Abstract We studied the hypotensive effects of OPC-21268, an orally effective nonpeptide vasopressin V₁ receptor antagonist, in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP). OPC-21268 was given intravenously to conscious, freely moving SHR and SHRSP. We used young and aged animals to examine the contribution of vasopressin to the development and maintenance of hypertension in both types of rats. In SHR, hypertension was fully established at 38 weeks of age, and intravenous injection of OPC-21268 produced no significant hypotensive effects in young rats at 15 weeks, but at 25 weeks a significant decrease in blood pressure was observed. Furthermore, in the malignant state of SHRSP (35 to 41 weeks), OPC-21268 significantly decreased mean blood pressure by 32.4±7.9 mm Hg (mean±SEM) at 3 mg/kg IV, and the decrease was dose dependent (0.3 to 3.0 mg/kg). Plasma vasopressin concentrations were increased in a more malignant phase of SHRSP at 45 weeks of age, whereas at other ages of SHRSP or in SHR, plasma vasopressin levels were not increased. These results suggest that vasopressin plays an important role through V₁ receptors in the maintenance of hypertension, at least in the malignant phase of SHRSP, and OPC-21268 may be therapeutically useful in the treatment of some types of hypertension.

Key Words • vasopressins • hypotension • rats, inbred SHR • hypertension, malignant

In recent years, there has been considerable interest in the possible role of arginine vasopressin (AVP) in the pathogenesis of hypertension. Numerous studies have attempted to demonstrate the contribution of AVP to the hypertension process in several forms of hypertension, particularly in deoxycorticosterone acetate (DOCA)-salt hypertension and in spontaneously hypertensive rats (SHR). Although it is likely that AVP is essential for the production of DOCA-salt hypertension, the contribution of AVP to genetic hypertension is still controversial. Plasma AVP concentrations, posterior pituitary AVP content, and urinary AVP excretion were elevated in SHR and stroke-prone SHR (SHRSP). Administration of specific AVP antiserum resulted in a marked fall in blood pressure in SHRSP, and long-term infusion of d(CH₂)₅-Tyr(Me)VAVP, which blocks actions of AVP on both vascular (V₁) and antidiuretic (V₂) receptors, significantly attenuated the development of hypertension in SHR. Furthermore, enhanced pressor responsiveness to AVP and impairment of baroreceptor reflex activity were also observed in SHR. In contrast, decreased or unchanged plasma AVP concentrations in young SHRSP and no hypotensive effects of short-term and long-term administration of a peptide V₁ antagonist, d(CH₂)₆-Tyr(Me)AVP, in SHR were reported. In SHRSP crossed with rats homozygous for hypothalamic diabetes insipidus of the Brattleboro strain, which are unable to synthesize AVP, mean arterial blood pressure was markedly increased as well as in SHRSP.

SHR and SHRSP are often considered to be a model of human essential and malignant hypertension. Therefore, a study that investigates the effects of AVP antagonists in these rats is the best means to define the role of AVP in hypertension. But all studies on AVP antagonists have been performed using peptide AVP antagonists, some of which are known to have partial antidiuretic activity. Recently, we developed a nonpeptide AVP V₁ receptor-specific antagonist, OPC-21268. We designed the present study to evaluate the hypotensive effects of OPC-21268 in conscious SHR and SHRSP at young and old ages. In addition, we measured plasma AVP concentrations to determine the contribution of AVP to the development of hypertension.

Methods

Animals Male Sprague-Dawley rats and SHR were obtained from Charles River Japan. Male SHRSP/Otk (SHRSP) were derived from our SHRSP/Otk breeding colony by brother-sister mating. The colony originated in the SHRSP obtained from the Department of Pathology, Shimane Medical University. The animals were housed in our research animal colony, where room temperature, humidity, and lighting were controlled, and were given commercial rat chow and tap water ad libitum.

