased circulating humoral vasodilators, which are proposed to contribute to the hyperdynamics [2]. Hyporesponsiveness to vasoconstrictors such as potassium chloride [3, 4], noradrenaline [5,6], vasopressin [7, 8] and angiotensin II [9, 10] in portal hypertension has been found in various in vivo and in vitro studies. Several recent studies have shown that preincubation with a nitric oxide synthase inhibitor in the organ bath can potentiate the vascular responsiveness of arterial vessels from animals with portal hypertension, implicating a mediator role for nitric oxide in such phenomena [3, 4, 11, 12]. Few studies have explored whether such hyporesponsiveness is amenable to drug treatment.

Octreotide is a synthetic eight amino acid analogue of somatostatin with prolonged action [13, 14]. It has been shown to be effective in controlling acute variceal bleeding [15, 16]. Haemodynamic studies in cirrhotic patients suggest that octreotide reduces hepatic and azygous blood flow and causes a modest reduction in portal venous pressure [17, 18]. In animals with portal hypertension, acute administration of octreotide has been demonstrated to reduce portal venous blood flow and cause splanchnic vasoconstriction [19–21]. Recently, there have been two studies investigating the possible haemodynamic effects of repeated, chronic octreotide treatment on rats with portal hypertension [22, 23]. Albillos et al. [22] observed that a 4-day course of octreotide treatment ameliorates peripheral vasodilatation and Na+ retention in rats with portal hypertension. In the same animal model, we [23] found that chronic octreotide treatment also reduces portal pressure and portal venous flow and enhances splanchnic vascular resistance. These studies suggest that chronic octreotide treatment could correct the hyperdynamic state in animals with portal hypertension. In a preliminary communication, Sieber et al. [24] reported that octreotide treatment could prevent vascular hyporeactivity in rats with portal hypertension in vitro. As vascular hyporeactivity is proposed to contribute to the hyperdynamic circula-
tion in portal hypertension [1, 2], the present study was undertaken to test whether chronic octreotide treatment can increase vascular hyporesponsiveness in portal vein-stenosed rats.

**METHODS**

**Animal groups**

Animals were allocated to one of two groups: the octreotide-treated group or the vehicle-treated group. The octreotide group received 100 μg/kg octreotide by subcutaneous injection every 12 h for 9 days. The vehicle-treated group received saline injections. One day after the first drug or saline treatment, both groups of rats underwent partial portal vein ligation (PVL) and were housed in animal cages after recovery.

**Portal hypertensive rats**

PVL was performed according to the method of Chojkier and Groszmann [25] and as previously reported [5]. Briefly, male Sprague–Dawley rats (200–250 g) were anaesthetized with ether. A midline incision was made, and the portal vein proximal to the bifurcation was exposed. A 3–0 silk ligature was made around the portal vein and a piece of PE 50 tubing (Clay Adams, Parsippany, NJ, U.S.A.). The PE tubing was then removed and the abdomen closed. In sham-operated rats, the portal vein was mobilized but not stenosed. Animal studies were approved by the Animal Experiment Committee of the National Yang-Ming University and conducted humanely.

**Pressure measurement**

Eight days after surgery, the rats were anaesthetized with 150 mg/kg ketamine after an overnight fast. The ileocolic vein was cannulated with PE 50 tubing to measure portal venous pressure and the femoral artery was cannulated to measure arterial blood pressure. Changes in pressures and heart rate were monitored with a polygraph (RS 3400, Gould, Valley View, OH, U.S.A.) via strain-gauge transducers. Immediately after the pressure recording, 2 ml of blood was collected for plasma glucagon measurement and the rats were killed with an overdose of sodium pentobarbital. The thoracic aorta (above the diaphragm and below the aortic arch) and mesenteric artery were then excised and placed in aerated (95% oxygen/5% carbon dioxide) Krebs–Ringer bicarbonate (KRB) solution and cleaned at room temperature. The composition of KRB solution was (in mmol/l): NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; and glucose, 11.1.

