Cholinergic Stimulation of Vasopressin Release in Spontaneously Hypertensive Rats

CELIA D. SLADEK AND MARTHA L. BLAIR

SUMMARY  Plasma vasopressin (VP) concentration is elevated in spontaneously hypertensive rats (SHRs) relative to their normotensive Wistar-Kyoto (WKY) controls. The possibility that this reflects altered responsiveness of the hypothalamo-neurohypophyseal system (HNS) in SHRs was examined by comparing VP release in response to acetylcholine from organ cultured HNS explants obtained from SHR and WKY donors. Explants were prepared from 5-, 8-, and 18-week-old animals. Blood pressure was significantly elevated in the 8- and 18-week-old SHR donors relative to their age-matched WKY donors. VP release was assessed on the 4th day of culture during a control hour and during the subsequent hour in the presence of acetylcholine. Acetylcholine caused a concentration-dependent stimulation of VP release from both types of explants, but the response was significantly greater in the explants from 5- and 8-week-old SHRs than in explants from age-matched WKYs. The explants from 18-week-old SHRs and WKYs demonstrated comparable sensitivity to acetylcholine. Basal VP release was not significantly different in explants from age-matched SHRs and WKYs, but it did increase with donor age in both strains. These studies indicate potential hyperresponsiveness of the HNS to excitatory stimuli in SHRs during the developmental phase of hypertension. The hyperresponsiveness disappears in the chronically hypertensive phase. Thus, increased sensitivity of the HNS during the development of hypertension may contribute to the elevation of plasma VP concentration in SHRs. (Hypertension 6: 855-860, 1984)

KEY WORDS • hypothalamo-neurohypophyseal system • supraoptic nucleus • acetylcholine

In the strain of spontaneously hypertensive rats (SHRs) developed by Okamoto,1 there is evidence of abnormalities in the regulation of vasopressin (VP) secretion. Plasma VP is elevated in SHRs from 5 through 10 weeks of age,2,3 and urinary excretion of VP is significantly greater in SHRs than in their normotensive Wistar-Kyoto (WKY) controls during this same period.2 Thus, it is possible that the HNS may respond inappropriately to excitatory or inhibitory signals for VP release. This possibility was evaluated by studying cholinergic stimulation of VP release from organ-cultured HNS explants prepared from either SHR or WKY donors of 5, 8, or 18 weeks of age. Acetylcholine previously has been shown to be a potent stimulus for VP release from HNS explants obtained from Sprague-Dawley rats4 (strain Crl: CD(SD)BR, Charles River Laboratories, Wilmington, Massachusetts).

Materials and Methods

Explant Preparation

The HNS explants were prepared as described previously4 from male SHRs and WKYs 5, 8, or 18 weeks of age. Each explant consisted of a triangular slice of tissue from the basal hypothalamus, which included the VP neurons of the supraoptic nucleus with their axonal projections extending through the median eminence and terminations in the attached neural lobe. The paraventricular nucleus was excluded due to its dorsal position in the hypothalamus. Explants also included hypothalamic tissue, which is extraneous to the HNS (arcuate, suprachiasmatic, and a portion of ventromedial hypothalamic nuclei). The paraventricular nucleus was excluded due to its dorsal position in the hypothalamus. Explants were maintained in culture for 4 days under the conditions used previously.5 On Day 4 of culture, VP release into the culture medium by each explant was measured by radioimmunoassay during a control hour (basal release) and during a subsequent test hour (stimulated release).
after the addition of varying concentrations of acetylcholine (10^{-8} \text{ to } 10^{-4} \text{ M}). The sampling protocol and the radioimmunoassay have been described previously.\(^4\) The sampling protocol allowed for evaluation of VP degradation in the culture medium. The VP degradation in the medium was not significantly different between explants from SHRs and WKYs at any age (F = 0.8). Any given experiment involved testing the response of a group of explants from SHRs of one age and a group of explants from WKYs of the same age to a single concentration of acetylcholine or vehicle (10^{-4} \text{ M} \text{ of } 0.9\% \text{ NaCl}). The pH and osmolality of the culture medium were not altered by the addition of acetylcholine. The pH was monitored by the presence of phenol red in the medium, and osmolality was determined by vaporpressure osmometry (Wescor, Inc., Logan, Utah) at the end of each experiment. The osmolality of the culture medium was 296 ± 5 mOsm.

