Vasopressin as a Possible Contributor to Hypertension

Yoshio Yazaki, M.D., Yasuyoshi Ohuchi, M.D., Terunao Ashida, M.D., Ron-Chi Tsai, M.D., and Masao Yoshizumi, M.D.

The role of vasopressin as a pressor agent to the hypertensive process was examined. Vasopressin plays a major role in the pathogenesis of DOCA-salt hypertension, since the elevation of blood pressure was not substantial in the rats with lithium-treated diabetes insipidus after DOCA-salt treatment. Administration of DDAVP which has antidiuretic action but minimal pressor effect failed to increase blood pressure to the levels observed under administration of AVP. Furthermore, the pressor action of vasopressin appears to be important in the development of this model of hypertension, since the enhanced pressor responsiveness to the hormone was observed in the initial stage of hypertension. Increased secretion of vasopressin from neurohypophysis also promotes the function of the hormone as a pathogenetic factor in hypertension. An unproportional release of vasopressin compared to plasma osmolality may be induced by the absence of an adjusting control of angiotensin II forming and receptor binding capacity for sodium balance in the brain. However, the role of vasopressin remains to be determined in human essential hypertension.

Evidence has accumulated that vasopressin may play a role in the development and maintenance of hypertension in rats. Elevations in plasma levels and urinary excretion of vasopressin have been observed in the rats with DOCA-salt hypertension, spontaneous hypertension and one-clip, two-kidney Goldblatt hypertension. Furthermore, blockade of plasma vasopressin by the intravenous injection of a vasopressin antiserum or competitive antagonists of vasopressin lowers arterial pressure transiently in these high vasopressin hypertensive states. Crofton et al. have also reported that Brattleboro rats which are homozygous for diabetes insipidus (DI rats), which totally lack vasopressin and the ability to synthesize the hormone, do not develop hypertension when treated with DOCA-salt. Despite these suggestions the mechanism by which vasopressin participates in the development of these types of hypertension is not completely understood.

Vasopressin has been considered to mainly have an antidiuretic action in the physiological concentration, rather than a vasoconstrictor action, and to play a major role in the fluid balance. However, recent findings that vasopressin contributes to the restoration of arterial blood pressure which is reduced during hemorrhage provide support for a physiological role of the pressor action of vasopressin in the arterial blood pressure control system. The administration of an antagonist which blocks the pressor but not the antidiuretic activity of vasopressin in hypertensive rats with high plasma levels of vasopressin, produces an acute reduction in arterial pressure suggesting also that the pressor action of

Key Words:
Vasopressin
DOCA-salt hypertension
Pressor responsiveness
Angiotensin II receptor
Rat

Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo
Supported by grant-in-aid for special project research (No.521105, 56570322) and grant-in-aid for scientific research (No.557209, 58480234) from Ministry of Education, Science and Culture.
Mailing address: Yoshio Yazaki, M.D., Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Japanese Circulation Journal Vol. 48, February 1984 188
vasopressin participates, in part, in maintaining high blood pressure in these hypertensive rats?

In the present study, we aimed to further clarify the role of vasopressin as a pressor agent in the development of DOCA-salt hypertension in the rat in which vasopressin is most likely involved. For this purpose, firstly, we studied effects of the administration of vasopressin and its analogy of the hypertensive properties of DOCA-salt in rats in which endogenous vasopressin was blocked by lithium chloride (LiCl) treatment. Secondly, we investigated the changes in the pressor responsiveness to vasopressin in the DOCA-salt hypertensive rats at different stages of hypertension. Furthermore, we discussed the high secretion rate of vasopressin in those hypertensive rats with special reference to the increased binding capacity of the brain angiotensin II receptors.

MATERIALS AND METHODS

Animals
Deoxycorticosterone acetate (DOCA)-salt hypertensive rats.

Male Wister rats, weighing 100--150g, were anesthetized with ether and their left kidneys were excised. Rats of Groups 1--3 were tested two weeks after surgery as follows:

Group 1: control rats. One half ml of sesame oil alone was subcutaneously injected once a week and tap water was given ad libitum.

