MECHANISMS OF CENTRAL HYPOTENSIVE ACTIVITY OF L-DOPA AND A PERIPHERAL DECARBOXYLASE INHIBITOR IN DOCA-SALT HYPERTENSIVE RATS*

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Abstract—The intraventricular administration of α-adrenoceptive blocker (phentolamine) or dopamine (DA)-β-hydroxylase inhibitor (diethyldithiocarbamate) was found to abolish the most pronounced fall in blood pressure (BP) of freely moving DOCA-salt hypertensive rats (DHR) observed among normotensive or various types of other hypertensive rats after L-DOPA with combination of a peripheral decarboxylase inhibitor (Ro 4-4602). L-DOPA plus Ro 4-4602 markedly reduced the pressor response to electrical stimulation of the posterior hypothalamus, midbrain reticular formation and dorsal medulla oblongata in freely moving, unanesthetized or anesthetized DHR. L-DOPA plus Ro 4-4602 potentiated the depressor component in BP response to the posterior hypothalamic stimulation of the anesthetized DHR. These results suggest that L-DOPA plus Ro 4-4602 markedly lowers BP of DHR due to stimulation of α-adrenoceptor in the brainstem with effluxed noradrenaline after dopamine accumulation in the brainstem noradrenergic neurons and also at least partly due to blockade of the brainstem pressor areas.

The administration of L-DOPA plus a peripheral decarboxylase inhibitor has been known to induce the fall in blood pressure (BP), accompanied with centrally mediated bradycardia and venous pooling (1), and a decrease in peripheral sympathetic outflows (2–4). The administration of L-DOPA plus a peripheral decarboxylase inhibitor, Ro 4-4602 (5), markedly reduced BP in DOCA-salt hypertensive rats (DHR), intermediary in spontaneously hypertensive rats (SHR) and least in hypertensive rats made by clipping the renal artery and uninephrectomy (RHR) (6). The fall in BP of DHR following L-DOPA plus Ro 4-4602 seemed to correlate with accumulation of dopamine (DA) and L-DOPA in the brainstem area, without involvement of DA neurons per se (6).

This paper was designed to present the mechanisms of pronounced fall in BP after the administration of L-DOPA plus Ro 4-4602 to DHR.

MATERIALS AND METHODS

Preparation of DHR

As described previously (6), DOCA-salt hypertension was produced in male Wistar rats, weighing 140–160 g. Animals were anesthetized with ether, uninephrectomized, and given deoxycorticosterone acetate (DOCA) (10 mg/kg once in a week s.c.) and 0.9% NaCl

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as drinking fluid *ad libitum*. They were used 4-5 weeks later and their systolic BP ranged from 170-210 mmHg. The BP was measured without anesthesia or restraint through a cannula intubated into the aorta as described previously (7).

**Drug administration**

Twenty mg/kg of L-DOPA homogenized with 1% CMC and 0.5% ascorbic acid solution was intraperitoneally administered to the rats 30 min after the intraperitoneal administration of Ro 4-4602, a peripheral decarboxylase inhibitor (8), in doses of 20 and 50 mg/kg, as a standard dosage schedule. Continuous infusion of phentolamine or diethyldithiocarbamate into the lateral ventricle was carried out by using the Stoelting micro-infusion instrument via an previously implanted cannula in freely moving rats as described formerly (9).

**Electrical stimulation of the brain region**

For induction of pressor or depressor response to electrical stimulation of the brain in freely moving or anesthetized DOCA-salt hypertensive rats, Cashew-coated bipolar electrodes (a diameter of 0.5 mm, 0.1 mm uninsulated tip, a resistance of 10 K ohm) was inserted stereotaxically with the David-Kopf instrument into the posterior hypothalamus.
(frontal 3.3 mm, lateral 0.5 mm, horizontal 2.7 mm), the midbrain reticular formation (f 1.3, l 1.5, h -1.5), the pressor area in the dorsal portion of the medulla oblongata (f 8.8, l 0.7, h -1.7) following the coordinates described in the rat brain (10). The area was the dorsal portion of A2 cells, adrenergic cell bodies, where the nucleus commissuralis is located and of which nerve endings terminated in the nucleus of the solitary tract and nucleus dorsalis n. vagi (11). Throughout the description of this study the active pressor sites have been designated the area around specific nuclei, since the electrodes are fairly large with respect to the size of these nuclei. The electrodes were advanced in 0.1 mm steps to explore the site in which pressor responses could be induced with the lowest stimulus frequency in the anesthetized animals before fixation of the electrode with a dental cement. The animals were used for the experiments 1–3 days later and electrical stimulation of 1–2 V, 5–200 Hz and 1 ms pulse duration was given for 5 sec without any anesthesia or with anesthesia (1 g/kg i.p. of urethane). Heart rate was recorded continuously with a tacograph pre-amplifier triggered by blood pressure or ECG.

