Effects of β_1 - and β_2 -Adrenoceptor Agonists Applied into the Hypothalamic Paraventricular Nuclei of Spontaneously Hypertensive Rats on Urine Production

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ABSTRACT—We investigated effects of β -adrenoceptor agonists (β_1 -selective: T-1583 and dobutamine, β_2 -selective: fenoterol, non-selective: isoproterenol) on urine outflow rate, blood pressure, heart rate, respiratory rate and rectal temperature. The drugs were applied into the paraventricular nuclei (PVN) of spontaneously hypertensive (SHR), Wistar-Kyoto (WKY) and Wistar rats. Fenoterol and isoproterenol markedly decreased the urine outflow rate, compared with T-1583 and dobutamine in the rats. There was no marked difference among the three strains in responsiveness to fenoterol and isoproterenol. The antidiuretic effects of fenoterol were inhibited by a β_2 -selective antagonist, butoxamine, more markedly than a β_1 -selective antagonist, atenolol, in SHR; and the inhibitory effects of these drugs were partial in WKY. In Wistar rats, the effect of fenoterol was inhibited by a non-selective β -antagonist, timolol, but not by atenolol or butoxamine. A vasopressin antagonist (i.v.) did not diminish the antidiuretic effect of fenoterol. Fenoterol reduced the blood pressure in SHR and WKY, but not in Wistar rats. It was suggested that there were predominantly β_2 -adrenoceptors mediating antidiuresis in SHR. In WKY and Wistar rats, however, the β -adrenoceptor subtypes mediating antidiuresis have yet to be determined. The ability of β -adrenoceptor agonists to decrease urine outflow rates in SHR was not altered as compared to that in the control rats. β -Adrenoceptor-mediated antidiuresis was not due to vasopressin release.

Keywords: Spontaneously hypertensive rat, Antidiuretic effect, Vasopressin, Paraventricular nucleus, β -Adrenoceptor subtype

The hypothalamic paraventricular nuclei (PVN) consists of the magnocellular region, which contains cell bodies of vasopressinergic neurons, and the parvocellular region, which is not fully understood. Histochemical studies have demonstrated the existence of terminals of noradrenergic neurons from the caudal ventrolateral and dorsomedial medulla in the PVN (1, 2). In addition, some neurons from the PVN innervate the nuclei of the tractus solitarius, the autonomic nervous system and the spinal cord (1, 2). Pharmacological studies have shown that the PVN plays important roles in the regulation of vasopressin release, the cardiovascular system or hemodynamics (3–7).

There are some differences between spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY, from which SHR had been derived) in norepinephrine (NE) concentrations, activities of NE synthetic enzymes

and vasopressin concentrations in the PVN or in the hypothalamus (8–13). In addition, blood pressure rises in WKY grafted with the hypothalamus of SHR (14). Electrical lesion of the PVN (15, 16) or intracerebroventricular injections (i.c.v.) of 6-hydroxydopamine (17) delay development of hypertension in SHR. It has been reported that the pressor response of SHR to β_2 -adrenoceptor agonists, applied into the cerebroventricle, is greater than that of WKY (18). Thus, catecholaminergic neurons in the PVN are suggested to play an important role in developing and/or maintaining hypertension in SHR.

The aim of the present experiments is to show pharmacological evidence for differences between SHR and the control rats in adrenergic neuron activities in the PVN. We investigated the effects of β_1 - and β_2 -adrenoceptor agonists, applied into the PVN, on important physiological functions of the nucleus; i.e., urine production and cardiovascular function in SHR.

