ROLE OF SYMPATHETIC NERVE INHIBITION IN THE VASODEPRESSOR EFFECT OF BROMOCRIPTINE IN NORMOTENSIVE AND HYPERTENSIVE RATS

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This study aimed to elucidate the mechanism of the hypotensive effect of bromocriptine (BRC), and to investigate whether or not the effects of BRC on the sympathetic nervous system are altered in hypertension. BRC was administered intravenously to normotensive and spontaneously hypertensive rats (SHR). It elicited hypotensive effects dose-dependently in urethane-anaesthetized normotensive rats, an effect which was antagonized with metoclopramide. Pretreatment with intravenous hexamethonium attenuated the hypotensive effect of BRC. BRC decreased plasma norepinephrine (NE) without inhibiting the sympathetic nerve spikes recorded from the postganglionic sympathetic nerve bundle. The hypotensive effect of BRC was significantly greater in SHR than in Wistar Kyoto Rats (WKY). Decrease in NE by BRC was also significantly greater in SHR than in WKY. These results suggest that the hypotensive effect of BRC is induced by suppression of NE release, not by inhibition of sympathetic nerve spikes, and that the dopaminergic presynaptic inhibition is attenuated in SHR.

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It has been demonstrated that dopamine (DA) can play an important role in the regulation of blood pressure! There are two types of dopamine receptor, DA₁ and DA₂. DA₁ receptors are distributed in the postsynaptic peripheral blood vessels and DA₂ receptors in the pre-synaptic nerve endings. Dopamine induces depressor responses due to the dilatation of peripheral vessels and natriuretic effects are mediated by DA₁ receptors, and reduction of peripheral resistance by inhibition of norepinephrine release by DA₂ receptor stimulation.

The dopaminergic system is known to be involved in the development or maintenance of hypertension. Prolactin release, which in under dopaminergic control, is reported to be altered in hypertension and the total dopamine content in plasma is elevated in a subset of essential hypertension. Bromocriptine (BRC), a DA₂ agonist, has a depressor effect. That hypotensive effect mediated by the central nervous system is shown by the fact that BRC alters plasma catecholamine and prolactin in hypertensive patients as well as in normotensives. However, domperidone, which does not cross the blood brain barrier, blocks the hypotensive effect of BRC. Thus, the exact mechanism of the

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Japanese Circulation Journal Vol.56, September 1992 943
hypotensive effect of BRC is not yet clear.

To elucidate the mechanism of the hypotensive action of BRC, it was injected intravenously into urethane-anaesthetized normotensive and hypertensive rats while measuring or recording blood pressure, sympathetic nerve firing, and plasma catecholamines.

MATERIALS AND METHODS

Thirty one normotensive male Wistar rats weighing 200—220 g, and 36 SHR and WKY (Wistar Kyoto rats) which were 9 weeks old were purchased from Oriental Animal Service (Kyoto, Japan). These rats were anaesthetized with urethane (1.2 g/kg, intraperitoneally), and catheters were inserted into the left femoral vein for the injection of drugs and into the left femoral artery for recording arterial blood pressure. Phasic blood pressure was recorded through a cannula connected to a low volume-displacement pressure transducer (MPV-290, NEC-Sanei Sokki Co.). Heart rate was measured from the phasic pressure signals by a heart rate meter (2140, NEC-Sanei Sokki Co.).

Sympathetic nerve activity was recorded in artificially ventilated rats. The abdominal plexus was exposed as previously described and a bipolar stainless steel electrode was placed on the major nerve bundle between the cardiac and coeliac ganglion. Spike potentials were amplified using a Grass PI5AC amplifier, monitored on a storage oscilloscope (VI34, Hitachi Co.) and recorded continuously, together with the blood pressure, on magnetic tape (R210B, TEAC). To quantify the nerve activity, the original analogue signals were played back, fed into a spike counter (DSE-325P, Dia Medical System Co.), and the spikes were counted every 10 sec by the spike counter. The low level control of the window discriminator was routinely set to filter out any background noise persisting after the crushing of the nerve bundle.

