Effects of Exogenous [Arg⁸]-Vasopressin on Borderline-Hypertensive Hiroshima Rats

Yasuhito Teranishi¹, Tsutomu Kumazaki², Ryoji Ozono³ and Hiromichi Tsuru⁴

¹Department of Physiology, ²Department of Biochemistry and Biophysics, Research Institute for Radiation Biology and Medicine, ³Department of Clinical Laboratory Medicine, Hiroshima University School of Medicine, Minami-ku, Hiroshima 734–8551, Japan ⁴Department of Pharmacology, Toho University School of Medicine, Ohta-ku, Tokyo 143–8540, Japan

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ABSTRACT—The interaction between [Arg⁸]-vasopressin and a vasopressin receptor antagonist, [d(CH₂)₅]-O-Me-Tyr², Arg⁸]-vasopressin, was examined in Hiroshima rats and normotensive control rats under pentobarbital anesthesia. [Arg⁸]-vasopressin dose-dependently increased the arterial pressure in both the Hiroshima and control rats, the pressor effect being greater in the Hiroshima rats. After the administration of a vasopressin antagonist (0.01 mg/kg), which by itself decreased arterial pressure only in the Hiroshima rats, the dose-response curve for [Arg⁸]-vasopressin was much more greatly shifted to the right in the control rats. These results indicate that with or without a vasopressin antagonist, the exogenous [Arg⁸]-vasopressin induced more powerful pressor actions in the Hiroshima rats compared to the control rats.

Keywords: Vasopressin system, Vasopressin antagonist, Borderline-hypertensive rat

Hiroshima rats (HRs) are a rat strain derived from the Wistar rat strain by repeated sib-mating for about 17 years, in which each mating was carried out using males and females with relatively high systolic blood pressure. Those male and female rats that exhibited normal systolic blood pressure were mated to obtain normotensive control rats (NCRs). In our previous study of HRs in the conscious state, the mean arterial pressure (MAP), body weight and age were 137±2.4 mmHg (mean±S.E.M.), 312±6.3 g and 17.9±0.7 weeks of age in male HRs (n=24) and 120±1.5 mmHg, 350±13.3 g and 17.0±0.8 weeks of age in male NCRs (n=26), respectively (1). The MAP values of the HRs were significantly higher than those of the NCRs (P<0.001). However, there was no significant difference in the heart rate between the HRs and NCRs. The body weights of the HRs were significantly lower than those of the NCRs (P<0.02). In a study of rats in the conscious state, the hypotensive effect of a ganglionic blockade with hexamethonium (C6) was not significantly different between HRs and NCRs (1). We also observed that the hypotensive effect of captopril, an angiotensin-converting enzyme (ACE) inhibitor, was not significantly different between HRs and NCRs, with or without a preceding treatment with C6 (Y. Teranishi et al., unpublished data). These results show that the vasoconstrictor actions mediated by the sympathetic nervous system and renin-angiotensin system are not significantly different between HRs and NCRs. However, the hypotensive effect of a vasopressin (VP) antagonist after the inhibition of the sympathetic nervous activity was significantly greater in HRs than in NCRs (1). These findings indicate that a compensatory vasopressor action of VP may be activated in HRs with the inhibition of the central nervous system, including the cardiovascular center in the medulla oblongata. To clarify the role of the VP system in maintaining the borderline-hypertension of HRs, we examined the antagonistic effect of a VP antagonist on the elevation of MAP by a VP agonist in HRs.

The present study was approved by the Animal Welfare Committee of the Hiroshima University School of Medicine. The HRs were inbred in the experimental animal center of the Hiroshima University School of Medicine. The controls consisted of NCRs and Wistar rats purchased from Charles River Japan, Inc. (Yokohama).

