Does Vasopressin Sustain Blood Pressure in Conscious Spontaneously Hypertensive Rats?

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SUMMARY To investigate the possible role of arginine vasopressin in maintaining high blood pressure of spontaneously hypertensive rats (SHR), the effect of two arginine vasopressin pressor antagonists on mean arterial pressure and the pressor responsiveness to exogenous arginine vasopressin were studied in conscious, freely moving SHR and in Wistar-Kyoto rats (WKY). Intravenous injections of either d(CH2)5Tyr(Me)arginine vasopressin, 10 μg/kg, or dPTyr(Me)arginine vasopressin, 20 μg/kg, had no effect on mean arterial pressure or heart rate of normohydrated SHR, although both antagonists almost completely abolished the pressor response to exogenous arginine vasopressin. Furthermore, dPTyr(Me)arginine vasopressin was ineffective in eliciting a depressor response, even after 24 or 48 hours of water deprivation. During converting enzyme inhibition with SQ 20881, mean arterial pressure and heart rate remained unchanged following arginine vasopressin blockade in both normohydrated and fluid-restricted animals. α-Adrenergic receptor blockade reduced the blood pressure of normohydrated SHR, from 160 ± 7 to 81 ± 8 mm Hg. When dPTyr(Me)arginine vasopressin was given during α-adrenergic receptor blockade there was a small, transient fall in mean arterial pressure. The pressor responsiveness to exogenous arginine vasopressin was similar in hypertensive and normotensive rats. These results suggest that arginine vasopressin does not function as an important pressor hormone in conscious SHR. (Hypertension 8: 514-519, 1986)

KEY WORDS • vasopressin pressor antagonists • pressor responsiveness • water deprivation • converting enzyme blockade • α-adrenergic receptor blockade • mean arterial pressure

THERE is no consensus on the role of arginine vasopressin (AVP) in the pathogenesis of spontaneous hypertension in rats. Studies of the plasma AVP concentrations in spontaneously hypertensive rats (SHR) are controversial: both elevated1 2 and decreased3 plasma AVP levels have been reported. Crofton et al.1 found that urinary AVP excretion is enhanced in SHR, but this has not been confirmed by others.2 Administration of a specific AVP antiserum resulted in a marked fall in blood pressure,4 5 and enhanced pressor responsiveness to AVP has also been observed in SHR.4 5 7 Nevertheless, the participation of AVP in the development of spontaneous hypertension is questionable, since when rats with hereditary diabetes insipidus and stroke-prone SHR (SHRSP) are crossed, the resulting strain, Heidelberg hypertensive drinkers (HHD), has hypertension as severe as that of SHRSP.8

The present study was designed to evaluate further the possible role of AVP in maintaining the high blood pressure of normally hydrated and water-deprived conscious SHR using two competitive AVP pressor antagonists in the presence and absence of converting enzyme inhibition or after α-adrenergic receptor blockade. We also investigated the pressor responsiveness to exogenous AVP in SHR and age-matched Wistar-Kyoto rats (WKY). A particular objective of our study was to ensure that our investigations were complicated as little as possible by surgical stress, anesthesia, and restraint.

Materials and Methods

Female SHR of the Okamoto-Aoki strain (13-16 weeks of age) and age-matched normotensive female WKY (Ivanovas, Kisslegg, Federal Republic of Germany) were housed in individual metabolic cages and given standard laboratory chow (Altromin, Lage, Federal Republic of Germany) containing 0.09 mmol/g of sodium and 0.26 mmol/g of potassium and tap water ad libitum. After a 2-week adaptation period, the animals were fitted with arterial and venous catheters according to methods detailed elsewhere.9 Briefly,
with the rats under anesthesia (ketamine, 75 mg/kg, and sodium pentobarbital, 15 mg/kg i.p.) catheters were implanted into the abdominal aorta and vena cava through the ventral tail artery and a lateral tail vein, respectively. The catheters and the wounds were protected by an acrylic cuff glued to the tail and connected to a stainless steel spiral. The other end of the metal spiral was fed through the top of the metabolic cage. The animals were allowed at least 5 days to recover from the operation. During this period they regained their initial body weights (SHR initial weight: 218 ± 3 g, postoperative weight: 216 ± 3 g, n = 19, p < 0.2; WKY initial weight: 228 ± 3 g, postoperative weight: 226 ± 3 g, n = 8, p < 0.4). During the experiments the animals could move freely and showed no signs of discomfort. All procedures followed were in accordance with guiding principles on the care and use of animals approved by the Council of the American Physiological Society.

