Enhanced Vasopressin Release upon Activation of the Central Neural Mechanism in Spontaneously Hypertensive Rats

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Summary Monopolar electrical stimulation of the intermediate portion of the solitary nucleus in spontaneously hypertensive rats (SHRs) with cervical cordotomy and vagotomy resulted in a 1.68 times greater increase in plasma vasopressin concentration than in Wistar rats. The result implies that such an enhanced function of the central neural mechanism to release vasopressin in SHR backs up the functionally aberrant cardiovascular receptors.

Key words: spontaneously hypertensive rats, solitary nucleus, plasma vasopressin.

Although a very small but significant difference in plasma vasopressin concentration (PVP) is often reported between spontaneously hypertensive rats (SHRs) and normotensive rats, the PVP in the resting state was found to be maintained at substantially normal levels in SHRs as in normotensive rats (e.g. RASCHER *et al.*, 1982; ROCKHOLD *et al.*, 1984). It is conceivable therefore that the overall function of the cardiovascular reflex mechanisms which tonically regulate vasopressin release, operates normally. On the other hand, it is an established fact that the baroreceptors are aberrant in SHRs (BROWN, 1980). A reduced sensitivity of carotid chemoreceptors has also been suggested in SHRs (PRZYBYLSKI, 1981).

Such findings inevitably raise the question of whether or not the central neural mechanism, which regulates vasopressin release and upon which the terminals of afferents of the receptor mechanisms about, backs up the functionally aberrant

cardiovascular receptors so that the overall function of the reflex mechanisms remains normal. The present study was undertaken therefore to assess the neurosecretory function of the central neural mechanism for releasing vasopressin in SHR. In an attempt to activate such a neural mechanism, we electrically stimulated the intermediate portion of the solitary nucleus upon which afferents of the cardiovascular reflexes are known to terminate (PALKOVITS and ZABORSZKY, 1977; SPYER, 1981). The stimulation is reported to elicit a consistent increase in PVP (YAMANE *et al.*, 1984; NAKAI *et al.*, 1985).

Experiments were conducted during the day on 19 male SHR and 25 male normotensive Wistar rats, aged 13 to 15 weeks. Details of the experimental procedure have been given in a previous paper (NAKAI *et al.*, 1985). Thin polyethylene cannulae were introduced into the femoral arteries and veins bilaterally under 2% halothane anesthesia. The animals were then artificially ventilated, immobilized with *d*-tubocurarine (0.5 mg/kg body weight, i.m.) and maintained in a homeothermic state. The bilateral vago-sympathetic trunks were cut. At this stage of the experiments, the baseline arterial blood pressure and heart rate of each rat were monitored at the end of a settling period of 30 min under 60% N₂O anesthesia. Cordotomy was then performed at C₂ on a stereotaxic frame under a second session of halothane anesthesia. The autonomic outflow to the systemic circulation was thus entirely eliminated and the experiment could be carried out under a stable arterial blood pressure upon stimulation of the solitary nucleus. Continuous infusion of phenylephrine via a venous catheter served to combat hypotension. The degree of blood loss during surgery was estimated by weighing the cotton pellets which had absorbed the shed blood. The animals were then replenished with an appropriate amount of blood obtained from outbred rats. On termination of the operative procedures, the anesthetic was again switched to 60% N₂O.

During a subsequent 60-min settling period, cross circulation for sampling of 1.4 ml of arterial blood over 2 min for determination of the PVP was set up with another anesthetized (Nembutal, 40 mg/kg, i.p.) donor rat (Wistar rat). This procedure allowed repetitive sampling of blood to be carried out without producing hypovolemia in the SHR (NAKAI *et al.*, 1985).

The monopolar electrode for stimulation consisted of an insulated stainless steel wire (0.15 mm in diameter) with only the bevelled tip exposed. An indifferent electrode (chlorided silver plate) was embedded in the muscles of the back. The electrode was lowered with a micromanipulator into the solitary nucleus and was fixed in place where the vasopressin-induced pressor response (NAKAI *et al.*, 1982, 1985; YAMANE *et al.*, 1984) was elicited with the lowest threshold current of stimulus. Arterial blood was then sampled for determination of the control PVP. Beginning 2 min before the start of the second sampling, the solitary nucleus was stimulated (NAKAI *et al.*, 1985) with intermittent (1 s on/1 s off) trains of pulses (50 Hz; 0.5 ms width) over 4 min. During this period of stimulation, the phenylephrine infusion was reduced in rate or discontinued so that the marked vasopressin-induced pressor response was minimal. Otherwise, considerable bleeding would

Table 1. Body weight, hemodynamic parameters, arterial blood gas values, phenylephrine (PE) infusion rate ($\text{ng} \cdot \text{min}^{-1} \cdot (100 \text{ g body weight})^{-1}$), and control plasma vasopressin concentration (PVP, pg/ml).

	Wistar rats	SHRs
Number of animals	25	19
Body weight (g)	389 ± 7	$297 \pm 5^{***}$
Threshold (μA)	9.6 ± 0.7	$6.6 \pm 0.7^{***}$
Blood pressure (mmHg) Control	123 ± 2	$182 \pm 3^{***}$
Stimulation	$141 \pm 3^{\dagger\dagger}$	$200 \pm 3^{***,\dagger\dagger}$
Heart rate (beats/min) Control	376 ± 5	$388 \pm 8^{\text{ns}}$
Stimulation	$351 \pm 5^{\dagger\dagger}$	$373 \pm 9^{*,\dagger}$
PE infusion rate (iv) Control	178 ± 23	$184 \pm 35^{\text{ns}}$
Stimulation	$15 \pm 9^{\dagger\dagger}$	$0 \pm 0^{***,\dagger\dagger}$
Control PVP	20.5 ± 2.5	$29.3 \pm 3^*$

Data are presented as mean $\pm$ S.E. * The difference from the Wistar rats was significant ($p < 0.05$, unpaired t -test); *** $p < 0.001$; ns not significant ($p > 0.05$). † The difference from the control was significant ($p < 0.05$, paired t -test); †† $p < 0.001$.

have taken place. The PVP was measured according to the radioimmunoassay technique proposed by HUSAIN *et al.* (1973). The vasopressin antiserum was a kind gift from Dr. T. Saito, Jichi Medical College, Tochigi, Japan. Data from the rats were abandoned when the control PVP exceeded a level of 50 pg/ml.