BRC (Sandoz Co.) was dissolved in a saline solution containing 2% of ethanol and brought to pH 3 using HCl. BRC was administered intravenously at doses of 62.5, 125 and 250 μg/kg. Metoclopramide (MCP) was administered intravenously at a dose of 5 mg/kg. Norepinephrine was injected intravenously at doses of 50, 100 and 200 ng/100 g body weight in SHR before and ten minutes after BRC injection (iv). Blood samples of 0.5 ml were drawn through the venous cannula inserted into the femoral vein before and after i.v. injection of 250 μg/kg BRC. The same volume of saline was injected immediately after each venous sampling. Blood samples were centrifuged to obtain plasma and then frozen until the time of catecholamines measurement. Plasma catecholamines were assayed by a radio-
Vasodepressor effect of BRC

Fig. 3. Bradycardic responses to i.v. injection of BRC (125, 250 μg/kg) in normotensive Wistar rats. Each group was significantly different from the others by using Newman-Keuls' multiple range test, except for vehicle vs. 125 μg/kg, and 62.5 μg/kg vs 125 μg/kg.

Fig. 4. Sympathetic nerve activity following i.v. injection of BRC (250 μg/kg) in normotensive Wistar rats (n=7).

Fig. 5. Effect of hexamethonium pretreatment (30 mg/kg) on cardiovascular responses to i.v. administration of BRC (250 μg/kg) in Wistar rats (n=5—7 in each group).

d enzymatic method. All data are expressed as averages ± SEM. Absolute values of dose responses to BRC injection were used for analysis, while percent values were used when assessing the effects of MCP or hexamethonium and when comparing responses in WKY and SHR since the baseline blood pressure and heart rate were different. Percent changes were verified, whether they were normally distributed or not, using ranked numbers. Time course responses to BRC in blood pressure, heart rate and sympathetic nerve activity were analyzed using a repeated measures analysis of variance. Catecholamine concentrations were compared using Student's t test. Percent changes of cardiovascular responses to BRC injection in WKY and SHR, percent changes in catecholamine concentrations and percent changes of blood pressure by BRC pretreatment in pressor responses to norepinephrine were compared using Wilcoxon's non-parametric test. Whenever F-ratios significant at 5% or less were obtained with the repeated measures analysis of variance, Neuman-Keuls' multiple range test was applied to determine the significance of differences between rat groups.

RESULTS

Cardiovascular and Sympathetic Nerve Responses to i.v. Injection of BRC in Normotensive Rats

BRC elicited depressor responses dose-de-
Fig. 6. Cardiovascular responses to i.v. injection of BRC (250 μg/kg) in WKY and SHR.

Effect of Hexamethonium on Depressor Responses

Intravenous (i.v.) injection of hexamethonium (30 mg/kg) reduced blood pressure and heart rate immediately. BRC was administered 10 min later, when blood pressure and heart rate became stable. Both vasodepressor and bradycardic responses to BRC injection were eliminated (Fig. 5).

Augmented Depressor Response to BRC in SHR

BRC (250 μg/kg) lowered blood pressure in both WKY and SHR significantly. In WKY, the maximum blood pressure drop occurred 3 min after injection and remained at that level up to 10 min after injection. In contrast, although the percent reduction in mean blood pressure was comparable in SHR and WKY 3 min after BRC injection, mean blood pressure tended to decrease further in SHR beyond the 3 min period. Thus, depressor responses were significantly greater in SHR than in WKY (10 min: −39 ± 2 vs −29 ± 2%, P < 0.05). There was no difference between the bradycardic responses of the 2 groups (10 min: −16 ± 2 vs −11 ± 3%) (Fig. 6).

Reduction of Plasma Norepinephrine by BRC in WKY and SHR

There were no significant differences in plasma norepinephrine (NE) and epinephrine (E) between SHR and WKY (NE: 401 ± 43 vs 398 ± 76 pg/ml, n.s., E: 328 ± 55 vs 352 ± 67 pg/ml, n.s.). After BRC injection (250 μg/kg), plasma NE was decreased significantly both in SHR (from 401 ± 43 to 221 ± 17 pg/ml, P < 0.01) and WKY (from 398 ± 76 to 305 ± 68 pg/ml, P < 0.05), and this decrease was significantly greater in SHR than in WKY (SHR: 44 ± 5 vs WKY: 23 ± 6%, P < 0.05) (Fig. 7).
E was increased both in SHR (from 328±55 to 530±56 pg/ml, P<0.01) and WKY (from 352±67 to 875±91 pg/ml, P<0.01), while the increase in epinephrine in SHR was not statistically different from that in WKY (Fig. 8).

