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The responses of the hepatic and splanchnic vascular beds to vasopressin in rats

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ABSTRACT

Vasopressin, a vasoactive peptide, causes vasoconstriction via V1a vasopressin receptors. Unlike other vasoconstrictor agents, vasopressin also has vasodilatory properties. The purpose of this study was to determine the effect of vasopressin on hepatic and splanchnic circulation in Sprague-Dawley rats. The experiments were conducted in not only isolated blood- and constant flow-perfused livers but also anesthetized spontaneously breathing rats. In anesthetized rats, portal venous pressure (Ppv), systemic arterial pressure (Psa), central venous pressure, and hepatic blood flow (HBF) of combined portal venous and hepatic arterial blood flow were continuously measured, and splanchnic vascular bed resistance (Rspl) defined by (Psa − Ppv) / HBF was determined. In perfused livers, vasopressin at 0.1–1,000 nM caused weak venoconstriction as evidenced by small increase in Ppv. In anesthetized rats, when vasopressin was injected into the portal vein as a bolus consecutively at 0.01–100 nmol/kg, Psa increased dose-dependently with the peak increment of 60 ± 18 mmHg at 100 nmol/kg. Ppv and HBF decreased, with resultant increase in Rspl, indicating splanchnic vasoconstriction. In conclusion, hepatic venoconstrictor action of vasopressin was weak in rats. Vasopressin causes splanchnic vasoconstriction, resulting in a decrease in HBF and Ppv in anesthetized rats.

Vasopressin, a vasoactive peptide, causes vasoconstriction via V1a vasopressin receptor of vascular smooth muscle cells (1, 5). Vasopressin has been a well-established therapeutic agent controlling severe hypotension such as hemorrhagic shock due to gastrointestinal bleeding (21), septic shock (6, 8), and anaphylactic shock (13). Its beneficial effects on hypotension are usually ascribed to its strong arteriolar constrictor activity in most peripheral vascular beds, especially splanchnic vascular beds. Vasopressin-induced vasoconstriction in the intestinal and splenic vascular beds results in a reduction of portal blood flow with a fall in hepatic portal venous pressure (Ppv).

Vasopressin causes vasoconstriction in most vascular beds. Paradoxically, vasopressin has also been demonstrated to cause vasodilation in numerous vascular beds (2, 3, 9, 10, 12, 16, 17, 19), a feature not shared by other vasoconstrictor agents. For rats which are one of the most frequently used animals for experiments, the investigations on the effect of vasopressin on the splanchnic vascular bed *in vivo* are limited (11): blood flow to liver was not measured, although a decrease in Ppv was reported. A decrease in Ppv could be theoretically induced by vasodilation, rather than vasoconstriction, in the hepatic vessels. On the other hand, there are reports on the hepatic vascular response of perfused rat livers to vasopressin, indicating absence of constriction or dilatation (14, 18). However, these experiments

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were performed by using blood-free saline as the perfusate (14, 18).

Thus, we determined the responsiveness of the splanchnic and hepatic circulation to vasopressin not only in *in vivo* anesthetized Sprague-Dawley rats, but also in isolated blood-perfused rat livers. In *in vivo* animals, we administered vasopressin consecutively at doses ranging 0.01–100 nmol/kg into the portal veins to determine its direct action on hepatic vessels.

MATERIALS AND METHODS

Animals. Ten male Sprague-Dawley rats (Japan SLC, Japan) were used. These animals were maintained at the room temperature of 23°C and under pathogen-free conditions on a 12 : 12-hour dark/light cycle, with food and water *ad libitum*. This experiment was approved by the Animal Research Committee of Kanazawa Medical University. We followed the principles of laboratory animal care (NIH publication No. 85–23, revised 1996).

