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Dopamine: Mediator of Brain Polysome Disaggregation after L-Dopa

Abstract. *The disaggregation of brain polysomes which is produced by giving large doses of* L-*dopa to rats is not reproduced by administering its metabolite, 3-*O-*methyldopa, by giving* D-*dopa, which also depletes the brain of* S-*adenosylmethionine but is not converted to catecholamines, or by giving the* L-*dopa after a decarboxylase inhibitor. Polysome disaggregation is potentiated by the prior administration of a monoamine oxidase inhibitor, indicating that formation of a catecholamine is an obligatory requirement. These observations suggest that the mechanism by which* L-*dopa disaggregates brain polysomes involves its conversion to dopamine within the majority of brain cells.*

Administration of L-dopa (500 mg per kilogram of body weight, intraperitoneally) to rats is followed between 40 and 60 minutes by the disaggregation of polysomes obtained from whole brain. This disaggregation is unaccompanied by a decrease in the concentrations of any free amino acid; brain tryptophan levels actually increase significantly (*1*).

We have attempted to identify L-dopa or one of its metabolites as the agent causing disaggregation of brain polysomes. Administered L-dopa is transformed by the brain to the catecholamines dopamine and norepinephrine, and their metabolites, and to the amino acid 3-*O*-methyldopa (Fig. 1) (*2*). The *O*-methylation of exogenous dopa, catalyzed by catechol *O*-methyltransferase, depletes the brain of *S*-adenosylmethionine (*3*). To identify which of the compounds formed or utilized after administration of dopa is involved in polysome disaggregation, we examined the state of polysome aggregation in animals given (i) 3-*O*-methyldopa; (ii) D-dopa, which forms 3-*O*-methyldopa in the brain but does not undergo decarboxylation to dopamine; (iii) L-dopa along with an inhibitor (RO4-4602) of its decarboxylation to dopamine; (iv) L-dopa along with an inhibitor (pheniprazine) of monoamine oxidase; or (v) intracisternal dopamine or norepinephrine. Changes in whole brain polysome aggregation were correlated with alterations in the concentrations of dopa, 3-*O*-methyldopa, *S*-adenosylmethionine, dopamine, and norepinephrine in the brain.

Male Sprague-Dawley rats (Charles River Laboratories) weighing approximately 50 g were exposed to light from 9 a.m. to 9 p.m. daily. They were housed four per cage and given free access to Purina Chow and water. L-Dopa, 3-*O*-methyldopa, and D-dopa (Hoffmann-LaRoche, Inc.) were dissolved in 0.05*N* HCl and administered intraperitoneally; control animals received only the acidic diluent. The decarboxylase inhibitor RO4-4602 (Hoffmann-LaRoche, Inc.) and the monoamine oxidase inhibitor pheniprazine (JB 516; Lakeside Laboratories, Inc.) were dissolved in water and administered intraperitoneally. Dopamine hydrochloride

Table 1. Effects of L-dopa and related drugs on brain polysome profiles and dopa metabolism of 50-g male rats. Data are presented as mean and standard error of the mean. Significance of differences was evaluated by Student's *t*-test. The number of determinations in each group is given in parentheses. If two determinations were made for a group, the two values are given in brackets. Abbreviations: i.p., intraperitoneal; i.c., intracisternal.