Arginine Vasopressin Pressor Blockade

The effects of [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(O-methyl)tyrosine]AVP (d(CH$_2$)$_5$Tyr(Me)AVP)$^{10}$ were investigated in eight normohydrated SHR. Each experiment was begun at 0830. Mean arterial pressure was monitored with an electromanometer (Hugo Sachs Elektronik, March-Hugstetten, Federal Republic of Germany) using a Statham P23dB strain gauge (Hato Rey, Puerto Rico). Heart rate was measured with a rate meter (Digi-Puls, Hugo Sachs Elektronik) triggered by the signal of the electromanometer. After a 3-hour equilibration period, d(CH$_2$)$_5$Tyr(Me)AVP, 10 µg/kg, was injected intravenously in a volume of 62.5 µl/100 g of body weight, followed by 60 µl of 0.85% NaCl. The effectiveness of the AVP pressor antagonist was tested in separate experiments by administering 4 and 40 µU/kg of d(CH$_2$)$_5$Tyr(Me)AVP (Grade VI, Sigma Chemical, St. Louis, MO, USA) before and 30 minutes after the administration of d(CH$_2$)$_5$Tyr(Me)AVP.

The effects of another AVP pressor antagonist, 1-deaminopenicillamine, 2-(O-methyl)tyrosine AVP (dPTyr(Me)AVP)$^{11}$ at a dose of 20 µg/kg were studied using the same protocol in the same rats under free water intake and after 24 and 48 hours of water deprivation, respectively.

Plasma AVP concentration was measured in six SHR before and during water deprivation. Blood (1 ml) was collected over a period of 35 to 45 seconds from the arterial catheter into a plastic tube containing heparin and was immediately centrifuged at 4°C. Red blood cells were resuspended in sterile 0.9% NaCl solution and were reinjected within 4 minutes in a final volume of 1 ml. Plasma samples were stored at −20°C until extracted and assayed according to methods described previously.$^{12}$ Briefly, vasopressin was extracted from the plasma with Sep-Pak C$_8$ cartridges (Waters Associates, Milford, MA, USA)$^{13}$ and measured by radioimmunoassay.$^{12}$ Arginine vasopressin triacetate (Ferring Arzneimittel GmbH, Kiel, Federal Republic of Germany) was used as standard, and AVP antiserum was provided by Dr. M.D. Lindheimer (Chicago, IL, USA). This antiserum has virtually no cross-reactivity with oxytocin and vasotocin.$^{14}$ The lower limit of detection with this assay was 0.4 fmol/ml. Intraassay and interassay coefficients of variation were 5.8 and 7.9%, respectively. Recovery of 3 to 12 fmol of AVP added to plasma of rats homozygous for diabetes insipidus was 69.8 ± 2.4% (n = 6). Plasma vasopressin measurements were corrected for recovery.

A protocol similar to that just described was followed in eight water-deprived SHR except that 40 minutes before the injection of dPTyr(Me)AVP, 20 µg/kg, an infusion of SQ 20881, a nonapeptide (Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) converting enzyme inhibitor (Squibb, Princeton, NJ, USA), at a rate of 100 µg/kg/min was started. The SQ 20881 was dissolved in 0.85% NaCl and was infused at a rate of 27 µl/min for 70 minutes.

The effects of dPTyr(Me)AVP, 20 µg/kg, were investigated after α-adrenergic receptor blockade in seven normohydrated SHR. Forty minutes before the antagonist was administered, phentolamine (Regitin), 10 mg/kg, was given intravenously during a 2-minute period.