In the first experiment of the present study, male HRs and NCRs at 13.9±0.26 (mean±S.E.M.) and 13.3±0.33 weeks of age, respectively, were used. The mean body weight was 342±7.7 g (mean±S.E.M.) in the NCRs (n=29) and 290±5.8 g in the HRs (n=30), the latter
being significantly less than the former (P<0.001). After each rat was anesthetized with pentobarbital sodium (Dainabot, Osaka; 50 mg/kg, i.p.), a polyethylene tube (PE-10 fused to PE-20) was inserted into the right femoral artery, and the tip of the tube was placed in the terminal aorta for the direct measurement of arterial pressure (AP). Another tube was inserted into the right femoral vein for drug administration. After the operation, the AP and heart rate were measured in the anesthetized condition by using pressure transducers (model TP-400T; Nihon Kohden, Tokyo), amplifiers (model AP-601G) and a tachometer (model AT-601G) and recorded on a pen-writing oscillograph (model WI-641G). The AP values were smoothed with a resistance and capacitance (RC) filter with a time constant of one second. We considered the smoothed AP values the MAP. [Arg⁴]vasopressin (Arg-VP; Sigma Chemical Co., St. Louis, MO, USA) was used as a VP receptor agonist in the present study. The rats with implanted catheters were divided into two groups. In one (the control) group, a bolus of Arg-VP was intravenously administered repeatedly in a cumulative manner at an interval of about 2 min after the effect of the previous dose reached a plateau. Thus, the dose-response curve for Arg-VP was obtained at the dose range from 10⁻¹¹ to 10⁻⁷ g/kg. In the other group, 0.01 mg/kg of [d(CH₂)₅, O-Me-Tyr², Arg⁴]-vasopressin (Bachem, Inc., Torrance, CA, USA), a V₁ receptor antagonist, was first administered intravenously as a bolus. About 5 min after the treatment with this VP antagonist, Arg-VP was applied intravenously to obtain a dose-response curve as described for the control group. In order to quantitatively evaluate the agonism and antagonism on VP receptors (vasoconstrictive V₁ receptors), the ED₅₀ value of Arg-VP, i.e., the dose that produced a 20-mmHg increase in MAP was obtained based on the dose-response curve from each experiment, and the pED₅₀ value was calculated as a negative logarithm of the ED₅₀ value.

In the second experiment, male HRs and NCRs both at 11 weeks of age were used. The mean body weight was 363±3.4 g (mean±S.E.M.) in the NCR group (n=5) and 299±8.9 g in the HR group (n=5), the latter being significantly less than the former (P<0.001). After the rat was anesthetized with pentobarbital sodium (50 mg/kg, i.p.), Arg-VP was injected intravenously as a bolus at the time points indicated by arrows, in a cumulative manner.
i.p.), the rat’s heart was exposed by thoracotomy. Blood was drawn from the right atrium, and then the heart was excised for measurement of the ratio of the heart weight (mg) to body weight (g) as an index of cardiac hypertrophy. The plasma concentration of VP was measured by radioimmunoassay.

The statistical analysis was performed with the paired or unpaired Student’s t-test. The unpaired t-test was used to determine the significance of differences between the HRs and NCRs, and the paired t-test was used for the significance of changes caused by Arg-VP and the VP antagonist. All values are expressed as the mean±S.E.M. The mean ED$_{20}$ value and its 95% confidence coefficient were obtained on the basis of the antilog of the mean pED$_{20}$ value and its S.E.M.

We observed that the MAP was significantly higher in the anesthetized HRs (131±2.1 mmHg, n=16) than in the anesthetized NCRs (105±2.2 mmHg, n=14) (P<0.001), although there was no significant difference in the heart rate between the HRs (370±9.8 beats/min) and NCRs (347±8.2 beats/min).

Figure 1 presents typical recordings of MAP and heart rate obtained in an NCR (top) and an HR (bottom). In this stretch of recording, a bolus of Arg-VP was administered repeatedly in a cumulative manner at doses ranging from $10^{-12}$ to $10^{-6}$ g/kg, showing that Arg-VP dose-dependently increased the MAP. The degree of increase in MAP in response to Arg-VP is graphically presented in Fig. 2A. The pressor response to Arg-VP was significantly greater in the HRs than in the NCRs at doses from $10^{-11}$ to $10^{-8}$ g/kg except for $10^{-9}$ g/kg, while the maximal responses to the highest dose ($10^{-7}$ g/kg) were similar between the strains.

The VP antagonist (0.01 mg/kg) induced a significant decrease in MAP in the HRs (−16.1±2.9 mmHg, n=14), whereas no appreciable effect was observed in the NCRs (−1.23±0.52 mmHg, n=13). The mean value of MAP (111±5.1 mmHg, n=14) in the HRs obtained after the injection of VP antagonist was not significantly different from the intact level of MAP in the conscious NCRs (120±1.5 mmHg, n=26). The heart rate was not altered by the VP antagonist in either strain of rats. In the presence of the VP antagonist, the degree of increase in MAP produced by Arg-VP was significantly greater in the HRs than in the NCRs at all Arg-VP doses from $10^{-10}$ to $10^{-6}$ g/kg, as shown in Fig. 2B. In addition, the dose-response curves for Arg-VP were markedly shifted by the VP antagonist (0.01 mg/kg) to the right in both the NCR and HR groups (Fig. 3).