Isolated perfused rat liver experiment. The basic methods for isolated perfused rat livers were described previously (15). Five rats (250 ± 5 g) were anesthetized with pentobarbital. After laparotomy, the hepatic artery was ligated; the bile duct was cannulated with the polyethylene tube. At 5 min after heparinization ($500\ U \cdot kg^{-1}$) via the right carotid artery, blood (9–10 mL) was withdrawn. The inferior vena cava (IVC) above the renal veins was ligated, and the portal vein was cannulated with a stainless steel cannula. After thoracotomy, the supradiaphragmatic IVC was cannulated through a right atrium incision with a stainless steel cannula, then portal perfusion was begun with the heparinized autologous blood diluted with 5% bovine albumin (Sigma-Aldrich Co., St. Louis, MO) in Krebs solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 25.5 mM $NaHCO_3$, and 5.6 mM glucose) at hematocrit of 12%. The liver was rapidly excised, weighed continuously with an isometric transducer (TB-652T; Nihon-Kohden, Tokyo, Japan), and perfused recirculatingly at a constant flow rate with blood (40 mL) oxygenated by bubbling with 95% O_2 and 5% CO_2. The Ppv and hepatic venous pressure (Phv) were measured with the reference points at the hepatic hilus. Portal flow rate was measured with an electromagnetic flow meter (MFV 1200; Nihon-Kohden). The hepatic vascular pressures and liver weight were displayed through a thermal physiograph, and digitized using a personal computer. Twenty minutes after the start of perfusion, an isogravimetric state (no weight gain or loss) was obtained by adjusting portal flow rate and the height of the reservoir at a Phv of 0–1 cmH_2O, and then vasopressin was injected cumulatively into the reservoir to gain the concentration of 0.1–1,000 nM.

Anesthetized rat experiment. Rats (n = 5, 436 ± 21 g) were anesthetized with $50\ mg \cdot kg^{-1}$ pentobarbital sodium ip, and placed on a heating pad (ATC-101B; Unique Medical, Japan) to maintain body temperature at 37°C throughout the experiment. The adequacy of anesthesia was monitored by the stability of the systemic arterial pressure (Psa) and respiration during a pinch of the hindpaw. Supplemental doses of anesthetic (10% of initial dose) were given intraperitoneally if necessary. The Psa was measured via the right femoral artery. For measurement of the central venous pressure (Pcv), the right external jugular vein was catheterized.

Following a midline incision of the abdominal wall, a catheter (ID 0.47 mm, OD 0.67 mm) was inserted directly into the main portal vein. This catheter was connected to a Y-type miniature plastic tube (7, 24), one twig end of which was connected via a water-filled polyethylene tube to the pressure transducer for measurement of Ppv, and another twig end was used to introduce a thin inner polyethylene tube, which was tapered to ~0.3 mm in diameter over hot air, for an intravenous injection of vasopressin. The total hepatic blood flow (HBF) was measured with an ultrasound transit-time flow probe (Transonics, Ithaca, NY), which was 2 mm in width, and placed around both the portal vein and the hepatic artery at the hepatic hilum. After closure of the abdomen, the baseline measurements were started.

The Psa, Ppv, and Pcv were continuously measured with pressure transducers (TP-400T; Nihon-Kohden) and the reference level was set at the level of the left atrium. These vascular pressures and HBF were digitally recorded at 40 Hz (PowerLab, AD Instruments). Mean values of Psa, Ppv and HBF recorded instantaneously were calculated using the Power Lab. The values of Pcv were the values measured at expiration. Splanchnic vascular bed resistance (Rspl) was computed by the following equation:

$$Rspl = (Psa - Ppv) / HBF \quad (1)$$

At 20 min after surgery, the baseline measurements were performed. Then vasopressin was injected into the portal vein as a bolus consecutively at

the doses ranging 0.01–100 nmol/kg. Each dose of vasopressin was dissolved in 300 μL saline. All drugs were purchased from Sigma Chemical Company (St Louis, MO).

Statistics. All results are expressed as the means ± SD. Statistical analyses were performed using analysis of variance followed by Dunnett post hoc test. A P value less than 0.05 was considered significant.

RESULTS

Isolated perfused rat liver experiment

The basal levels of hepatic hemodynamic variables in perfused livers were as follows: Ppv, 6.5 ± 0.5 cmH_2O; Phv, 0.2 ± 0.2 cmH_2O; and portal blood flow, 42 ± 1 mL/min/10 g liver weight. Figure 1 shows the changes in Ppv and weight of perfused rat livers after injections of vasopressin into the perfusing blood. In rat livers, vasopressin at 0.1–1,000 nM caused a slight but significant increase in Ppv, which was accompanied by a decrease in liver weight. Maximal increase in Ppv was only 0.4 ± 0.1 cmH_2O at 10 nM vasopressin. This small increase in Ppv corresponds to only 6% increase in the transhepatic vascular resistance. The liver weight loss was also small with the greatest decrease of 0.15 ± 0.02 g at 1 nM.