**In vitro tension experiments**

The blood vessels were prepared as previously reported [5]. The aorta and mesenteric artery were cut into 4 mm segments and care was taken not to damage the endothelium. Each vessel segment was equilibrated in a 15-mm tissue chamber (maintained at 37°C and bubbled with a gas mixture of 95% oxygen/5% carbon dioxide) for 1 h under optimal tension of 2 g for aorta and 1.2 g for mesenteric artery. Two fine stainless-steel wires were gently inserted through the aortic lumen; the lower one was anchored to a stationary support and the upper one to a force–displacement transducer (FT 03, Grass Instrument Co., Quincy, MA, U.S.A.). Tension was recorded on a Gould physiograph (RS 3400). The readiness of tissue was indicated by consistent responses on two consecutive tests with 60 mmol/l potassium chloride. Thereafter, the cumulative concentration–response curves to phenylephrine (10⁻⁹ to 10⁻³ mol/l) in each group were obtained. The tissue was then rinsed and allowed to recover for 45 min and the cumulative concentration–response curves to vasopressin (10⁻¹⁰ to 10⁻⁷ mol/l) were obtained. At the end of the experiments, the tissue was challenged with 60 mmol/l potassium chloride. The final response to potassium chloride was not significantly different from the initial response. At the end of the experiment, the (wet) tissue weight of each segment was determined. On each study day, one PVL rat from the octreotide-treated group and one from the vehicle-treated group were killed for comparative contractile study. Overall, there were eight contractile study days. The study was conducted with knowledge of the sample tested and not blindly.

To exclude the possibility that octreotide may have a direct effect on smooth muscle contractility, a separate study was conducted in sham-operated rats undergoing the same protocol of octreotide treatment as the PVL rats. The aorta and mesenteric artery were removed and prepared as described above. Cumulative concentration–response curves to phenylephrine and vasopressin were obtained.

Octreotide (Sandostatin) was from Sandoz Pharma (Basle, Switzerland). Vasopressin was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.) and dissolved in distilled water. All other chemicals were purchased from Sigma (St. Louis, MO, U.S.A.). Phenylephrine was dissolved in 0.1% ascorbic acid. Plasma glucagon concentrations were determined by radioimmunooassay (Daichi Radioisotope Laboratories, Tokyo, Japan) as described previously [23].

**Data analysis**

Data are expressed as means ± SEM. Significance was determined by the one-way analysis of variance (ANOVA) for the concentration–response curves and otherwise by Student's t-test at P < 0.05.
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Table 1. General characteristics of PVL rats after octreotide or vehicle treatment. Statistical significance: *P < 0.01, **P < 0.05 compared with vehicle group (means ± SEM, n = 8 in each group).

<table>
<thead>
<tr>
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<th>Octreotide-treated group</th>
<th>Vehicle-treated group</th>
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<tbody>
<tr>
<td>Portal venous pressure (mmHg)</td>
<td>12.8 ± 0.3*</td>
<td>14.6 ± 0.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>120 ± 5</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>285 ± 13</td>
<td>298 ± 8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>260 ± 3</td>
<td>265 ± 4</td>
</tr>
<tr>
<td>Plasma glucagon levels (pg/ml)</td>
<td>521 ± 23**</td>
<td>624 ± 37</td>
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</table>

RESULTS

General data

As shown in Table 1, the vehicle-treated group showed typical features of portal hypertension, including increased portal venous pressure (PVP; 14.6 ± 0.4 mmHg) and reduced mean arterial pressure (MAP; 111 ± 3 mmHg). The PVP in the octreotide-treated group (12.8 ± 0.3 mmHg) was significantly lower than that in the vehicle-treated group, whereas MAP, heart rate and body weight were similar in the two groups. Octreotide also significantly decreased the high plasma glucagon levels found in PVL rats (521 ± 23 versus 624 ± 37 pg/ml in the octreotide and vehicle-treated groups respectively).

Contractile responses in aorta in vitro

The tissue weights of aorta from the octreotide- (2.5 ± 0.2 mg) and vehicle-treated (2.4 ± 0.2 mg) groups were similar. The tension responses to potassium chloride (60 mmol/l) were also similar in the octreotide- (2.16 ± 0.10 g) and vehicle-treated (1.96 ± 0.15 g) groups. Both phenylephrine (10^{-9} to 10^{-5} mol/l) and vasopressin (10^{-10} to 10^{-7} mol/l) induced concentration-dependent contractile responses in the aorta from both groups. The maximal contractile responses to both phenylephrine (1.46 ± 0.14 versus 0.93 ± 0.09 g) and vasopressin (1.22 ± 0.10 versus 0.85 ± 0.08 g) were significantly greater in the octreotide-treated group than in the vehicle-treated group (Figs 1 and 2). The EC50 values (expressed as -log mol/l) for phenylephrine (6.49 ± 0.12 versus 6.14 ± 0.12, P = 0.06) were almost significantly different between the two groups. The EC50 values (expressed as -log mol/l) for vasopressin (8.23 ± 0.09 versus 8.06 ± 0.09) were not different between the two groups (Table 2).

Contractile responses in sham-operated rats in vitro after octreotide treatment

Octreotide treatment in sham-operated rats induced no change in haemodynamic parameters.