Neurochemical determination

Medulla-pons, hypothalamus, thalamus-midbrain, striatum and the residual part of the brain except the cerebellum were dissected according to the method of Glowinsky and Iversen (12). NA was determined fluorimetrically by the method of Shellenberger and Gordon (13) after separation with alumina column (14).

RESULTS

Effect of intraventricular infusion of phentolamine on BP fall after 1-DOPA plus Ro 4-4602 in freely moving NR and DHR

The BP lowering effect of 1-DOPA (20 mg/kg i.p.) and Ro 4-4602 (50 mg/kg i.p.) in NR and DHR was completely abolished by continuous infusion of phentolamine (5.2 µg/1.2 µl/10 min) into the lateral ventricle (Fig. 1). Phentolamine alone caused no significant influence on BP of NR, whereas the drug slightly but not significantly elevated BP of DHR.

Effect of intraventricular administration of diethyldithiocarbamate (DDC) on BP fall after 1-DOPA plus Ro 4-4602 in DHR

Repeated administration of DDC (200
TABLE 1. Effects of the administration of diethyldithiocarbamate (DDC) into the lateral ventricle on the brain NA contents in DHR

| Brain region       | Control (I) | l-DOPA + Ro 4-4602 (II) | DDC (III) | DDC (IV) |
|--------------------|-------------|-------------------------|-----------|----------|
| Medulla-pons       | 670±32      | 850±85 p<0.05           | 361±37    | p<0.01   |
| Hypothalamus       | 1,597±77    | 1,883±167 p<0.05        | 683±61    | p<0.0025 |
| Thalamus-midbrain  | 497±20      | --                      | 263±50    | 371±76   |
| Striatum           | 398±38      | 387±48 n.s.             | 337±33 n.s.| 261±27 n.s.|
| Residual brain     | 268±26      | 343±34 a               | 196±14    | <0.025   |

The values indicated represent mean±s.e. obtained from 8 rats.
a includes those of thalamus-midbrain and does not differ from the corresponding controls.

\( \mu g/2 \mu l \) once in every 15 min for 3 times) prevented the BP fall after l-DOPA plus Ro 4-4602 in DHR (Fig. 2). DDC alone was found to have no significant effect on BP levels of NR or DHR. The same doses of DDC reduced the endogenous NA contents in the hypothalamus and medulla-pons of NR and DHR. l-DOPA plus Ro 4-4602 did not change the reduced NE contents in the brainstem with DDC. The results are shown in Table 1.

**Effects on BP changes after electrical stimulation of the brainstem**

Electrical stimulation of either posterior hypothalamus, midbrain reticular formation or dorsal medulla oblongata produced a frequency-dependent pressor response in

![Fig. 3. Effects on BP changes induced by electrical stimulation of the posterior hypothalamus in DOCA-salt hypertensive rats. The data shown indicate mean ±s.e. obtained by 5-6 rats. The symbols, open squares and closed circles mean initial values, those of 20-30 min after Ro 4-4602 (20 mg/kg i.p.) and those of 40-60 min after l-DOPA (20 mg/kg i.p.), respectively. Broken lines indicate depressor responses. s means a statistically significant difference from those after Ro 4-4602, p<0.05.](image)
Fig. 4. Effects on pressor response induced by electrical stimulation of the medulla oblongata in DOCA-salt hypertensive rats. The data presented mean±s.e. calculated from 5-6 rats. The symbols, open circles, open squares and closed circles indicate initial, 20-30 min after Ro 4-4602 (20 mg/kg i.p.) and 40-60 min after L-DOPA (20 mg/kg i.p.), respectively. s indicates a significant difference from initial or Ro 4-4602, p<0.05.