Group 2: salt rats. One half ml of sesame oil alone was subcutaneously injected once a week and 1% saline was given to drink.

Group 3: DOCA-salt hypertensive rats. Thirty mg/kg of DOCA suspended in 0.5 ml of sesame oil was subcutaneously injected once a week and 1% saline was given to drink.

The systolic blood pressure was measured every week by tail plethysmography in the conscious state at a temperature of 30°C for 15 to 20 minutes.

Lithium Treated Rats
After left unilateral nephrectomy, the rats were divided into the following four groups:

DOCA-salt group. After surgery, each rat received intramuscular DOCA, 30 mg/kg and were given 1% saline to drink for 5 weeks.

DOCA-salt-lithium group. The same as DOCA-salt group but also receiving daily intraperitoneal injections of 3.5 mEq of lithium/kg from a solution of 150 mM LiCl.

DOCA-salt-lithium group administered with arginine vasopressin (AVP). Administration of AVP was done at a constant infusion rate of 3.4 µg/24h for the remaining 2 weeks of the study by using an osmotic minipump (Alzet model 2002) implanted subcutaneously on Day 21 of DOCA-salt-lithium treatment.

DOCA-salt-lithium group administered with 1-deamino-8-D-arginine vasopressin (DDAVP). DDAVP was infused instead of AVP with an osmotic minipump. The constant infusion rate was 0.625 µg/24h which was equivalent to the antidiuretic action of 3.4 µg of AVP10

Pressor Responsiveness to Vasopressin
Changes in pressor responsiveness to vasopressin was studied in DOCA-salt hypertensive rats. The rats were anesthetized with urethane and injected with 1 mg/kg of phenoxybenzamine intravenously. Catheters were inserted into the femoral artery and vein. Mean blood pressure was recorded from the femoral artery. After blood pressure had stabilized, the pressor responsiveness to graded intravenous injections of arginine vasopressin was determined in each rat. Doses ranged from 5 to 320 ng/kg. The doses on a dose-response curve were randomized.

Angiotensin II Binding Study
Rats were decapitated and the brains were immediately removed and rinsed in chilled Krebs Ringer-Phosphate buffer (ph 7.4). A tissue block consisting of hypothalamus was excised, weighed and used to study the binding of 125I-angiotensin II by the method of Bennett and Snyder11 with some modifications. The tissue was homogenized with 20 volumes of 20 mM sodium bicarbonate in a glass homogenizer with 10 strokes of a tight teflon pestle, and centrifuged at 1,500 × G for 10 minutes. Supernatants were centrifuged at 20,000 × G for 30 minutes. This 20,000 × G pellets were studied as angiotensin II receptor sites as described before.

Bound and unbound hormones were separated by filtration through Milipore nitrocellulose HAWP filters. The radioactivity on filters was measured in an automatic γ spectrometer with a counting efficiency of 50% for 125I. Proteins were determined by the method of Lowry et al.12 Monoiodinated 125I-angiotensin II was obtained from The New England Nuclear Co., Boston. The specific activity was 1.5 mCi/µg.
TABLE 1  TIME-COURSES OF MEAN BLOOD PRESSURE IN THE DOCA-salt RATS

<table>
<thead>
<tr>
<th></th>
<th>Start</th>
<th>3 weeks</th>
<th>5 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control DOCA-salt group</td>
<td>118.3 ± 3.3</td>
<td>174.0 ± 5.3</td>
<td>178.7 ± 4.4</td>
</tr>
<tr>
<td>LiCl-treated group</td>
<td>115.3 ± 2.9</td>
<td>139 ± 4.3</td>
<td>134.6 ± 10.1</td>
</tr>
<tr>
<td>Administration of AVP</td>
<td>134.7 ± 5.1</td>
<td>168 ± 5.2</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Administration of DDAVP</td>
<td>143.7 ± 4.8</td>
<td>149 ± 5.4</td>
<td>(n = 6)*</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to administration of AVP (means ± SD)

RESULTS

Blood Pressure

Blood pressure remained relatively constant in the rats, after unilateral nephrectomy, given 1% NaCl to drink without DOCA treatment (Group 2) similar to those in the control group (Group 1). However, the rats treated with DOCA and given a 1% NaCl solution to drink (Group 3) became hypertensive with a significant increase in blood pressure occurring two weeks after DOCA treatment (p < 0.01). Systolic blood pressure increased progressively throughout the 5 weeks of treatment to a level 60 mmHg above its initial value (Table 1).

