Vasopressin in the rat with spontaneous hypertension

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CROFTON, JOAN T., LEONARD SHARE, ROBERT E. SHADE, CYNTHIA ALLEN, AND DEBORAH TARNOWSKI. Vasopressin in the rat with spontaneous hypertension. Am. J. Physiol. 235(4): H361-H366, 1978 or Am. J. Physiol.: Heart Circ. Physiol. 4(4): H361-H366, 1978.—Because vasopressin is one of the most potent naturally occurring pressor agents, and because of its importance in the regulation of blood volume and composition, we have undertaken a study of the role of vasopressin in the pathogenesis of the hypertension in the Okamoto-Aoki spontaneously hypertensive (SH) rat. In SH rats, systolic blood pressure increased from 135 ± 3 (SE) mmHg at age 33 days to 184 ± 3 mmHg at age 75 days (P < 0.01). In the Wistar-Kyoto (WKY) control rats, blood pressure increased from 100 ± 2 to 120 ± 2 mmHg (P < 0.01). The differences in blood pressure between the SH and WKY rats at all ages were significant (P < 0.01). During the age period 33-75 days, the 24-h urinary excretion of vasopressin in the SH rat was consistently more than twofold greater (P < 0.01) than in the WKY rat. Plasma vasopressin concentration and pituitary vasopressin content were also increased in the SH rat (P < 0.01 and P < 0.02, respectively). Changes in systolic blood pressure in the SH rat, however, were not paralleled by changes in the urinary excretion of vasopressin. The data indicate that the secretion of vasopressin is elevated in the SH rat. However, the magnitude of this elevation, in and of itself, may not be sufficient to account for the rising blood pressure in the young SH rat.

METHODS

Four-week-old male SH rats derived from the Okamoto-Aoki strain, normotensive male WKY controls, and normal male Wistar rats were obtained from Charles River Breeding Laboratories. Eight SH and eight WKY rats were randomly selected from the above groups and housed individually in stainless steel metabolism cages for measurements of 24-h urinary vasopressin excretion and systolic blood pressure. The remaining SH and WKY rats were housed 6-10 to a cage and were later used for plasma and pituitary vasopressin determinations. Six normal Wistar and six WKY rats were also housed individually in metabolism cages for urinary vasopressin measurements. All rats were allowed to acclimate for four days before any measurements were begun. The rats were kept in a Biocon Room (Dwyer Instruments) in which temperature was kept at 23-24°C. The automatic lighting cycle was 12 h on and 12 h off. Animals were given Purina laboratory chow and tap water ad libitum.

Protocol

Experiment I. Twenty-four hour urinary excretion of vasopressin (U_{ADH}V), systolic blood pressure, and body weight were measured twice weekly in SH and WKY groups. Rats were 33 days old when measurements were begun. Systolic blood pressure and body weight were measured at the end of the 24-h urine collections. The experiment was concluded when the rats were 10 wk old.

Experiment II. Groups of SH and WKY rats were killed at ages 33, 55, and 75 days for determination of plasma vasopressin (P_{ADH}) and plasma sodium (P_{Na}).

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concentrations, hematocrit, and pituitary vasopressin content.

Experiment III. Twenty-four hour excretion of vasopressin and body weight were measured once a week in the normal Wistar rats and a second group of WKY rats. No blood pressure measurements were made in these rats.

Systolic Blood Pressure

Systolic blood pressure was measured by tail plethysmography in conscious rats. The rats were trained by subjecting them to the procedure three times before experimental measurements were begun. They were warmed at 37°C for 15-20 min and then restrained in a Nareo Gram animal warming unit to maintain tail arterial vasodilation. Recordings were made with a pneumatic pulse transducer connected to a Physiograph Six-B recorder (Nareo Bio-Systems). Pressure was measured randomly between groups and among rats within a group to minimize sequencing effects, always at the same time of day by the same person. A daily mean consisting of at least six measurements was calculated for each rat.

Vasopressin Measurement: Extraction and Radioimmunoassay

Urine was collected for 24 h under mineral oil in 50-ml polypropylene centrifuge tubes containing 0.2 ml glacial acetic acid. At the end of the collection, samples were centrifuged at 2,500 rpm for 30 min at 4°C to remove food. Urine was separated from the mineral oil, and urine volume was measured. The sample was stored at -14°C for 1-4 days until vasopressin was extracted with Amberlite CG-50 ion exchange resin (10).