Fig. 5. Effects on pressor response induced by electrical stimulation of the midbrain reticular formation in normal and DOCA-salt hypertensive rats. The data presented mean±s.e. calculated from 5 rats. The symbols, open circles, open squares and closed circles indicate initial, 20-30 min after Ro 4-4602 (20 mg/kg i.p.) and 40-60 min after L-DOPA (20 mg/kg i.p.), respectively. s indicates a statistically significant difference from initial or Ro 4-4602, p<0.05.
both unanesthetized and anesthetized DHR. Under urethane anesthesia, electrical stimulation of the posterior hypothalamus induced biphasic BP changes; pressor and depressor responses. Ro 4-4602 (20 mg/kg i.p.) transiently reduced the BP response to electrical stimulation. After the responses had returned to normal, L-DOPA (20 mg/kg i.p.) was administered. This procedure significantly decreased the pressor response after stimulation of either of three brain regions in freely moving DHR (Figs. 3-5). In urethane anesthetized animals, depressor response to electrical stimulation of the posterior hypothalamus was significantly enhanced with L-DOPA plus Ro 4-4602. The reduction of the pressor effects in anesthetized animals was less than in freely moving animals.

**DISCUSSION**

**Role of noradrenergic neurons**

The previous observation (6) that the marked accumulation of DA in the medulla-pons and hypothalamus and of DOPA in the medulla-pons of DHR suggests that β-hydroxylation of DA may be rate limiting in the NA synthesis in these noradrenergic neurons areas and that consequent accumulation of DA or DOPA may affect the activity of noradrenergic neurons: enhancement or suppression. The present results that blockade of either central α-adrenoceptor with phentolamine or NA synthesis from DA with a DA-β-hydroxylase inhibitor in the brain completely abolished the BP fall in DHR after L-DOPA plus Ro 4-4602, may indicate an involvement of α-adrenoceptor of central noradrenergic neurons with the marked BP fall. Blockade of α-adrenoceptors in the brain also reduced the activity of clonidine (15, 16). L-DOPA enhances NA turnover in the brainstem (17) as well as in the whole brain (18, 19). L-DOPA-enhances NA turnover in the brainstem may be partly explained by the increased release of NA from nerve endings with newly accumulated DA, since there is evidence to indicate that L-DOPA exerts enhancement of the spontaneous efflux of NA after conversion of L-DOPA to DA in the brain homogenate particles (20). Therefore, enhancement of NA turnover in the brainstem after L-DOPA plus Ro 4-4602 may cause a pronounced BP fall in DHR of which NA turnover in the brainstem was markedly lowered (21, 22). Furthermore, one can consider the existence of noradrenergic inhibitory pathway between the nucleus of the tractus solitarius and the vasomotor centers (23), since the activation of the nucleus of the solitary tract with clonidine diminishes the pressor responses to the posterior hypothalamic stimulation (24). It is reasonably assumed that phentolamine-induced suppression of a BP fall in DHR after L-DOPA plus Ro 4-4602 may be explained by blockade of α-adrenoceptor to the effluxed NA with accumulated DA in the brainstem.

**Electrical stimulation of the pressor areas in the brainstem**

The pressor response to electrical stimulation of the pressor area in the brain is known to be due to an increased sympathetic nerve activity (25), accompanied by an increased release of catecholamine into the adrenal vein (26). The present results obtained in DHR show that L-DOPA and Ro 4-4602 markedly decrease the BP response following stimulation in the pressor area of the posterior hypothalamus, the midbrain reticular for-
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mation or the dorsal medulla oblongata. The onset and duration of the effects coincided with hypotension obtained by the same dose of both drugs. The suppression of pressor responses occurred much more in conscious rats than the animals anesthetized with urethane. Also, histochemical fluorescence analysis showed that the noradrenergic nerve endings of the posterior hypothalamus at least partly originate from cell bodies located at A6 cells, the locus coeruleus in the pons and A2 cells, the nucleus commissuralis in the medulla oblongata, through midbrain reticular formation (11, 27, 28). Moreover, pressor responses induced by the locus coeruleus stimulation were diminished after destruction of the posterior hypothalamus (29) and also pressor response to the posterior hypothalamic stimulation was abolished by infusion of clonidine into the nucleus of the solitary tract (24). They also stated that the pressor response to the posterior hypothalamic stimulation might be mediated by stimulation of \( \alpha \)-adrenoceptors. These results suggest that the posterior hypothalamaus may be considered to be functional BP terminals. Therefore, it seems that suppression of the pressor response with L-DOPA and Ro 4-4602 may be explained by depression of the pressor noradrenergic neurons after marked accumulation of DA or DOPA. Accordingly, these results suggest that BP may be under the control of both inhibitory and excitatory noradrenergic neurons in the brainstem.

