V1 Receptor Activation Induced by Hemorrhage and Sympathoinhibition in the Mesentery and Hindquarters of Spontaneously Hypertensive Rats

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ABSTRACT
The aim of this study was to determine the effects of vasopressin V1 receptor antagonism on regional hemodynamics in spontaneously hypertensive rats (SHR/Izm). Changes in blood flow in the superior mesenteric artery or terminal aorta were measured in rats with a chronically implanted electromagnetic flowmeter. The combination of a non-hypotensive hemorrhage (0.3 ml/100 g weight) and ganglionic blockade with hexamethonium bromide (C6; 25 mg/kg weight) had no effect on mesenteric resistance. On the other hand, subsequent intravenous administration of a peptide vasopressin V1 receptor antagonist (V1A; 10 µg/kg: [d(CH2)51-O Methyl-Tyr2-Arg8]-vasopressin) significantly reduced mesenteric resistance in SHR/Izm but had no effect on hindquarter resistance. Furthermore, the infusion of C6 (after pretreatment with hemorrhage plus V1A) induced a marked reduction of blood pressure and a significant decrease in superior mesenteric resistance only in SHR/Izm. Thus, we showed an altered reactivity to V1A in the superior mesenteric and/or hindquarter vascular regions of SHR/Izm, suggesting that maintenance of elevated resistance in the mesenteric vascular bed mainly relates to a potential vasopressin-mediated vasoconstriction and that a new sympathetic vasoconstrictor tone is generated within the superior mesenteric vascular bed to compensate for hypotensive intervention (minor hemorrhage plus V1A) in conscious SHR/Izm.

Key words: Regional blood flow, Hemorrhage, Ganglionic blockade, V1 receptor antagonist

In spontaneously hypertensive rat strain (SHR), sympathetic vasoconstrictor tone, which is estimated by the decrease in regional peripheral resistances after a ganglionic blockade with hexamethonium bromide (C6), does not exist in the mesenteric vascular area but exists in the common carotid, renal, and hindquarter vascular areas4). Therefore it is not clear what contributes to the elevation of mesenteric resistance in SHR. In two-kidney, 1-clip (2K1C) renovascular hypertensive rats, the superior mesenteric resistance was decreased by injection of a selective V1 receptor antagonist (V1A) subsequent to the administration of C613). Our recent studies also showed that administration of V1A after infusion of C6 significantly attenuated not only renal but also mesenteric resistances in inbred borderline-hypertensive Hiroshima rats14).

On the other hand, several studies have indicated that vasopressin may play an important role in the development and maintenance of hypertension in spontaneously hypertensive rats8,9,10,17). Vasopressin secretion is thought to be regulated by baro- and volume receptor impulses9). Volume depletion by blood withdrawal can trigger secretion of vasopressin. Endogenous vasopressin itself does not change arterial pressure. We hypothesized that sensitivity to vasopressin in SHR is high in the mesenteric artery.

Therefore, the present study was designed to assess the interactions of V1 receptor activation and sympathetic nerves in SHR and Wistar rat (WKY). We evaluated the effects of minor blood loss (to enhance secretion of vasopressin), administration of C6, administration of a specific peptide V1A, and combinations of these interventions on regional blood flow in SHR and WKY.

METHODS
The present study was conducted in accordance...
with the guidelines of the Animal Welfare Committee of the Hiroshima University Graduate School of Medicine and with the Guide for Care and Use of Laboratory Animals in the Field of Physiological Science of the Physiological Society of Japan.

Animal Preparations

Twenty-one male Wistar-Izumo rats (normotensive control rat strain; WKY/Izm) and 18 spontaneously hypertensive-Izumo rats (SHR/Izm) at 14–17 weeks of age were each anesthetized with 50 mg/kg of thiamylal sodium (Yoshitomi Pharmaceuticals, Osaka, Japan) by intraperitoneal injection, and an electromagnetic flow probe (1.5 or 2.0 mm ID, Nihon Kohden Co., Tokyo, Japan) was implanted along the superior mesenteric artery or at the terminus of the abdominal aorta (most of blood flow measured there is considered to be that of the lower-extremity muscles). A polyethylene catheter (PE-10) was inserted from the right common carotid artery and placed near the aortic arch to measure arterial pressure. Another cannula (PE-10 fused to PE-20) was inserted into the right external jugular vein for intravenous administration of the drugs.