Treatment	Route of administration	Dose (mg/kg)	Time after injection (min)	Polysomes (percent of profile)	Dopa (μg/g)	3-*O*-methyldopa (μg/g)	*S*-Adenosylmethionine (percent of control)*	Dopamine (ng/g)	Norepinephrine (ng/g)
Control	i.p.	0	60	65 ± 1.9 (8)	< 0.1 (11)	< 0.1 (9)			
L-Dopa	i.p.	500	40		18.7 ± 4.3 (7)†	0.5 ± 0.1 (7)†			
			60	43 ± 3.6 (7)†	6.8 ± 2.0 (7)†	1.2 ± 0.4 (8)‡			
3-*O*-methyldopa	i.p.	500	60	60 [64, 56]	0.3 ± 0.1 (7)§	4.6 ± 1.9 (7)§			
			120	65 [67, 63]	0.3 ± 0.05 (6)†	11.8 ± 1.9 (8)†			
Control	i.p.	0	60		< 0.1 (6)	< 0.1 (6)	100 ± 12.4 (6)	505 ± 32 (6)	300 ± 19 (6)
L-Dopa	i.p.	500	60		9.5 ± 1.8 (6)†	3.4 ± 0.4 (6)†	32 ± 7.9 (6)†	2,230 ± 423 (5)‡	330 ± 22 (6)
D-Dopa	i.p.	500	60	61 ± 4.0 (3)	10.7 ± 0.8 (6)†	2.9 ± 0.1 (6)†	27 ± 5.0 (6)†	640 ± 47 (6)	310 ± 23 (6)
RO4-4602	i.p.	800	90	65 ± 4.4 (3)	2.9 ± 1.0 (6)§	0.1 ± 0.02 (6)	45 ± 10.3 (6)‡	346 ± 34 (6)‡	188 ± 10 (6)†
RO4-4602 plus L-dopa	i.p. i.p.	800 500	90 60	60 ± 2.1 (3)	40.2 ± 2.5 (6)†	4.3 ± 0.9 (6)†	39 ± 4.0 (6)‡	298 ± 50 (6)‡	200 ± 10 (6)†
Control	i.p.	0	60	73 [73, 74]	< 0.1 (5)			503 ± 90 (5)	305 ± 29 (4)
L-Dopa	i.p.	100	60	75 ± 3.1 (3)	2.3 ± 0.8 (6)†			780 ± 24 (6)§	348 ± 12 (6)
Pheniprazine	i.p.	10	180	62 ± 1.5 (4)	< 0.1 (5)			779 ± 92 (5)	398 ± 20 (5)§
Pheniprazine plus L-dopa	i.p. i.p.	10 50	180 60		0.3 ± 0.03 (5)†			1,860 ± 185 (4)†	521 ± 24 (5)†
Pheniprazine plus L-dopa	i.p. i.p.	10 100	180 60	32 ± 7.3 (4)†	1.2 ± 0.2 (5)†			2,890 ± 397 (5)†	618 ± 63 (5)‡
Pheniprazine plus L-dopa	i.p. i.p.	10 150	180 60		1.3 ± 0.1 (3)†			7,940 [7,910; 7,980]†	691 ± 67 (3)‡
Pheniprazine plus L-dopa	i.p. i.p.	10 500	180 60	41 ± 9.0 (3)‡	17.0 ± 2.4 (4)†			30,000 ± 2,310 (4)†	801 ± 63 (4)†
		(micrograms per rat)							
Control	i.c.	0	45	60	< 0.1 (3)	< 0.1 (3)	100 ± 22.6 (3)	470 ± 34 (3)	289 ± 20 (3)
Dopamine	i.c.	100	15	59 [60, 57]	< 0.1 (5)	< 0.1 (5)	92 ± 2.6 (5)	6,020 ± 1,260 (5)§	308 ± 32 (5)
			45	58 [60, 56]	< 0.1 (5)	< 0.1 (5)	86 ± 1.7 (5)	903 ± 390 (5)	212 ± 18 (5)§
Norepinephrine	i.c.	100	15	57 [63, 52]	< 0.1 (5)	< 0.1 (5)	94 ± 4.6 (5)	555 ± 26 (5)	20,800 ± 2,470 (5)†
			45	54 [52, 57]	< 0.1 (5)	< 0.1 (5)	82 ± 6.7 (5)	593 ± 53 (5)	10,200 ± 826 (5)†

* Control brains contained an average of 13.4 μg of *S*-adenosylmethionine per gram. † $P < .001$ differs from control group. ‡ $P < .01$ differs from control group. § $P < .05$ differs from control group.

and norepinephrine (Regis Chemical Co.) were dissolved in 0.05*N* HCl and administered intracisternally; animals received one of the catecholamines or only the vehicle.

