The Importance of Vasopressin in the Mechanism Maintaining Hypertension in the Rat

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The further role of vasopressin in the pathogenesis of hypertension was studied in two different types of hypertensive rats in which the intravenous injection of a vasopressin antiserum reduced arterial blood pressure substantially. The increased secretion of vasopressin was demonstrated in deoxycorticosterone acetate (DOCA)-salt hypertensive and spontaneously hypertensive rats with high salt intake. Angiotensin II binding of the brain receptor which has been postulated to modify osmotically stimulated vasopressin release from neurohypophysis was not affected by sodium balance in these types of hypertensive rats, whereas the decrease in the brain receptor binding of angiotensin II was observed in the control rats. The lack of the adjusting control system in the brain angiotensin II receptors for sodium balance may be, at least in a part, responsible for the enhancement of vasopressin secretion in the hypertensive rats compared to that in the control rats with high salt intake. Since pressor responsiveness to vasopressin was increased in the rats with DOCA-salt hypertension, vasopressin may function as a direct pressor agent in the maintenance of high blood pressure in this type of hypertension.

The primary function of vasopressin has been shown to alter the permeability of the renal tubule to water and to play a major role in fluid balance for many years. However, support for a physiological role of vasopressin in arterial blood pressure control system has been recently provided by Cowley et al., who reported a significant contribution of vasopressin in the restoration of arterial blood pressure during hemorrhage. Furthermore, it has been proposed that vasopressin participates in maintaining high blood pressure in hypertensive rats, since plasma levels of the hormone are elevated and intravenous injection of an antagonist of the pressor action of vasopressin or a vasopressin antiserum resulted in an acute reduction in arterial blood pressure in these animals. Despite these suggestions, the quantitative importance of vasopressin in the maintenance of hypertension in rats has remained to be assessed.

The present study was designed not only to measure vasopressin secretion but to investigate pressor responsiveness to the hormone in the rats with deoxycorticosterone acetate (DOCA)-salt and spontaneously hypertension in which vasopressin has been most likely proposed to participate in the maintenance of hypertension. Furthermore, since evidence suggested that angiotensin II mediates osmotically stimulated vasopressin release from neurohypophysis via specific receptors in the brain, we brought out

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how central angiotensin II receptors adapt to changes in sodium balance and demonstrated a distinctive behavior of brain angiotensin II receptors in these types of hypertension.

MATERIALS AND METHODS

Animals
Deoxycorticosterone Acetate (DOCA)-Salt Hypertensive Rats
Male Wistar rats, weighing 100–150 g, were anesthetized with ether and their left kidneys were excised. Rats of Groups 1–3 were tested 6 weeks after surgery.

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

Group 2: salt rats. One half ml of sesame oil alone was subcutaneously injected once a week and 1% saline was offered as drinking fluid.

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 offered to drink.

Spontaneously Hypertensive Rats
Spontaneously hypertensive rats, 10 weeks old, were maintained on different salt intakes for 4 weeks.

Low salt group: 120 g of low sodium rat pellets (Japan Clea Co., Tokyo) containing 7 mEq of sodium per 100 g were offered. All rats had deionized water ad libitum.

High salt group: 120 g of ordinary rat pellets (Japan Clea Co.) which contained 14 mEq of sodium per 100 g and 1% saline as drinking fluid were offered.

Systolic blood pressure was measured by tail plethysmography in the conscious rat warmed at 30°C for 15 to 20 minutes.

Measurement of Vasopressin
Vasopressin concentrations were determined using a specific and highly sensitive radioimmunoassay procedure developed by Ishikawa et al. Blood samples were collected in Na₂EDTA tubes after decapitation, and plasma was stored at −20°C until the time of assay. Vasopressin content in hypopituitary was also measured. Plasma osmolalities and electrolytes were determined by osmometer and flame photometer, respectively.

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 Snyder with some modification. 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. The 20,000 × G pellets were washed once with 20 mM sodium bicarbonate and subsequently suspended in ice-cold 20 mM Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM EDTA disodium salt, 5 mM dithiothreitol and 10⁻⁴ M phenylmethylsulfonyl fluoride (assay buffer) to give a protein concentration of 2–4 mg/ml.