**Blood Pressure Measurement**

Before sacrifice, systolic blood pressure was monitored in the 8- and 18-week-old explant donors by tail plethysmography (IITC, Inc., Landing, New Jersey) twice a week for 2 weeks. Litter mates of the 5-week-old animals were saved, and their blood pressure was measured at 8 weeks of age. The blood pressure in the donor animals was compared to the blood pressure in another group of SHR (n = 10) and WKY (n = 10) rats twice a week from 5 through 18 weeks of age. Animals were warmed in a 30\(^\circ\) C box for 10 to 20 minutes before and during blood pressure recording.

**Data Analysis**

For each explant, stimulated VP release was compared to basal release, and a paired t test (two-tail) was performed to evaluate whether the response of that group of explants to acetylcholine was statistically different from basal release. Comparison of group means was evaluated by the least significant difference test following a one-way analysis of variance (ANOVA) with unequal sample size (ONEWAY, Statistical Package for the Social Sciences).\(^5\) The least significant difference test was only applied when the ANOVA F ratio indicated a difference between groups at \(p < 0.05\).\(^6\) Analyses of strain, age, and acetylcholine concentration effects were performed by using a nonparametric two-way ANOVA with interaction for unbalanced groups. This data analysis was performed with the General Linear Models statistical package developed by the SAS Institute (Carey, North Carolina).\(^7\) Data are expressed as means ± SEM.

**Results**

Systolic blood pressure increased rapidly in the SHRs from 5 to 12 weeks of age and subsequently stabilized at approximately 195 mm Hg (Figure 1). Blood pressure also increased in WKYs at 8 to 12 weeks, but the increment was less, and pressure stabilized at a significantly lower value (approximately 130 mm Hg; \(p < 0.001\)).

Blood pressure in the 8- and 18-week-old SHR donors was significantly greater than in the age-matched WKY donors (\(p < 0.001\), Student’s t test). The blood pressures of the animals used as explant donors were comparable to those of the animals measured sequentially (Figure 1). At 5 weeks of age, both SHRs and WKYs are known to be in the normotensive range. Thus, to be certain that the SHRs used at 5 weeks of age would have developed hypertension and the WKYs would have remained normotensive, litter mates were saved and their blood pressure was measured when they were 8 weeks old. At 8 weeks of age, the blood pressure in these litter mates was 156 ± 5 mm Hg in SHRs and 133 ± 4 mm Hg (\(p < 0.01\)) in WKYs and was comparable to that observed in other rats of the respective strains at 8 weeks of age.

Explants from 8-week-old donor SHRs and WKYs were exposed to one of three different concentrations of acetylcholine or vehicle on Day 4 of culture. As shown in Figure 2, the magnitude of the VP response to acetylcholine was concentration-dependent (F = 8.03, \(p = 0.0015\); two-way ANOVA). The addition of vehicle did not significantly alter VP release from explants from either SHRs (116% ± 21% of control, \(n = 6\)) or WKYs (110% ± 32% of control, \(n = 5\)). All explants from SHRs responded to acetylcholine at all concentrations tested, with an increase in VP release compared to basal VP release (\(p < 0.05\) at 10^{-8} \text{ M} and \(p < 0.001\) at 10^{-6} \text{ M}, paired t test). Explants obtained from WKYs showed a significant increase in VP release when exposed to 10^{-6} and 10^{-4} \text{ M} acetylcholine (\(p < 0.05\) and/\(p < 0.001\), respectively, paired /

![FIGURE 1. Systolic blood pressure in spontaneously hypertensive rats (SHRs [●], \(n = 10\)) and Wistar-Kyoto (WKYs [○], \(n = 8\)) rats monitored on consecutive weeks from 5 to 18 weeks of age. Values are mean blood pressures ± SEM. The mean blood pressures of the groups of rats actually used in the experiments reported here are indicated by +, §y:](image-url)
FIGURE 2. Concentration-dependent effects of acetylcholine on vasopressin (VP) release from hypothalamic-neurohypophysial (HNS) explants obtained from 8-week-old spontaneously-hypertensive rats (SHRs) and Wistar-Kyoto (WKY) donors. The response to acetylcholine by explants from SHR donors was significantly greater than that by explants from normotensive donors, *p < 0.001. Acetylcholine stimulated VP release at all concentrations tested on the explants from SHRs (*p < 0.05; **p < 0.001; paired t test of basal vs stimulated release). On explants from WKYs, however, acetylcholine was only effective at 10^{-6} and 10^{-5} M (**p < 0.01; ***p < 0.001; paired t test). Values are means ± SEM.