Pressor Responsiveness to Arginine Vasopressin, Angiotensin II, and Norepinephrine

The pressor responses to exogenous AVP, angiotensin II (Hypertensin), and norepinephrine (Arterenol) were studied in seven SHR and eight normotensive WKY. Doses ranged from 2 to 32 mU/kg for AVP, from 0.156 to 10 µg/kg for norepinephrine, and from 3.12 to 200 ng/kg for angiotensin II. All drugs were dissolved in sterile 0.85% NaCl and were injected in a volume of 0.5 µl/kg of body weight followed by 60 µl of 0.85% NaCl. Both the order in which the dose-response curves were run and the doses within each dose-response curve were randomized. Each new injection was administered only after blood pressure had returned to baseline for at least 10 minutes following the last injection. The animals were allowed to recover for 30 to 40 minutes between dose-response curves. Vehicle alone was administered four times throughout each experiment.

Statistical Analysis

Results are expressed as means ± SEM. Data were evaluated by one-way analysis of variance and Student’s paired and unpaired t tests.

Results

Five days after operation mean arterial pressure was 159 ± 4 mm Hg in normohydrated SHR (n = 19) and 106 ± 1.2 mm Hg in control WKY (n = 8, p < 0.001). Heart rate was 349 ± 8 and 342 ± 15 beats/min in hypertensive and normotensive rats, respectively. Figure 1 shows the time course of changes in mean arterial pressure and heart rate of normohydrated SHR after administration of d(CH$_2$)$_5$Tyr(Me)AVP. As can be
seen, this antagonist had no effect on mean arterial pressure or heart rate.

The effectiveness of the AVP pressor antagonists was tested in separate experiments. Both antagonists completely abolished the pressor response to AVP, 4 mU/kg. Furthermore, dPTyr(Me)AVP and d(CH2)5Tyr(Me)AVP reduced the pressor response to AVP, 40 mU/kg, by 94 and 97%, respectively (Table 1). Neither antagonist had any effect on the pressor response to exogenous angiotensin II or norepinephrine (see Table 1).

During water deprivation, plasma AVP concentrations increased from a control value of 1.9 ± 0.3 fmol/ml to 11.1 ± 1.7 fmol/ml on the first day and to 17.7 ± 4.1 fmol/ml on the second day. Body weight decreased 8.3% on the first day and was 87.4% of its control value on the second day, while mean arterial pressure and heart rate did not change significantly (Table 2). Administration of dPTyr(Me)AVP was ineffective in eliciting a depressor response even after 24 or 48 hours of water deprivation. Similarly, the other AVP pressor antagonist, d(CH2)5Tyr(Me)AVP, also failed to produce a depressor response in fluid-deprived animals (data not shown).

Infusion of the converting enzyme inhibitor SQ 20881 had no significant effect on mean arterial pressure or heart rate during free water intake and water deprivation, but it did reduce mean arterial pressure by 1.3 and 4.7% and increase heart rate by 3.7 and 9.3% following 24 and 48 hours of water deprivation, respectively. Administration of dPTyr(Me)AVP to rats pretreated with SQ 20881 failed to exert any significant effect (see Table 2). In preliminary experiments SQ 20881, 100 µg/kg/min, reduced the pressor response to angiotensin I (50 ng/kg) by about 96%.

After phentolamine administration, blood pressure fell from 160 ± 7 to 80 ± 7 mm Hg (p < 0.001) and was accompanied by an increase in heart rate from 338 ± 12 to 404 ± 15 beats/min (p < 0.001). When dPTyr(Me)AVP was injected following α-adrenergic receptor blockade, a small decrease in mean arterial pressure was observed in all animals. The average decrease in mean arterial pressure was 4 ± 1.5 mm Hg (p < 0.025). The peak response occurred within 3 minutes after the administration of dPTyr(Me)AVP and was reversible within 6 minutes. This fall in blood pressure was accompanied by a transient increase in heart rate (Figure 2). Forty minutes after the injection of the AVP antagonist, an additional dose of phentolamine (10 mg/kg) was given. No further reduction of mean arterial pressure was observed, which indicates that α-adrenergic receptors were completely blocked during the experiments.