In the DOCA-salt rats treated together with 0.15M LiCl (DOCA-salt-lithium group), systolic blood pressure also increased substantially during the first 3 weeks of the experiment and then reached a plateau in the remaining 2 weeks of the study. However, the increase in systolic blood pressure found in the DOCA-salt rats treated with lithium was much less (p < 0.01) than in the DOCA-salt rats as shown in Table I. After the lithium treatment, urinary volume was markedly increased.

The administration of arginine vasopressin (AVP) for two weeks resulted in a marked increase in systolic blood pressure up to the level of the DOCA-salt rat without lithium treatment. Again, the administration of 1-deamino-8-D-arginine vasopressin (DDAVP), which is a potent AVP analogue with a prolonged and specific antidiuretic action, showed a significant elevation of systolic blood pressure (p < 0.05) in the DOCA-salt rats treated with lithium. A marked decrease in the urinary volume followed. However, as shown in Fig. 1, this increase in systolic pressure was much smaller (p < 0.05) compared to that observed in the case of the administration of arginine vasopressin which also has a vasoconstrictor effect.

Fig. 1. Elevations of mean arterial blood pressure after DOCA-salt treatment. Columns are means and vertical lines ± 1 SD. Li (-): DOCA-salt group, Li (+): DOCA-salt-lithium group, Li (+) + AVP: DOCA-salt-lithium group administered with AVP, Li (+) + DDAVP: DOCA-salt-lithium group administered with DDAVP. Details are described in Methods. Values for Li (-) and Li (+) groups are increases in blood pressure after 3 weeks of DOCA-salt treatment. Values of dotted columns for Li (+) + AVP and Li (+) + DDAVP groups are added elevations of blood pressure during 2 weeks of administration of AVP or DDAVP, respectively.

Pressor Responsiveness to Vasopressin

Pressor responsiveness to vasopressin was examined in the anesthetized DOCA-salt hypertensive rats as described in Methods. Prior to testing with vasopressin, mean blood pressure fell by intravenous injection of α-adrenoceptor blockade (phenoxybenzamine) in both the DOCA-salt hypertensive and the control rats (186 to 68 mmHg and 124 to 51 mmHg, respec-
Brain Receptor Binding of Angiotensin II

Angiotensin II binding to brain membranes at various concentrations of angiotensin II was compared among the DOCA-salt hypertensive rats, the salt rats, after unilateral nephrectomy, given a 1% NaCl solution to drink and the control rats. As shown in Fig. 4, the brain membranes from the salt rats bound less angiotensin II at each concentration than those of the control rats. Scatchard analysis of these data showed a 36.1 ± 1.2% lower angiotensin II binding sites in the salt rats compared to the control rats (7.8 ± 0.5 vs 12.2 ± 2.2 fmol/mg-protein, p < 0.02). Despite greater sodium loading, the binding capacity of the brain membranes of the DOCA-salt hypertensive rats increased to the level of the control rats (11.4 ± 1.1 fmol/mg-protein). The binding affinity was not significantly different among three groups.

Levels of the angiotensin-forming enzyme activity at the physiological pH were also determined in the hypothalamic region of these groups of the rats. The plasma renin activity of the salt rats was decreased compared to that of the control rats (1.4 ± 0.5 vs 3.9 ± 0.5 ng/ml/hr). Moreover, in the DOCA-salt hypertensive rats the plasma renin activity was further decreased to a level undetectable in the assay system.
In contrast, the renin-like activity in the brain from the DOCA-salt hypertensive rats was not decreased compared to that from the control rats (1.57 ± 0.10 vs. 1.46 ± 0.12 ng/g/hr), although in the salt rats the renin-like activity was significantly decreased (1.06 ± 0.05 ng/g/hr).