For determination of plasma concentrations of vasopressin and sodium, hematocrit, and total pituitary content of vasopressin, rats were brought into the laboratory the night before they were to be killed. The next morning they were given an injection of 0.25 ml sodium heparin (1,000 U/ml ip) and were decapitated by guillotine 20-25 min later. Trunk blood was collected into heparinized plastic tubes packed in ice. Blood samples were centrifuged at 1,800 rpm for 30 min at 4°C to separate the plasma. For each sample, plasma was pooled: 10 rats per sample at age 33 days, 8 rats per sample at age 55 days, and 3 rats per sample at age 75 days. At age 75 days, the samples included plasma from the eight SH and eight WKY rats used for urinary vasopressin measurements in experiment I. Six to eight samples for each type of rat were taken at each age.

Plasma samples that were to be used for the measurement of vasopressin were acidified by adding 0.1 ml of 1 N HCl per milliliter of plasma, and were stored at -40°C until they were extracted. Samples were extracted by precipitating the plasma proteins with trichloroacetic acid (TCA), removing the TCA by extraction with ether, and adsorbing the vasopressin on an Amberlite CG-50 column (2). Samples were eluted from the columns with 75% ethanol adjusted to pH 2 with 50% HCl. Urine and plasma extracts were dried under vacuum with rotary evaporators and then redissolved in a solution containing 0.9% NaCl, 0.1% bovine serum albumin, and 0.03% glacial acetic acid. All samples were stored at -14°C.

Immediately after decapitation, one animal representative of those used for each plasma vasopressin sample was randomly selected; the pituitary was removed, placed into a 50-ml conical plastic centrifuge tube containing 10 ml of 0.25% acetic acid, and fragmented with a glass rod. When all the pituitaries were obtained, they were boiled for 5 min in a water bath and then filtered through a Buchner funnel (Pyrex 3600) into a siliconized 50-ml cylinder. The original tube was rinsed with three 10-ml aliquots of the 0.25% acetic acid, which were then filtered. The filtrate was brought to a final volume of 50 ml with 0.25% acetic acid. This was further diluted to 1:2,500 with the solution used for redissolving plasma and urine extracts, and stored at -40°C.

The antiserum used in the vasopressin radioimmunoassay was raised by the method of Skowsky and Fisher (19), in which arginine vasopressin (Bachem) is coupled to thyroglobulin. This antiserum is highly specific for arginine vasopressin and did not cross-react with lysine vasopressin, oxytocin, arginine vasotocin, or angiotensins I and II. Labeled vasopressin (125I-AVP) was prepared by iodinating arginine vasopressin (Sigma) with Na125I (Union Carbide), as described by Skowsky et al. (20). 125I-AVP was purified first by passing the iodination reaction mixture through a 0.8 x 15 cm column of O-(diethylaminoethyl)cellulose (DEAE-cellulose) resin, with 0.02 M ammonium acetate buffer (pH 5.5) as the solvent. One-milliliter fractions were collected in tubes containing 0.1 ml of 10% bovine serum albumin (BSA) in the ammonium acetate buffer. The peak tube was mixed with 0.3 ml human serum and then passed through a 1.5 x 20 cm column of G-25 fine Sephadex in 1.2% glacial acetic acid. This was further diluted to 1:2,500 with the solution used for redissolving plasma and urine extracts, and stored at -40°C.

The antiserum used in the vasopressin radioimmunoassay was prepared by diluting USP Posterior Pituitary Reference Standard with 0.9% NaCl-0.1% BSA-0.03% glacial acetic acid, so that doses ranging from 0.5 to 100 μU were delivered in 0.1 ml Standard (0.1 ml) or sample extract, 0.1 ml 125I-AVP (5,000 cpm), 0.2 ml diluted (1:150,000) antiserum, and sufficient assay buffer to bring the final volume to 1 ml were added simultaneously to each tube with a Micromedic automatic pipetting station. Each point on the standard curve was run in triplicate. Since, in a large assay, total time for preparing the tubes may be as much as 5 h, separate standard curves were run at the beginning and end of the assay, to ensure that no differences that occurred were due to differences in incubation time. Extracted urine and plasma samples were run in duplicate at three different doses (0.05, 0.1, and 0.2 ml). All re-
agents, samples, standards, and assay tubes were kept in ice throughout the assay. After completion of the assay, the samples were incubated at 4°C for 20–22 h. The bound $^{125}\text{I}$-AVP was then separated from the free by addition of 1 ml of BSA-coated charcoal (0.83% Norit "A" (Amend Drug and Chemical Co.) in assay buffer, in which the BSA concentration was increased to 0.167%). Supernatant and charcoal fractions were separated by centrifugation at 2,500 rpm for 20 min at 4°C. The interval between the addition of charcoal and separation of the fractions was held constant. Both free and bound fractions were counted, and the log free/bound counts were plotted against dose of vasopressin for the standard curves. The line of best fit and the concentrations of vasopressin in the samples were calculated by computer. A correction was made for the amount of $^{125}$I AVP the charcoal was unable to bind in the absence of antiserum and standard or sample. Excess binding (0.2 ml of 1:100 antiserum in the absence of standard or sample) was also calculated with each curve. When excess binding deviated from the usual 89–91%, fresh $^{125}$I-AVP was prepared.

