ATTENUATED DEVELOPMENT OF HYPERTENSION BY CHRONIC ADMINISTRATION OF BROMOCRIPTINE IN DOCA-SALT HYPERTENSIVE RATS

SEIICHI KAMBARA, M.D., MANABU YOSHIMURA, M.D.
HIDEKO OKABAYASHI, M.D., HAKUO TAKAHASHI, M.D.
AND HAMAO IJICHI, M.D.

The aim of the study was to investigate whether or not the development of hypertension is influenced by chronic treatment with bromocriptine and/or domperidone. Rats treated with DOCA-salt were divided into 4 groups: control with vehicle, bromocriptine, bromocriptine with domperidone, and domperidone. Increased blood pressure by DOCA-salt treatment was significantly suppressed by treatment with bromocriptine and this bromocriptine suppression was significantly blocked by treatment with domperidone. Increased urinary excretion of norepinephrine by DOCA-salt treatment was significantly suppressed by bromocriptine and the inhibiting effect of bromocriptine disappeared with domperidone. In the four groups of rats, there were significant correlations between systolic blood pressure and urinary excretion of norepinephrine, systolic blood pressure and urinary excretion of epinephrine, and urinary excretion of dopamine and sodium. These results suggest that the chronic effect of bromocriptine is to suppress development of DOCA-salt hypertension, mainly through peripheral mechanisms which are involved in the decreased release of norepinephrine.

CLINICAL and experimental studies have shown that bromocriptine, a dopamine agonist, has a depressor effect in humans1-4 and experimental hypertensive animals5-7. Bromocriptine also reduces the pressor response to posture and isometric exercise in normotensive and hypertensive subjects3. Moreover, bromocriptine decreases prolactin2-5, norepinephrine3,4, epinephrine4 and aldosterone3,5 levels in the blood in basal or in response to stimuli in humans and animals. These studies suggest that the depressor action of bromocriptine may be due, in part, to the suppression of prolactin8,9 norepinephrine3,4 epinephrine4 and aldosterone3,5. Lokhandwala10 suggested that the depressor action of bromocriptine is mediated mainly through the presynaptic inhibition of norepinephrine release. Taylor et al11 observed that the depressor action of other dopamine analogs is attenuated by treatment with domperidone, which does not cross the blood brain barrier12,13 and suggested that these depressor actions are mediated through peripheral dopaminergic mechanisms.

In contrast, several investigators1-5,7 have suggested that the depressor effect of bromocriptine is mediated mainly through central dopaminergic mechanisms. Nagahama et al7 demonstrated that the depressor effect of bromocriptine is attenuated by treatment with the dopamine antagonist metoclopramide, which

Key Words:
Bromocriptine
Domperidone
Dopaminergic mechanism
DOCA-salt hypertension

(Received April 25, 1986; accepted August 15, 1986)
The Second Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan
Mailing address: Manabu Yoshimura, M.D., The Second Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-hirokoji, Kamikyo, Kyoto 602, Japan

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crosses the blood brain barrier, and is not attenuated by treatment with domperidone. Thus, it is not clear whether the hypotensive action of bromocriptine is related mainly to its central or peripheral mechanisms.

To clarify the depressor action of bromocriptine in hypertensive animals, this study investigated the effect of the chronic administration of bromocriptine and/or domperidone on the development of hypertension using deoxycorticosterone acetate (DOCA)-salt hypertensive rats.

MATERIALS AND METHODS

Animal preparation

Male outbred 3-week-old Wistar rats were obtained from Nihon Animals Co. (Osaka) and housed in a room kept at a constant temperature of 24 ± 2°C. One week after left nephrectomy, the DOCA-salt treatment, which consisted of weekly subcutaneous injections of 10 mg DOCA suspension with 1% methyl cellulose solution and a high sodium diet (Oriental Yeast Inc.) containing 3.14% sodium, was given for 3 weeks. Water and feed were provided ad libitum.

Animals (body weight: 92 ± 2 g) were divided into four groups matching weight. One group of rats was subcutaneously injected daily with 1 mg/kg of bromocriptine (Sandoz) solution. The second group of rats was subcutaneously injected daily with 1 mg/kg of bromocriptine and 3 mg/kg of domperidone (Kyowa Hakko Ind.). The third group of rats was subcutaneously injected daily with domperidone alone. Control animals received an equal volume of injection vehicle. These treatments were initiated concomitantly with DOCA-salt treatment.