**DISCUSSION**

In the present study, we demonstrated that vasopressin participates as a pressor agent, in part, in the development of DOCA-salt hypertension in rats, and that the enhanced pressor responsiveness to vasopressin is possibly involved in the initial stages of this form of hypertension. Furthermore, we found here that the increased secretion of vasopressin observed in the hypertensive rats results from the lack of the adjusting control in the brain renin-angiotensin-receptor system for sodium balance.

The primary function of vasopressin has been shown many times to alter the water permeability of the renal tubule and to play a major role in fluid balance. However, recent reports have revealed that vasopressin has a direct pressor role in the control of blood pressure. Furthermore, administration of an antagonist which blocks the pressor but not the antidiuretic activity of vasopressin in the DOCA-salt hypertensive rats produces an acute reduction in arterial blood pressure. These observations, together with the finding of the increased plasma level of vasopressin, support the possibility that vasopressin participates as a direct pressor agent in the maintenance of DOCA-salt hypertension in rats.

Recently, Crofton et al. have shown the approaches which are available for evaluating the role of vasopressin in hypertension. They have found that DOCA-salt hypertension cannot be produced in the Brattleboro rat with hereditary hypothalamic diabetes insipidus (DI). Of particular interest are the effects of the replacement of vasopressin and its analogues on arterial blood pressure in these non-hypertensive DI rats with DOCA-salt treatment. In the present experiments, we used the rats with diabetes...
insipidus produced by lithium treatment which blocks the action of endogenous vasopressin. Blood pressure significantly increased 3 weeks after DOCA-salt treatment in the rats with this form of diabetes insipidus. However, the increased levels of blood pressure were much lower compared to those observed in the control DOCA-salt hypertensive rats (Fig. 1), and blood pressure was not increased in the next two weeks of DOCA-salt treatment. The administration of high doses of arginine vasopressin (AVP) for the remaining two weeks of the experiment increased blood pressure up to the almost same level as that in the control DOCA-salt hypertensive rats. On the other hand, the administration of AVP analogue, DDAVP, which has only an antidiuretic action in the concentration we used in this study increased blood pressure to a lesser extent. Saito et al. have also reported that the increase in blood pressure was depressed in the rats with hereditary hypohalamic diabetes insipidus after a combined treatment of DOCA-salt and DDAVP, although they became hypertensive. On the basis of these results, it would appear that vasopressin contributes to the development of DOCA-salt hypertension by virtue of its pressor activity as well as its antidiuretic activity.

However, plasma vasopressin concentrations in DOCA-salt hypertensive, although elevated, are far below the hormone levels sufficient to induce hypertension. Some other factors may participate in the development of hypertension with relation to an increased secretion of vasopressin per se. To test this possibility, we measured the pressor responsiveness to vasopressin in rats both in the later stage and in the initial stage of DOCA-salt hypertension. A marked increase in the pressor responsiveness to vasopressin was demonstrated in all six rats examined after 6 weeks of DOCA-salt treatment. Furthermore, the pressor responsiveness to vasopressin was increased in 4 of 10 rats 5 days after the commencement treatment while their blood pressure was not significantly increased. The very rapid development of increased pressor responsiveness to vasopressin in our experiment compared to other reports may reflect the use of younger animals, and larger doses of DOCA. Incidence of enhanced responsiveness seems to be increased with the duration of the treatment. These observations strongly suggest that the enhanced pressor responsiveness to vasopressin is involved not only in the maintenance of DOCA-salt hypertension in rats but also during the development of this form of hypertension. The mechanism by which pressor responsiveness to vasopressin was increased in the initial stages of DOCA-salt hypertension was not discussed. Cox has reported that the passive stiffness of carotid and tail arteries of the DOCA-salt hypertensive rats increased monotonically with time and that significant changes were shown in carotid arteries after two weeks of treatment. Therefore, it is unlikely that organic changes in arteries are responsible for the enhancement of pressor responsiveness. Another possible explanation of the very early changes in pressor responsiveness is the involvement of the action through the sympathetic nerve and baroreceptors of vasopressin. In the present study, although an α receptor blocker was given, neither baroreceptor denervation nor β receptor block was done, and so the effects through the baroreceptor or β receptor should not be excluded.