Recovery of Vasopressin from Plasma and Urine

Urine. Pooled urine, collected from an earlier group of SH and WKY rats, was divided into 10 ml samples and stored at −40°C. Two WKY and two SH samples were run with each extraction. A known amount of USP Posterior Pituitary Reference Standard was added to one of each pair, and the other served as a blank. Percent recovery was 94.5 ± 3.5% (SE) for WKY and 94.6 ± 3.7% for SH urine (n = 7 for each group). The coefficient of variability within assay was 6.3 ± 1.7% for the WKY group and 4.9 ± 0.6% for the SH group. Between-assay variability was 8.7% for the WKY group and 14.9% for the SH group. No correction for recovery was made.

Plasma. Normal male Wistar rats (200–300 g) were decapitated, and plasma was collected, acidified with 1 N HCl, pooled, and stored at −40°C. Recovery of vasopressin from plasma was determined each time experimental plasma samples were extracted. A known amount of Posterior Pituitary Reference Standard was added to 10 ml samples (5–11 samples per assay at each of the three times when vasopressin was measured). Three 10-ml aliquots served as blanks and were run with each recovery. The coefficient of variability within assay was 19.2 ± 2.6% (SE); between-assay variability was 14.3%. Average recovery (n = 30) was 90.4 ± 6%. Plasma vasopressin measurements were corrected for recovery.

Sodium and Hematocrit

Sodium was measured with an IL343 flame photometer. Hematocrit was measured by a microcapillary technique.

Statistics

To correct for nonhomogeneity of variance, a logarithmic transformation was performed on all vasopressin data. For the SH and WKY groups, a three-factor analysis of variance (type of rat × week × day within week) for repeated measures was performed on UAIV, systolic blood pressure, and body weight. No statistical difference was found for the day-within-week interaction, so all analyses were done on weekly means. When the analysis of variance showed a significant interaction with time within a group, a Newman-Keuls test was performed to isolate differences. Second, week-by-week comparisons were made between the two groups by use of multiple $t$ tests. An alpha level of $P < 0.01$ was set to keep the overall level of significance at $P < 0.05$ to ensure that no type I errors were made. A two-factor analysis of variance (age × rat) was performed on plasma vasopressin and plasma sodium concentrations, total pituitary vasopressin, body weight, and hematocrit. Multiple $t$ tests were run between groups, and the alpha level was again set at $P < 0.01$. A two-factor analysis of variance (age × rat) was also performed on UAIV and body weight for the comparison of normal Wistar with WKY rats. Multiple $t$ tests were done between groups when significant differences were found with time within a group. Means and standard errors are presented in the text, figures, and table, although these standard errors were not used in the statistical analyses.

RESULTS

Experiment I

There were no differences in body weight between WKY and SH rats (Fig. 1) until the final 2 wk of the experiment, when the WKY rats were significantly heavier ($P < 0.01$) than the SH rats. As expected, body weight increased significantly ($P < 0.01$) in both groups throughout the experiment.

Systolic blood pressure increased ($P < 0.01$) in the WKY rats from 100 ± 2 mmHg at age 5 wk to 120 ± 1 mmHg by age 7 wk and remained constant throughout the remainder of the experiment (Fig. 2A). In the SH
rat, systolic blood pressure rose rapidly and markedly \((P < 0.01)\) from 135 ± 3 mmHg at age 5 wk to 188 ± 4 mmHg at age 9 wk and then dropped slightly during the final week of the experiment (184 ± 3 mmHg). Blood pressure in the SH rats was greater than in the WKY \((P < 0.01)\) at all ages.