Cardiovascular and metabolic studies

Blood pressure and heart rate were measured weekly using the tail-cuff method and a programmed electro-sphygmomanometer (Narco) 24 hours after administration of vehicle, bromocriptine, or domperidone. The rats were housed in individual metabolic cages and a 24-hour urine collection was made in flasks containing 1 ml of 6N-HCl for determination of catecholamine content after a 24-hour period of acclimatization. Urine collection for sodium followed the urine collection for catecholamines. Sodium content was determined by flame photometry (Corning, type 480), and results were expressed as mEq/day.

Determination of catecholamines

Urinary free norepinephrine, epinephrine, and dopamine contents were determined with high performance liquid chromatography (HPLC) with electrochemical detection (Bioanalytical System Inc.) by a modification of the method of Riggan and Kissinger14 using Dowex AG ion exchange resin, 50–100 mesh (Muromac, KWC-1, Muromachi Chemical Co.) for extraction of catecholamines from the urine. Results were expressed as µg/day or ng/day (24 hours).

After completion of these studies, the rats were sacrificed by decapitation without anesthesia. The kidneys were rapidly removed and frozen on dry-ice and stored at −70°C for later determination of catecholamines. Renal catecholamine content was determined using HPLC with electrochemical detection. The kidneys were homogenized with 3 ml/g of cold 0.05M perchloric acid solution containing 0.1 ml of 0.1M EDTA, 0.1 ml of 1M NaHSO4, and 45 µl of 0.14 mM DHBA as an internal standard. Catecholamines were extracted by a modification of the method of Felice et al.15

Statistical analysis

The experimental results, expressed as mean ± SEM, were analyzed by one-way analysis of variance; when differences were noted, the means were compared by the Tukey test. Correlation coefficients were calculated by standard regression techniques.16

RESULTS

Body weight, blood pressure and heart rate

There was no significant change in body weight in the four groups of DOCA-salt rats treated with bromocriptine (BEC), bromocriptine and domperidone (BEC + DMP), domperidone (DMP), or vehicle as the control for 3 weeks (BEC: 187 ± 5, BEC + DMP: 188 ± 5, DMP: 190 ± 5, control: 189 ± 7 g).

Blood pressure in all groups increased in the following weeks. Bromocriptine administration resulted in a significantly attenuated development of hypertension as compared to the control rats with the vehicle (BEC vs control; 114 ± 3 vs 130 ± 2 at 1 week; 131 ± 2 vs 147 ± 2 at 2 weeks; 138 ± 4 vs 163 ± 3 mmHg at 3 weeks; p < 0.01, respectively). Contrarily, domperidone significantly inhibited the bromocriptine-induced attenuation in the development of hypertension (BEC + DMP vs BEC; 126 ± 3 vs 114 ± 3 at 1 week, p < 0.05; 145 ± 3 vs 131 ± 2 at 2 weeks, p < 0.01; 166 ± 2 vs 138 ± 4 mmHg at 3 weeks,
Blood pressure of the domperidone group was significantly higher than that of the bromocriptine group (DMP vs BEC; 129 ± 3 vs 114 ± 3 at 1 week; 149 ± 2 vs 131 ± 2 at 2 weeks, 165 ± 2 vs 138 ± 4 mmHg at 3 weeks, p < 0.01, respectively). There was no significant change in the development of hypertension between the control group and the bromocriptine with domperidone group. Domperidone administration alone did not show any effect on blood pressure as compared to that of the control group (Fig. 1). The heart rate of rats decreased in the following weeks. The differences in heart rates in the four groups were insignificant.

Urinary excretion of sodium

Urinary excretion of sodium in the four groups of rats was enhanced by the DOCA-salt treatment. Bromocriptine administration resulted in a significant increase in urinary excretion of sodium as compared to that of the domperidone group. (BEC vs DMP; 18.9 ± 0.5 vs 15.5 ± 0.7 mEq/day at 1 week, p < 0.05). Augmented excretion of sodium in bromocriptine rats was abolished by treatment with domperidone (BEC vs BEC + DMP; 20.0 ± 0.9 vs 17.5 ± 0.4 mEq/day at 3 weeks, p < 0.05). There were no significant differences in urinary excretion of sodium between the control and the bromocriptine group, the control and the bromocriptine-domperidone group, or the control and the domperidone group (Fig. 2).