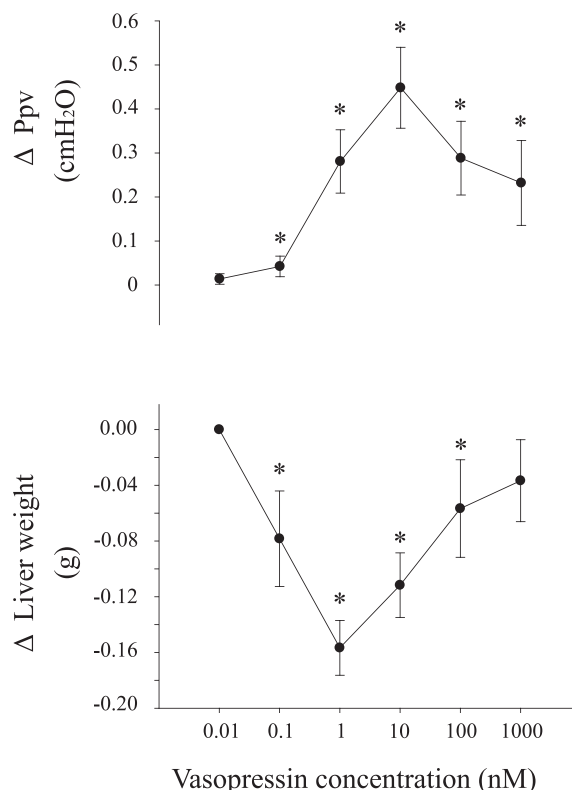

Fig. 1 The summary of the maximum changes at each concentration in the portal venous pressure (Ppv), and liver weight after an injection of vasopressin into the perfusate of isolated blood-perfused rat livers (n = 5). Values are means ± SD; *P < 0.05 vs. the baseline values.

Anesthetized rat experiment

The baseline values just before an injection of 0.01 nmol/kg vasopressin into rats were as follows: Psa, 112 ± 19 mmHg; Pcv, 1.1 ± 0.8 cmH_2O; and HBF, 27 ± 6 mL/mint; Ppv, 8.5 ± 1.2 cmH_2O. Based on these values, Rspl was determined as 4.0 ± 0.9 mmHg min/mL where the Ppv values (mmHg) were calculated as 0.739 times the Ppv values (cmH_2O). There were no significant differences between these baseline values and those just before administration of each dose of vasopressin (1–100 nmol/kg) in any hemodynamic variables (data not shown). Figure 2 shows the representative recordings of Psa, Pcv, HBF and Ppv after intravenous injections of vasopressin at various doses in an anesthetized rat. Figure 3 shows the time course of changes in Psa, Rspl, Ppv and HBF after consecutive injections of vasopressin. Psa tended to increase at 0.01 nmol/kg of vasopressin, but statistically significant increases were observed at 0.1 nmol/kg or more. Psa increased considerably in a dose-dependent manner with the peak increment of 60 ± 18 mmHg at 100 nmol/kg (Figs. 2 and 3).

As shown in Figs. 2 and 3, Rspl and HBF dose-dependently increased and decreased, respectively, after injection of vasopressin: at 10 nmol/kg vasopressin, Rspl increased 9-fold from the baseline of 4.8 ± 1.2 to 43.4 ± 22.1 mmHg/mL/min, while HBF decreased by 84 ± 6% from the baseline of 23.2 ± 4.4 to 3.8 ± 1.4 mL/min. Ppv decreased in parallel with the decrease in HBF: Ppv significantly decreased by 1.3 ± 0.4, 3.3 ± 0.9, 3.8 ± 0.9 and 4.2 ± 0.8 cmH_2O at the doses of 0.1, 1, 10, and 100 nmol/kg, respectively (Figs. 2 and 3). In response to vasopressin, Pcv increased significantly at 10 nmol/kg, but did not change at other doses.