Measurement of arterial pressure and regional blood flow

After completion of the instrumentation operation described above, the rats were housed separately in polyethylene cages that were lined with wood chips. The rats were allowed food pellets and drinking water ad libitum. On the third day after the operation, arterial pressure and blood flow of the superior mesenteric artery or terminal abdominal aorta were measured simultaneously. The arterial pressure values were smoothed with a resistance and capacitance filter with a time constant of one second. The smoothed arterial pressure value was regarded as mean arterial pressure (MAP). The flow probe was regularly calibrated before implantation by passing known amounts of saline through excised arteries of other rats. After implantation of a probe around an artery, the point of zero flow was defined by momentarily occluding the artery by digital compression directly applied to the artery through the skin. Flow signals were also integrated with a time constant of one second to obtain the mean flow. The vascular resistance was calculated as MAP divided by respective regional mean blood flow. The regional blood flow and vascular resistance were normalized to values per 100 g of body weight for statistical analysis.

Experimental protocol

First, baseline hemodynamics in each rat were determined in the conscious resting state in the home cage. After baseline values had been obtained, each rat was subjected to acute hemorrhage, the extent of which did not affect arterial pressure, to assess sympathetic effects in various vascular regions. Using a syringe, 0.3 ml of blood per 100 g body weight was withdrawn through the arterial catheter over a period of 1–2 min. The acute minor hemorrhage was followed by equilibration over a period of about 10 min and a ganglionic blockade with 2.5% (w/v) hexamethonium bromide (C6; Nacalai Tesque, Kyoto, Japan) injected at a rate of 0.8 mg/min for a total dose of 25 mg/kg. Finally, a peptide V1A, [d(CH2)3, O-Methyl-Tyr2, Arg8]-vasopressin (Manning compound; Bachem Inc., Torrance, CA) was injected (10 μg/kg) as a bolus to see whether vasopressin was being secreted in an amount sufficient to influence MAP and regional vascular resistances. In some experiments, we altered the order of administration of these drugs; i.e. 10 min after minor hemorrhage, V1A was injected and about 10 min after, C6 was infused. In a separate series of experiments using 34 WKY/Izm and 24 SHR/Izm, we assessed the effects of V1A on the only arterial pressure of rats preconditioned with hemorrhage, ganglionic blockade with C6 or hemorrhage plus C6 and compared those effects with the direct effects of V1A in intact SHR/Izm and WKY/Izm.

Statistical analysis

All hemodynamic values are expressed as means ± S.E.M., and comparisons among groups were performed using ANOVA with a repeated measure design when appropriate. Statistical analysis was performed with the unpaired t-test to determine the significance of differences between WKY/Izm and SHR/Izm treated with the same drug and also with the paired t-test to determine the differences in rats before and after treatment with each drug. A value of p<0.05 was considered to indicate a statistically significant difference.

RESULTS

Basal hemodynamics

Hemodynamic parameters were measured and compared in SHR/Izm and WKY/Izm to assess their involvement in the regulation of MAP in different local vascular beds, such as the superior mesenteric artery and hindquarter artery (terminal aorta), in the conscious state. The parameters measured were MAP, regional blood flow of the mesentery (SMF) and of the hindquarters (HQR), and the calculated vessel resistance of the mesentery (SMR) and that of the hindquarters (HQR) (Table 1). The MAP was significantly higher in SHR/Izm than in WKY/Izm. The values of SMF in the two rat groups were similar, while HQR in SHR/Izm was significantly less than in WKY/Izm. Accordingly, both SMR and HQR were higher in
Table 1. Basal hemodynamics in spontaneously hypertensive rats (SHR/Izm) and normotensive control rats (WKY/Izm)