Apart from the animal group that was used for studying hypotensive effects of OPC-21268, 27 male SHRSP were maintained in the same conditions until 45 weeks of age to investigate their characteristics. Body weight was measured every week.
General Procedures

At 38 and 70 weeks of age in SHR and at 15, 25, and 35 to 41 weeks of age in SHRSP, rats were fitted with arterial and venous catheters for measurement of arterial blood pressure and for intravenous injection according to the following methods. Rats were anesthetized with pentobarbital sodium (40 mg/kg IP), and an abdominal midsection was performed on each. A heparinized polyethylene catheter (SP-55, Narumi, Tokyo, Japan; tip outer diameter of 1.2 mm and inner diameter of 0.8 mm pulled to an outer diameter of 0.5 mm and J-shaped by heating) was introduced into the abdominal aorta through an incision with a 26-gauge needle, and the right jugular vein was cannulated. The catheters were passed subcutaneously to the dorsal side of the neck and exteriorized. On the following days, the rats were placed in individual boxes, and blood pressure was measured with a pressure transducer (T12AD, NEC San-ei Instruments, Tokyo, Japan) and recorded on a thermal pen recorder (Recti-Horiz 8K, NEC San-ei). In every experiment, after at least 1 hour of equilibration, OPC-21268 dissolved in dimethylformamide (Wako Pure Chemicals, Osaka, Japan) was injected intravenously in a volume of 100 µL/kg, followed by 0.3 mL saline. The highest dose of OPC-21268 was 3 mg/kg, and this dose was considered to cause complete antagonistic effects to AVP.16 In SHRSP at 35 to 41 weeks of age, OPC-21268 was given at 0.3, 1, or 3 mg/kg. Control animals received the same volume of dimethylformamide and saline. The measurement was performed for 1 hour after administration. Only at 9 weeks of age in SHRSP, blood pressure was measured by the tail-cuff method.

Plasma Vasopressin Concentration

Another group of SHR and SHRSP was used. At 13 weeks of age in normotensive Sprague-Dawley rats, 35 weeks of age in SHR, and 7, 22, and 45 weeks of age in SHRSP, the animals were killed by decapitation, and trunk blood was collected in chilled tubes containing EDTA. After centrifugation, plasma AVP was extracted with the method described by LaRochelle et al,19 who used small columns packed with octadecasilyl silica, and was measured by radioimmunoassay with AVP RIA kits20 (Mitsubishi Yuka Bio-chemical Laboratories, Ltd, Tokyo, Japan).

Statistical Analysis

Results are presented as mean±SEM. The statistical significance of the difference between two groups was determined by means of the Student's t test. Multiple-group comparisons were done with one-way ANOVA, followed by Dunnett's test. A value of P<.05 was considered significant.

Results

Body weight of SHRSP increased to 370 g until approximately 35 weeks of age, and weight loss was observed from 40 weeks (Fig 1A). The first SHRSP died at 38 weeks, and half of them died at 45 weeks (Fig 1B). We therefore considered that hypertension was in a malignant phase around 38 weeks of age. Basal blood pressure (resting blood pressure) at each age in SHR and SHRSP is shown in the Table. In SHR, systolic blood pressure was 195±6 mm Hg at 35 weeks and 185±7 mm Hg at 70 weeks, indicating that hypertension was fully established at 38 weeks of age. Systolic blood pressure of SHRSP at 9, 15, 25, and 35 to 41 weeks of age was 150±1, 213±4, 248±8, and 248±7 mm Hg, respectively. Thus, blood pressure was elevated with the advance of age and reached a maximum level at 25 weeks.

The time course of changes in mean blood pressure in SHR after intravenous administration of 3 mg/kg OPC-21268 is shown in Fig 2. Mean blood pressure was slightly decreased after intravenous administration of OPC-21268 in either age group compared with vehicle treatment, but systolic blood pressure did not change (data not shown). As shown in Figs 3 and 4, intravenous injection of OPC-21268 caused different effects on blood pressure depending on the development of hypertension in SHRSP. Vehicle injection had no effect on blood pressure at any age. At 15 weeks of age, no significant hypotensive effect was observed. However, at 3 mg/kg IV, OPC-21268 significantly lowered mean blood pressure at 25 weeks of age (Fig 3). Furthermore, at 35 to 41 weeks (Fig 4), OPC-21268 produced marked hypotensive effects in a dose-dependent manner. At a dose of 1 mg/kg IV of OPC-21268, mean blood pressure was decreased by 20.4±2.9 mm Hg at 15 minutes (P<.01) and returned to the pretreatment value after 60 minutes. At 3 mg/kg IV, a more marked hypotensive effect of 32.4±7.9 mm Hg was obtained at 25 minutes and lasted more than 60 minutes. In contrast to the changes in blood pressure, heart rate did not change significantly at any dose (Fig 4). On the other hand, 3 mg/kg IV of OPC-21268 produced almost complete inhibition of the pressor response to exogenous AVP (30 mU/kg IV) until 30 minutes and approximately 50% inhibition at 60 minutes after administration in conscious SHRSP at 25 weeks of age (data not shown). Therefore, it was considered that plasma OPC-21268 concentrations were kept high enough to inhibit vasocostriction induced by AVP over the measurements.