In experiments comparable to those described for explants from 8-week-old donors, explants from 18-week-old SHR and WKY donors were exposed to one of three different concentrations of acetylcholine (10^{-8} and 10^{-6} M, *p < 0.05 by least-significant difference method for evaluating differences) and was diminished below statistical significance at a maximally effective concentration (10^{-4} M). Basal VP release from WKY explants was 48 ± 11 pg/hr • explant and 116 ± 23 pg/hr • explant, respectively, for those exposed to 10^{-8} M acetylcholine; 165 ± 27 pg/hr • explant and 213 ± 48 pg/hr • explant for those exposed to 10^{-6} M; and 62 ± 18 pg/hr • explant and 121 ± 48 pg/hr • explant for those exposed to 10^{-4} M.

In experiments comparable to those described for explants from 8-week-old donors, explants from 18-week-old donor SHRs and WKYs were exposed to one of three different concentrations of acetylcholine on Day 4 of culture. The data from these experiments are presented in Table 1. The VP response to acetylcholine was concentration-dependent (F = 11.22, *p = 0.0021, two-way ANOVA), but the response of explants from SHR donors was comparable to the response of explants from WKY donors (F = 0.13, two-way ANOVA). The concentration-response relationship in explants from 18-week-old WKY donors was essentially identical to that of explants from 8-week-old WKYs: For explants from 8- and 18-week-old donors, the response to 10^{-6} M acetylcholine was, respectively, 156% ± 69% of control and 167% ± 21%, and the response to 10^{-4} M was 327% ± 55% and 347% ± 108% (Figure 2). Basal VP release from WKY and SHR explants was, respectively, 370 ± 61 pg/hr • explant and 417 ± 78 pg/hr • explant for explants exposed to 10^{-4} M; 313 ± 101 pg/hr • explant and 435 ± 61 pg/hr • explant for those exposed to 10^{-6} M; and 163 ± 25 pg/hr • explant and 185 ± 28 pg/hr • explant for those exposed to 10^{-4} M acetylcholine.

Explants from 5-week-old donor SHRs and WKYs were exposed to 10^{-6} M acetylcholine on Day 4 of culture. Explants from the SHR donors showed a hyperresponsiveness to acetylcholine relative to the response of explants from age-matched WKY explants (*p < 0.05; Figure 3); all SHR explants responded, and four of five WKY showed increased VP release. Basal VP release by these explants is presented in Table 2.

To evaluate the effect of donor age on cholinergic stimulation of VP release, the previously discussed data on the response of explants to 10^{-6} M acetylcholine from 5-, 8-, and 18-week-old SHR and WKY donors are presented in Figure 3. Analyzed in this manner, there is a significant difference between the response of explants from SHR and WKY donors (F = 7.35, *p = 0.01, two-way ANOVA). There is also significant interaction between the strain and age effects (F = 3.79, *p = 0.03, two-way ANOVA). The least-significant difference method for evaluating differences between means (p < 0.05) indicated that the response of the explants from 5- and 8-week-old SHR donors was different from that of explants obtained from age-matched WKY donors. Furthermore, the response of explants from 8-week-old SHR explants was significantly different from that observed in explants from 18-week-old SHRs. Thus, the hyperresponsiveness that characterizes explants from 5- and 8-week-old SHRs is not apparent in explants from 18-week-old donors.

