Osmotic Regulation of Vasopressin and Renin in Spontaneously Hypertensive Rats

CELIA D. SLADEK, YIH-HUEY CHEN, PAUL F. ARAVICH, AND MARTHA L. BLAIR

SUMMARY  Abnormalities in the vasopressin and renin systems have been reported in spontaneously hypertensive rats (SHR). Therefore, studies were performed to evaluate the responsiveness of these systems to changes in plasma osmolality and sodium concentration. These variables were manipulated in vivo by intraperitoneal administration of distilled water, isotonic saline, or hypertonic saline to 8- and 18-week-old SHR and normotensive Wistar-Kyoto rats (WKY). Animals were decapitated 30 minutes later, and trunk blood was collected. The hypertonic saline injections resulted in an increase in plasma osmolality and serum sodium at both ages (p< 0.001). Serum vasopressin was higher in all groups of animals receiving hypertonic saline (1200 mosm/kg H$_2$O; p<0.05), but the magnitude of increase was not significantly different in the SHR and WKY at either age. Serum renin activity was lower in SHR than in WKY following acute decreases in serum sodium at 8 weeks, but it was the same for both strains at 18 weeks. Both kidney renin content and concentration were lower in SHR than in WKY at 18 weeks but not at 8 weeks. Therefore, the suppressed renin response to acute osmotic challenge in 8-week-old SHR is not the consequence of reduced kidney renin content. The vasopressin response to osmotic stimulation also was evaluated in vitro using hypothalamoneurohypophyseal explants obtained from 5-, 8-, and 18-week-old SHR and WKY. Vasopressin release was significantly increased in response to an increase in osmolality of 12 mosm/kg H$_2$O in all groups (p<0.01), but the response was not significantly different in explants of SHR and WKY at any age. The comparable response of the vasopressin system to osmotic challenge in SHR and WKY is in contrast to the previously observed hyperresponsiveness of the vasopressin system to an acute decrease in plasma volume and to acetylcholine in vitro. Thus, it indicates that the previously observed hyperresponsiveness of the vasopressin system is specific to the stimulus rather than a generalized phenomenon. (Hypertension 10: 476-483, 1987)

KEY WORDS • hypertension • renin-angiotensin system • renal renin content

ALTERATIONS in the responsiveness of the vasopressin (VP) and renin systems to an acute decrease in plasma volume have been observed in spontaneously hypertensive rats (SHR). Specifically, during the development of hypertension in SHR the VP response to an acute decrease in plasma volume is exaggerated, but the renin response is suppressed when compared with that in age-matched normotensive Wistar-Kyoto rats (WKY). The present studies were performed to evaluate whether the VP and renin systems show altered responsiveness to extracellular fluid osmolality and sodium concentration, respectively. VP release is stimulated by increased plasma osmolality detected by hypothalamic osmoreceptors, while renin release is suppressed by elevated plasma sodium due to direct renal effects and, possibly, as a central nervous system-mediated response to changes in cerebrospinal fluid (CSF) sodium concentration. Enhanced VP sensitivity to increases in osmolality have been observed in sheep with deoxycorticosterone acetate (DOCA)-induced hypertension, and a correlation between elevated sodium intake and elevated plasma VP exist in hypertensive men, but not normotensive men, over 50 years of age. In contrast, the increase in CSF VP elicited by intraventricular perfusion with hypertonic saline was re-
duced in SHR relative to WKY. Thus, altered VP responsiveness to osmotic stimuli may exist in the SHR.

In the current studies, the VP and renin responses to acute changes in plasma osmolality and sodium concentration were evaluated in vivo in SHR and WKY at 8 and 18 weeks of age. Alterations in plasma osmolality and sodium were achieved by intraperitoneal injections of distilled water, isotonic saline, or hypertonic saline according to the protocol of Dunn et al. The VP response to osmotic stimulation was also evaluated in vitro using organ-cultured explants of the hypothalamo-monoendothelial system (HNS) obtained from 5-, 8-, and 18-week-old SHR and WKY. These ages represent early (5 weeks of age) and middle (8 weeks of age) stages of the development of hypertension and chronic hypertension (18 weeks of age).