Bound and unbound hormones were separated by filtration through Milipore nitrocellulose HAWP filters. Radioactivity on filters was measured in an automatic γ spectrometer with a counting efficiency of 50% for ¹²⁵I as previously reported. Proteins were determined by the method of Lowry et al. Monoiodinated ¹²⁵I-angiotensin II was obtained from New England Nuclear Co., Boston. The specific activity was 1.5 Ci/g.

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 1 mg/kg of phenoxymethylamine intravenously. Catheters were inserted into a 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 were determined in each rat. Doses ranged from 5 to 320 ng/kg. The doses within a dose-response curve were randomized.

RESULTS

Blood Pressure
The rats, post unilateral nephrectomy, treated with DOCA and given 1% saline as drinking fluid, became hypertensive with a significant increase in blood pressure occurring one week after DOCA treatment (p < 0.05). The systolic pressure rose up to 188 ± 7 mmHg 6 weeks later. However, blood pressure remained relatively constant in the rats given 1% saline to drink without DOCA treatment.
Normal Wistar rats

![Graph showing blood pressure (BP) over time for normal Wistar rats](image)

DOCA hypertensive rats

![Graph showing blood pressure (BP) over time for DOCA hypertensive rats](image)

Fig. 1. Effects of intravenous injection of a vasopressin antiserum on blood pressure in DOCA-salt hypertensive and control rats. Values given are the mean ± SEM.

In spontaneously hypertensive rats, systolic blood pressure was elevated to 185 ± 6 mmHg by the age of 10 weeks. Between the two different amounts of salt intake described in Methods, no significant difference in blood pressure was observed.

As previously reported by Yajima et al., the intravenous injection of a vasopressin antiserum resulted in an acute reduction in arterial blood pressure in DOCA-salt hypertensive and spontaneously hypertensive rats. In the rats with DOCA-salt hypertension, mean blood pressure substantially fell from 179 ± 8 mmHg to 159 ± 12 mmHg (p < 0.05) (Fig. 1). The reduction in blood pressure was slight in spontaneously hypertensive rats (185 ± 7 to 171 ± 9 mmHg) but significant (p < 0.05).

Vasopressin Concentrations

Plasma concentrations of vasopressin and sodium, plasma osmolality and pituitary vasopressin content were determined in the hypertensive rats as described in Methods. The plasma vasopressin concentration in DOCA-salt hypertensive rats was twice that in control rats (34.3 ± 3.0 vs 16.3 ± 2.1 pg/ml, p < 0.01) (Fig. 2). Plasma sodium concentration was also significantly increased in DOCA-salt hypertensive rats (149.1 ± 0.6 vs 145.0 ± 0.5 mEq, p < 0.01). On the other hand, plasma osmolality was not significantly increased, although the variables tended to be higher in DOCA-salt hypertensive rats compared with control rats (286 ± 2.5 vs 283 ± 2.7 mOsm/L). In contrast, pituitary vasopressin content was significantly lower in DOCA-salt hypertensive rats than in control rats (116 ± 21 vs 313 ± 67 ng/100 g BW, p < 0.01) (Fig. 2). These changes in plasma and pituitary vasopressin concentrations in DOCA-salt hypertensive rats appear to be induced, at least in a part, by sodium loading. This effect of sodium loading on vasopressin concentration was also seen in spontaneously hypertensive rats. Plasma vasopressin concentration was significantly higher in the rats with high salt intake than in the rats with low salt intake (23.8 ± 0.8 vs 17.6 ± 3.4 pg/ml, p < 0.05), whereas pituitary vasopressin content was lower in the rats with high salt intake (101.8 ± 2.4 vs 244.4 ± 6.0 ng/100 g BW, p < 0.01).

Brain Receptor Binding of Angiotensin II

Brain membranes of salt rats, post unilateral

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*Figure 2. Plasma concentration and pituitary content of vasopressin in DOCA-salt hypertensive and control rats. Values given are the mean ± SEM.*
nephrectomy, given 1% saline as drinking fluid bound less angiotensin II than those of control rats. Scatchard analysis of these data showed a 36.1 ± 1.2% lower angiotensin II binding sites in salt rats compared to control rats (7.8 ± 0.5 vs 12.2 ± 2.2 fmoles/mg-protein, p < 0.02) (Fig. 3). However, the binding capacity of brain membranes of the rats was increased to the control level by treatment with DOCA (11.4 ± 1.1 fmoles/mg-protein). The apparent binding affinity calculated from the slope of the regression lines was not significantly changed among these three groups of the rats. In spontaneously hypertensive rats, the difference in the amount of salt intake failed again to act on the binding capacity of the brain angiotensin II receptors. In high salt intake, the rats showed relatively high binding capacity of brain membranes compared with that in low salt intake (13.4 ± 2.0 vs 12.4 ± 0.9 fmoles/mg-protein).