Basal VP release was not significantly different between explants from SHR and WKY donors in any of the individual experiments. Furthermore, as shown

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**TABLE 1. Vasopressin Response to Acetylcholine by Explants from 18-Week-Old Donors**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Acetylcholine concentration</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>10^{-8} M</td>
<td>172±19</td>
<td>7*</td>
<td>347±99</td>
<td>6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>206±53</td>
<td>166±26</td>
<td>8t</td>
<td>435±87</td>
<td>8t</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*p < 0.005, paired t test of basal vs stimulated release.

tp < 0.025, paired t test of basal vs stimulated release.
TABLE 2. Basal Vasopressin (VP) Release and Degradation and Neural Lobe Content of Hypothalamo-neurohypophyseal (HNS) Explants on Day 4 of Culture

<table>
<thead>
<tr>
<th></th>
<th>5 weeks old</th>
<th>8 weeks old</th>
<th>18 weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>VP release (pg/hr)</td>
<td>56±8</td>
<td>43±6</td>
<td>101±17</td>
</tr>
<tr>
<td>VP degradation (pg/hr)</td>
<td>38±11</td>
<td>38±12</td>
<td>39±18</td>
</tr>
<tr>
<td>Number of explants</td>
<td>9</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>VP content (ng)*</td>
<td>296±29</td>
<td>489±21</td>
<td>435±19</td>
</tr>
<tr>
<td>Number assayed</td>
<td>3</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

*Two-way analysis of variance indicated significant strain (p < 0.005) and age effects (p < 0.001).

in Table 2, when the release rates from all the experiments were evaluated together, there was no significant strain effect on basal VP release (F = 0.8, two-way ANOVA); however, basal VP release increased with donor age in explants from both SHRs and WKYs (F = 53.04, p < 0.001; two-way ANOVA). The increase in basal VP release with age paralleled the increase in VP content of the neural lobe. As shown in Table 2, the VP content of the neural lobe increased with age (F = 52.54, p = 0.0001; two-way ANOVA). The VP content of the neurointermediate lobe was greater in explants from SHR than WKY donors after 4 days in culture (F = 9.48, p < 0.001; two-way ANOVA).

Discussion

The elevation in VP content of the posterior pituitary and decreased hypothalamic VP content in SHRs relative to normotensive WKYs suggest that abnormalities in the synthesis and/or release of VP by SHRs may be responsible for the elevation in plasma and urinary VP in these animals. The observations reported herein support this hypothesis, because cholinergic stimulation of VP release from HNS explants obtained from SHR donors in the developmental phase of hypertension was significantly greater than that of explants from age-matched WKY donors. This observation is consistent with the report by DeVito et al. that potassium-stimulated VP release is greater from perifused HNS preparations obtained from SHRs between 6 and 8 weeks old than from HNS preparations obtained from WKY donors. In our studies, the disappearance of cholinergic hyperresponsiveness in explants obtained from chronically hypertensive SHRs (18 weeks old) relative to age-matched WKYs suggests that either the system becomes inhibited by the higher blood pressure present at this age or that the chronic exposure to this high blood pressure has permanently altered the responsiveness of the supraoptic-neurohypophyseal system to the signals controlling VP release. The in vitro nature of these culture experiments lends credence to the latter possibility, because once placed in culture, the explants no longer receive information from the cardiovascular receptors.

The VP content of the HNS explants at the end of the culture period reflects the previously reported elevation in neural lobe VP content in SHRs relative to WKYs. The elevated VP content may contribute to the exaggerated response of explants from 5- and 8-week-old SHRs to acetylcholine. However, VP release from these explants is not uniformly correlated with HNS
VP content, as evidenced by the similar rates of basal VP release from HNS explants of both strains at all ages and by the similar response of explants from 8-week-old SHR and WKY donors to $10^{-4}$ M acetylcholine, a maximally effective concentration for stimulation of VP release. Furthermore, the hyperresponsiveness is not observed when an osmotic stimulus is used to evoke VP release from HSN explants. Although the difference in VP content of HNS explants between SHR and WKY explants was reduced in explants from 18-week-old donors, neural lobe VP content is still elevated in vivo in chronically hypertensive SHRs. Therefore, the VP content of the neural lobe probably contributes to the differences that exist between SHRs and WKYs in plasma and urinary VP levels and in VP release from HNS explants, but it is not the only determinant of the rate of the VP release.