Materials and Methods

Male SHR and WKY were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA) at 3, 6, and 16 weeks of age. Animals were maintained in group cages in the University of Rochester vivarium (Rochester, NY, USA) for 1 week, and during the subsequent week blood pressure was monitored on 2 separate days in all animals by tail plethysmography. Animals were warmed in a box for 10 minutes preceding and during the blood pressure measurement. The box was maintained at either 30°C (renin experiments) or 27°C (VP experiments). Animals were used during the following week at 5, 8, and 18 weeks of age.

In Vivo Experiments

On the morning of the in vivo experiment, rats were transported to the laboratory in their home cages and injections were performed between 0900 and 1100. Rats were decapitated 30 minutes after intraperitoneal injection of 2 ml/100 g body weight of either distilled water or NaCl solution at concentrations of 290 (isotonic; control group), 600, 800, or 1200 mosm/kg H₂O. Trunk blood was collected for radioimmunoassay (RIA) of serum arginine VP (SVP) or serum renin activity (SRA) and for determination of hematocrit and sodium. Explants were prepared for in vitro experiments by tail plethysmography, and during the blood pressure measurement. The explants were maintained in culture for 3 days under conditions described previously. Data are expressed as means ± SEM.

In Vitro Experiments

For the in vitro experiments, HNS explants were prepared as described previously from male SHR and WKY at 5, 8, and 18 weeks of age. Each explant included the neurons of the supraoptic nucleus with their axonal projections extending through the median eminence and terminating in the neural lobe. Explants were maintained in culture for 3 days under conditions described previously. On Day 3 of culture, VP release into the culture medium by each explant was measured by RIA during a control hour (basal release) and during a subsequent test hour (stimulated release) after the addition of NaCl to increase the osmolality of the culture medium. The sampling protocol has been described previously and allows for evaluation of VP degradation in the culture medium. The rate of VP degradation is not significantly different between explants from SHR and WKY at any age.

For each explant, stimulated release was compared with basal release and a paired t test (two-tailed) was performed to evaluate whether the response of that group of explants to the osmotic stimulus was statistically different from basal release. Two-way ANOVA was used to compare the responses between SHR and WKY at different ages.

Vasopressin RIA

For the VP RIA, serum (≤ 1 ml) was extracted immediately following collection using the acetone-ether method of Robertson et al. Extracts were frozen at −20°C and assayed within 2 weeks. The percentage of recovery from the extraction procedure was determined for every group of samples extracted and averaged 61 ± 12%. All VP values were corrected for percentage of recovery. Duplicate 300-μl and single 150-μl aliquots of each serum extract were assayed for VP as described previously using an antiserum provided by Drs. Jacques Durr and Marshall Lindheimer (University of Chicago, Chicago, IL, USA). Synthetic arginine VP (Ferring, Kiel, West Germany), 400 IU/mg, was used as the standard. Interassay coefficient of variation was 7.2%. Tissue and culture medium VP content was determined using antisera developed in conjunction with Amel Products (Brooklyn, NY, USA) as described previously. Hypothalamic and posterior pituitary homogenates were diluted 100-fold and 10,000-fold, respectively, with assay buffer, and duplicate 100-μl and 25-μl aliquots were assayed. VP content of culture medium was determined on duplicate 12-μl and 6-μl aliquots as described previously.

Renin Assay

Renin activity was determined by RIA for angiotensin I (ANG I), using a modification of the method of Haber et al. Rat serum and kidneys were prepared for RIA as previously described. The interassay coefficient of variation for the ANG I assay was 13%.
Results

Systolic pressure was elevated in SHR relative to WKY at all three ages in all the groups used in these studies (Table 1).

In Vivo Vasopressin Release

Plasma osmolality was significantly altered by the distilled water, isotonic saline, and hypertonic saline injections at both ages (8 weeks: $F_{3,32} = 23.89, p < 0.001$; 18 weeks: $F_{3,32} = 32.25, p < 0.001$), but both strains were comparably affected. Figure 1 shows the relationship between SVP and plasma osmolality in SHR and WKY. Two-way ANOVA showed significant treatment effects on SVP at both 8 and 18 weeks of age (8 weeks: $F_{3,32} = 25.28, p < 0.001$; 18 weeks: $F_{3,32} = 46.21, p < 0.001$). However, strain differences were apparent at 8 ($F_{1,32} = 15.8, p < 0.001$), but not at 18 ($F_{1,32} = 0.89, p = 0.35$), weeks of age. Specifically, the SRA was lower in the 8-week-old SHR receiving distilled water and 290 mosm/kg H$_2$O NaCl than in the WKY. This difference was reflected in a significant strain-treatment interaction effect at 8 weeks ($F_{1,32} = 4.19, p < 0.01$). As in the VP experiments, there was no effect on hematocrit.