Urinary excretion of catecholamines

Urinary excretion of norepinephrine increased with the DOCA-salt treatment in the following weeks. Urinary excretion of norepinephrine in bromocriptine rats decreased significantly as compared to that of control rats (BEC vs control; 207 ± 9 vs 281 ± 18 at 1 week, p < 0.05; 302 ± 9 vs 418 ± 15 ng/day at 3 weeks, p < 0.01). Decreased urinary output of norepinephrine in rats with bromocriptine was inhibited by the additional administration of domperidone (BEC vs BEC + DMP; 302 ± 9 vs 386 ± 10 ng/day at 3 weeks, p < 0.01). Urinary output of norepinephrine in rats with bromocriptine was significantly lower than that in rats with domperidone (BEC vs DMP; 207 ± 9 vs 284 ± 21 at 1 week, p < 0.05; 272 ± 18 vs 384 ± 29 at 2 weeks, p < 0.05; 302 ± 9 vs 424 ± 12 ng/day at 3 weeks, p < 0.01). There were no significant changes in urinary
Fig. 2. Effect of bromocriptine, domperidone, and bromocriptine and domperidone on urinary excretion of sodium (U-Na). Open circle: control rats treated with vehicle (n = 7). Closed circle: rats treated with bromocriptine (n = 7). Closed triangle: rats treated with domperidone (n = 7). Open triangle: rats treated with bromocriptine and domperidone (n = 7). +: p < 0.05, significant difference between the rats with bromocriptine and the rats treated with domperidone at 1 week, and the rats treated with bromocriptine and domperidone at 3 weeks.

Fig. 3. Effect of bromocriptine, domperidone, and bromocriptine and domperidone on urinary excretion of norepinephrine (U-NE). Open circle: control rats treated with vehicle (n = 7). Closed circle: rats treated with bromocriptine (n = 7). Closed triangle: rats treated with domperidone (n = 7). Open triangle: rats treated with both bromocriptine and domperidone (n = 7). **: p < 0.01, significant difference between the rats treated with bromocriptine and the other three groups. +: p < 0.05, significant difference between the rats treated with bromocriptine and the rats treated with vehicle or domperidone at 1 week, and the rats treated with domperidone at 2 weeks.

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Fig. 4. Effect of bromocriptine, domperidone, and bromocriptine and domperidone on urinary excretion of epinephrine (U-E). Open circle: control rats treated with vehicle (n = 7). Closed circle: rats treated with bromocriptine (n = 7). Closed triangle: rats treated with domperidone (n = 7). Open triangle: rats treated with bromocriptine and domperidone (n = 7).

Fig. 5. Effect of bromocriptine, domperidone, and bromocriptine and domperidone on urinary excretion of dopamine (U-DA). Open circle: control rats treated with vehicle (n = 7). Closed circle: rats treated with bromocriptine (n = 7). Closed triangle: rats treated with domperidone (n = 7). Open triangle: rats treated with bromocriptine and domperidone (n = 7).

excretion of norepinephrine in the control, domperidone, or bromocriptine with domperidone groups (Fig. 3).

Urinary excretion of epinephrine increased with the DOCA-salt treatment in the following weeks. There were no significant changes in

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TABLE I CORRELATIONS BETWEEN SYSTOLIC BLOOD PRESSURE (SBP) AND URINARY EXCRETION OF NOREPINEPHRINE (U-NE), EPINEPHRINE (U-E) AND SODIUM (U-Na) AND BETWEEN URINARY EXCRETION OF SODIUM (U-Na) AND DOPAMINE (U-DA) IN FOUR GROUPS OF RATS TREATED FOR 3 WEEKS

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urinary excretion of epinephrine in the four groups (Fig. 4).

Urinary excretion of dopamine increased with the DOCA-salt treatment. There were no changes in urinary dopamine excretion in the four groups (Fig. 5).

Correlation

Parameters obtained at 3 weeks after treatment showed significant positive correlations between systolic blood pressure and urinary excretion of norepinephrine (p < 0.01), systolic blood pressure and urinary excretion of epinephrine (p < 0.05), and urinary excretion of sodium and dopamine (p < 0.01). There was a significant negative correlation between systolic blood pressure and urinary sodium (p < 0.05), shown in Table 1.