In the present study, we also observed the lack of the adjusting control in the brain renin-angiotensin system for sodium balance. As previously reported, we confirmed the findings that plasma levels of vasopressin are elevated, in the rats with DOCA-salt hypertension. Furthermore, we demonstrated that high plasma concentrations of vasopressin are induced by an increased release of the hormone from the neurohypophysis into the blood stream, since the pituitary vasopressin content was decreased in these animals.

The initial stimulus for the secretion of vasopressin is a consequence of increased salt intake, i.e., an increased plasma osmolality. However, we observed an unproportional increase in plasma vasopressin concentration compared with the increase in plasma osmolality in the DOCA-salt hypertensive rats. These findings suggest the presence of other factors responsible for the further increase in vasopressin secretion.

There is evidence that angiotensin II mediates or modulates osmotically stimulated vasopressin release from neurohypophysis through a specific receptor in the brain. Our data in the present study demonstrated that an increase in salt intake results in a reduction in the binding capacity of brain angiotensin II receptors in the control rats followed by a decrease in the renin-like activity of the hypothalamic region. These observations suggest that the decrease in angiotensin II forming and receptor binding in the brain dulled the osmotic stimulus for an increased release of vasopressin induced by salt intake.
and prevented a further increase in the secretion of the hormone. However, heavy salt loading failed to decrease angiotensin II forming and receptor binding capacity in the brain of the DOCA-salt hypertensive rats. These results indicated that the further increase in vasopressin secretion observed in the rats with this form of hypertension may be, at least in part, due to the lack of adjusting control in brain angiotensin II forming and receptor binding capacity for sodium intake.

As we reported, vasopressin apparently contributes to other modes of hypertension such as spontaneously hypertensive rats, although the hormone may not be essential for the production of the hypertension. However, the question of whether vasopressin is involved in human essential hypertension is controversial, since the enhanced secretion of the hormone is not certain.18–20

In conclusion, evidence accumulated in the past several years that vasopressin contributes to the hypertensive process, especially the pathogenesis of DOCA-salt hypertension in the rat. Our present study provides evidence that vasopressin is involved as a direct pressor agent in the development of DOCA-salt hypertension as well as in its maintenance. We demonstrated that the administration of DDAVP could not substantially increase blood pressure in the rat with DOCA-salt-lithium treatment and that pressor responsiveness to vasopressin was enhanced in the initial stage of DOCA-salt hypertension. Furthermore, we showed that heavy salt loading failed to lower brain angiotensin II forming and receptor binding capacity which adjusts the plasma concentrations of vasopressin for sodium balance in this form of hypertension. This may be related to the unproportional vasopressin secretion compared to the increase in plasma osmolality.

Acknowledgements

The authors are grateful to Prof. Fumimaro Takaku, Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo and Dr. Toshikazu Saito, Division of Endocrinology and Metabolism, Jichi Medical School for their kind suggestions and criticisms of the manuscript.

REFERENCES

17. SLADEK CD, JOYNT RJ: Role of angiotensin II in the osmotic control of vasopressin release by the organ-cultured rat hypothalamo-neurohypophysial system. Endocrinol 106: 173, 1980
18. PADFIELD PL, LEVER AF, BROWN JJ, MORTON JJ, ROBERTSON JIS: Change of vasopressin in hypertension: Cause or effect? Lancet 1: 1255, 1976

Japanese Circulation Journal Vol. 48, February 1984