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MATERIALS AND METHODS

The experimental procedures have been described in detail elsewhere (19). Male SHR (230–385 g, 11- to 19-week-old), WKY (255–400 g, 10- to 20-week-old) and Wistar rats (280–420 g, 9- to 11-week-old) were starved for about 18 hr, but allowed free access to water. All animals were loaded with water (5 ml/100 g, p.o.) and 45 min later, anesthetized with 12% ethanol (5 ml/100 g, p.o.). After cannulae were inserted into the trachea, the jugular vein and the bladder, the rats were placed in a stereotaxic frame (Takahashi Co., Tokyo). The indwelling jugular venous cannula was used to infuse Locke's solution containing 3% ethanol. Ethanol infusion partially inhibits vasopressin release and so allows a constant rate of urine outflow. A stainless steel cannula (outer diameter: 200 μ m) was inserted into the PVN (coordinate: 5.6 mm anterior to the lamda, 0.25 mm lateral to the midline, 8.0 mm below the dura) according to the atlas of König and Klippel (20). One microliter of drug solution followed by an artificial cerebrospinal fluid (2 μ l) for washing out the dead space was microinjected into the PVN through a microsyringe attached to the stainless steel cannula. The microinjection of the drugs was carried out for 10 min. Vasopressin and a vasopressin antagonist were intravenously injected.

Numbers of urinary drops from the bladder cannula were counted by a photoelectric drop counter (Unique Medical Inc., Tokyo). The percentage of urine outflow rate after drug administration with respect to the control level (urine outflow rate for 10 min before the drug administration) was calculated. Since decreases in urine outflow rates induced by the tested drugs became maximal at 30 min, dose-response curves for the drugs were made from the values obtained at 30 min after the drug injection.

Osmotic pressure of urine was measured by the freezing point depression method (The Fiske Osmometer, Model G-62; Fiske Associate, Inc., Uxbridge, MA, USA).

In some rats, mean blood pressure, heart rate, respiratory rate and rectal temperature were monitored throughout the experiments. A polyethylene cannula, attached to a transducer (MPU-0.5-290-III; Nihon Kohden Kogyo Co., Ltd., Tokyo), was inserted into the carotid artery for direct measurement of the blood pressure. The heart rate was measured by an electrocardiograph (FD-14; Fukuda, Tokyo). Respiratory rate and rectal temperature were measured by thermister probes (SR-115S, Nihon Kohden Kogyo Co., Ltd. and MGA III-219, Nihon Kohden Kogyo Co., Ltd.) (19).

Injection sites were verified by the following methods: 1) Lesioning was made with an electric current (3 mA, 60 sec, Lesion Generator Model RFG-4A; Radionics, Inc.,

Burlington, MA, USA). Coronal 15- μ m sections were cut using a freezing microtome (Tissue-Tek II; Miles Inc., Erkhart, IN, USA), and sections were stained with Hematoxylin-Eosin; 2) Microinjection of 2% methylene blue (1 μ l) was performed at the injection sites. Coronal sections were cut with the freezing microtome.

All data are expressed as the mean $\pm$ S.E. Statistical analysis was performed by Student's *t*-test. A significant difference was assumed when the *P* value was less than 0.05.

The following drugs were dissolved in 0.9% NaCl: β_1 -selective agonists: dobutamine HCl (Shionogi Co., Ltd., Osaka) and T-1583 HCl (Tanabe Pharmaceutical Co., Ltd., Osaka); a β_2 -selective agonist: ($\pm$)fenoterol HBr (Boehringer Ingelheim, Kawanishi); a non-selective agonist: ($\pm$)isoproterenol HCl (Sigma Chemical Co., St. Louis, MO, USA); a β_1 -selective antagonist: ($\pm$)atenolol HCl (ICI Pharmaceuticals, Wilmslow, UK); a β_2 -selective antagonist: ($\pm$)butoxamine HCl (Wellcome, Osaka); a non-selective antagonist: ($\pm$)timolol malate (Sumitomo Chemical and Industrial Co., Tokyo) and vasopressin (Sigma Chemical Co.). A vasopressin antagonist, 1-(mercapto- β , β -cyclopentamethylene propionic acid) 2-(*O*-ethyl) D-tyrosine, 4-valine, arginine vasopressin, was a gift from Prof. K.G. Hofbauer (Cardiovascular Division, Ciba-Geigy, Ltd., Basel, Switzerland).