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mmHg)</th>
<th>SMF (ml/min/100 g)</th>
<th>HQF (ml/min/100 g)</th>
<th>SMR (mmHg/ml/min/100 g)</th>
<th>HQR (mmHg/ml/min/100 g)</th>
</tr>
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<tbody>
<tr>
<td>SHR/Izm</td>
<td>175 ± 5.0 (18)*</td>
<td>4.43 ± 0.3 (10)</td>
<td>3.38 ± 0.3 (8)*</td>
<td>43.4 ± 2.8 (10)*</td>
<td>52.9 ± 5.2 (8)*</td>
</tr>
<tr>
<td>WKY/Izm</td>
<td>115 ± 2.2 (21)</td>
<td>5.15 ± 0.3 (10)</td>
<td>4.85 ± 0.3 (11)</td>
<td>23.0 ± 1.2 (10)</td>
<td>24.4 ± 1.5 (11)</td>
</tr>
</tbody>
</table>

Mean values ± S.E.M. of the number of rats shown in parentheses. *indicates a significant difference between SHR/Izm and WKY/Izm by the unpaired t-test at p<0.01. Mean arterial pressure (MAP), superior mesenteric flow (SMF), hindquarter flow (HQR), superior mesenteric resistance (SMR) and hindquarter resistance (HQR).

SHR/Izm than in WKY/Izm.

**Hypotensive effects of a peptide V1 receptor antagonist (V1A)**

We compared the hypotensive effects of V1A (10 μg/kg body weight) in SHR/Izm and WKY/Izm under 4 different conditions: no pretreatment, hemorrhage, ganglionic blockade with hexamethonium and hemorrhage plus C6. The hypotensive effect of V1A alone or hemorrhage plus V1A was small in both SHR/Izm and WKY/Izm (∆MAP = -16.3 ± 2.0 vs. -2.4 ± 0.9 mmHg for WKY/Izm, n=12–19, p<0.001) or after pretreatment with hemorrhage plus C6 (∆MAP = -26.3 ± 1.9 vs. -3.1 ± 1.1 mmHg for WKY/Izm, n=17–21, p<0.001) (Fig. 1).

**Effects of a peptide V1 receptor antagonist (V1A) on regional hemodynamics**

We assessed the effects on SMF and HQF of hemorrhage, the extent of which did not affect the MAP, because we have observed that certain hypotensive maneuvers potentiate the sympathetic vasoconstricting tone in the hindquarter vascular region in WKY/Izm. In order to obtain an insight into the mechanism underlying the difference in properties of vascular beds of SHR/Izm and WKY/Izm, SMF and HQF were measured simultaneously with MAP.

Examples of the results of bleeding experiments in an WKY/Izm (top) and an SHR/Izm (bottom) are presented in Fig. 2. A summary of the data obtained from the above experiments in WKY/Izm and SHR/Izm is presented in Figs. 3–5.

**Superior mesenteric vascular region**

The hemorrhage did not affect MAP, resulting in almost no changes in SMR in both SHR/Izm (closed circles) and WKY/Izm (open circles) (Fig. 3). Infusion of C6 after the blood depleton resulted in a significant reduction in MAP (∆MAP for SHR/Izm = -59.4 ± 4.2 vs. -22.0 ± 2.7 mmHg for WKY/Izm, n=10, p<0.01), but the calculated resistance (SMR) did not change during these interventions either in SHR/Izm or WKY/Izm (∆SMR for SHR/Izm = 1.1 ± 2.6 vs. 0.7 ± 1.7 mmHg/ml/min/100 g for WKY/Izm, n=10). In contrast, injection of V1A after the hemorrhage plus C6 treatment resulted in a further decrease in MAP only in SHR/Izm (∆MAP for SHR/Izm = -23.9 ± 2.0 vs. -2.5 ± 1.4 mmHg for WKY/Izm, n=10, p<0.01), and the combination of hemorrhage, C6 and V1A resulted in significant attenuation of SMR in SHR/Izm but had no effect on SMR in WKY/Izm (∆SMR for SHR/Izm = -15.1 ± 2.6 vs. 1.6 ± 2.3 mmHg/ml/min/100 g for WKY/Izm, n=10, p<0.01) (Fig. 3). It is likely that disruption of the sympathetic vasoconstrictor tone induced vasoconstricting actions of vasopressin, which may be involved in the higher magnitude of vessel resistance observed in SHR/Izm than in WKY/Izm.