DISCUSSION

In the present study, the effects of vasopressin on hepatic and splanchnic circulation in Sprague-Dawley rats were examined by using isolated perfused livers and *in vivo* anesthetized rats. The isolated perfused liver experiments revealed that the venoconstrictor response to vasopressin was present but very weak. In the anesthetized rats, at 0.1–100 nmol/kg

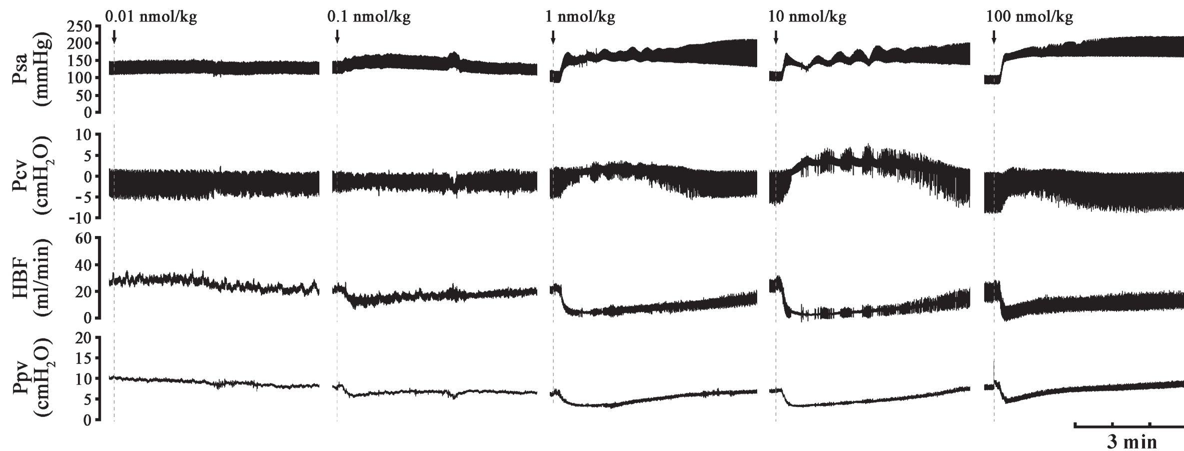

Fig. 2 The representative recordings of systemic arterial pressure (Psa), central venous pressure (Pcv), hepatic blood flow (HBF) and portal venous pressure (Ppv) after an intraportal injection of vasopressin in an anesthetized rat.