Kidney renin content and concentration of control rats are presented in Table 3. Two-way ANOVA indicated significant strain ($p<0.001$) and strain-age interaction ($p<0.05$) for both kidney renin content and concentration. In particular, kidney renin content and concentration were the same for SHR and WKY at 8 weeks of age but were reduced in 18-week-old SHR relative to WKY ($F_{1,32} = 54.94, p<0.001$ kidney content; $F_{1,32} = 4.41, p<0.05$ kidney concentration).

In Vitro Vasopressin Release

The response of HNS explants to an osmotic stimulus is shown in Figure 3. NaCl was added to the culture medium at the beginning of the test hour on Day 3 of culture and resulted in an increase in osmolality (from 295 to 307 mosm/kg H$_2$O). VP release was significantly increased in explants from both SHR and WKY of 5, 8, and 18 weeks of age relative to basal release, but there was no significant strain ($F_{2,32} = 0.5$) effect. As shown in Table 1, the basal rate of VP release was not different between SHR and WKY ($F_{1,32} = 0.75$), but it did increase with age ($F_{2,32} = 10.38, p<0.001$), as reported previously.$^{30}$

Discussion

The responses of the VP and renin-angiotensin systems to alterations in osmolality and sodium concentration of the extracellular fluid were compared in SHR.

<table>
<thead>
<tr>
<th>TABLE 1. Body Weights and Systolic Blood Pressure Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>In vivo VP experiment</strong></td>
</tr>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
</tr>
<tr>
<td><strong>In vivo renin experiment</strong></td>
</tr>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
</tr>
<tr>
<td><strong>In vitro experiment</strong></td>
</tr>
<tr>
<td>No. of rats</td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Basal VP release (pg/hr)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. SBP = systolic blood pressure; VP = vasopressin.

*p<0.001, t*p<0.05, compared with values in age-matched SHR, by Student's t tests.
and WKY. The response of the VP system to osmotic manipulation was similar in SHR and WKY during the development and maintenance stages of hypertension in SHR, as was demonstrated by a similar relationship between plasma osmolality and SVP in SHR and WKY at 8 and 18 weeks of age. In contrast, during the developmental phase of hypertension in SHR (8 weeks of age), the renin response to a decrease in SNa was suppressed. A difference between SHR and WKY in the renin response was not observed during the chronic phase of hypertension (18 weeks of age).

Suppression of the renin response to a decrease in SNa in the 8-week-old SHR could reflect either a generalized suppression of renin responsiveness or a specific decrease in sensitivity to changes in extracellular fluid sodium concentration. The previous observation that the renin response to an acute decrease in plasma volume is also suppressed in young SHR indicates that renin release is suppressed in response to multiple stimuli. However, in contrast to the continued responsiveness of renin release to a decrease in SNa observed in chronically hypertensive SHR in the current study, the degree of suppression of the renin response to a decrease in plasma volume increased with age such that 18-week-old SHR showed no change in SRA in response to an acute isonatremic decrease in plasma volume. Thus, during chronic hypertension renin release maintains responsiveness to hyponatremia, but not to hypovolemia.

The decreased renal renin content and concentration in 18-week-old, but not 8-week-old, SHR compared with WKY observed in this study and a previous study are consistent with a generalized suppression of renin release. However, the suppressed renin response to decreased SNa was present in the 8-week-old SHR and therefore preceded the decreased renal renin content. Thus, the abnormality of the renin response was not the consequence of reduced kidney renin content. Furthermore, the reduced renal renin content evident at 18 weeks did not compromise the ability of SHR to respond to acute changes in SNa.