### Table 1. Pressor Responsiveness to Arginine Vasopressin, Angiotensin II, and Norepinephrine in SHR in the Absence and Presence of Vasopressin Pressor Antagonists

<table>
<thead>
<tr>
<th>Pressor antagonist</th>
<th>Change in mean arterial pressure (mm Hg)</th>
<th>AVP (mU/kg)</th>
<th>ANG II (ng/kg)</th>
<th>NE (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>14 ± 5</td>
<td>13 ± 3</td>
<td>16 ± 6</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>Present</td>
<td>0 ± 1</td>
<td>12 ± 3</td>
<td>16 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>d(CH2)5Tyr(Me)AVP (n = 5)</td>
<td>17 ± 3</td>
<td>10 ± 2</td>
<td>15 ± 6</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>Absent</td>
<td>0 ± 1</td>
<td>10 ± 2</td>
<td>17 ± 7</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Present</td>
<td></td>
<td>35 ± 2</td>
<td>40 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. AVP = arginine vasopressin; ANG II = angiotensin II; NE = norepinephrine.
The dose-response curves to exogenous AVP, angiotensin II, and norepinephrine were similar in the hypertensive and normotensive animals (Figure 3). Vehicle alone had no effect on mean arterial pressure or heart rate.

### Discussion

Spontaneous hypertension in rats is often considered to be a model of human essential hypertension. Although the pathogenesis of the hypertension in these animals is poorly understood, there are reports of central dysfunction resulting in increased sympathetic activity and increased secretion of AVP. Since AVP is one of the most potent naturally occurring pressor agents in mammals, it is not surprising that AVP has been suspected of being involved in the pathogenesis of essential hypertension. Elevated plasma AVP concentrations and increased urinary AVP excretion have been found in young SHR and adult SHRSR. Furthermore, a direct role for plasma AVP in the elevation of blood pressure was suggested by two observations: that blood pressure could be lowered temporarily by administration of either a specific AVP antisera or an AVP pressor antagonist and that the pressor responsiveness to AVP is enhanced in both SHR and SHRSR. On the other hand, depressed plasma AVP levels were found in young SHRSR. Recently, spontaneous hypertension has been shown to develop in rats with hereditary diabetes insipidus; this finding suggests that AVP is not essential for the development of this type of hypertension.

Interpretation of these results is complicated, however, by the fact that different substrains of SHR were used in these studies and the experiments were performed on anesthetized rats or shortly after anesthesia and operation. Since these latter conditions are known to elevate plasma AVP levels and to alter cardiovascular control mechanisms, the actual role of AVP in the maintenance of blood pressure may have been overestimated. To overcome these difficulties, the present experiments were performed in conscious, chronically prepared, freely moving rats.

In the present study, both AVP pressor antagonists markedly inhibited the vasopressor response to exogenous AVP. When the antagonists were injected into normohydrated SHR, no changes in mean arterial pressure and heart rate were observed. Nevertheless, the failure to observe a decrease in blood pressure may have been due to a parallel increase in the activity of other pressor systems. To test this possibility, we also studied the effect of AVP antagonists in the presence of the converting enzyme inhibitor SQ 20881 and after \( \alpha \)-adrenergic receptor blockade with phentolamine. The lack of response after administration of AVP antagonists probably is not due to an increased activity of renin-angiotensin system, as SQ 20881 failed to unmask any depressor response. Blood pressure dropped

### Table 2: Effect of dPTyr(Me) Arginine Vasopressin on Mean Arterial Pressure and Heart Rate of SHR in the Presence and Absence of the Converting Enzyme Inhibitor SQ 20881

