DIFFERENCE IN HYPOTENSIVE RESPONSE TO L-DOPA AND A DECARBOXYLASE INHIBITOR IN VARIOUS FORMS OF HYPERTENSIVE RATS*

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Abstract—In unanesthetized and unrestraint rats, the administration of L-DOPA (20 mg/kg i.p.) with a peripheral decarboxylase inhibitor, Ro 4-4602 (50 mg/kg i.p.), markedly lowered blood pressure in DOCA-salt hypertensive rats (DHR) much more than it did in spontaneously hypertensive rats (SHR) or three types of normotensive controls; normal (NR), uninephrectomied and DOCA only. L-DOPA plus Ro 4-4602 did not change the renal hypertension induced by clipping a renal artery and uninephrectomy (RHR). The marked fall in blood pressure of DHR after L-DOPA plus Ro 4-4602 seemed to correlate with the accumulation of dopamine in the medulla-pons and hypothalamus, and of DOPA content in the medulla-pons. In RHR, accumulation of dopamine in the medulla-pons and hypothalamus was markedly lower than that in DHR, SHR and NR. Bilateral destruction of dopaminergic neurons in the striatum with 6-hydroxydopamine did not change blood pressure lowering activity of L-DOPA plus Ro 4-4602 in NR and DHR. Spiroperidol (0.01 mg/kg i.p. × 2) caused no effect on the fall in blood pressure of DHR after L-DOPA plus Ro 4-4602, whereas the same doses of spiroperidol reduced amantadine-induced stereotyped behavior in DHR. These data suggest that the pronounced fall in blood pressure of DHR after L-DOPA plus Ro 4-4602 may be related to the marked accumulation of DA or DOPA in the brainstem, most probably in its noradrenergic neurons, but involvement of DA neurons per se may be minimal. Hypotensive re-activity of brainstem neurons with the DA accumulation is in the order of: DHR > SHR = NR > RHR.

The possible contribution of the central nervous system to the control of blood pressure (BP) has been recently re-emphasized. Much experimental data have accumulating to indicate that central noradrenergic neurons contribute to the regulation of BP. The hypotensive action of clonidine is known to occur by enhancement of activity of central noradrenergic neurons (1, 2). Reduction of noradrenaline (NA) turnover was observed in the brainstem of DOCA-salt hypertensive rats (DHR) (3, 4). L-DOPA-induced fall in blood pressure was observed only with the presence of a peripheral decarboxylase inhibitor (PDI), which indicated the action to be mediated through the central nervous system (5). The fall in BP with L-DOPA plus PDI was accompanied with centrally mediated bradycardia and venous pooling (6) and the decrease in peripheral sympathetic outflows (7–9).

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MATERIALS AND METHODS

Hypertensive animals

DOCA-salt hypertension was induced in male Wistar rats weighing 140-160 g. The animals were uninephrectomized and given desoxycorticosterone acetate (DOCA) (10 mg/kg once in a week s.c.) and 0.9% NaCl as drinking fluid ad libitum. They were used 4-5 weeks later and their systolic BP ranged from 170 to 210 mmHg. Renal hypertension was induced by clipping the left renal artery with a silver ribbon and removing the right kidney a week later in Wistar rats weighing 140-160 g. These RHR were used 4-5 weeks after clipping and their systolic BP ranged from 170 to 200 mmHg. Spontaneously hypertensive rats (SHR) were F25, 17 to 20 weeks of age, weighing 220-250 g and were from the colony of the Iyaku-Shigen Institute for Medical Research. Their systolic BP ranged from 160 to 220 mmHg. Normal, only DOCA treated and unilaterally nephrectomized rats matched in age and body weight were used as three different controls. Their systolic BP ranged from 100 to 130 mmHg. The BP was measured without anesthesia or restraint through a cannula intubated into the aorta as described previously (11).

Neurochemical determination

Hypothalamus, medulla-pons, striatum and residual parts of the brain except the cerebellum were dissected according to the method of Glowinsky and Iversen (12). NA and DA were determined fluorimetrically by the method of Shellenberger and Gordon (13), after separation on alumina column by the method of Anton and Sayre (14). When L-DOPA was administered, NA, DA and DOPA were separated by Dowex 5-x4 column (15, 16) and, NA, DA and DOPA were determined fluorimetrically by the method of Shellenberger and Gordon (13).