RESULTS

Effects of β -adrenoceptor agonists on urine outflow rate

Locke's solution containing 3% ethanol was infused at a constant rate of 0.1 ml/min. Urine outflow rates obtained were 0.090 ± 0.004 ml/min in Wistar rats (*n*=80), 0.115 ± 0.004 ml/min in WKY (*n*=95) and 0.104 ± 0.004 ml/min in SHR (*n*=81). The urine outflow rate in a given rat was constant during the experiment. The microinjection of vehicle (saline) into the PVN did not change the urine outflow rates.

Isoproterenol (2–40 nmol) microinjected into the PVN decreased urine outflow rates in a dose-dependent fashion in SHR, WKY (Fig. 1a) and Wistar rats. The antidiuretic response to 20 nmol isoproterenol appeared within 20 min after the injection, became maximal at 30 min and lasted for 80 min. Dose-response curves for isoproterenol are shown in Fig. 2; isoproterenol at 5 nmol induced more marked antidiuresis in SHR than in WKY and Wistar rats (*P*<0.05), but the responses to 10 and 20 nmol isoproterenol were similar in the three groups (Fig. 2).

The microinjection of fenoterol (1–10 nmol) into the PVN also decreased the urine outflow rates (Fig. 1b). The time-course of the fenoterol (10 nmol)-induced effect was similar to that of the isoproterenol (20 nmol)-induced effect. The antidiuretic potency of fenoterol in SHR was

Fig. 1. Time-courses of the antidiuretic effects of isoproterenol and fenoterol in SHR and WKY. Open and closed symbols are for SHR and WKY, respectively. a) isoproterenol, $\circ$: 2 nmol; $\triangle$, $\blacktriangle$: 5 nmol; $\square$, $\blacksquare$: 10 nmol; ∇ , $\blacktriangledown$: 20 nmol. b) fenoterol, $\circ$, $\bullet$: 1 nmol; $\triangle$, $\blacktriangle$: 2 nmol; $\square$, $\blacksquare$: 10 nmol. Ordinate: urine outflow rates expressed as a percentage of the control level. Abscissa: time in min after the microinjection of the drugs. Symbols indicate the mean $\pm$ S.E. ($n=3-7$).

Fig. 2. Dose-response curves for the antidiuretic effects of isoproterenol, fenoterol, dobutamine and T-1583 in SHR, WKY and Wistar rats. Ordinate: urine outflow rates at 30 min after the microinjection of the drugs, expressed as a percentage of the control level. Abscissa: doses in nmol of the drugs. $\circ$: Isoproterenol, $\triangle$: Fenoterol, $\square$: Dobutamine, ∇ : T-1583. Symbols indicate the mean $\pm$ S.E. from 4-17 experiments.

similar to those in WKY and Wistar rats (Fig. 2). The antidiuretic effects of fenoterol at 5 and 10 nmol were significantly more marked than those of equimolar concentrations of isoproterenol in SHR, WKY and in Wistar rats (Fig. 2).

Dobutamine at 80 nmol slightly decreased the urine outflow rate in WKY, but not in SHR and Wistar rats. On the other hand, 80 or 160 nmol T-1583 caused antidiuresis in SHR and Wistar rats, but not in WKY (Fig. 2). Dobutamine and T-1583 were less potent as antidiuretic agents than fenoterol and isoproterenol.

Effect of fenoterol on osmotic pressure of urine

To determine whether the fenoterol-induced antidiuresis was due to an increased secretion of vasopressin, effects of fenoterol (applied into the PVN) and vasopressin (applied into the vein) on urinary osmotic pressure were compared. Percent decrease by 5 nmol fenoterol in urine outflow rate at 30 min was similar to that induced by 400 μ U vasopressin injected intravenously at 20 min. Fenoterol increased urinary osmotic pressure to $160 \pm 14\%$ of the control values ($n=4$, Fig. 3a), whereas vasopressin increased it to $344 \pm 37\%$ of the control values ($n=3$, Fig. 3b).