When the order of drug administration was altered, the hemorrhage again did not affect MAP, resulting in almost no changes in SMR in both
Fig. 2. In the period during which tracing of mean arterial pressure (MAP) was interrupted, 0.3 ml/100 g body weight of blood was withdrawn from the arterial catheter. MAP, SMF and HQF were minimally affected by the bleeding, and they all reached new levels within about 10 min. C6 (25 mg/kg body weight) was then infused (during the underlined period shown in Fig. 2) and finally a peptide V1 receptor antagonist (V1A; 10 µg/kg body weight) was injected as a bolus.

SHR/Izm (closed circles) and WKY/Izm (open circles) (Fig. 4). Infusion of V1A after the blood depletion resulted in no significant reduction in MAP (ΔMAP for SHR/Izm = -8.42 ± 2.5 vs. -0.2 ± 1.8 mmHg for WKY/Izm, n = 5–7) and the calculated resistance (SMR) did not significantly change during these interventions either in SHR/Izm or WKY/Izm (ΔSMR for SHR/Izm = -4.17 ± 1.8 vs. 0.68 ± 1.4 mmHg/ml/min/100 g for WKY/Izm, n = 5–7). In contrast, injection of C6 after the hemorrhage plus V1A treatment resulted in marked potentiation of the reduction in MAP in both rat groups (ΔMAP for SHR/Izm = -99.1 ± 6.6 vs. -37.0 ± 8.6 mmHg for WKY/Izm, n = 5–7; p<0.01), and the combination of hemorrhage, C6 and V1A resulted in significant attenuation of SMR in SHR/Izm but had no effect on SMR in WKY/Izm (ΔSMR for SHR/Izm = -8.1 ± 2.7 vs. 1.6 ± 2.3 mmHg/ml/min/100 g for WKY/Izm, n = 5–7; p<0.05) (Fig. 4).
Fig. 3. Changes in mean arterial pressure (MAP) and resistance (SMR) of the mesentery vascular region in both groups of rats following hypotensive interventions: hemorrhage (blood loss of 0.3% body weight), ganglionic blockade by hexamethonium bromide (C6, 25 mg/kg body weight), and a peptide V1 receptor antagonist (V1A, 10 µg/kg). † Significant difference between hemorrhage and C6 or between C6 and V1A by one-way ANOVA.

Fig. 4. Changes in mean arterial pressure (MAP) resistance (SMR) of the mesentery vascular region in both groups of rats following hypotensive interventions: hemorrhage (blood loss of 0.3% body weight), a peptide V1 receptor antagonist (V1A, 10 µg/kg), and ganglionic blockade by hexamethonium bromide (C6, 25 mg/kg body weight). † Significant difference between V1A and C6 by one-way ANOVA.

Fig. 5. Changes in mean arterial pressure (MAP) and resistance (HQR) of the hindquarter vascular region in both groups of rats following hypotensive interventions: hemorrhage (blood loss of 0.3% body weight), ganglionic blockade by hexamethonium bromide (C6, 25 mg/kg body weight), and a peptide V1 receptor antagonist (V1A, 10 µg/kg). † Significant difference between hemorrhage and C6 or between C6 and V1A by one-way ANOVA.
Hindquarter vascular region

After the blood depletion, C6 significantly attenuated MAP in both rat groups (ΔMAP for SHR/Izm = -50.4 ± 6.8 vs. -27.8 ± 1.3 mmHg for WKY/Izm, n = 7–11; p < 0.01) and the hemorrhage plus ganglionic blockade reduced HQR in both rat groups. The magnitude of reduction in HQR was greater in SHR/Izm than in WKY/Izm (ΔHQR for SHR/Izm = -32.4 ± 5.0 vs. -8.9 ± 1.7 mmHg/ml/min/100 g for WKY/Izm, n = 7–11; p < 0.01). In contrast, after the hemorrhage plus C6 treatment, subsequent injection of V1A did not induce a significant reduction in HQR in either SHR/Izm or WKY/Izm (ΔHQR for WKY/Izm = -8.3 ± 2.3 vs. -4.2 ± 0.7 mmHg/ml/min/100 g for WKY/Izm, n = 7–11) (Fig. 5).