**Pressor Responsiveness to Vasopressin**

Pressor responsiveness to vasopressin was studied 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-adrenoreceptor blocker (phenoxycbenzamine) in both DOCA-salt hypertensive and control rats (186 to 68 mmHg and 124 to 51 mmHg, respectively), but the elevated blood pressure was remained in DOCA-salt hypertensive rats when compared to that in control rats. The pressor responsiveness to graded intravenous injections of vasopressin was substantially increased in the rats with DOCA-salt hypertension (Fig. 4).

**DISCUSSION**

Vasopressin has been shown to have potentially important actions on renal function and the key role which it plays in fluid balance has been known for many years. However, evidence accumulated in the past several years indicated that vasopressin has a direct pressor role in the control of blood pressure! Furthermore, administration of either a vasopressin antiserum or a competitive antagonist of the pressor activity of vasopressin resulted in an acute reduction in blood pressure in the rats with hypertension, such as DOCA-salt hypertension\(^2\)–\(^3\) spontaneous hypertension\(^4\) and one-clip, two-kidney Goldblatt hypertension\(^5\). These observations, together with the findings of the increased plasma level of vasopressin\(^1\) have proposed that vasopressin participates in the maintenance of hypertension in rats. Yajima et al\(^6\) also observed that mean blood pressure was substantially reduced by intravenous injection of a vasopressin antiserum in DOCA-salt hypertensive rats and to a larger extent when compared to spontaneously hypertensive rats. In the present experiments, we have confirmed the findings that plasma levels of

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vasopressin are elevated in the rats with DOCA-salt hypertension and spontaneously hypertensive rats with high salt intake. Since pituitary vasopressin content was decreased in these animals, high plasma concentrations of vasopressin indicate an increased release of the hormone from neurohypophysis into the bloodstream.

The initial stimulus for this increased secretion of vasopressin appears to be a consequence of the increased salt intake, i.e., an increased plasma osmolality. However, an unproportional increase in plasma vasopressin concentrations compared with the increase in plasma osmolality that occurred in these types of hypertensive rats suggested presence of other factors responsible for the further increase in vasopressin secretion.

Of particular interest is the possible relationship between sodium balance and brain receptor binding of angiotensin II. Our data in the present study demonstrated that an increase in salt intake resulted in a reduction in the binding capacity of brain angiotensin II receptors in control rats. Since a recent report by Sladek and Joynt suggested that angiotensin II mediates or modulates osmotically stimulated vasopressin release from neurohypophysis through the specific receptor in the brain, it could be proposed that the decrease in brain receptor binding of angiotensin II blunted osmotic stimulus for an increased release of vasopressin induced by salt intake and prevented a further increase in the secretion of the hormone. However, high salt intake failed to decrease the binding capacity of brain angiotensin II receptors in DOCA-salt hypertensive and spontaneously hypertensive rats. These observations indicated that the further increase in vasopressin secretion that occurred in the rats with these types of hypertension may have been due, at least in part, to the lack of an adjusting control system in brain angiotensin II receptors for sodium balance.

The data presented here also demonstrated that pressor responsiveness to vasopressin was substantially enhanced in DOCA-salt hypertensive rats in addition to the increased plasma concentrations of the hormone. Thus, vasopressin may function as a direct pressor agent in the maintenance of high blood pressure in the rats with this form of hypertension.

In conclusion, evidence accumulated in the past several years indicates that vasopressin may play a significant role in the pathogenesis of hypertension in rats, especially DOCA-salt hypertension. We demonstrated the increased secretion of vasopressin and the enhanced pressor responsiveness to the hormone in DOCA-salt hypertensive rats. Furthermore, our present study provides evidence that increased salt intake fails to lower brain receptor binding of angiotensin II, which appears to modify vasopressin release from neurohypophysis, in both DOCA-salt hypertensive and spontaneously hypertensive rats. This may be related to the substantial increase in vasopressin secretion and of pathological significance in these forms of hypertension in rats.

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