Acetylcholine was chosen as a stimulus for VP release in these experiments because it has previously been shown to be a potent stimulus for VP release both in vivo and in vitro, and there is evidence supporting cholinergic innervation of the VP neurons. Acetylcholine stimulates VP release from HNS explants in a concentration-dependent manner. Furthermore, it increases VP release in vivo when administered by way of the carotid artery or into the cerebroventricles, and it increases the electrical activity of supraoptic neurons when iontophoresed onto them in vivo, or when added to the superfusion medium in vitro. These electrophysiological studies suggest that cholinergic receptors are localized on supraoptic neurons, and this is further supported by autoradiographic experiments that demonstrated that ($^{3}H$)-alpha-bungarotoxin, a cholinergic receptor ligand, was localized around supraoptic neurons. Cholinergic neurons are located immediately adjacent to the supraoptic nucleus and probably innervate the VP neurons. The proximity of these cells to the supraoptic nucleus suggests that they may be included in the HNS explant. In addition to acting within the supraoptic nucleus to stimulate VP release, acetylcholine may also act on the nerve terminals in the posterior pituitary. Thus, these cholinergic pathways probably play an important role in the regulation of VP secretion. In demonstrating altered cholinergic sensitivity of explants from SHR donors, these studies demonstrate a change in the responsiveness of the VP neuron itself to an excitatory input.

The concentration-response relationship between acetylcholine and VP release in the explants from WKY donors was comparable at all ages evaluated. Furthermore, it was similar to that observed in explants from Sprague-Dawley rats. Thus, the hyperresponsiveness of explants from 5- and 8-week-old SHR donors appears to reflect an abnormality in the sensitivity of these explants to acetylcholine rather than a reduced sensitivity of the explants from age-matched WKY donors. Also, the similar responsiveness of explants from 18-week-old SHR and WKY donors appears to reflect a normalization of cholinergic sensitivity in the SHR rather than a change in the responsiveness of explants from WKY donors.

The role of VP in the development and maintenance of hypertension is poorly understood and may vary substantially in different forms of hypertension. In the SHR, plasma VP is elevated slightly, but urinary excretion of VP is significantly greater during the developmental phase of the hypertension. Since the urinary excretion measurements reflect the integrated 24-hour plasma measurements, this may indicate a lability in the 24-hour plasma VP concentration; this lability may not be detected in plasma samples collected under standardized procedures and could result from hyperresponsiveness of the HNS. In vivo evidence that the VP system in young SHRs is hyperresponsive to at least one physiological stimulus has been obtained in studies evaluating the VP response to a decrease in plasma volume. Thus, a hyperexcitable HNS might result in significantly greater circulating VP concentrations over a 24-hour period, which could potentially contribute to the development of hypertension.

The development of a strain of SHR with diabetes insipidus does not eliminate the possibility that VP plays a causal role in the development of hypertension in SHRs, because the stroke-prone strain of SHRs was used to develop the strain of rats with genetic hypertension and diabetes insipidus. The abnormalities in VP in the stroke-prone SHR strain are different from the VP abnormalities described above for nonstroke-prone SHRs. In the stroke-prone strain of SHRs, VP does not contribute to the development of the hypertension, but may be important in the chronic phase. This is consistent with the ability to develop a strain of stroke-prone SHRs that do not produce VP, but may not clarify the role of VP in the development of hypertension in nonstroke-prone SHRs.

In conclusion, the current studies present evidence for hyperresponsivity of the mechanisms controlling VP release from the neural lobe during the developmental phase of hypertension in HNS explants from SHRs. This hyperresponsiveness was not evident in explants from chronically hypertensive SHRs. Further studies are required to evaluate the relevance of the hyperactivity of the VP system to the development of hypertension, and also to evaluate the role of the hypertension in attenuating the hyperresponsiveness in the older animals.

**Acknowledgments**

We thank Margaret Mudd, Patricia Wantinger, and Carol Sterling for technical assistance; and Dr. Michael Akritas, Assistant Professor of Statistics, University of Rochester, for consultation on the data analysis.

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Cholinergic stimulation of vasopressin release in spontaneously hypertensive rats.
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Hypertension. 1984;6:855-860
doi: 10.1161/01.HYP.6.6.855

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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