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 injection of 50 mg/kg of Ro 4-4602, the dose of peripheral decarboxylase inhibition (10), as a standard dosage schedule. A combination of the dose of both drugs was found to be suitable to enhance DA accumulation in the brain (17). Twenty μg of 6-hydroxydopamine hydrobromide (6-OHDA) dissolved in 2 μl of artificial cerebrospinal fluid (18) was injected slowly into the bilateral caudate nuclei of the brain fixed with a David-Kopf instrument as described by Nakamura et al. (19) 1-2 days before the operation for induction of DOCA-salt or renal hypertension.

RESULTS

Effects of L-DOPA plus Ro 4-4602 on BP of various forms of freely moving hypertensive rats

Administration of L-DOPA (20 mg/kg i.p.) 30 min after Ro 4-4602 (50 mg/kg i.p.)
slightly reduced the BP of three types of normotensive controls: normal (N), uninephrectomized and only DOCA treated (Fig. 1, left panel). The combination of both drugs markedly decreased BP of DHR, whereas there was no significant fall in BP of the RHR. The BP fall in SHR after L-DOPA plus Ro 4-4602 was less than that of DHR. The fall in BP was associated with a decrease in heart rate of any group of rats. L-DOPA alone caused no significant change in BP of both N and DHR (Fig. 1, right panel). Ro 4-4602 alone induced a slight fall in BP, which returned to the initial level within 10-20 min (Fig. 1, left panel). The direct administration of Ro 4-4602 (150 μg/10 μl) into the lateral ventricle of DHR through an implanted cannula 20 min before L-DOPA completely prevented a fall in BP induced with L-DOPA plus Ro 4-4602. The initial BP of 6 DHR was 198±11 mmHg, whereas the BP values of treated DHR were 189±17 mmHg at an expected peak time of 40 min after L-DOPA. The results indicated that cerebral conversion of L-DOPA to DA was prerequisite for the fall in BP.

Effects on DA, NA and DOPA contents in the brain regions

There was no marked difference in endogenous NA and DA contents in the brain regions of the normotensive and 3 types of hypertensive rats, except that there was slight
TABLE 1. Endogenous catecholamine contents in the brain regions of normotensive and hypertensive rats

| Brain regions     | NA contents in ng/g | DA contents in ng/g | N | DH | RH | SH |
|-------------------|---------------------|---------------------|---|----|----|----|
| Medulla-pons      |                     |                     | 21| 15 | 8  | 24 |
|                   | 599                 | 670<sup>a</sup>     | 602| 89 | 69 | 77 |
|                   | 18                  | 70                  | 40 | 6  | 9  | 26 |
| Hypothalamus      | 1,429<sup>b</sup>   | 1,597               | 1,932<sup>b</sup> | 1,782<sup>b</sup> | 470 | 420 | 369 | 509 |
|                   | 53                  | 77                  | 42 | 118| 28 | 22  | 66  | 64  |
| Thalamus-midbrain | 485                 | 497                 | 509| 287| 241| 264 |
|                   | 34                  | 20                  | 13 | 23 | 41 | 37  |
| Striatum          | 326                 | 398                 | 399| 372| 10,493| 9,409| 9,392| 10,004 |
|                   | 18                  | 38                  | 55 | 25 | 679 | 461 | 820  | 889  |
| Residual brain    | 242                 | 268                 | 280| 356| 256 | 309 | 319  | 286  |
|                   | 15                  | 26                  | 2  | 20 | 19  | 16  | 10   | 44   |

The values given represent mean and s.e. calculated individually from numbers of animals shown in parentheses.

<sup>a</sup>, a significant difference from normotensive controls, p<0.05; <sup>b</sup>, p<0.025

