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Dopa-induced locomotor stimulation after inhibition of extracerebral decarboxylase

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In rats, a combination of small doses of the dopa decarboxylase inhibitor Ro 4–4602 [*N*-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)hydrazine] with dopa causes a marked enhancement of the spontaneous locomotor activity which is not seen with dopa alone. If high instead of low doses of Ro 4–4602 are used, locomotor stimulation does not occur. Low doses of Ro 4–4602, owing to selective inhibition of extracerebral decarboxylase, enhance the dopa-induced rise of dopa and catecholamines in the brain, whereas high doses of Ro 4–4602, which also inhibit the cerebral dopa decarboxylase, increase only the level of dopa but not that of the catecholamines. It is concluded that the locomotor activation after small doses of Ro 4–4602 in combination with dopa is due to cerebral accumulation of catecholamines which consist mainly of dopamine.

It has previously been reported that small doses of Ro 4–4602 [*N*-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)hydrazine], an inhibitor of the decarboxylase of aromatic amino-acids, selectively inhibit the decarboxylation of administered L-3,4-dihydroxyphenylalanine (dopa) in extracerebral tissues (e.g. blood and heart). As a consequence, the dopa-induced increase of catecholamines and phenolic carboxylic acids in the blood is diminished, whereas the concentration of dopa is greatly raised, leading to an increased supply of the amino-acid to the brain. Since the cerebral dopa decarboxylase is not significantly affected by Ro 4–4602, an enhanced formation of catecholamines (particularly dopamine) takes place in the brain (Bartholini, Bates & others, 1967; Bartholini & Pletscher, 1968). By fluorescence microscopy it has been demonstrated that the formation of catecholamines occurs in the parenchyma and not in the capillary walls of the brain (Constantinidis, Bartholini & others, 1968).

Since the cerebral catecholamines can be selectively increased by small doses of Ro 4–4602 plus dopa, the effect of this combination on locomotor activity has been investigated and is now reported.

EXPERIMENTAL

Male albino rats, weighing 80–100 g, received Ro 4–4602 (50 or 500 mg/kg, i.p.) alone or followed after $\frac{1}{2}$ h by L-dopa (200 mg/kg i.p.). Controls were given L-dopa only. In some experiments, the animals were treated with reserpine (5 mg/kg, i.p.) 16 h before Ro 4–4602. The locomotor activity of 3 rats per experiment was measured in activity cages (Lehigh-Valley Electronics, Inc., Mod. A 1497), the number of interruptions of light beams during 1 h being recorded for 6 consecutive hours.

In parallel series, rats treated as indicated above were killed by decapitation 2 h after administration of L-[2-^{14}C]dopa (specific activity 2·07 mCi/mmol), and the radioactive metabolites of dopa were isolated from the brain and measured as previously described (Bartholini & Pletscher, 1968).

RESULTS

L-Dopa (200 mg/kg) does not markedly affect either the locomotor activity (Fig. 1) or the brain catecholamine concentration. Ro 4–4602 (50 and 500 mg/kg) also has no major influence. However, the combination of 50 mg/kg of the inhibitor with 200 mg/kg of dopa strongly increases the number of movements. A maximum is

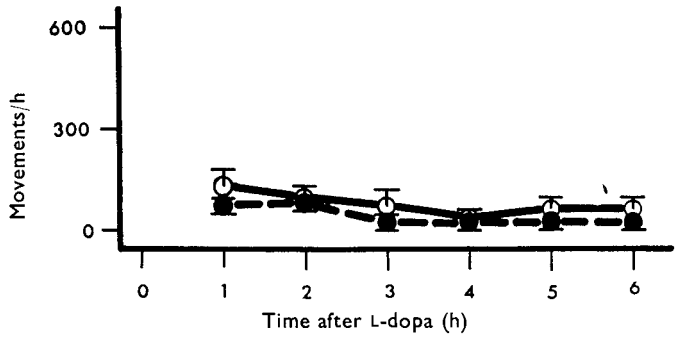

FIG. 1. Effect of L-dopa on locomotor activity of rats. In each experiment, the locomotor activity of 3 rats was measured for 6 h after injection of 200 mg/kg L-dopa i.p. Untreated rats were used as controls. ——— controls; - - - - - - L-dopa. Each value represents an average with s.e. of 3 experiments.