Contractile responses in mesenteric artery in vitro

The tissue weights of mesenteric artery from the octreotide- (0.9 ± 0.1 mg) and vehicle-treated (0.8 ± 0.1 mg) groups were similar. The tension responses to potassium chloride (60 mmol/l) were also similar in the octreotide- (1.80 ± 0.49 g) and vehicle-treated (1.46 ± 0.22 g) groups. Both phenylephrine (10^{-9} to 10^{-5} mol/l) and vasopressin (10^{-10} to 10^{-7} mol/l) induced concentration-dependent contractile responses in the mesenteric artery from both groups. The maximal contractile responses to both phenylephrine (1.46 ± 0.14 versus 0.93 ± 0.09 g) and vasopressin (1.22 ± 0.10 versus 0.85 ± 0.08 g) were significantly greater in the octreotide-treated group than in the vehicle-treated group (Figs 3 and 4). The EC50 values (expressed as -log mol/l) for phenylephrine (6.49 ± 0.12 versus 6.14 ± 0.12, P = 0.06) were almost significantly different between the two groups. The EC50 values (expressed as -log mol/l) for vasopressin (8.23 ± 0.09 versus 8.06 ± 0.09) were not different between the two groups (Table 2).
Table 2. *E*max and *EC*50 values for agonists in the octreotide- and vehicle-treated groups. Statistical significance: *P* < 0.05 compared with vehicle-treated group (means ± SEM, n = 8 in each group).

<table>
<thead>
<tr>
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<th>Octreotide-treated group</th>
<th>Vehicle-treated group</th>
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<tbody>
<tr>
<td>Aorta</td>
<td></td>
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<tr>
<td>Phenylephrine</td>
<td><em>E</em>max (g) 2.37 ± 0.17*</td>
<td>1.89 ± 0.11</td>
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<td></td>
<td><em>EC</em>50 (-log mol/l) 7.39 ± 0.10*</td>
<td>7.12 ± 0.03</td>
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<tr>
<td>Vasopressin</td>
<td><em>E</em>max (g) 1.46 ± 0.15*</td>
<td>0.94 ± 0.10</td>
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<td></td>
<td><em>EC</em>50 (-log mol/l) 8.09 ± 0.04*</td>
<td>7.87 ± 0.07</td>
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<tr>
<td>Mesenteric artery</td>
<td></td>
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<tr>
<td>Phenylephrine</td>
<td><em>E</em>max (g) 1.46 ± 0.14*</td>
<td>0.93 ± 0.09</td>
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<td><em>EC</em>50 (-log mol/l) 6.49 ± 0.12</td>
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<td>Vasopressin</td>
<td><em>E</em>max (g) 1.22 ± 0.10*</td>
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such as PVP, MAP or heart rate compared with vehicle-treated rats. Phenylephrine induced concentration-dependent contractile responses in both aorta and mesenteric artery, and there was no difference in the *E*max (2.01 ± 0.14 g versus 2.06 ± 0.17 g in aorta; 1.49 ± 0.14 g versus 1.63 ± 0.06 g in mesenteric artery) or *EC*50 values (expressed as −log mol/l: 7.07 ± 0.16 versus 7.25 ± 0.08 in aorta; 6.48 ± 0.13 versus 6.40 ± 0.15 in mesenteric artery) between octreotide- and vehicle-treated sham-operated rats (*n* = 8 in each group). Similarly, vasopressin induced concentration-dependent contractile responses in both aorta and mesenteric artery, and there was no difference in the *E*max (1.07 ± 0.24 g versus 1.34 ± 0.21 g in aorta; 1.06 ± 0.11 g versus 1.04 ± 0.11 g in mesenteric artery) or *EC*50 values (expressed as −log mol/l: 8.05 ± 0.07 versus 7.93 ± 0.18 in aorta; 8.16 ± 0.03 versus 8.29 ± 0.05 in mesenteric artery) between octreotide- and vehicle-treated sham-operated rats.

**DISCUSSION**

Somatostatin and octreotide have gained increasing recognition as a first-choice drug in the management of acute variceal bleeding with good efficacy and few side-effects [26–28]. Although the acute haemodynamic effects of somatostatin or octreotide are better known, few studies have explored their chronic effects in patients or animals with portal hypertension. Unlike propranolol, octreotide is currently not a chronic prophylactic drug for variceal bleeding. Nevertheless, chronic study of octreotide may provide insight into the pathobiology of portal hypertension and its haemodynamic circulation. Moreover, it remains to be assessed whether vascular hyporeactivity, one of the characteristics of portal hypertension [1, 2], is as amenable to octreotide treatment as is hyperdynamics [22, 23]. In the present study, we show that chronic administration of octreotide enhances the
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contractile responsiveness of the mesenteric artery and aorta and reduces portal venous pressure in PVL rats. The rationale for starting octreotide treatment 1 day before the induction of portal vein stenosis was that early administration of portal hypotensive drugs is more effective in ameliorating hyperdynamics and portosystemic shunting in portal hypertension [29-31]. Recently, it was also observed that a 4-day [22] or 14-day [23] course of octreotide immediately after portal vein stenosis ameliorates vasodilatation in PVL rats. Taken together, these studies suggest that early chronic administration of portal hypotensive drugs could partially correct the systemic or splanchnic hyperdynamics in animals with portal hypertension.