DISCUSSION

The present study demonstrated that the chronic subcutaneous administration of bromocriptine lowered blood pressure and inhibited the development of hypertension with DOCA-salt treatment. The attenuated development of hypertension with DOCA-salt was inhibited by domperidone, which does not cross the blood brain barrier. The blocking effect of domperidone against the depressor action of bromocriptine is mediated mainly through peripheral dopaminergic mechanisms. These results are in agreement with previous observations that presynaptic inhibition against norepinephrine release is involved in the depressor action of bromocriptine. Taylor et al. observed that the depressor effects of dopamine analogs were attenuated by treatment with domperidone mediated mainly by the presynaptic DA2 receptors.

In contrast, it has been reported that intravenous administration of bromocriptine lowered blood pressure in SHR and this depressor effect was blocked by metoclopramide but not by domperidone, which suggests that the depressor action of bromocriptine is mediated mainly through a central dopaminergic mechanism. On the other hand, we observed that the depressor effect of bromocriptine by intravenous administration was clearly blocked by intravenous pretreatment with domperidone in sino-aortic denervated rats, which suggests that the depressor action of bromocriptine is mediated mainly through peripheral dopaminergic mechanisms. We also confirmed that the chronic depressor effect of bromocriptine was mediated mainly through peripheral dopaminergic mechanisms. The chronic depressor effect of bromocriptine, estimating blood pressure 24 hours after subcutaneous administration, rules out the secondary responses of sympathetic and adrenomedullary activity caused by the reduction of blood pressure.

The present study demonstrated that bromocriptine reduced the urinary excretion of norepinephrine in DOCA-salt hypertensive rats in the developing stage. The bromocriptine-induced reduction of urinary norepinephrine was abolished by additional treatment with domperidone, which suggests that bromocriptine activates the presynaptic receptors to inhibit norepinephrine release from sympathetic nerve terminals. Inhibiting release of norepinephrine by bromocriptine has already been reported in humans and animals and in part, it participates in the reduction of blood pressure.

Epinephrine release from the adrenal medulla has been reported to be inhibited by dopamine via the DA2 receptors in the adrenal medulla. However, we did not find differences in urinary epinephrine excretion in the four groups in this study.

There were significant correlations between systolic blood pressure and urinary excretion of norepinephrine and epinephrine in this study. These results suggest that the dopaminergic modulation of norepinephrine and epinephrine release contributes, in part, to the elevation of blood pressure by DOCA-salt treatment via DA2 receptors.

Urinary excretion of sodium was insignifi-

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cantly enhanced in rats with bromocriptine and the enhanced natriuresis was significantly abol-
ished by additional treatment with domperidone. There was a negative correlation between systolic blood pressure and urinary excretion of sodium.

These results suggest that dopamine increases renal blood flow mediated by vasodilatation through DA₁ receptors at the renal arteries and an inhibiting release of norepinephrine via presynaptic DA₂ receptors. The natriuresis may also contribute, in part, to the attenuated development of hypertension in this study.

Natriuresis is regulated by aldosterone secretion, which is also modulated by dopaminergic tone. Peripheral infusion of the dopamine agonist reduced the aldosterone response to angiotensin II and, in contrast, the dopamine antagonist enhanced to release aldosterone in response to angiotensin II. The dopaminergic modulation of aldosterone release may also have contributed to the reduction of blood pressure in our study.

A positive correlation between urinary excretion of sodium and dopamine was observed although neither bromocriptine nor domperidone influenced the urinary excretion of dopamine. These results suggest that endogenous dopamine formed in the kidneys contributes, in part, to the excretion of sodium.

In summary, the chronic depressor effect of bromocriptine was demonstrated in the developing stage of DOCA-salt hypertensive rats. Chronic administration of bromocriptine lowered the blood pressure and urinary excretion of norepinephrine. The effects of bromocriptine, such as a decrease in blood pressure and a suppressed excretion of norepinephrine, were inhibited by treatment with domperidone. These results suggest that the depressor effect of bromocriptine is due mainly to the inhibiting excretion of norepinephrine via the peripheral DA₂ receptors. These results do not support the theory of depressor action of bromocriptine by central dopaminergic mechanisms, but rather suggest an involvement of peripheral dopaminergic mechanisms.

Acknowledgments

The authors gratefully acknowledge the assistance of Miss Yasuko Araki and Miss Judith Clancy. This work was supported in part by a grant in aid for scientific research from the Ministry of Education, Science and Culture.

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