In conclusion, the marked fall in BP of DHR after L-DOPA and Ro 4-4602 is probably due to activation of \( \alpha \)-adrenoceptor in the brainstem after the enhanced efflux of NA with the accumulation of DA and due to blockade of pressor areas in the brainstem. Here with one can consider the presence of both inhibitory and excitatory noradrenergic neurons in the brainstem to maintain control of BP.

REFERENCES

1) OSPERNE, M.W., WENGER, J.I. AND WILEMS, W.: J. Pharmacol. exp. Ther. 168, 517 (1971)
2) WATANABE, A.M., PARKS, L.C. AND KOPIN, I.J.: J. clin. Invest. 50, 132 (1971)
3) SCHMITT, H., SCHMITT, H. AND FERNAND, S.: Europ. J. Pharmacol. 17, 293 (1972)
4) BAUM, T. AND SHROPSHIRE, A.T.: Neuropharmacology 12, 49 (1973)
5) BARTELOLINI, G. AND PLETSCHER, A.: J. Pharmacol. exp. Ther. 161, 14 (1968)
6) NAKAMURA, K., MISOGAMI, S. AND NAKAMURA, K.: Japan. J. Pharmacol. 25, 85 (1975)
7) MISOGAMI, S., SHIBAYAMA, F., KIKUCHI, H. AND SOKABE, H.: Japan. J. Const. Med. 32, 59 (1969)
8) PLETSCHER, A. AND BARTELOLINI, G.: Clin. Pharmacol. Ther. 12, 344 (1971)
9) NAKAMURA, K. AND THOREN, H.: Psychopharmacologia 24, 359, (1972)
10) KÖNIG, J.F.R. AND KLIPPEL, R.A.: The Rat Brain R.E. Krieger Publ. Co., New York (1970)
11) DAHLESTROM, A. AND FUSE, K.: Acta physiol. scand. 62, suppl. 232, 1 (1964)
12) GLOWINSKI, J. AND IVIersen, L.L.: J. Neurochem. 13, 655 (1966)
13) SHELENBERGER, M.K. AND GORDON, J.H.: Analyt. Biochem. 39, 356 (1971)
14) ANTON, A.H. AND SAYRI, D.F.: J. Pharmacol. exp. Ther. 138, 360 (1962)
15) SCHMITT, H., SCHMITT, H. AND FERNAND, S.: Europ. J. Pharmacol. 14, 98 (1971)
16) SCHMITT, H., SCHMITT, H. AND FERNAND, S.: Europ. J. Pharmacol. 22, 212 (1973)
17) ROMERO, J.A., CHALMERS, J.P., COTTMAN, K., LYTLE, L.D. AND WURTMAN, R.J.: J. Pharmacol. exp. Ther. 180, 277 (1972)
18) GERSHON, E.S., GOODWIN, F.K. AND GOLD, P.: Pharmacologist 12, 268 (1970)
19) CHALMERS, J.P., BALDASSARINI, R.J. AND WURTMAN, R.J.: Proc. natn. Acad. Sci. 68, 662 (1971)
20) NG, L.Y.K., COLBURN, W.R. AND KOPIN, I.J.: J. Pharmacol. exp. Ther. 183, 316 (1972)
21) Nakamura, K., Gerold, M. and Thoenen, H.: Arch. Pharmacol. 268, 125 (1971)
22) De Champlain, J., Van Ameringen, M.R., Gauthier, P. and Nadeau, R.: Life Sci. 13, XV (1973)
23) Haeusler, G. and Finch, L.: Extract J. Pharmacol. 3, 16 (1972)
24) Philippu, A., Rosenberg, W. and Przuntek, H.: Arch. Pharmacol. 278, 373 (1973)
25) Folkow, B. and Rubinstein, E.H.: Acta physiol. scand. 68, 48 (1966)
26) Ueda, H., Iizuka, T., Yasuda, H., Takabatake, Y., Iizuka, M., Ihori, M. and Yamamoto, M.: Japan. Heart J. 7, 277 (1966)
27) Loizou, L.A.: Brain Res. 15, 563 (1969)
28) Ungerstedt, U.: Acta physiol. scand., suppl. 367, (1971)
29) Przuntek, H., Guimaraes, S. and Philippu, A.: Arch. Pharmacol. 271, 311 (1971)