The mechanisms of action of octreotide or somatostatin in reversing the hyperdynamics of portal hypertension have been proposed to be chiefly through the suppression of gastrointestinal hormones, especially glucagon [14, 32, 33]. Indeed, Pizcueta et al. [32] have shown that in PVL rats the splanchnic haemodynamic effects of somatostatin can be hindered by glucagon. Several studies, including ours [21, 23, 32, 33], have reported that the splanchnic haemodynamic or portal hypotensive effects of somatostatin or octreotide after acute as well as chronic administration are associated with a reduction in plasma glucagon levels. Glucagon, as one of the mediators in the hyperdynamics of portal hypertension, has been recognized [1, 2]. Findings, such as the fact that plasma infusion of glucagon into normal rats leads to reduced splanchnic resistance [34] and vascular responsiveness [7, 35], simulating portal hypertension, and that glucagon antisera is capable of reducing splanchnic hyperaemia by about 40% [36] lend support to a mediator role for glucagon in portal hypertension.

In the present study, the maximum contractile responses to phenylephrine and vasopressin of the aorta as well as mesenteric artery were significantly enhanced in octreotide-treated PVL rats, whereas the contractile sensitivity (EC50) was enhanced only in the aorta and not in the mesenteric artery. Also, the contractile responses to potassium chloride in both aorta and mesenteric artery were not significantly changed. This suggests that octreotide treatment could not completely correct the hyporesponsiveness in PVL rats. This may be related to the following two points: (a) chronic administration of octreotide reduced portal pressure and portal tributary blood flow but the splanchnic arterial resistance was still lower than that in sham-operated rats, i.e. the hyperdynamics was not completely reversed by octreotide [23]; and (b) octreotide is known to act mainly through suppression of gastrointestinal hormones such as glucagon [14, 32, 33]. However, there are other important independent mediators, such as nitric oxide, prostacyclin, bile acid, which are known to contribute to the hyperdynamics in portal hypertension [1, 2]. In a preliminary communication, Sieber et al. [24] also reported that octreotide enhanced the splanchnic vascular responses to potassium chloride without direct effect upon nitric oxide secretion. In their report, contractile responses in vitro to very high concentrations of potassium chloride (130 and 300 mmol/l) were enhanced in octreotide-treated PVL rats, whereas contractile responses to lower concentrations of potassium chloride (30 and 60 mmol/l) were not significantly changed. In this study, we also found that the contractile responses to 60 mmol/l potassium chloride were similar in octreotide- and vehicle-treated PVL rats. It is interesting to note that the responsiveness of aorta (a systemic vessel) is more susceptible to octreotide treatment than that of mesenteric artery (a splanchnic vessel). In our previous study [23], we also found that the splanchnic vascular beds were less affected than systemic vascular beds by chronic octreotide treatment. In the present study, we found that chronic octreotide treatment induced no change in the vascular responsiveness of sham-operated rats. It has also been shown that acute administration of octreotide has no direct contractile effect on splanchnic vascular beds [37]. Previous studies [22, 23] have indicated that octreotide has no haemodynamic effect on sham-operated animals.

Recently, there have been several reports showing that the vascular hyporesponsiveness in PVL or cirrhotic rats can be reversed by preincubation with nitric oxide synthase inhibitors [3, 4, 11, 12, 38], suggesting a role for nitric oxide in vascular hyporesponsiveness. On the other hand, another report indicates that vascular hyporesponsiveness cannot be reversed by preincubation with a nitric oxide synthase inhibitor [39]. The issue of nitric oxide as a mediator for portal hypertension remains controversial [40]. It remains to be investigated whether chronic octreotide administration can eventually lead to ‘nitric oxide related’ change in vascular responsiveness in PVL rats [24].

In summary, our results show that chronic administration of octreotide causes an increase in contractile responsiveness to phenylephrine and vasopressin in the aorta and mesenteric artery of portal vein-stenosed rats, together with a reduction in PVP and plasma glucagon level.

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