The osmotic pressure of urine collected for the control period was 267 ± 16 mOsm/kg ($n=11$), and the osmotic pressure was not changed by the PVN injection of vehicle (saline) ($n=4$).

Effects of fenoterol on the other functions

As shown in Table 1, the microinjection of fenoterol (5 nmol) into the PVN resulted in decreased blood pressure in SHR and WKY, but not in Wistar rats. The depressor effect in SHR was less marked than that in WKY. Heart rate, respiratory rate and rectal temperature were not changed by fenoterol in the three groups.

Effects of β -adrenoceptor antagonists and a vasopressin antagonist on the fenoterol-induced antidiuresis

Fenoterol at 5 nmol was injected into the PVN at 30–50 min after intra-PVN injection of β -adrenoceptor antagonists or after i.v. injection of a vasopressin antagonist. Butoxamine (25 nmol) inhibited more potently the antidiuretic effect of fenoterol in SHR than in WKY (Fig. 4). Atenolol (5 nmol) partially inhibited the effect of fenoterol in SHR as well as in WKY. In SHR, butoxamine was more effective than atenolol. On the other hand, in Wistar rats, the fenoterol-induced antidiuresis was inhibited by timolol (100 nmol), but not by atenolol or butoxamine (Fig. 4). The microinjection of butoxamine or atenolol alone did not change urine outflow rates, but timolol increased urine outflow rates to $163 \pm 15\%$ and $188 \pm 28\%$ of the control values ($n=4$) in WKY and

Fig. 3. Effects of fenoterol and vasopressin on urine outflow rates and urinary osmotic pressure. Osmotic pressure of urine and urine outflow rates were expressed as a percentage of the control levels. a) 5 nmol fenoterol microinjected into the PVN and b) 400 μ U vasopressin injected intravenously. The control values of osmotic pressure are a) 258 ± 28 mOsm/kg ($n=4$) and b) 300 ± 20 mOsm/kg ($n=3$). The abscissa indicates time in min after the administration of fenoterol or vasopressin. Symbols indicate the mean $\pm$ S.E. *Significantly different from the control values ($P < 0.05$).

Wistar rats, respectively.

The vasopressin antagonist at a dose of 16.7 μ g inhibited vasopressin (4 mU, i.v.)-induced antidiuresis, but did not inhibit the fenoterol (5 nmol)-induced antidiuresis, although the drugs at the doses used produced similar antidiuresis (Fig. 4). The vasopressin antagonist alone did not change urine outflow rates.

DISCUSSION

It has been reported that there are β -adrenoceptor binding sites in the PVN of rats (21). It was suggested that β -adrenoceptors in the PVN or in the hypothalamus were

Table 1. Effects of fenoterol on urine outflow, blood pressure, heart rate, respiratory rate and rectal temperature