In all experiments, animals were decapitated at 1 p.m., and brain polysome profiles were prepared (*1*). For each determination of a profile, two whole brains were pooled, and the profiles were run in duplicate. For the metabolic assays, single brains were homogenized in 10 ml of 10 percent trichloroacetic acid in 0.05*N* HCl. After centrifugation at 10,000*g*, samples of the supernatant fluid were analyzed by fluorimetric assays for dopa (*4*), 3-*O*-methyldopa (*5*), and dopamine and norepinephrine (*6*, *6a*). *S*-Adenosylmethionine was measured in portions of the supernatant fluid by a double-label, isotope dilution technique (*7*).

In agreement with previous data (*1*), administration of 500 mg of L-dopa per kilogram to 50-g male rats caused extensive polysome disaggregation in the brain 60 minutes after injection, with reduction of polysome abundance from 65 percent (controls) to 43 percent of the total ribosome profile (Table 1). Forty minutes after L-dopa injection, dopa concentrations in the brain were considerably elevated (Table 1) above the insignificant concentration found in the brains of controls. By 1 hour after injection of L-dopa, the concentration of dopa in the brain had decreased, whereas that of 3-*O*-methyldopa had doubled. This evidence that polysome disaggregation was coincident with accumulation of 3-*O*-methyldopa suggested that the *O*-methylated amino acid might be the effector metabolite. Injection of 500 mg of 3-*O*-methyldopa per kilogram resulted in considerable levels of brain 3-*O*-methyldopa at 1 hour and 2 hours (Table 1). At both of these times, concentrations of brain dopa were low but were higher than those in controls. Although the concentrations of brain 3-*O*-methyldopa were even higher than those obtained after injection of L-dopa, the polysomes were unaffected (Table 1).

Even though the product of L-dopa methylation was not the cause of polysome disaggregation, it was possible that the disaggregation resulted from the accompanying depletion of *S*-adenosylmethionine. To test this hypothesis, the effects of D-dopa and L-dopa on polysome aggregation were compared. D-Dopa also accepts a methyl group from *S*-adenosylmethionine (*3*); how-

ever, it is not converted to catecholamines (*8*). One hour after intraperitoneal injection of 500 mg of D-dopa or of L-dopa per kilogram, the concentrations of brain dopa in the two groups were similar (Table 1), and comparable brain concentrations of 3-*O*-methyldopa were formed, accompanied by similar depletions of *S*-adenosylmethionine. On the other hand, D-dopa failed to raise dopamine and norepinephrine concentrations (*8*), whereas L-dopa elevated brain dopamine concentrations to four times those of the controls and raised norepinephrine slightly. D-Dopa failed to disaggregate brain polysomes (Table 1), which implies that the formation of catecholamine metabolites from dopa is prerequisite to disaggregation.

The conversion of L-dopa to dopamine was blocked by administration of large doses (800 mg/kg) of RO4-4602. In rats treated only with this inhibitor, brain dopa concentrations rose (Table 1), thus confirming that the decarboxylase was actually inhibited. When the rats were given 500 mg of L-dopa per kilogram 30 minutes after the decarboxylase inhibitor, brain dopa attained concentrations four times greater than those obtained with L-dopa treatment alone (Table 1). In accordance with the known action of the inhibitor, this extensive increase in brain dopa was accompanied by a decrease in the concentrations of dopamine and norepinephrine to below normal values. In the presence of the decarboxylase inhibitor, L-dopa also failed to disaggregate the brain polysomes, confirming the hypothesis that a high brain concentration of L-dopa is not by itself directly responsible for polysome disaggregation.

Thus either dopamine or one of its metabolites must be responsible for the phenomenon. Norepinephrine is a less likely candidate, since only relatively few neurons can transform exogenous dopa to this catecholamine. Moreover, the effect of L-dopa on brain norepinephrine is slight (Table 1). In order to prove that the subsequent metabolism of dopamine and norepinephrine to deaminated products is not responsible for the polysome disaggregation, pheniprazine, an inhibitor of monoamine oxidase, was administered prior to L-dopa. The inhibitor alone caused the concentrations of dopamine and norepinephrine to rise (see Table 1). When L-dopa was given in graded doses 2 hours after the inhibitor, the concentrations of dopamine achieved with small