increase in hypothalamic NA contents in RHR and SHR and that NA contents in the medulla-pons were slightly elevated in DHR, as shown in Table 1. The administration of L-DOPA (20 mg/kg i.p.) 30 min after Ro 4-4602 (50 mg/kg i.p.) to the normal rats markedly elevated DA contents in all parts of the brain when determined 40 min after L-DOPA, the time of maximal fall in BP, more than did in those after L-DOPA alone (Fig. 2, upper panel). L-DOPA alone which caused no effect on BP levels increased DA contents of the heart and kidney (Fig. 2, upper panel). In the heart and kidney, accumulation of DA was significantly (p<0.001) prevented by the prepreament with Ro 4-4602 (Fig. 2, right side of the upper panel). L-DOPA or L-DOPA plus Ro 4-4602 slightly decreased NA contents in the hypothalamus and kidney (Fig. 2, middle panel). These results are in agreement with previous reports using labelled L-DOPA (10, 17). There were no detectable contents of DOPA in the brain of untreated rats except in the neostriatum, as reported previously (15). After L-DOPA treatment, DOPA contents were found to increase uniformly in the brain regions, 400-750 ng/g, which was not reflected correlation with the endogenous catecholamine contents of each regions (Table 2). L-DOPA plus Ro 4-4602 significantly (p<0.001) accumulated DOPA in all parts of the brain, heart and kidney as compared with those of L-DOPA alone (Fig. 2, lower panel; Table 2). A significant (p<0.05) enhancement of DA accumulation was characteristic in the medulla-pons and hypothalamus of DHR (Fig. 3). Only DHR exhibited enhanced accumulations of DA, DOPA and NA (less extent) in the medulla-pons area after L-DOPA and Ro 4-4602 (Fig. 3; Table 3). The results suggest that accumulation of DA, DOPA or NA in the brainstem area may be involved in the marked fall in BP of DHR. In contrast, accumulation of DA following the administration of L-DOPA plus Ro 4-4602 to RHR was found less in the medulla-pons and hypothalamus than in those of DHR, SHR and NR (Fig. 3). SHR exhibited a more marked accumulation of NA and less of DA in the striatum than those of others.
Fig. 2. Effects of L-DOPA alone or L-DOPA plus Ro 4-4602 on the catecholamine and DOPA contents in the brain and peripheral organs of normal rats. The data shown indicate mean ± s.e. DOPA contents were measured in 3 groups in which 8 rats were pooled per each group. DA and NA contents obtained individually from 8 rats are shown as percent of untreated controls. DOPA contents (lowest panel) are shown as percent of those treated with DOPA alone.

Contents in MP, HT, TM, CN and RB of normotensive rats treated with L-DOPA were MP, medulla-pons; HT, hypothalamus; TM, thalamus-midbrain; CN, striatum RB, residual parts of the brain except the cerebellum.

a, significant different from each left column p<0.05 ; b, p<0.025 ; c, p<0.01 ; d, p<0.001

TABLE 2. Effects of L-DOPA or L-DOPA plus Ro 4-4602 on DOPA contents in the brain regions of normotensive rats

| Brain regions       | DOPA contents in μg/g | L-DOPA | L-DOPA + Ro 4-4602 |
|---------------------|------------------------|--------|--------------------|
| Medulla-pons        | 0.503±0.051            | 4.25 ±0.17 |
| Hypothalamus        | 0.563±0.071            | 5.02 ±0.49 |
| Thalamus-midbrain   | 0.747±0.041            | 5.15 ±0.34 |
| Striatum            | 0.549±0.096            | 3.20 ±0.11 |
| Residual brain      | 0.392±0.029            | 2.43 ±0.09 |
| Heart               | 0.406±0.052            | 2.231±0.20 |
| Kidney              | 0.295±0.022            | 2.544±0.06 |

The values given represent mean ± s.e., calculated from 3 samples, each sample contained 8 pooled tissues.

The animals were sacrificed 40 min after the administration of L-DOPA. There was a significant difference (p<0.0005) in L-DOPA contents in all tissues between L-DOPA and L-DOPA plus Ro 4-4602 in normotensive rats.
Fig. 3. Accumulation of catecholamine in the brain regions after administration of L-DOPA and Ro 4-4602 to normotensive and hypertensive rats. The data represent mean±s.e. calculated from 8 rats. DA and NA contents are shown as per cent of normotensive controls; p<0.05 ; b, p<0.025 ; c, p<0.001. MP, medulla-pons ; HT, hypothalamus ; TM, thalamus-midbrain ; CN, striatum ; RB, residual parts of the brain except cerebellum.

Table 3. Effects of L-DOPA plus Ro 4-4602 on DOPA contents in the brain regions of various forms of hypertensive rats

| Brain regions        | NR     | DHR    | % change | NR     | SHR    |
|----------------------|--------|--------|----------|--------|--------|
|                      | μg/g   | μg/g   |          | μg/g   | μg/g   |
| Medulla-pons         | 4.25±0.17 | 6.90±0.47a | +62      | 4.78±0.23 | 4.57±0.37 |
| Hypothalamus         | 5.02±0.49 | 6.47±1.40 | +29      | 6.25±0.20 | 5.37±0.49 |
| Thalamus-midbrain    | 5.15±0.34 | 6.35±0.38 | +23      | 6.76±1.11 | 4.79±0.87 |
| Caudate nucleus      | 3.20±0.11 | 3.15±0.06 | +93      | 4.15±0.57 | 4.33±0.44 |
| Residual brain       | 2.43±0.09 | 7.12±0.34b | +193     | 3.06±0.41 | 4.51±0.72 |

The values given represent mean±s.e. calculated from 3 samples, each sample contained 8 pooled tissues. The animals were sacrificed 40 min after the administration of L-DOPA.