	Time after microinjection of fenoterol (min)						
	0	10	20	30	40	50	60
SHR							
Blood pressure (mmHg)	164 $\pm$ 7	162 $\pm$ 7	146 $\pm$ 10	138 $\pm$ 8*	157 $\pm$ 6	162 $\pm$ 9	150 $\pm$ 6
Heart rate (beats/min)	410 $\pm$ 14	408 $\pm$ 14	440 $\pm$ 5	434 $\pm$ 7	424 $\pm$ 8	420 $\pm$ 7	418 $\pm$ 9
Respiratory rate (beats/min)	123 $\pm$ 7	126 $\pm$ 9	132 $\pm$ 15	136 $\pm$ 17	134 $\pm$ 19	129 $\pm$ 16	127 $\pm$ 16
Rectal temperature ($^{\circ}$ C)	36.3 $\pm$ 0.5	36.4 $\pm$ 0.2	36.5 $\pm$ 0.3	36.7 $\pm$ 0.3	36.8 $\pm$ 0.3	36.8 $\pm$ 0.3	36.8 $\pm$ 0.3
WKY							
Blood pressure (mmHg)	134 $\pm$ 11	140 $\pm$ 14	103 $\pm$ 5*	97 $\pm$ 6*	103 $\pm$ 5*	124 $\pm$ 7	127 $\pm$ 8
Heart rate (beats/min)	390 $\pm$ 28	408 $\pm$ 30	446 $\pm$ 30	432 $\pm$ 34	426 $\pm$ 30	416 $\pm$ 33	422 $\pm$ 32
Respiratory rate (beats/min)	102 $\pm$ 10	93 $\pm$ 8	112 $\pm$ 11	100 $\pm$ 13	107 $\pm$ 14	113 $\pm$ 12	114 $\pm$ 9
Rectal temperature ($^{\circ}$ C)	36.1 $\pm$ 0.2	36.2 $\pm$ 0.3	36.5 $\pm$ 0.3	36.7 $\pm$ 0.4	36.9 $\pm$ 0.5	36.8 $\pm$ 0.4	36.9 $\pm$ 0.4
Wistar rat							
Blood pressure (mmHg)	105 $\pm$ 7	107 $\pm$ 8	99 $\pm$ 7	103 $\pm$ 8	99 $\pm$ 6	88 $\pm$ 8	96 $\pm$ 8
Heart rate (beats/min)	443 $\pm$ 10	442 $\pm$ 8	450 $\pm$ 17	465 $\pm$ 6	457 $\pm$ 6	463 $\pm$ 5	457 $\pm$ 6
Respiratory rate (beats/min)	94 $\pm$ 7	92 $\pm$ 6	91 $\pm$ 7	97 $\pm$ 9	93 $\pm$ 10	93 $\pm$ 10	96 $\pm$ 9
Rectal temperature ($^{\circ}$ C)	37.0 $\pm$ 0.1	37.0 $\pm$ 0.1	37.1 $\pm$ 0.1	37.3 $\pm$ 0.1	37.3 $\pm$ 0.1	37.4 $\pm$ 0.2	37.5 $\pm$ 0.2

Fenoterol at 5 nmol was microinjected into the PVN. Values are the mean $\pm$ S.E. of 5–6 experiments. Significance compared with the 0 min-value: * $P < 0.05$.

Fig. 4. Effects of the antagonists on the fenoterol-induced antidiuresis. Ordinate: urine outflow rates expressed as a percentage of the control levels. Abscissa: time in min after the microinjection of fenoterol. ●: Fenoterol (5 nmol), ○: Butoxamine (25 nmol) + Fenoterol, △: Atenolol (5 nmol) + Fenoterol, ▽: Timolol (100 nmol) + Fenoterol, □: The vasopressin antagonist (16.7 μ g) + Fenoterol. The antagonists were pretreated at 20–50 min before fenoterol was microinjected into the PVN. Symbols indicate the mean $\pm$ S.E. from 4–17 experiments. *Significantly different from the changes in urine outflow rates by fenoterol alone at each time-point (●, $P < 0.05$).

involved in regulations of blood pressure, urine production and vasopressin release (4, 6, 18, 22–26). In addition, elevated blood pressure in SHR was suggested to be partly due to changed catecholaminergic neuron activity in the PVN or in the hypothalamus (14–17). Therefore, we studied the roles of β -adrenoceptor subtypes on the regulation of urine outflow rate in the PVN of SHR.

Urine outflow rates were decreased by β -adrenoceptor agonists injected into the PVN of rats, consistent with our previous findings (6). It was found that fenoterol was more potent as an antidiuretic agent than isoproterenol. The β_1 -agonists (T-1583 and dobutamine) slightly reduced urine outflow rates; the β_1 -agonists behaved as extremely weak agents. The order of antidiuretic potencies for the agonists used was similar to that of the relaxation potencies for these drugs in peripheral arteries, which contained predominantly β_2 -adrenoceptors (27). There was no obvious difference among SHR, WKY and Wistar rats in their responsiveness to fenoterol and isoproterenol.