**Pressor Responses to i.v. Injection of Norepinephrine in SHR**

Pressor responses to i.v. injection of norepinephrine were obtained dose-dependently in urethane-analasthetized SHR. These responses were not altered after BRC (250 μg/kg) administration (Fig. 9).

**DISCUSSION**

The present results show that intravenous administration of BRC elicited depressor responses dose-dependently, and reduced plasma NE without decreasing sympathetic nerve spikes. Since MCP blocked the hypotensive effect of BRC, the effect of BRC could be mediated by DA receptors. In the present study, because the sympathetic nerve activity recorded from the post ganglionic fibers was not inhibited, it was suggested that the sympathetic nervous system was not suppressed at the level from the brain to the ganglion. Thus, it seems likely that the decrease in plasma NE was due to the suppression of NE release induced by DA₂-receptor stimulation in the sympathetic nerve ending. The inhibition of neurotransmitter release by DA₂-receptor stimulation has been demonstrated.

As to the hypotensive mechanisms of BRC, central suppression of the sympathetic nerve activity such as reduction of peripheral NE and attenuation of response to stress is suggested. In these studies, the reduction of norepinephrine may be due to pre-synaptic inhibition by BRC. In contrast, our results indicate that BRC does not centrally inhibit the sympathetic nervous system because sympathetic nerve activity was not altered.

Plasma E elevation after i.v. administration of BRC may be due to direct stimulation of the adrenal gland through DA receptors, as reported by Nagahama. On the other hand, Hamilton suggested that the hypotensive effect of BRC in SHR is due not to DA receptor stimulation but to E release.
from the adrenal gland, because the hypotensive effect was eliminated by the resection of the adrenal medulla. In the present study, it is not clear whether or not E plays any role in the hypotensive effect. The reduction of norepinephrine was independent of the increase in E, since E accelerates neurotransmitter release in sympathetic nerve endings. Nagahama and Ractz reported that E release from the adrenal medulla was not involved in the depressor action of BRC. These findings suggest that the increase in plasma E may not have played any important role in the depressor effect in our study. In the present study, heart rate did not increase in spite of the elevation of E. The lack of increases in heart rate may be due to anaesthesia and inhibition of sympathetic cardiac nerve endings.

Hamilton et al. suggested that BRC has a blocking effect on α-adrenoceptors. However, our results did not support this effect of BRC. BRC injection did not change pressor response to norepinephrine in SHR.

This study showed that the depressor response to i.v. injection of BRC and the percent reduction in NE were greater in SHR than in WKY. Thus, it is suggested that inhibition of NE release by DA₂ activation in nerve endings is greater in SHR. In contrast, it has been reported that in vitro the suppression of NE release by dopamine is smaller in SHR. The details of this discrepancy are not clear. In this study, it is suggested that sensitivity of DA₂ receptors in sympathetic nerve endings is augmented in SHR as shown by the result that the reduction of NE release was greater when BRC was administered exogenously. This hypersensitivity of DA₂ receptors may be caused by up-regulation through the paucity of DA in sympathetic nerve endings.

A mechanism other than the influence of the sympathetic nervous system, relaxation of peripheral vascular smooth muscle, has also been reported. It is unlikely that this effect contributes to the depressor response in SHR, because BRC did not alter the pressor response to i.v. injection of norepinephrine.

In summary, (1) in urethane-anaesthetized normotensive Wistar rats, BRC, a dopamine agonist, elicited dose-dependent hypotensive effects which were antagonized with MCP. Sympathetic nerve activity was not altered following BRC injection.

(2) Pretreatment with intravenous hexamethonium attenuated the hypotensive effect of BRC.

(3) The hypotensive effect of BRC was greater in SHR than in WKY.

(4) BRC reduced plasma NE both in WKY and SHR, and the reduction of NE was significantly greater in SHR than in WKY. Plasma E was increased both in SHR and WKY after BRC administration.

(5) Pressor response to i.v. injection of NE was not altered after BRC administration.

In conclusion, these findings suggest that the hypotensive effect of BRC is mainly due to the inhibition of NE release at sympathetic nerve endings via DA₂ receptors, not to central inhibition of sympathetic nerve activity. Also, the response to dopaminergic agonist is augmented in rats with spontaneous hypertension.

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