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before dPTyr(Me)AVP</th>
<th>After dPTyr(Me)AVP (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10</td>
<td>10-20</td>
</tr>
<tr>
<td>SQ 20881 absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free water intake (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>149 ± 6</td>
<td>150 ± 5</td>
</tr>
<tr>
<td>HR</td>
<td>331 ± 12</td>
<td>337 ± 10</td>
</tr>
<tr>
<td>24-hr water deprivation (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>153 ± 6</td>
<td>153 ± 5</td>
</tr>
<tr>
<td>HR</td>
<td>351 ± 15</td>
<td>360 ± 18</td>
</tr>
<tr>
<td>48-hr water deprivation (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>147 ± 4</td>
<td>144 ± 4</td>
</tr>
<tr>
<td>HR</td>
<td>364 ± 17</td>
<td>367 ± 16</td>
</tr>
<tr>
<td>SQ 20881 present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free water intake (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>152 ± 7</td>
<td>154 ± 6</td>
</tr>
<tr>
<td>HR</td>
<td>370 ± 17</td>
<td>366 ± 12</td>
</tr>
<tr>
<td>24-hr water deprivation (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>163 ± 11</td>
<td>163 ± 11</td>
</tr>
<tr>
<td>HR</td>
<td>363 ± 21</td>
<td>368 ± 18</td>
</tr>
<tr>
<td>48-hr water deprivation (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>143 ± 5</td>
<td>141 ± 4</td>
</tr>
<tr>
<td>HR</td>
<td>354 ± 19</td>
<td>364 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SEM. AVP = arginine vasopressin; MAP = mean arterial pressure; HR = heart rate.
dramatically after α-adrenergic receptor blockade, and dPTyr(Me)AVP caused a further small, transient fall.

Before concluding that AVP plays any role in maintaining the hypertensive levels of blood pressure in SHR one must remember that during α-adrenergic receptor blockade AVP secretion is dramatically stimulated\(^\text{21}\) and pressor responsiveness to AVP is also enhanced.\(^\text{22}\) Thus, the transient drop in blood pressure following AVP blockade in phentolamine-treated animals may be due to shifting baseline values to extreme, nonphysiological levels (e.g., mean arterial pressure was about 80 mm Hg and plasma AVP concentration increased by more than 30-fold\(^\text{21}\)). In order to apply a physiological stimulus, the effects of fluid restriction were also investigated. Water deprivation for 24 and 48 hours resulted in a fivefold and more than eightfold increase in plasma AVP concentration, respectively. However, AVP presor blockade failed to alter mean arterial pressure and heart rate following water deprivation for up to 48 hours and was ineffective even if combined with SQ 20881. In contrast to these findings, previous studies have suggested that water deprivation releases AVP in sufficient concentration to affect cardiovascular tone in both conscious, restrained\(^\text{22}\) and anesthetized\(^\text{23}\) normotensive rats. When these experiments were repeated on truly conscious, unrestrained animals, however, no effect of AVP antagonists on blood pressure could be detected.\(^\text{12, 23}\)

Another possible mechanism through which AVP may contribute to spontaneous hypertension is an enhanced pressor responsiveness to this hormone. In previous studies, increased pressor responsiveness has been reported after administration of AVP,\(^\text{4-6, 7}\) angiotensin II,\(^\text{7}\) and norepinephrine.\(^\text{26}\) In contrast, in our experiments the dose-response curves of these vaso-
constrictors were similar in hypertensive and normotensive animals. However, the previous and the present experiments are not readily comparable, since the previous studies used stressed male rats6–8 after phenylephrine treatment7 whereas we used conscious, nonstressed female rats. Recent articles report a dromic pattern of hypertension development in SHR7 and androgen-mediated sex differences of cardiovascular responses.28,29 On the other hand, differences in experiments (i.e., degree of surgical stress, restraint and presence or absence of anesthesia) have been suggested more recently as the primary source of the divergent results.12

In summary, our present findings strongly suggest that AVP does not function as an important pressor hormone in conscious SHR. The possibility that AVP contributes to essential hypertension by virtue of its anti-diuretic activity,30 however, remains to be determined.

Acknowledgments

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