Plasma AVP concentrations in normotensive rats, SHR, and SHRSP are shown in Fig 5. In SHR, plasma AVP concentrations were slightly elevated but were nearly equal to normal levels. In SHRSP, marked elevation of AVP concentrations was observed only at 45 weeks of age, when one half of the SHRSP died. The mean value was 6.7±1.5 pg/mL and was significantly higher than the values of normotensive rats, SHR, and SHRSP at the other ages. The maximum level of 19.3 pg/mL reached the level responsible for vasocostriction in vivo.22

Discussion

Recently, we developed a nonpeptide AVP V₁ receptor antagonist, OPC-21268,16 which is more than 1000 times more potent in inhibiting the pressor response to exogenous AVP than AVP itself, and it is more than 1000 times more potent in blocking the pressor responses to AVP in vivo than the AVP V₁ receptor antagonist OPC-19279.17,19 In the present study, OPC-21268 produced marked hypotensive effects at 15 and 25 weeks of age, and the effect was marked at 35 weeks and 45 weeks of age. At 25 weeks of age, OPC-21268 produced a significant hypotensive effect at 3 mg/kg IV, and at 35 weeks and 45 weeks of age, OPC-21268 produced a significant hypotensive effect at 1 mg/kg IV. These effects suggest that OPC-21268 is effective in inhibiting AVP action in SHRSP at 25 weeks of age and in SHRSP at 35 and 45 weeks of age.
times as selective for V<sub>1</sub> receptors as for V<sub>2</sub> receptors. In vivo, OPC-21268 competitively and specifically antagonized the pressor response to AVP.

The present study clearly demonstrates that a non-peptide AVP V<sub>1</sub> receptor antagonist, OPC-21268, produced significant hypotensive effects in conscious SHRSP in the malignant phase (at 35 to 41 weeks of age) in a dose-dependent manner. Although there was a report that when rats with hereditary diabetes insipidus and SHRSP were crossed, the resultant strain had hypertension as severe as SHRSP, almost no studies have investigated the effects of AVP antagonists in SHRSP. The present results suggest the importance of V<sub>1</sub> receptors in the maintenance of hypertension at least in the malignant phase of SHRSP.

There is evidence that administration of a specific AVP antiserum lowered blood pressure in SHRSP. In contrast, short-term administration of a V<sub>1</sub> antagonist, d(CH<sub>2</sub>)<sub>5</sub>-Tyr(Me)AVP or dPTyr(Me)AVP, failed to lower blood pressure in SHR. Although chronic blockade of the pressor effects of endogenous AVP by long-term administration of d(CH<sub>2</sub>)<sub>5</sub>-Tyr(Me)AVP did not alter the course of hypertension in SHR, long-term administration of a peptide V<sub>1</sub> and V<sub>2</sub> antagonist, d(CH<sub>2</sub>)<sub>5</sub>-d-Tyr(Me)AVAP, resulted in attenuation of the development of hypertension. The diverse results of these studies suggest that multiple actions of AVP cooperate in its contribution to hypertension. However, these studies used peptide AVP antagonists, and some of them are known to possess partial agonistic activity. Similarly, it was reported that a peptide angiotensin II receptor antagonist, saralasin, caused a transient increase in blood pressure. In contrast, the nonpeptide angiotensin II antagonist DuP 753 did not cause a transient increase in blood pressure and produced a greater fall in blood pressure than saralasin in SHR.

In SHR at both 38 and 70 weeks we could observe no changes in systolic blood pressure but a slight decrease...
in diastolic blood pressure after administration of OPC-21268. A small increase in plasma AVP concentrations was observed at 35 weeks, but these levels were within the normal range, and so AVP might not contribute to hypertension. In the present study, we did not carry out an investigation in Wistar-Kyoto rats because there were almost no hypotensive effects and no changes in plasma AVP levels in SHR.