DISCUSSION
Effects of sympathetic tone on regional vascular resistances

Many studies have shown that sympathetic activity is enhanced in SHR [7-9,11]. We also observed that the level of sympathetic activity is higher in SHR/Izm than in WKY/Izm. Iriuchijima et al [10] reported that no sympathetic tone exists in the mesenteric artery of either SHR/Izm or WKY/Izm. We confirmed in the present study that the resistances of both the mesenteric and hindquarter arteries are significantly higher in SHR/Izm than in WKY/Izm (Table 1). However, the infusion of C6 with pretreatment of hemorrhage did not reduce mesenteric resistance in either SHR/Izm or WKY/Izm (Fig. 3). These findings show that no basal sympathetic tone exists in the mesentery of either SHR/Izm or WKY/Izm and suggest that the elevated mesenteric resistance in SHR/Izm is maintained by another factor.

Effects of a V1 antagonist on regional vascular resistances

It is important to show whether V1 receptor activation is differentially involved in elevations of regional vascular resistances in SHR. It is well known that acute hemorrhage triggers a tonic effect in both the neural (sympathetic nerve) and hormonal (vasopressin) systems. We therefore evaluated the involvement of these two systems after sub-threshold depletion of blood in arterial blood pressure.

Superior mesenteric vascular region

After a preceding hemorrhage plus ganglionic blockade, subsequent injection of V1A resulted in a significant decrease only in the mesenteric resistance of SHV/Izm. The drastic decrease in mesenteric resistance caused by V1A is parallel to the significant drop in blood pressure in SHR/Izm (Fig. 3). On the other hand, V1A (after pretreatment with hemorrhage plus C6) had no effect in the hindquarter vascular bed in either rat group (Fig. 5). These results suggest that there is heterogeneity in the properties of regional vascular beds and that the activation of V1 receptors is responsible for the elevation of artery resistance at least in the mesentery of SHR/Izm. We speculate that the pronounced effect of the minor blood depletion is sufficient to accelerate either the release of vasopressin or the potential action of the V1 receptor in SHR/Izm, in which the peptide is one of the key vasoactive hormones involved in maintenance of the elevation of mesenteric resistance and/or high blood pressure.

The infusion of C6 (after pretreatment with hemorrhage plus V1A) induced a marked reduction of blood pressure and a significant decrease in SMR only in SHR/Izm. These results suggest that a new sympathetic vasoconstrictor tone is generated within the superior mesenteric vascular area to compensate for hypotensive intervention in conscious SHR/Izm (Fig. 4).

Many studies have shown that the effects of vasopressin in central and renal systems play important roles in the development and maintenance of high blood pressure in genetically hypertensive humans and animals [1-11]. Feng et al [13] reported that VP-induced vasoconstriction is mediated predominantly by the V1 receptor in the rat kidney. Furthermore, Cowley et al [12] recently reported that the effect of long-term vasopressin V1 receptor stimulation resulted in a chronic reduction in renal medullary blood flow and hypertension. In contrast, some investigators have reported that V1A caused little reduction in arterial pressure in intact SHR [12,13]. This discrepancy in findings may be due to the fact that the vasoconstricting effect of endogenous vasopressin is apparently concealed under the condition in which the sympathetic vasoconstrictor tone is intact.

Hindquarter vascular region

In the present study, we also found that ganglionic blockade (C6) after hemorrhage resulted in a significant decrease in HQR not only in WKY/Izm but also in SHR/Izm. In addition, the magnitude of the percent decrease in HQR induced by C6 was significantly greater in SHR/Izm with hemorrhage than in SHR/Izm with no hemorrhage [16]. Thus, our findings show that compensatory vasoconstrictor tone is activated within the limits of the hindquarters to compensate for hypotensive intervention in conscious SHV/Izm.

In conclusion, sympathetic and/or vasopressinergic vasoconstrictor effects are exerted specifically in local vasculature and complementarily lead to elevation of vascular resistance in SHR/Izm.

(Received July 20, 2005)
(accepted October 4, 2005)
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