α -Adrenoceptor Subtype in the Hypothalamic Paraventricular Nucleus Involved in the Regulation of Urine Production: Comparison between Wistar Kyoto and Spontaneously Hypertensive Rats

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ABSTRACT—Microinjections of α -adrenoceptor agonists into the paraventricular nucleus (PVN) of Wistar Kyoto rats (WKY) decreased the urine outflow rate in dose- and time-dependent manners. The order of the antidiuretic potency is norepinephrine (an α -agonist) > phenylephrine (an α_1 -agonist) $\gg$ clonidine (an α_2 -agonist). The phenylephrine-induced effect was inhibited by WB4101 (an α_1 -antagonist), but not by yohimbine (an α_2 -antagonist). $d(\text{CH}_2)_5\text{-D-Tyr(Et)VAVP}$ (a vasopressin antagonist) blocked the antidiuretic effect of phenylephrine. In spontaneously hypertensive rats (SHR), norepinephrine and phenylephrine produced weaker antidiuretic effects than in WKY. These findings suggest that the α_1 -subtype of the PVN decreases urine production mediated through vasopressin release. This mechanism is more feeble in SHR than WKY.

Keywords: α_1 -Adrenoceptor, Paraventricular nucleus, Vasopressin

Spontaneously hypertensive rats (SHR) have frequently been used as a model animal for essential hypertension in humans. It had been suggested that catecholaminergic neurons of the hypothalamic paraventricular nucleus (PVN) are involved in the development and/or maintenance of hypertension in SHR (1–5). In the PVN of SHR, there are increased synthesis and release of norepinephrine as compared to Wistar Kyoto rats (WKY) (2, 3, 5). Since the PVN localizes the cell bodies of the vasopressin-containing neurons and synthesizes precursors of vasopressin, we have studied effects of β -adrenoceptor agonists injected into the PVN of SHR on the urine outflow rate (6). We found that 1) the β -adrenoceptor whose stimulation decreased urine production in the PVN of SHR was the β_2 -subtype, although it was unclear in the control rats, 2) β -adrenoceptor-mediated decrease in urine outflow in SHR was similar to that in WKY and 3) this mechanism was not mediated through vasopressin release. In this study, we compared the effects of α -adrenoceptor agonists injected into the PVN on urine outflow in SHR and WKY.

Male SHR (17- to 23-week-old) and age-matched WKY inbred in our laboratory (12-hr light/dark cycle, $23 \pm 1^\circ\text{C}$) were used. Their origin was the Okamoto-Aoki strain. The rats, starved for 17 hr, were orally administered tap water (5 ml/100 g body weight) followed

by, 45 min later, the same volume of 12% ethanol for anesthesia. After the trachea, urinary bladder and jugular vein of the animals were cannulated with polyethylene tubes, they were immobilized in a stereotaxic instrument. During the experiment, Locke's solution containing 3% ethanol was infused at 0.1 ml/min through the jugular vein cannula to maintain anesthesia and urinary volume for several hours. Urinary drops flowing from the bladder cannula were continuously counted with a photoelectric drop counter. When the urine outflow rate became a constant level, a stainless steel cannula (o.d.: 200 μm) connected to a microsyringe was inserted into the PVN (coordinates: 5.6 mm anterior from the lambda, 0.3 mm to the right from the midline, 7.8 mm ventral from the dural surface) for the administrations of norepinephrine bitartrate (NE), phenylephrine hydrochloride (PHE), methoxamine hydrochloride (MET), clonidine hydrochloride (CLO), yohimbine hydrochloride (YOH) (Sigma Chemical Co., St. Louis, MO, USA) and 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB4101; Research Biochemicals, Inc., Natick, MA, USA) (1 μl). 1-(Mercapto- β , β -cyclopentamethylene propionic acid)2-(*O*-ethyl)-D-tyrosine, 4-valine, arginine-vasopressin ($d(\text{CH}_2)_5\text{-D-Tyr(Et)VAVP}$; a gift from Prof. K.G. Hofbauer, Cardiovascular Division, Ciba-Geigy, Ltd., Basel, Switzerland) (7) and Arg-

Fig. 1. Dose-effect curves for antidiuresis induced by microinjection of the α -adrenoceptor agonists into the PVN of SHR and WKY. The abscissa shows the dose in nmol of phenylephrine (SHR: ○, WKY: ●), norepinephrine (SHR: △, WKY: ▲) and clonidine (SHR: □, WKY: ■). The ordinate indicates the maximum decrease in the urine outflow rate, at 20 or 30 min after the administration, expressed as a percentage of the control level (urine outflow for 10 min before drug administration). The values are the mean $\pm$ S.E.M. of 3–9 experiments.

vasopressin (Grade IV, Sigma) were intravenously injected through the jugular vein cannula (0.1 ml). All drugs were dissolved in saline.