The observation that SRA was lower in the control

### Table 2. Posterior Pituitary and Hypothalamic Vasopressin Content

<table>
<thead>
<tr>
<th>Variable</th>
<th>8-week-old</th>
<th>18-week-old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Posterior pituitary VP (μg/gland)</td>
<td>1.18±0.04</td>
<td>0.97±0.04*</td>
</tr>
<tr>
<td>Hypothalamic VP (ng/homogenate)</td>
<td>66.73±4.86</td>
<td>90.48±9.24*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. VP = vasopressin.

* p<0.001, compared with values in age-matched SHR, by Student's t test.
group of SHR than in the control group of WKY at 8 weeks of age suggests that basal renin levels are suppressed in young SHR. However, no statistically significant difference in control group SRA was observed between young SHR and WKY in another study from this laboratory,1 in which the experimental protocol was identical except that rats were decapitated 3 hours (rather than 30 minutes) after 290 mosm/kg H2O saline injection. Inconsistent observations on plasma renin levels in SHR exist in the literature. Basal plasma renin in SHR is reported to be elevated,17 normal,18-20 or suppressed21,22 during the development of hypertension and either to remain normal,23 become suppressed,20 24,25 or become elevated18 during the established phase of hypertension. Some of this inconsistency may be due to variability in the normotensive strain.22,23 Another source of this inconsistency may arise from considering data obtained from control groups as representative of basal renin values. In the present experiments, SRA in the control group probably is not representative of basal SRA, because the 290 mosm/kg H2O saline solution was apparently slightly hypertonic to the extracellular fluid. This is indicated by the finding that SNa was less than 145 mEq/L in both the 8- and 18-week-old animals receiving the 290 mosm/kg H2O solution. Therefore, these data should not be used as evidence that basal SRA is suppressed in SHR. Instead, the data indicate a differ-

### Table 3. Kidney Renin Content in 8- and 18-Week-Old Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>8-week-old</th>
<th>18-week-old</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (n = 5)</td>
<td>WKY (n = 5)</td>
<td>SHR (n = 5)</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>627 ± 40</td>
<td>640 ± 42</td>
<td>906 ± 44</td>
</tr>
<tr>
<td>Renin content (µg ANG 1/hr/kidney)</td>
<td>29.3 ± 1.9</td>
<td>35.2 ± 4.2</td>
<td>26.9 ± 3.4*</td>
</tr>
<tr>
<td>Renin concentration (ng ANG 1/hr/mg tissue)</td>
<td>47.2 ± 4.4</td>
<td>57.6 ± 9.8</td>
<td>29.6 ± 3.9†</td>
</tr>
</tbody>
</table>

Values are means ± SEM. × = interaction; NS = not significant.
*p<0.001, †p<0.05, for comparison between strains at each age.
The absence of enhanced osmosensitivity of VP also is significant, because it demonstrates that the abnormality underlying the hyperresponsiveness of the VP system to hypovolemia and acetylcholine is selective to specific stimuli rather than a generalized phenomenon affecting the response of the system to all stimuli. Several different phenomena could account for this differential hyperreactivity in the VP system. One possibility that is attractive, because of the obvious presence of abnormalities in the cardiovascular regulatory system in SHR, is that the abnormality responsible for hyperresponsiveness of the VP system to decreases in plasma volume resides in the cardiovascular receptors or neural pathways transmitting this stimulus to the VP neuron. Although abnormalities in the cardiovascular receptors have been reported in SHR, the resetting of these receptors that occurs in SHR would be expected to render the system hyporesponsive rather than hyperresponsive. Thus, abnormalities in the pathways responsible for transmitting information about blood volume and pressure to the VP neurons are more likely. Although these pathways have not been conclusively identified, it is clear that they are largely distinct from the pathways involved in osmotic regulation of VP release. Specifically, the signals from the cardiovascular volume and baroreceptors are transmitted over multisynaptic pathways from the brainstem to the hypothalamus. In contrast, osmotic control of VP release involves osmosensitive elements located in the hypothalamus (see References 2 and 3 for review). Thus, an abnormality in the projections from the brainstem to the VP neurons could confer selective hyperresponsiveness of the VP system to cardiovascular regulatory signals.