Although \(\text{U}_{\text{ADH}} \text{V} \) (Fig. 2B) showed a slight tendency to fall with time in the WKY group, this was not statistically significant. In the SH rats, \(\text{U}_{\text{ADH}} \text{V} \) decreased one-third \((P < 0.01)\) from age 5 wk to ages 7 and 8 wk and then rose to near initial values at ages 9 and 10 wk. Urinary excretion of vasopressin was always two to three times greater in the SH group than in the WKY \((P < 0.01)\).

**Experiment II**

Plasma, urine, and pituitary samples for the measurement of vasopressin were obtained when rats were 33, 55, and 75 days old (Fig. 3). At these three ages, the plasma vasopressin concentration (Fig. 3A) in the SH rats was 13 28% higher than in the WKY \((P < 0.01)\). In both groups, the plasma vasopressin concentration fell \((P < 0.01)\) at age 55 days and then returned to approximately initial levels at age 75 days. As in experiment I, \(\text{U}_{\text{ADH}} \text{V} \) (Fig. 3B) in the SH rats exceeded that in the WKY rats \((P < 0.02)\), and was lower in both groups at age 55 days \((P < 0.01)\) than at ages 33 or 75 days. Pituitary vasopressin content, which did not change significantly with time, was also higher in the SH than the WKY rats (Fig. 3C; \(P < 0.02)\).

The WKY rats were significantly heavier than the SH rats \((P < 0.02)\) at each of the three ages (Table 1). Body weight increased significantly \((P < 0.01)\) in both groups with time. Plasma sodium (Table 1) was also found to increase significantly with time \((P < 0.01)\); there were no differences between the two groups. Hematocrit (Table 1) increased with age \((P < 0.01)\) in the WKY group, but was significantly elevated \((P < 0.01)\) only at age 75 days in the SH group. A statistical difference existed between the two groups only at age 33 days \((P < 0.02)\).

![Fig. 2. Changes with time in systolic blood pressure (A) and the 24-h urinary excretion of vasopressin (B) in the SH and WKY rats of \(\text{expt I} \) (means & SE). Differences between groups are shown by asterisks between lines; differences from initial values are shown by asterisks above or below lines.](image)

![Fig. 3. Changes with time in plasma vasopressin concentration (A), urinary vasopressin excretion (B), and pituitary vasopressin content (C) in SH and WKY rats of \(\text{expt II} \) (means & SE). Statistically significant differences between groups are shown by asterisks between bars; differences from initial observations are shown by asterisks above bars.](image)

<table>
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<th>Age, days</th>
<th>33</th>
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<tr>
<td>Body wt, g</td>
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<td>255±4</td>
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<td>(P_{\text{Na}} )</td>
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<td>146.3±0.4</td>
<td>151.7±0.4</td>
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<td>Hct, %</td>
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<td>37.2±0.8</td>
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</tbody>
</table>

Values are means ± SE. * Significantly different from observation at 33 days \((P < 0.01)\). + SH significantly different from WKY \((P < 0.01)\).
VASOPRESSIN IN SH RAT

Experiment III

Because the WKY rats appear in some ways to be different from other "normal" rats, \( U_{ADH} \) \( V \) was measured in a group of normal Wistar rats and a group of WKY rats to see if they were comparable (Fig. 4A). \( U_{ADH} \) \( V \) in the normal Wistar rats was more than twice that in the normal WKY rats at age 5 wk (\( P < 0.05 \)). In the latter group, \( U_{ADH} \) \( V \) increased with time, so that at age 12 wk there was no longer a significant difference between the two groups. Body weight (Fig. 4B) increased significantly (\( P < 0.01 \)) throughout the experiment (Fig. 4B), and the normal Wistar group was significantly heavier than the WKY group (\( P < 0.01 \)), except during the last 2 wk of the experiment.

DISCUSSION

Vasopressin is one of the most potent naturally occurring pressor agents in mammals. Its pressor potency is as great as, if not greater than, that of angiotensin II in the rat prepared for pressor assay (12). It is, then, not surprising that the possibility that vasopressin is an etiologic factor in hypertension has been examined sporadically for many years. Section of the pituitary stalk was found to decrease blood pressure in six of eight dogs with Goldblatt hypertension (13) and in five hypertensive patients (7). Permanent diabetes insipidus was produced in the former, but not in the latter study. Ellis and Grollman (4) found an elevated urinary excretion of an antidiuretic substance in dogs with Goldblatt hypertension, as well as in hypertensive patients. However, the importance of vasopressin in the pathogenesis of at least one form of hypertension was not convincingly demonstrated until recently. Möhring et al. (11) reported that plasma levels of vasopressin were elevated in rats with DOC-salt hypertension, and that in these rats, blood pressure could be transiently lowered by the intravenous injection of an antiserum to vasopressin. We have subsequently shown (Crofton et al., unpublished) that blood pressure in rats with DOC-salt hypertension can be transiently decreased by a synthetic analog of vasopressin that blocks its pressor action. We also found that the urinary excretion of vasopressin rises when DOC-salt hypertension first develops and that rats with hereditary hypothalamic diabetes insipidus do not become hypertensive when treated with the DOC-salt regimen.