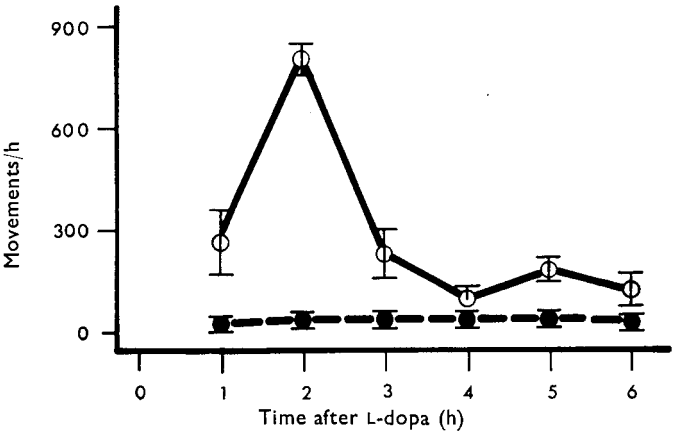

FIG. 2. Effect of two different doses of Ro 4–4602 combined with 200 mg/kg L-dopa on the locomotor activity of rats. Ro 4–4602 (50 or 500 mg/kg) was administered i.p. 30 min before L-dopa i.p. In each experiment, the locomotor activity of 3 rats was measured for 6 h. ——— Ro 4–4602 (50 mg/kg) + dopa; - - - - - - Ro 4–4602 (500 mg/kg) + dopa. Each value represents an average with s.e. of 3 experiments.

reached 2 h after dopa administration (Fig. 2). At the same time, a large rise of catecholamines, dopa and *O*-methyldopa occurs in the brain of animals treated with Ro 4–4602 plus dopa compared with rats injected with dopa alone. The dopa-induced increase of phenolic carboxylic acids is also enhanced by Ro 4–4602, but less markedly than the rise of amino-acids and catecholamines (Table 1).

The administration of 500 mg/kg of Ro 4–4602 followed by 200 mg/kg of dopa does not change the locomotor behaviour of the animals (Fig. 2). Compared to rats treated with dopa alone, only the amino-acid fraction strongly increases after this combination, whereas the catecholamine content does not significantly change

Table 1. *Effect of* L-*dopa and its combination with different doses of Ro* 4–4602 *on the concentration of catechol derivatives in rat brain*

Dopa metabolites	Dopa	Ro 4–4602 (50 mg/kg) + dopa	Ro 4–4602 (500 mg/kg) + dopa
Dopa	0·18 ± 0·03	22·54 ± 1·97	36·13 ± 7·98
O-Methyldopa	2·03 ± 0·13	17·60 ± 0·17	7·65 ± 1·27
Catecholamines	0·59 ± 0·06	3·35 ± 0·25	0·74 ± 0·08
Phenolic carboxylic acids ..	8·12 ± 0·88	13·47 ± 1·15	1·21 ± 0·02

L-[2-^{14}C]Dopa (200 mg/kg, i.p.) was injected alone or 30 min after 50 or 500 mg/kg of Ro 4–4602, i.p., and the animals were killed 2 h later.

The values are expressed in % of the radioactivity injected per gram of body weight and represent averages with s.e. of 3 experiments.

Table 2. *Effect of dopa and Ro* 4–4602 + *dopa on the catechol concentration in brain of reserpinized rats*

Dopa metabolites	Dopa	Ro 4–4602 (50 mg/kg) + dopa
Dopa	0·17 ± 0·01	17·65 ± 1·25
O-Methyldopa	2·30 ± 0·20	26·70 ± 0·50
Catecholamines	0·02 ● 0·00	2·26 ± 0·04
Phenolic carboxylic acids ..	6·60 ± 0·20	17·30 ± 0·50

Reserpine pretreated animals (5 mg/kg i.p. 16½ h before administration of [L-2-^{14}C]dopa) received 50 mg/kg Ro 4–4602 followed after 30 min by 200 mg/kg [L-2-^{14}C]dopa or were given [L-2-^{14}C]dopa only. The animals were killed 2 h after [L-2-^{14}C]dopa.

The values are expressed in percent of the radioactivity injected per gram of body weight and represent averages with s.e. of 3 experiments.

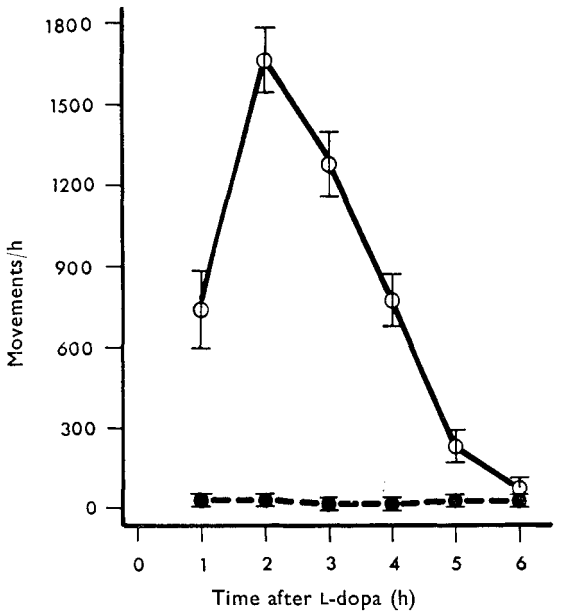

FIG. 3. Effect of dopa and Ro 4–4602 plus dopa on the locomotor activity of reserpinized rats. Reserpine-pretreated animals (5 mg/kg i.p. 16½ h before administration of dopa) received 50 mg/kg Ro 4–4602 followed after 30 min by 200 mg/kg L-dopa, or were given L-dopa only. In each experiment, the locomotor activity of 3 rats was measured for 6 h. ——— Reserpine + Ro 4–4602 + dopa; - - - - - - reserpine + dopa. Each value represents an average with s.e. of 3 experiments.