In SHRSP at 25 weeks of age, when hypertension was fully established, a slight but significant decrease in blood pressure was observed after administration of OPC-21268. Furthermore, at 35 to 41 weeks when the animals were considered to be in the malignant phase, marked hypotensive effects were observed in a dose-dependent manner soon after administration of OPC-21268. At 45 weeks of age, plasma AVP concentrations were significantly elevated. Thus, it appears that there is a relation between plasma AVP levels and the acute blood pressure-lowering effect of OPC-21268. These results strongly suggest that AVP contributes to the maintenance of high blood pressure in the malignant phase of SHRSP.

Both elevated^{4-6} and decreased^{10} plasma AVP concentrations have been reported in SHR and SHRSP. Our results are in accordance with the former case. This could be due to enhanced AVP release or decreased total clearance. The former is more reasonable because there are reports on increased secretion of AVP,^{25} increased AVP content in the pituitary,^{26,27} and increased mRNA of AVP in the suprapoctic and paraventricular nuclei in SHR.^{26} The latter reason is also probable because a prolonged pressor response to exogenous AVP was observed in SHRSP compared with normotensive rats (our unpublished data). However, the specific mechanism responsible for the participation of AVP in hypertension remains to be elucidated, because including our results, all studies have failed to confirm that plasma AVP concentrations reached pressor levels in vivo. Therefore, there may be abnormalities in which AVP could contribute to hypertension even if AVP concentrations do not reach pressor levels. One possibility is that pressor responsiveness to AVP may be enhanced in both SHR and SHRSP.^{5,8} Another possibility is that an impairment of baroreceptor reflex activity may contribute to the enhanced pressor activity of AVP. Despite an enhanced pressor response to AVP in SHR, heart rate fell much more for any given elevation of blood pressure in Wistar-Kyoto rats than SHR during infusion of AVP.9 In the present study, heart rate did not change despite a considerable decrease in blood pressure (Fig 4). This result may suggest a dysfunction in the baroreceptor reflex. Furthermore, it is important to consider interaction with other pressor factors such as angiotensin II and norepinephrine.8,28 But most of these studies were carried out in SHR, and further evaluation in SHRSP is required.

In the present study, we did not consider the central effects of OPC-21268, because OPC-21268 was distributed to the central nervous system at only a small percentage of plasma concentration (unpublished data). Reports conflict concerning the central role of AVP in genetic hypertension,^{29} but hypotensive effects of centrally administered AVP in SHRSP^{30} and lower hypothalamic AVP contents in SHR^{37} were reported, and these findings suggest that AVP acts centrally to depress cardiovascular activities and that low concentrations of AVP in hypothalamic nuclei contribute to hypertension in SHR or SHRSP. Therefore, if OPC-21268 antagonizes to AVP in the central nervous system, the blood pressure should be further elevated.

Concerning other types of hypertension, DOCA-salt hypertension has been most intensively studied with respect to AVP, and the contribution of AVP to DOCA-salt hypertension has been supported by many studies. It is likely that AVP is required in the early stages of this model for its antidiuretic activity.1,2 Further investigation is required to clarify the role of AVP in other types of hypertension.
thermore, blockade of AVP with a peptide V₂ antagonist lowered blood pressure in rats with established DOCA-salt hypertension, and it appears that AVP contributes to DOCA-salt hypertension in the established phase by virtue of its pressor activity. On the other hand, acute oral administration of OPC-21268 lowered blood pressure, and chronic treatment with OPC-21268 led to a fall in blood pressure in DOCA-salt hypertensive rats. Furthermore, chronic treatment with OPC-21268 limited the development of proteinuria and hypertension in DOCA-salt and adriamycin-treated rats with chronic renal failure. These results are consistent with previous studies and suggest an important role of V₂ receptors in DOCA-salt hypertension.

In conclusion, a nonpeptide AVP V₂ receptor antagonist, OPC-21268, caused a significant and dose-dependent decrease in blood pressure in SHRSPrats in the malignant phase (35 to 41 weeks). Plasma AVP concentrations were increased in a more malignant phase of SHRSPrats (45 weeks). These results suggest that AVP plays an important role through V₂ receptors in the control of high blood pressure in SHRSPrats. Furthermore, OPC-21268 was reported to antagonize V₂ receptor-mediated vasoconstriction in humans. Therefore, although the mechanism underlying the contribution of AVP to hypertension remains to be elucidated, OPC-21268 may be therapeutically useful in the treatment of some forms of hypertension.

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