Effects of spiroperidol on BP fall after L-DOPA plus Ro 4-4602 in freely moving DHR

For further investigation to determine whether or not DA or NA neurons participate in the fall of BP after L-DOPA plus Ro 4-4602, the effect of spiroperidol, which blocked DA receptor in the brain (Bolme and Fuxe, 1971), was examined. The marked fall in BP of DHR after L-DOPA plus Ro 4-4602 was found to be unaffected by spiroperidol (0.01 mg/kg i.p.), administered 10 min before and 20 min after L-DOPA. The same doses of spiroperidol markedly suppressed amantadine (100 mg/kg i.p.) induced stereotyped behaviors: sniffing, catalepsy and enhanced exploration, and occasional clonic convulsion in DHR.
**DOPA AND HYPOTENSION**

**Effects of 6-OHDA on the development of DOCA-salt and renal hypertension or on BP levels after L-DOPA with an inhibition of peripheral decarboxylase**

The drug, 6-OHDA, was applied into the bilateral striatum to determine whether or not there was relationship between the neostriatal DA neurons and a fall in BP after L-DOPA plus Ro 4-4602.

Pretreatment of the bilateral striatum with 6-OHDA (20 \( \mu \)g/2 \( \mu \)l) did not affect the development of hypertension in DHR or RHR, and BP levels of normals for a period of 5 weeks. The pretreatment of 6-OHDA had no influence on the BP fall induced by L-DOPA plus Ro 4-4602 in N, DHR and RHR. The 6-OHDA administration destroyed the neostriatal DA neurons by 40\%. The DA content in striatum 10.5 \( \pm \) 0.7 \( \mu \)g/g (n=5), was reduced to 6.3 \( \pm \) 0.5 \( \mu \)g/g (n=5) at 5 weeks after 6-OHDA.

**DISCUSSION**

**Differences in BP response in various hypertensions**

These studies demonstrated that L-DOPA with a peripheral decarboxylase inhibitor, Ro 4-4602, caused a marked fall in BP in DHR, intermediary in SHR or 3 types of normotensive controls, and least in RHR. The results are consistent with those of normal rats (5), SHR (20) and anesthetized normotensive dogs (6, 21). The greater reduction in BP of DHR was apparently not related to the initial BP levels. The results in RHR suggested that central noradrenergic neurons in renal hypertension might be less responsible for maintaining hypertension. However, the peripheral sympathetic nervous system may be involved in the development or maintenance of RHR (22, 23). The difference in BP responses to L-DOPA plus Ro 4-4602 between DHR and SHR as found in the present studies may partly be explained by the difference in the involvement of noradrenergic neurons of the brainstem: decreased NA turnover in DHR (3, 4) and unchanged state in SHR (24–26).

**Role of catecholaminergic neurons**

The marked BP fall in DHR seemed to correlate with enhanced accumulation of DA and L-DOPA in the medulla-pons area (Fig. 3). The small doses of spiroperidol which were considered to block DA but not NA receptor sites in the rat brain (2) did not change the BP fall after L-DOPA plus Ro 4-4602 in DHR. The same doses of spiroperidol blocked amantadine-induced stereotyped behavior, which provided additional evidence that amantadine stimulates central DA neurons as reported by others (27–29). Furthermore, destruction of neostriatal DA neurons with 6-OHDA did not affect the BP fall induced by L-DOPA plus Ro 4-4602, although intraventricular administrations of 6-OHDA are known to produce a post-synaptic supresensitivity to DA in the neostriatum (30, 31). Therefore, the present results suggest that the marked BP fall after L-DOPA plus Ro 4-4602 in DHR may be not mediated through stimulation of the neostriatal DA neurons. Further, apomorphine is known to lower BP due to an increased efferent activity of the vagal nerves but not mediated by central dopaminergic receptors (32). Therefore, the results suggest that a pronounced fall in BP of DHR with L-DOPA plus Ro 4-4602 may be due
to the marked accumulation of DA and DOPA in noradrenergic neurons of the brainstem vasomotor area.

In conclusion, the pronounced fall in BP of DHR induced with L-DOPA plus Ro 4-4602 may be due to functional changes of the brainstem vasomotor center by marked accumulation of DA and DOPA. Involvement of DA neurons per se may be minimal.