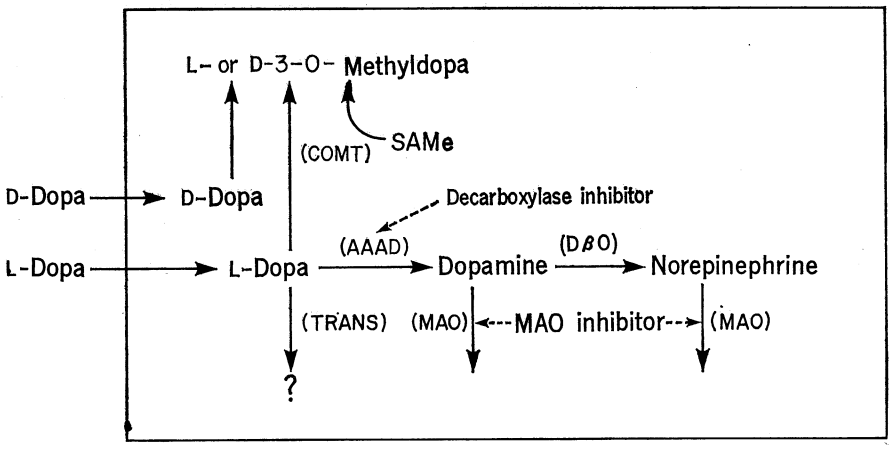

Fig. 1. Metabolism of L-dopa and D-dopa in rat brain. *COMT*, catechol-*O*-methyltransferase; *AAAD*, aromatic L-amino acid decarboxylase; *DβO*, dopamine-β-oxidase; *MAO*, monoamine oxidase; *TRANS*, dopa transaminase; and *SAMe*, *S*-adenosylmethionine. The decarboxylase inhibitor was RO4-4602; the monoamine oxidase inhibitor was pheniprazine.

doses of L-dopa (50 to 100 mg/kg) equaled those induced by much larger doses (for example, 500 mg/kg) in rats not treated with the inhibitor. This accumulation of dopamine at a low dose of L-dopa following treatment with pheniprazine was accompanied by extensive polysome disaggregation, a phenomenon not obtained when the same dose of L-dopa was given to animals that did not receive the enzyme inhibitor (Table 1).

This correlation of increased concentrations of dopamine and norepinephrine with polysome disaggregation prompted us to apply these compounds directly to the brain, since neither readily crosses the blood-brain barrier. Fifteen minutes after injecting 100 μg of dopamine intracisternally, rats had brain dopamine concentrations at least as high as those after intraperitoneal injection of L-dopa (Table 1). By 45 minutes after intracisternal treatment, dopamine had fallen to near control values. At 15 and 45 minutes after injection of 100 μg of norepinephrine, brain norepinephrine concentrations remained very high. Rats receiving either catecholamine intracisternally failed to exhibit disaggregated polysomes (Table 1) (*9*).

The failure of intracisternal administration of catecholamines to cause polysome disaggregation does not necessarily exclude these compounds as effector substances: The cerebrospinal fluid route may distribute the active agent only to superficial brain cells, leaving the major cell population unexposed (*10*); alternatively, the catecholamines placed in the cisterna magna may be so effectively concentrated within catecholaminergic neurons as to be unavailable to the other cells in the brain (*11*). Another of our studies (*12*) indicates that the accumulation of brain dopamine after administration of L-dopa is not significantly decreased in rats whose central catecholamine-containing neurons have been largely destroyed by prior intracisternal administration of 6-hydroxydopamine; hence dopamine probably is formed from exogenous L-dopa in all brain cells, even glia. It seems likely that, for the extensive polysome disaggregation to occur after the administration of L-dopa, the majority of brain cells, neurons and glia, must participate. In this connection, catecholamines have recently been shown to influence adenyl cyclase activity in glia (*13*).

Disaggregation of polysomes is usually evidence of disturbed protein synthesis; however, it remains to be demonstrated that the L-dopa–induced disaggregation described here and earlier (*1*) is actually associated with changed synthesis of brain proteins. The ability of catecholamines formed intracerebrally from exogenous L-dopa to disaggregate brain polysomes raises the possibility that catecholamine neurotransmitters released at synapses may also exert some of their effects by modifying brain polysome function.

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