($P > 0{\cdot}05$), and the phenolic carboxylic acid fraction considerably decreases (Table 1). The ratio of dopa to 3-*O*-methyldopa is higher than in the experiments in which 50 mg/kg of Ro 4–4602 plus dopa were used (Table 1).

The well-known reserpine-induced depression of the locomotor activity is not modified by 50 mg/kg of Ro 4–4602 or by 200 mg/kg of dopa, whereas the combination of the two substances induces a marked hyperactivity for at least 2 h. This increase in locomotor activity is greater and longer-lasting than that induced by the combination of Ro 4–4602 plus dopa without reserpine pretreatment (compare Figs 2 and 3). In the brain, the amino-acids, catecholamines and phenolic carboxylic acids are much increased compared to their levels in reserpinized rats administered dopa alone (Table 2).

DISCUSSION

The present experiments confirm previous findings that 50 mg/kg of Ro 4–4602, intraperitoneally, strongly enhances the dopa-induced rise of amino-acids in the brain as well as of the catecholamines and their breakdown products, the phenolic carboxylic acids (Bartholini & others, 1967; Bartholini & Pletscher, 1968). Furthermore, they show that administration of 500 mg/kg of Ro 4–4602 plus dopa causes only the amino-acid fraction to increase. The relatively marked rise of dopa, compared to 3-*O*-methyldopa, possibly indicates a partial inhibition of catechol-*O*-methyltransferase by the high doses of Ro 4–4602.

As mentioned above, the action of small doses of Ro 4–4602 (50 mg/kg) on the metabolism of dopa is probably due to a selective inhibition of extracerebral dopa decarboxylase in consequence of which dopa, 3-*O*-methyldopa, catecholamines and phenolic carboxylic acids accumulate in the brain. High doses of Ro 4–4602 (500 mg/kg) also inhibit the cerebral dopa decarboxylase enhancing the dopa-induced accumulation of amino-acids, especially dopa, without major formation of catecholamines and phenolic carboxylic acids in the brain (Bartholini, Tissot & Pletscher, 1968).

The marked increase of locomotor activity induced by dopa after pretreatment with 50 mg/kg Ro 4–4602 is in agreement with previous findings according to which low doses of Ro 4–4602 + 200 mg/kg of dopa markedly increased lever pressing by rats in a continuous avoidance experiment (Scheckel, Boff & Pazery, 1965). The locomotor activation seems to be caused by the cerebral accumulation of catecholamines rather than of dopa. Thus, after high doses (500 mg/kg) of Ro 4–4602 plus dopa, the level of dopa in the brain is at least as high as after the combination with the low dose of Ro 4–4602, but no increase of locomotor activity occurs nor are the cerebral catecholamines elevated. A peripheral effect of catecholamines can be excluded since it has been previously shown (Bartholini & Pletscher, 1968) that the rise of the catecholamines as well as of the phenolic carboxylic acids in the blood is markedly less pronounced after the combination than after dopa alone. The latter, in the doses used in the present experiments, does not enhance locomotor activity.

The more marked and prolonged hyperactivity induced by 50 mg/kg Ro 4–4602 plus dopa in reserpinized, compared to non-reserpinized, rats is not due to a higher increase of the total catecholamines in the brain (compare Table 1 and 2). It may, however be the consequence of an enhanced catecholamine concentration at the receptor sites after reserpine pretreatment. Thus, reserpine probably inhibits the uptake of newly formed catecholamines into the storage granules where the amines are protected from

monoamine oxidase (Carlsson, 1966). The significant increase of phenolic carboxylic acids after reserpine pretreatment compared to non-reserpinized controls (compare Table 1 to Table 2) is compatible with this view since this finding indicates an increased exposure of catecholamines to monoamine oxidase.

It has previously been shown that low doses of Ro 4–4602 plus dopa increase the cerebral dopamine much more than the noradrenaline (Bartholini & Pletscher, 1968) Preliminary experiments show that, in the present experiments with non-reserpinized animals, the dopamine and noradrenaline rose by a factor 7 and 1·5 respectively with the combination Ro 4–4602 (50 mg/kg) plus dopa compared to dopa alone. It may therefore be assumed that the increase of cerebral dopamine is a major causative factor in the enhancement of locomotor activity although a possible role of noradrenaline cannot be excluded. The enhanced locomotor activity might also be connected with a liberation of endogeneous cerebral monoamines, e.g. by the dopamine formed from the exogenous dopa. Thus, Ro 4–4602 (50 mg/kg) plus dopa (25–200 mg/kg) decrease the 5-hydroxytryptamine (Bartholini, Da Prada & Pletscher, 1968) and according to preliminary results also the noradrenaline content of the brain. A major involvement of endogenous monoamines is, however, unlikely since locomotor stimulation also occurs in animals whose endogenous monoamine depots have been depleted by previous reserpinization.

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