In the present experiments, we have found that in SH rats as young as 33 days, there is an increased plasma vasopressin concentration, pituitary vasopressin content, and increased urinary excretion of vasopressin. Systolic blood pressure in these 33-day-old rats was elevated when compared to WKY controls, but was not at hypertensive levels. These elevated levels of plasma and urinary and pituitary vasopressin were maintained in SH rats until the experiment was terminated when the rats were 75 days old. Taken individually, these data are somewhat difficult to interpret. The increased plasma vasopressin concentration could be due either to increased secretion or decreased metabolism of this hormone. The latter seems unlikely as a long term cause of an elevated plasma level of vasopressin; a resultant increased water retention by the kidney in that event should decrease the secretion rate of vasopressin, returning its plasma concentration to normal. An increased pituitary content of vasopressin can be due to increased synthesis, decreased release, or an increased release of a lesser magnitude than a concomitant increased synthesis. Finally, since the kidneys and liver are the primary sites for clearance of vasopressin from the circulation (8), an increased urinary excretion could reflect either a shift in clearance from the liver to the kidneys or an increased release of the hormone from the neurohypophysis. Taken together, however, all these data support the hypothesis that there is an increased release of vasopressin in the SH rat, beginning at an early age.

It is difficult at this time to assess the role of the elevated plasma vasopressin concentration in the development and maintenance of hypertension in the SH rat. The magnitude of this elevation is small, but Hoffman et al. (6) have shown that the SH rat has an increased sensitivity to the pressor action of vasopressin. Furthermore, vasopressin in suppressor doses potentiates the pressor action of catecholamines (1), and increased circulating levels of norepinephrine have been reported in the SH rat (5). On the other hand, our observation that \( U_{ADH} \) \( V \) fall substantially at a time when systolic blood pressure was rising suggests that other factors are of major importance in the developing hypertension. Consistent with this interpretation is our finding that an analog of vasopressin that blocks its pressor action reduced blood pressure in only three of six SH rats tested (unpublished observations). However, the SH rats in which this blocker was tested had well developed hypertension; the blocker might have been more effective in younger rats.

If one accepts that there is an elevated release of...
vasopressin in the SH rat, what is the cause of this increase? Unfortunately, this question, too, cannot yet be adequately answered. Since the plasma sodium concentration in the SH rats was identical with that in the WKY rats at ages 33, 55, and 75 days, it is unlikely that the increased vasopressin secretion was due to increased osmotic stimulation. Indeed, the elevated blood pressure should inhibit vasopressin release. An intriguing possibility is that the increased secretion of vasopressin is the consequence of increased secretion of prostaglandin E₂ (PGE₂) in the kidney (9). An elevated intrarenal PGE₂ could block the ability of vasopressin to promote water reabsorption by the kidney. This would lead in turn to an increased vasopressin release and plasma concentration. However, although Limas and Limas (9) have reported an increased PG synthetic activity and a decreased PG degradative enzyme activity in the SH rat kidney, Sirois and Gagnon (18) and Stygels et al. (21) found that there was a decreased release of PGE₂ into the medium when renal medullary tissue from SH rats was incubated in vitro.

Although the secretion of vasopressin is elevated in the SH rat, its role in the pathogenesis of the hypertension cannot yet be stated with certainty. The possibility that the vasopressin is functioning as a pressor agent will be determined with more extensive studies with agents that block the pressor activity of this hormone and of the cardiovascular responsiveness of the SH rat to vasopressin. Furthermore, we should not overlook the possibility that vasopressin may contribute to the hypertension by virtue of its ability to increase water retention and expand blood volume.

This work was supported by Public Health Service Grant HL-19209. Computer assistance was provided by Public Health Service Grant HL-19495.

Received 21 February 1978; accepted in final form 18 May 1978.

REFERENCES