The baseline mean arterial pressure observed before cordotomy (data not shown) was approximated well by the control pressure (Table 1) which was set artificially by adjusting the phenylephrine infusion after cordotomy and observed shortly before stimulation of the solitary nucleus. The pressure during stimulation was moderately elevated, despite the phenylephrine infusion being reduced in rate or discontinued. It was found that the threshold current, which increased the mean arterial pressure by 10 mmHg, was significantly lower in the SHRs. In both the SHRs and Wistar rats, the control PVP was considerably higher than reported previously (e.g. RASCHER *et al.*, 1982; ROCKHOLD *et al.*, 1984), in spite of the replenishment of blood. Figure 1 shows the stimulation-evoked increase in PVP in Wistar rats and in SHRs. At a stimulus intensity of 50 μA , the PVP in the SHRs was found to be increased to a 1.68 times higher level than in the Wistar rats. We conclude therefore that the central neural mechanism which emanates from the solitary nucleus possesses an enhanced sensitivity for regulating vasopressin release in SHR.

Our study to observe the changes in PVP upon stimulation of the solitary nucleus was performed under a different phenylephrine infusion rate and arterial blood pressure from those in the control study (Table 1). These differences themselves might modify the degree of vasopressin release (SHARE, 1976; SCHRIER *et al.*, 1979; BRUNNER *et al.*, 1983) upon stimulation. In a previous paper (NAKAI *et al.*,

Fig. 1. Increase in plasma vasopressin concentration (Δ PVP; mean $\pm$ S.E.) upon electrical stimulation of the intermediate portion of the solitary nucleus in normotensive Wistar rats (solid lines with closed circles) and in age-matched SHR rats (broken lines with open circles). Numerals in parentheses indicate the number of animals. *** The difference from the Wistar rats was significant ($p < 0.001$, two-way analysis of variance); ns not significant ($p < 0.05$).

et al., 1985), however, we demonstrated that with our experimental setup, modification of the increase in vasopressin release upon stimulation was not detected regardless of the rate of phenylephrine infusion or level of blood pressure.

Although the blood loss at the time of surgery was compensated by blood transfusion, the control PVP observed shortly before stimulation of the solitary nucleus was very high. Such a high control PVP may be accounted for by the surgery, artificial ventilation, and sectioning of the vagus and aortic nerves. The high PVP itself might then modulate the degree of increase in PVP upon stimulation through some negative feedback mechanism (SKLAR and SCHRIER, 1983). However, the high control PVP may not invalidate our data since the averaged control PVP values obtained in Wistar rats and in SHR rats were at the same general level as each other. In the present report, we found a lower threshold current in SHR rats than in Wistar rats. This result, however, may not imply an enhanced sensitivity of central neural mechanism to release vasopressin in SHR rats, since the arteries of SHR rats are known to possess an enhanced responsiveness to vasopressin (SUETA *et al.*, 1983).

It is an established fact that the optimum work range of cardiovascular receptors of SHR rats is reset towards a higher blood pressure level in atrial stretch receptors (THOREN *et al.*, 1979) and in arterial baroreceptors (BROWN, 1980). In addition to resetting, the arterial baroreceptors, which inhibitorily regulate vasopressin release (SHARE, 1976; SCHRIER *et al.*, 1979), are known to possess a reduced sensitivity to a change in pressure in SHR rats when compared with that of normotensive control rats (BROWN *et al.*, 1976; SAPRU and WANG, 1976; ANDRESEN *et al.*,

1980). It is conceivable therefore that such reduced sensitivity of the baroreceptors in SHRs could lead to less sufficient disinhibition of vasopressin release upon hypotension than in normotensive rats. The carotid chemoreceptors, whose activation increases vasopressin release (SHARE and LEVY, 1966), have also been suggested to be less sensitive in SHRs (PRZYBYLSKI, 1981). Stimulation of the chemoreceptors in SHRs would then fail to adequately raise plasma vasopressin concentration. However, it was reported in SHR (ROCKHOLD *et al.*, 1984) that an exaggerated response of vasopressin release resulted from hemorrhagic hypotension down to such a low level that the chemoreceptors would be amply stimulated (EYZAGUIRRE *et al.*, 1983) together with a suppression of the baroreceptor activity. Further, despite such aberrant function of the cardiovascular receptors, the PVP in the resting state was found to be maintained more or less within the normal range in SHRs as in normotensive rats (e.g. RASCHER *et al.*, 1982; ROCKHOLD *et al.*, 1984).

To reconcile these conflicting findings, it may be logical to assume as follows: (a) the central ascending neural mechanism which emanates from the solitary nucleus and upon which the terminals of the afferents of the cardiovascular receptors terminate (PALKOVITS and ZABORSZKY, 1977; SPYER, 1981), possesses an enhanced sensitivity for regulating vasopressin release in SHR; (b) the central neural mechanism may then back up the reduced sensitivity of the cardiovascular receptors; (c) thus, the overall function of the entire cardiovascular reflex mechanisms may operate adequately in the resting state, and even hyperactively upon hemorrhagic hypotension.

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