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REFERENCES
1) ANDÉN, N.-E., CORRODI, H., FUXE, K., HÖKFELT, B., HÖKFELT, T., RYDIN, C. AND SVENSSON, T.: Life Sci. 9, 513 (1970)
2) BOLME, P. AND FUXE, K.: Europ. J. Pharmacol. 13, 168 (1971)
3) NAKAMURA, K., GEROLD, M. AND THOENEN, H.: Arch. Pharmacol. 268, 125 (1971)
4) DE CHAMPLAIN, J., VAN AMERINGEN, M.R., GAUTHIER, P. AND NADEAU, R.: Life Sci. 13, XV (1973)
5) HENNING, M. AND RUBINSON, A.: J. Pharm. Pharmacol. 22, 241 (1970)
6) OSBORNE, M.W., WENGER, J.J. AND WILLEMES, W.: J. Pharmacol. exp. Ther. 178, 517 (1971)
7) WATANABE, A.M., PARKS, L.C. AND KOPIN, I.J.: J. clin. Invest. 50, 132 (1971)
8) SCHMITT, H., SCHMITT, H. AND FENARD, S.: Europ. J. Pharmacol. 17, 293 (1972)
9) BAUM, T. AND SHROPSHIRE, A.T.: Neuropharmacology 12, 49 (1973)
10) BARTHOLINI, G. AND PLETSCHER, A.: J. Pharmacol. exp. Ther. 161, 14 (1968)
11) MIZOGAMI, S., SHIBAYAMA, F., KIKUCHI, H. AND SOKABE, H.: Japan. J. Const. Med. 32, 59 (1969)
12) GLOWINSKI, J. AND IVERSSEN, L.L.: J. Neurochem. 13, 655 (1966)
13) SHEFFENBERGER, M.K. AND GORDON, J.H.: Analyt. Biochem. 39, 356 (1971)
14) ANTON, A.H. AND SAYRE, D.F.: J. Pharmacol. exp. Ther. 138, 360 (1962)
15) CARLSSON, A. AND WALDECK, B.: Acr. pharmacol. tox. 20, 47 (1963)
16) ROMERO, J.A., CHALMERS, J.P., COTTMAN, K., LTLYLE, L.D. AND WURTMAN, R.J.: J. Pharmacol. exp. Ther. 180, 277 (1972)
17) PLETSCHER, A. AND BARTHOLINI, G.: Clin. Pharmacol. Ther. 12, 344 (1971)
18) MFRLIS, J.K.: Am. J. physiol. 131, 67 (1940)
19) NAKAMURA, K., KENTZMAN, R., MAGGIO, A.C., AUGULIS, V. AND CONNEY, A.H.: Psychopharmacologia 31, 177 (1973)
20) YAMORI, T., DE JONG, W., YAMABE, H., LOVENBERG, W. AND SIOERDSMA, A.: J. Pharm. Pharmacol. 24, 690 (1972)
21) ROBSON, M.W., WENGER, J.J. AND WILLEMES, W.: J. Pharmacol. exp. Ther. 178, 517 (1971)
22) VOLICER, L., SCHIE, E., HILSE, H. AND VISVESWARAN, D.: Life Sci. 7, 525 (1968)
23) GREWAL, R.S. AND KAUL, C.L.: Br. J. Pharmacol. Chemother. 42, 497 (1971)
24) NAKAMURA, K., GEROLD, M. AND THOENEN, H.: Arch. Pharmacol. 271, 157 (1971)
25) NAKAMURA, K., GEROLD, M. AND THOENEN, H.: Spontaneous Hypertension, ed. OKAMOTO, K., pp. 51–58, Igaku Shoin Ltd., Tokyo (1972)
26) YAMABE, H., DE JONG, W. AND LOVENBERG, W.: Europ. J. Pharmacol. 22, 91 (1973)
27) STROMBERG, U. AND SVENSSON, T.H.: Acta pharmacol. tox. 30, 161 (1971)
28) SCATTON, B., CHERAMY, A., BISSON, M.J. AND GLOWINSKI, J.: Europ. J. Pharmacol. 13, 131 (1970)
29) COX, B. AND TBA, S.J.: Europ. J. Pharmacol. 24, 96 (1973)
30) UNGERSTEDT, J.: Acta physiol. scand., suppl. 367, 69 (1971)
31) URETSKY, J.H. AND SCHOENFELD, R.I.: Nature New Biol. 234, 157 (1971)
32) FINCH, L. AND HAUSLER, G.: Europ. J. Pharmacol. 21, 264 (1973)