The heart weight / body weight index was significantly greater in the HRs (3.75±11.8 mg/g) than in the NCRs (2.99±8.9 mg/g) (P<0.001). This result shows that the hearts of the HRs were hypertrophied by about 25%. The plasma concentration of VP was significantly higher in the HRs (1.60±0.20 ng/ml) than in the NCRs (1.09±0.13 ng/ml) (P<0.05).

VP is a potent vasoconstrictor (V$_1$ receptor-mediated), but VP-induced pressor responses are minimal in intact animals (2). The dose of VP that induces the pressor effect in various species is about 10–100 times higher than the antiduretic dose (3). Iriuchijima et al. determined the conditions for VP secretion in pressor amounts in NCRs and several kinds of hypertensive rats (4–7) by using a

![Diagram](https://via.placeholder.com/150)

**Fig. 2.** Changes in mean arterial pressure (MAP) after each injection of Arg-VP in normotensive control (NCRs) and Hiroshima (HRs) rats without (A) and with (B) VP antagonist treatment (0.01 mg/kg, i.v.). Each column and bar represent the mean±S.E.M. Asterisks indicate the level of significant difference between HRs and NCRs: *P<0.05, **P<0.01 and ***P<0.001.
VP antagonist. In water-repleted NCRs, VP in amounts sufficient to elevate AP is secreted when baroreceptor impulses are interrupted and when catecholamine concentrations in the blood are lowered (4). In spontaneously hypertensive rats (SHRs), VP is secreted in pressor amounts after acute spinal transection or sinoaortic denervation (6). The facile secretion of pressor amounts of VP also occurs in deoxycorticosterone acetate (DOCA)-salt hypertensive rats only after inhibition of the sympathetic activity (7). Taken together, these results indicate that VP is secreted in pressor amounts much more readily in the DOCA-salt hypertensive rats, and more so in SHRs than in NCRs.

In the present study of rats under pentobarbital anesthesia, we observed that a VP antagonist induced a significant decrease in MAP in HRs, but not in NCRs. This finding indicates that when the central nervous system of HRs including the cardiovascular center in the medulla oblongata and pituitary gland is inhibited by pentobarbital, a compensatory vasopressor action of the VP system may be activated.

Arg-VP dose-dependently increased the MAP in both HRs and NCRs (Fig. 1). Although the threshold dose of Arg-VP seemed to be $10^{-11}$ g/kg in both strains, the degree of the increase in MAP was significantly greater in the HRs (Fig. 2A), indicating that vasoconstrictor V$_1$ receptor activity was facilitated in the HRs. There was no significant difference in the mean ED$_{50}$ values between the NCR and HR groups (NCRs: mean ED$_{50}=1.66 \times 10^{-8}$ g/kg and its 95% confidence coefficient = $1.07-2.57 \times 10^{-8}$ g/kg, n=14; and HRs: mean ED$_{50}=7.76 \times 10^{-9}$ g/kg and its 95% confidence coefficient = $0.55-1.10 \times 10^{-8}$ g/kg, n=14), while the dose-response curve for Arg-VP in the HRs was slightly left to that in the NCRs (Fig. 3).

After the administration of a VP antagonist, the degree of increase in MAP produced by Arg-VP was significantly greater in the HRs than in the NCRs at all doses from $10^{-10}$ to $10^{-4}$ g/kg (Fig. 2B), so that the dose-response curve for Arg-VP in the HRs was shifted much more to the right than that in the NCRs (Fig. 3). The mean ED$_{50}$ value was greatly increased by about 690-fold in the presence of a VP antagonist in the NCRs ($1.15 \times 10^{-5}$ g/kg), and by about 68-fold in the HRs ($5.25 \times 10^{-7}$ g/kg). Thus, it was noticed that the ED$_{50}$ value of NCRs was about 20-fold greater than that of HRs. In other words, much more Arg-VP was necessary to produce the same pressor effect in NCRs than in HRs. It might be that V$_2$ receptor-mediated endothelium-dependent vasodilation (8) decreased the V$_1$ receptor-mediated pressor effect of VP in NCRs, in contrast to HRs. It was also noted that a VP antagonist by itself decreased MAP in HRs, suggesting that the VP system is activated in HRs. The notion might be supported by the fact that the concentration of VP in plasma was higher in the HRs than in NCRs.

In conclusion, the present results indicate that with or without a VP antagonist, exogenous Arg-VP induces more powerful pressor actions in HRs than in NCRs. However, the exact role of VP in the maintenance of the borderline-hypertension in HRs still remains to be elucidated.
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REFERENCES