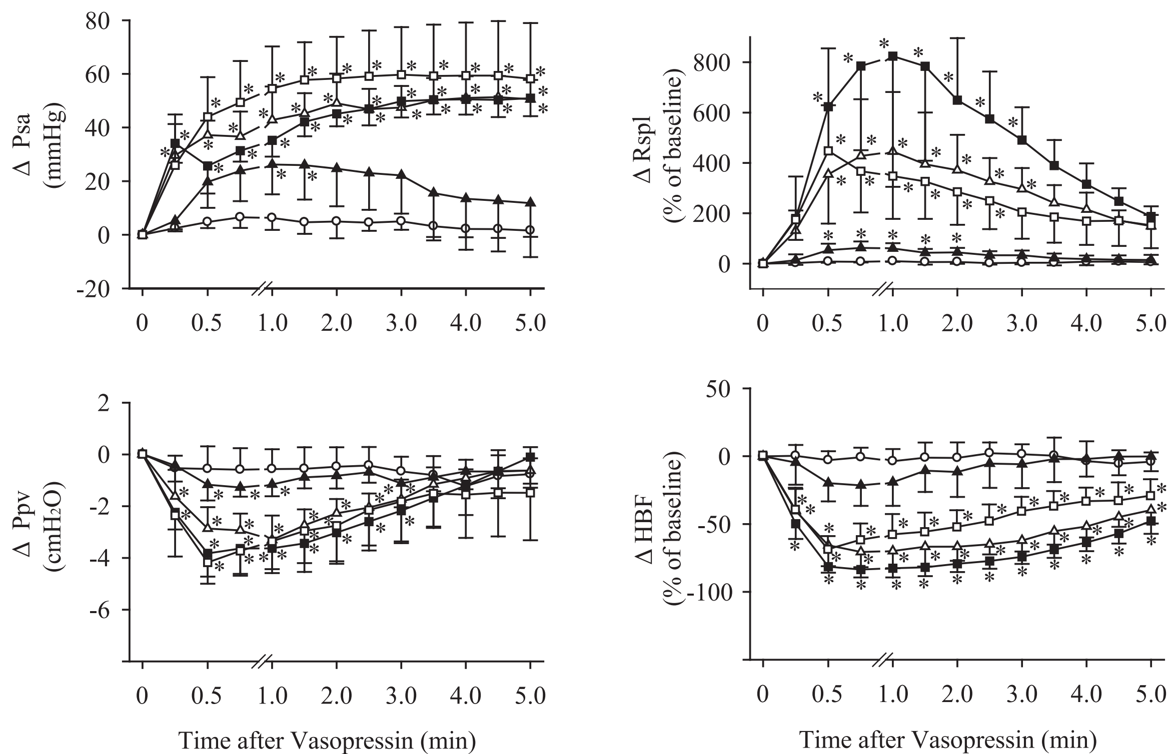

Fig. 3 The summary of the changes in the systemic arterial pressure (Psa), splanchnic bed vascular resistance (Rspl), portal venous pressure (Ppv) and hepatic blood flow (HBF) after intraportal consecutive injections of vasopressin at various doses in anesthetized rats (n = 5). Open circle, 0.01 nmol/kg; closed triangle, 0.1 nmol/kg; open triangle, 1 nmol/kg; closed square, 10 nmol/kg; open square, 100 nmol/kg. Values are means ± SD; $^*P < 0.05$ vs. the baseline values.

vasopressin, Psa and Rspl dose-dependently increased, where both HBF and Ppv decreased. These *in vivo* experiments revealed that in rats, vasopressin at 0.1–100 nmol/kg causes systemic hypertension and splanchnic vasoconstriction, but not vasodilation, along with decreased Ppv.

The results of the isolated perfused rat livers suggest that vasopressin causes venoconstriction albeit only slightly: the increase in the hepatic vascular resistance was only 6% of the baseline. This hepatic venoconstriction was accompanied by a liver weight loss, suggesting presinusoidal constriction (15). This

finding is not consistent with that of the previous studies in which no significant changes in Ppv were observed when vasopressin was challenged in isolated rat livers perfused with blood-free crystalloids (14, 18). The absence of an increase in Ppv in the previous reports (14, 18) may be ascribed to the low viscosity of the crystalloid perfusate, with which weak portal venous constriction induced by vasopressin might have been missed: the vasopressin-induced constriction of the portal vein was too weak to be detected in rat livers with low vascular resistance.

It is well known that Ppv levels are determined by not only portal vascular resistance but also portal blood flow. The absence of substantial venoconstriction or venodilatation in response to vasopressin, as revealed by isolated rat livers, suggests that the changes in Ppv of *in vivo* anesthetized rats mainly depend on the changes in HBF. Indeed, in agreement with this assumption, Ppv changed in parallel with HBF, as shown in Fig. 3: the vasopressin-induced decrease in Ppv was associated with a decrease in HBF.

In animals such as dogs (22), cats (4), rabbits (11) and pigs (20), Ppv decreased when vasopressin was intravenously administered. In the present study, a decrease in Ppv was also observed in anesthetized rats with 0.1–100 nmol/kg vasopressin intraportally administered. This study clearly demonstrated by measuring blood flow to liver that the decrease in Ppv was caused by splanchnic vasoconstriction, as evidenced by an increase in Rspl.

The vasopressin-induced decrease in Ppv, along with systemic hypertension and splanchnic vasoconstriction in anesthetized rats, contrasts with the effects of other vasoconstrictors, such as norepinephrine, angiotensin II, and endothelin-1, all of which increase not only Psa, but also Ppv (24). It is indicated that these vasoconstrictors, but not vasopressin, have a property to constrict hepatic vasculature. The lack of substantial hepatic vasoconstrictor action of vasopressin is its specific characteristic and may provide its therapeutic usefulness for circulatory shock via redistributing blood flow from splanchnic vascular beds without splanchnic congestion due to hepatic venoconstriction.

In summary, the results of the isolated perfused liver experiments revealed that hepatic venoconstrictor action of vasopressin was weak in Sprague-Dawley rats. In anesthetized rats, vasopressin of 0.01–100 nmol/kg injected into the portal vein does not cause splanchnic vasodilation but splanchnic vasoconstriction.

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