To provide strong evidence to support the predominance of β_2 -adrenoceptors regulating urine outflow in the PVN, we determined whether the fenoterol-induced antidiuresis was inhibited by β_1 - or β_2 -selective antagonists. Doses at which atenolol and butoxamine selectively blocked β_1 - and β_2 -adrenoceptors in the central nervous system, respectively, are not well-known. However, it was shown that pK_i values of atenolol to β_1 -sites and butoxamine to β_2 -sites for inhibition of [125 I]-iodocyanopindolol binding were 7.0 and 6.3, respectively (28). In addition, Pearson et al. (29) injected 40 nmol atenolol into the cisterna magna of rats to observe the central effects of the drug. In the present experiments, therefore, 5 nmol atenolol and 25 nmol butoxamine were injected into the PVN to block β_1 - and β_2 -adrenoceptors, respectively.

The antidiuretic effect of fenoterol was significantly inhibited by timolol, atenolol and/or butoxamine, suggesting that the effect was β -adrenoceptor-mediated. The inhibitory effects of the β_2 -antagonist butoxamine on the fenoterol-induced effects were more marked than those of the β_1 -antagonist atenolol in SHR. The results thus suggest that there are β_2 -adrenoceptors mediating urine production in the PVN of SHR, consistent with previous findings that β_2 -adrenoceptors in the central nervous system mediated changes in blood pressure, urine outflow rates and sodium excretion induced by β -adrenoceptor agonists (18, 30). On the other hand, butoxamine and atenolol did not influence the fenoterol-induced effects in Wistar rats and partly inhibited it in WKY. In other words, WKY and Wistar rats were less susceptible to butoxamine than SHR. Therefore, we could not demonstrate that the PVN of WKY and Wistar rats had predominantly β_2 -adrenoceptor subtypes. At present, we

do not know the reason why atenolol at a dose as low as 5 nmol could inhibit the antidiuretic effect of fenoterol. However, this may be explained by the possible presence of β_1 -adrenoceptors in the PVN. Alternatively, it is also possible to assume that in the central nervous system, fenoterol could act as a non-selective β -adrenoceptor agonist. In this case, the antidiuresis could be inhibited completely by timolol, but butoxamine or atenolol alone could not.

The vasopressin antagonist did not affect the reduced rate of urine outflow in the presence of fenoterol in WKY. In addition, fenoterol did not increase urinary osmotic pressure as much as vasopressin did. Therefore, it was suggested that the fenoterol-induced antidiuresis was not due to an increased release of vasopressin. It must be neuronal, since there is some information to support this hypothesis. For instance, electrophysiological studies have suggested that hypothalamic nuclei neuronally influence the kidney to increase reabsorption of water (30, 31). Koepke et al. (31) suggest that the antidiuretic effect of a β_2 -agonist injected into the posterior hypothalamus was due to increased activity of renal sympathetic neurons.

The blood pressure of anesthetized and conscious SHR was higher than that of WKY or Wistar rats (this study; 32, 33). Intra-PVN injection of fenoterol resulted in a decrease in blood pressure in both WKY and SHR. The possibility should not be excluded that the decrease in blood pressure by fenoterol caused secondarily antidiuresis in SHR and WKY. On the other hand, in Wistar rats, fenoterol at the dose at which fenoterol decreased the urine outflow rate did not alter the blood pressure, suggesting that mechanisms involved in the antidiuresis may be different from those in SHR and WKY. The depressor effects of fenoterol applied into the PVN were opposite to the pressor effects induced by i.c.v. injection of β_2 -agonists, sulbutamol and orciprenaline, in conscious SHR (18). This discrepancy may be due to differences in experimental conditions. For instance, they injected the drugs into the lateral ventricle. Consequently, the drugs must reach rapidly multiple sites.

In conclusion, the injection of the β -adrenoceptor agonists into the PVN resulted in the decrease in urine outflow rates, which was not due to vasopressin release. β -Adrenoceptors mediating the antidiuretic response were predominantly of the β_2 -subtype in the PVN of SHR. The antidiuretic response of SHR to the β -agonists was similar to that of the control rats. The ability of butoxamine to inhibit the β_2 -agonist-induced antidiuresis appears to be greater in SHR than in WKY and Wistar rats.

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