In WKY, NE (a non-selective α -adrenoceptor agonist) and PHE (an α_1 -agonist), when injected into the PVN, produced dose-dependent antidiuresis (Fig. 1). The antidiuretic effect of NE at 10 nmol began to appear within 5 min after the administration, the maximum effects were observed at 20–30 min after the administration, and the urine outflow rate recovered to the control level (the urine outflow rate before the drug administration) at 60 min after the administration. Microinjection of CLO (an α_2 -agonist) at 160 nmol into the PVN was also antidiuretic. The order of their potencies was NE > PHE >> CLO. In SHR, NE and PHE were less potent as antidiuretic agents. The antidiuretic effect of CLO in SHR was similar to that in WKY (Fig. 1). In WKY, WB4101 (an α_1 -antagonist), injected at 20 nmol into the PVN, inhibited the PHE (40 nmol)-induced antidiuresis. The small antidiuretic response still remained at 20 min, and the diuretic response appeared at 40 and 50 min after the administration (Fig. 2A). Microinjection of WB4101 alone into the nucleus slightly decreased the urine outflow rate ($54 \pm 16.3\%$ at 30 min, $75 \pm 18.6\%$ at 40 min, $86 \pm 17.6\%$ of the control level at 50 min after the administration; $*P < 0.05$ vs the saline-injected group at the corresponding time-points). Intra-PVN injection of YOH (an α_2 -antagonist) at 10 nmol did not inhibit the PHE-induced antidiuretic effect (Fig. 2A). When $d(\text{CH}_2)_5\text{-D-Tyr(Et)VAVP}$ (16.7 μg , a non-specific vasopressin receptor antagonist, Ref. 7) was injected intravenously, PHE

did not cause antidiuresis, but caused marked diuresis having a 70-min duration (Fig. 2B). Another α_1 -agonist, MET at 40 nmol elicited the antidiuretic effect with a time course similar to that of the PHE (40 nmol)-induced effect. The MET-induced antidiuresis was converted to diuresis after intravenous injection of $d(\text{CH}_2)_5\text{-D-Tyr(Et)VAVP}$. The vasopressin receptor antagonist alone showed no effect on the urine outflow rate. We confirmed that the vasopressin antagonist completely blocked antidiuresis induced by intravenous injection of 4 mU vasopressin under the same experimental condition (8).

The present results suggested that the α -adrenoceptor in the PVN for the decrease in urine production was the α_1 -subtype in ethanol-anesthetized hydrated rats. The weak antidiuresis of WB4101 was supposed to be non-specific. The α_1 -adrenoceptor agonist-induced antidiuresis was mediated through vasopressin release since the PHE- and MET-induced antidiuretic effects were inhibited by the vasopressin receptor antagonist. Veltmar et al. suggested that the third α -adrenoceptor subtype, neither α_1 - nor α_2 -subtype, of the PVN decreases vasopressin release in unanesthetized rats (9). This shows a possibility that vasopressin release is regulated by various mechanisms and a certain condition activates the appropriate, suitable mechanism for regulation. That is, α_1 - and the third α -adrenoceptors seem to predominantly function under low and high/normal concentration of vasopressin in plasma, respectively. The α_1 -agonists were diuretic in the rats pretreated with $d(\text{CH}_2)_5\text{-D-Tyr(Et)VAVP}$. Antidiuretic effects of oxotremorine (10), substance P (11), angiotensin II (12) and the peptidase inhibitors (13) were

Fig. 2. Inhibitory effects of the α_1 -adrenoceptor antagonists (A) and the vasopressin receptor antagonist (B) on the α_1 -adrenoceptor agonist-induced antidiuresis in WKY. The abscissa and ordinate show the time in min after administration of α_1 -adrenoceptor agonists and the change in the urine outflow rate expressed as a percentage of the control level, respectively. A) ●: 40 nmol phenylephrine (PHE), ○: 20 nmol WB4101 + PHE, △: 10 nmol yohimbine + PHE, □: saline; B) ●: 40 nmol PHE, ▲: 40 nmol methoxamine (MET), ○: 16.7 μ g $d(\text{CH}_2)_5$ -D-Tyr(Et)VAVP + PHE, △: $d(\text{CH}_2)_5$ -D-Tyr(Et)VAVP + MET, □: saline. Pretreatment with the antagonists was performed at 30–50 min before the agonist administration. The values are the mean $\pm$ S.E.M. of 3–6 experiments. ^a $P < 0.05$ vs the closed symbols, ^b $P < 0.05$ vs the saline-treated group (□) at the corresponding time-points. Significant differences were determined by Scheffe's test following ANOVA.

completely blocked by the vasopressin receptor antagonist and no diuretic effect appeared. Also, PHE caused the diuretic effects following the weak antidiuretic effects after the pretreatment with WB4101. These phenomena do not seem simply a rebound effect. We speculated that the α_1 -agonists have two opposite effects, antidiuretic and diuretic, and that diuretic effects are exposed under certain conditions which only inhibit the antidiuretic effects. The antidiuretic effects were mediated through increased vasopressin release via α_1 -adrenoceptors as described above; however, mechanisms underlying the diuresis are yet undefined. At least, the diuretic effects did not involve activation of α_1 -adrenoceptors. The following can be proposed as possible mechanisms: 1) indirect effects to neurons in the hypothalamus that modulate function of the kidney (14), 2) effects resulting from the interaction between the anesthetic, ethanol, and the α_1 -agonists and 3) non-specific effects of the α_1 -agonists. The regulation via the α_1 -subtype in SHR is weaker than that in WKY. The reason is probably down-regulation of α_1 -receptors in the PVN of SHR. It was demonstrated that activities of catecholaminergic neurons (1) and tyrosine hydroxylase (3) and catecholamine release (2, 5) increase in the PVN of SHR more than WKY. Therefore, α_1 -receptors in the

PVN of SHR may be down-regulated.

In conclusion, 1) stimulation of the α_1 -adrenoceptor subtype in the hypothalamic PVN decreases the urine production via vasopressin release and 2) this mechanism is more feeble in SHR than WKY.

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