A second possibility that could account for differential hyperreactivity in the VP system is the possibility that the VP neuron itself is hyperreactive to selective neurotransmitters. The observation that HNS explants from 5- and 8-week-old SHR release more VP in response to low concentrations of acetylcholine suggests this possibility. This observation is not readily correlated with the differential responsiveness of the VP system to osmotic and volume stimuli, because previous studies have suggested participation of a cholinergic mechanism in the osmotic control of VP release, and cholinergic transmission has not been hypothesized in the cardiovascular control of VP release. Thus, hyperresponsiveness of the system to acetylcholine should render the system hyperresponsive to osmotic stimulation rather than to volume stimulation. The apparent discrepancy between the results of these two studies suggests that acetylcholine is not the only mediator of osmotic stimulation of VP release from the HNS. This suggestion is consistent with the evidence for involvement of multiple osmosensitive systems in the regulation of VP release.

It is interesting that chronically elevated blood pressure did not result in suppression of the VP response to osmotic stimulation in the 18-week-old SHR relative to the WKY, since the hyperresponsiveness of the VP system to an acute decrease in plasma volume disappeared at 18 weeks of age in SHR. The normalization of this response and a reduction in urinary VP excretion observed in SHR during the development of hypertension previously had been interpreted as indica-
active that the system was responsive to the elevated blood pressure and that VP release was suppressed as a result. In humans, Robertson33 has suggested that, although the neural pathways subserving osmotic and cardiovascular regulation of VP release are separate, a single population of VP neurons responds to both types of regulation, and therefore, VP release is an integrated response to combined signals such that the response to changes in either extracellular fluid volume or osmolality is modified by alterations in the other parameter. However, the absence of suppression of the VP response to osmotic stimulation in 18-week-old SHR indicates that chronic elevations in arterial pressure do not serve to inhibit osmotic regulation of plasma VP. This observation is consistent with other data that indicate that osmotic signals can preempt signals from cardiovascular receptors in determining plasma VP concentrations.34

Conservation of the osmotic responsiveness of plasma VP in chronically hypertensive SHR contrasts with the report by Morris et al.4 that the increase in CSF VP elicited by CSF hypertonicity is reduced in SHR. This discrepancy could reflect either differential responsiveness of separate osmoreceptive elements for arterial and CSF osmolality or differential alterations in the VP neurons giving rise to plasma and CSF VP. Morris et al.35 observed that VP release into the CSF was not stimulated by an intravenous infusion of hypertonic saline that increased plasma VP. This finding suggests differential control of VP release into the plasma and CSF. The source of CSF VP is not clearly established, but one potential source is the paraventricular nuclei. VP content in the paraventricular nuclei is reduced in SHR.36 This reduction may underlie the reduced CSF VP response to CSF hypertonicity. Plasma VP is released from the posterior pituitary. In the current study, posterior pituitary content was elevated at all ages in SHR relative to WKY. These observations confirm the previous reports by Crofton et al.32 and Morris et al.,37 as well as our previous observations.1

In the current study, the VP response to an osmotic stimulus was comparable in SHR and WKY at 8 and 18 weeks of age while the renin response to a decrease in SNa was less in SHR than WKY at 8, but not 18, weeks of age. Although the VP system maintains normal response characteristics to osmotic stimuli, it is hyperresponsive to decreases in plasma volume during the early stages of hypertension.1 Similarly, the responsiveness of the renin system is dependent on the stimulus. Renin shows suppressed responses to decreases in both SNa and plasma volume during the early stage of hypertension, but during the chronic phase of hypertension, the renin response to a decrease in SNa is the same as in WKY while the response to an isotonicemic decrease in plasma volume is suppressed.1 Thus, the responsiveness of the VP and renin systems to regulatory stimuli is differentially altered in SHR, and the altered response characteristics of the VP and renin systems in SHR are stimulus-specific. These characteristics of the VP and renin systems may be permissive to the maintenance of hypertension, because in the chronic stage of hypertension both of these systems conserve their sensitivity to regulation by sodium-dependent signals, even in the face of elevated blood pressure.

Acknowledgments

The authors acknowledge the expert technical assistance of Margaret Mudd, Carol Sterling, Mark Gallagher, and Barbara Harvey in the performance of these studies.

References

Osmotic regulation of vasopressin and renin in spontaneously hypertensive rats.
C D Sladek, Y H Chen, P F Aravich and M L Blair

Hypertension. 1987;10:476-483
doi: 10.1161/01.HYP.10.5.476

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/10/5/476

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/