L-Dopa: Disaggregation of Brain Polysomes and Elevation of Brain Tryptophan

Abstract. *One hour after administration of* L-*dopa (50 to 300 milligrams per kilogram), there is a marked disaggregation of brain polysomes in immature rats. Adult animals show a similar response, but require larger doses of the amino acid (500 milligrams per kilogram). Single doses of* L-*dopa significantly elevate amounts of tryptophan in the brain; hence their effect on polysomes does not result from the unavailability of this amino acid.*

Many patients with Parkinson's disease are now given prolonged treatment with large (8 to 10 g/day) oral doses of the amino acid L-dihydroxyphenylalanine (L-dopa) (*1*). The theoretical basis for this use of L-dopa rests upon two observations. First, endogenously synthesized L-dopa is a metabolic intermediate within brain neurons that convert L-tyrosine to the catecholamine dopamine (*2*), and second, brains of Parkinsonian patients examined at autopsy contain subnormal amounts of dopamine (*3*). Administration of L-dopa raises amounts of dopamine in brains of experimental animals (*4*) and also influences the metabolic fate of other putative brain neurotransmitters. It lowers amounts of serotonin in brains of mice (*5*), possibly because the newly synthesized dopamine competes for intracellular storage sites of serotonin (*6*). It also raises amounts of norepinephrine, accelerates turnover of [^{3}H]norepinephrine, and suppresses *O*-methylation of brain norepinephrine (*7*). The latter effect probably results from the depletion of brain *S*-adenosylmethionine (*8*) caused by the *O*-methylation of L-dopa itself which forms the metabolites 3-*O*-methyl dopa and homovanilic acid (*9*).

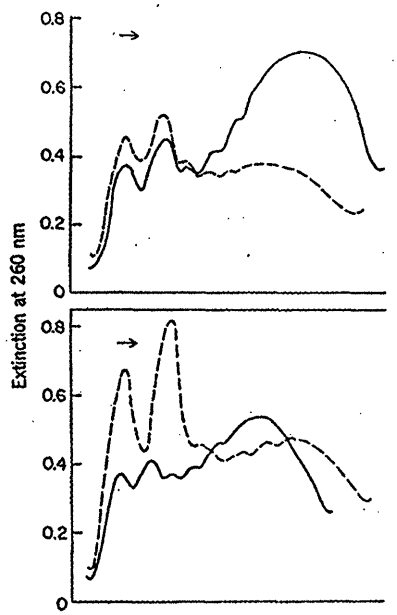

Fig. 1. Effects of L-dopa on brain polysome profiles in 20- and 100-g rats. Brains were taken 60 minutes after the administration of either L-dopa dissolved in 0.05*M* HCl, or the diluent alone. Twenty-gram rats (upper graph, 7- to 9-day-old) received 200 mg of the amino acid per kilogram; 100-g rats (lower graph) received 500 mg/kg. Polysomes were isolated with discontinuous sucrose gradients, and samples were spun on continuous 10 to 40 percent sucrose density gradients for 70 minutes at 38,000 rev/min to obtain polysome profiles (*14*). Solid line, controls; dashed line, L-dopa. The arrow indicates top of gradient.

In addition to serving as a precursor for brain catecholamines, L-dopa might also affect metabolic changes because of its structural similarity to circulating aromatic amino acids. Although exogenous L-dopa is apparently not incorporated into proteins (*9*), the transport system that carries it into brain cells is probably shared with other neutral α-amino acids, including phenylalanine and tyrosine (*10*). It is thus possible that exogenous L-dopa might modify the availability of other amino acids needed for neuronal protein synthesis, either by competitively inhibiting their transport or by inhibiting their binding to specific transfer RNA's.

There is abundant evidence that intracellular amounts of free amino acids exert a major influence on protein synthesis in mammalian tissues, exemplified in liver and brain. In rat liver, exogenous amino acids delivered by the portal circulation both stabilize polysomes and stimulate protein synthesis; tryptophan seems to be the limiting amino acid (*11*). Experiments on 7-day-old rat brains have also suggested that amounts of tryptophan in the brain are crucial in determining the extents of polysome aggregation and protein synthesis. Intraperitoneal injections of L-phenylalanine caused the depletion of brain tryptophan, the disaggregation of brain polysomes, and the inhibition of brain protein synthesis estimated in vitro (*12*). These effects did not occur if tryptophan was administered at the same time. They were also absent in older animals treated with only L-phenylalanine, although amounts of tryptophan decreased.

Since L-dopa is structurally related to phenylalanine, we have examined the effects of its administration on brain tryptophan content and polysome profiles of rats of various ages. We find that, like phenylalanine, L-dopa causes the disaggregation of brain polysomes. However, this effect is associated with an increase in the amount of tryptophan in the brain.

Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) were exposed to light from 9 a.m. to 9 p.m. daily (*13*); they were given free access to Purina Chow and water. All animals were killed at 1 p.m. Pregnant rats were obtained on the 14th day after conception and housed in individual cages. On the day after delivery, each litter was adjusted to eight pups which were killed 7 to 9 days after birth (15

Table 1. Effects of L-dopa on brain polysome profiles and tryptophan content. Rats 7 to 9 days old and weighing 15 to 20 g received L-dopa intraperitoneally; they were killed at various times thereafter. The polysome abundance is given as the percentage of the total ribosomal RNA present in the polysome peaks as described in the text. Brains from several rats were pooled for each determination. The number of such determinations at each dosage is given in parentheses. Because the number of determinations for each group is small, analysis of variance and calculation of fiducial limits between sets of observations were used in preference to Student's *t*-test. The *F* values for such analyses showed that L-dopa has a highly significant action on both polysome abundance and brain tryptophan content. Since the fiducial interval for significance of polysome abundance at different dosages was 19, this indicates that the value for the 20 mg/kg dose did not differ from the control value, but all higher doses were significantly below the control value and were indistinguishable from one another. A similar pattern emerged for tryptophan which did not rise significantly until a dose of 50 mg/kg was given (fiducial limit 3.9). In the case of a dose of 300 mg/kg given at various times, a significant depression of polysome aggregation occurred only at 40 and 60 minutes after injection (fiducial limit 23); whereas, tryptophan was significantly elevated at 20, 40, and 120 minutes (fiducial limit 5.7).

Dose (mg/kg)	Time after injection (minute)	Polysomes (% of profile)	Tryptophan content (μg/g of brain
0	60	69 (5)	6.5 (3)
20	60	84 (2)	7.2 (2)
50	60	45 (2)	12.4 (2)
100	60	46 (2)	9.7 (3)
200	60	47 (2)	10.7 (3)
300	60	42 (2)	11.3 (3)
0	60	69 (5)	6.5 (3)
300	20	52 (2)	16.3 (2)
300	40	45 (2)	20.4 (2)
300	60	42 (2)	11.3 (3)
300	120	54 (2)	14.8 (2)

to 20 g) or 19 to 22 days after birth (45 to 55 g). Older male rats weighing 100 to 120 g were housed two per cage. The L-dopa (Hoffmann-LaRoche, Inc., Nutley, N.J.) was dissolved in 0.05*M* HCl and administered intraperitoneally; control animals received only the diluent. L-Phenylalanine (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in 0.42 percent NaCl, and L-tyrosine (Calbiochem, Los Angeles, Calif.) was suspended in 0.05*M* HCl.

In preliminary experiments, the effect of administering L-dopa on brain polysome aggregation was examined in the 7- to 9-day-old group. Total ribosomes were isolated, and polysome profiles were prepared by a modification of the techniques of Roberts *et al.* (*14*). Quantitative measurements of polysome profiles were made with a compensating planimeter which measured the areas under the monosome, disome, and polysome peaks. The percentage of the total area attributable to polysomes or to monosomes plus disomes was calculated. Polysomes in these rats were significantly disaggregated 60 minutes after a 50 to 300 mg/kg dose of L-dopa (Table 1; Fig. 1); the extent of the disaggregation was not related to dosage. Since the area of monosomes and disomes increased much less than that of polysomes decreased (Fig. 1), it can be concluded that some increase in subunit population (not measured) took place. In control animals and rats receiving 20 mg of L-dopa per kilogram, the polysomes appeared highly aggregated and comprised the major fraction of total ribosomes. To examine the time course of this effect of L-dopa, we killed rats that received 300 mg/kg 20, 40, 60, or 120 minutes after the injection. Polysome disaggregation was maximum between 40 and 60 minutes after injection (Table 1), and not significant at the other times examined.

The disaggregation of brain polysomes after administration of L-phenylalanine has been reported to be dependent on age. Polysomes were not disaggregated by the administration of L-phenylalanine to 28-day-old rats, whereas there was disaggregation in the 7-day-old group (*12*). Accordingly, the effect of L-dopa on polysome profiles was examined in rats of various ages. Nineteen-day-old animals weighing 45 to 55 g were given 200 mg of L-dopa per kilogram or the diluent 60 minutes before being killed. The brain polysomes of control and experimental groups were aggregated to the same extent (67 ± 9.2 percent polysomes in control group; 66 ± 13.4 percent polysomes in treated group). Rats weighing 100 to 120 g received 500 mg of L-dopa per kilogram or the diluent and were killed after 60 minutes. The polysomes of the treated group were significantly disaggregated (33 ± 4.8 percent polysomes in treated group compared to 61 ± 2.2 percent in control brains; $P < .01$, as shown in Fig. 1). In order to examine dose-response relationships in older rats in more detail, we made a series of studies on rats weighing about 100 g. At each dosage, four rats were injected with L-dopa; their brains were pooled in pairs to give two samples for each polysome profile analysis. The proportions of polysomes in the profiles were, respectively, 54 and 58 percent for the two sets of control animals, 49 and 54 percent for the rats given 200 mg of L-dopa per kilogram, 50 percent for one set of rats given 350 mg/kg, and 27 and 39 percent for the two sets given 500 mg/kg. Hence, L-dopa can produce polysome disaggregation in older animals, but larger doses of the amino acid are required to produce this effect than are required in 7- to 9-day-old rats. Preliminary observations suggest that the actual concentration of L-dopa in the brain may be correlated with the state of aggregation of the polysomes, and that the age-related change in sensitivity to administered L-dopa may be related to the difference in the resultant amount of L-dopa in the brain.

Table 2. Effect of L-phenylalanine, L-tyrosine, or L-dopa on brain tryptophan content. Rats weighing 100 to 120 g were killed 60 minutes after receiving a single intraperitoneal injection of the aromatic amino acid. 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.

Dose (mg/kg)	Tryptophan content (μg/g of brain)
Control	
0	3.7 ± 0.16 (12)
L-Dopa	
100	5.9 ± 0.64* (7)
200	4.7 ± 0.31† (7)
500	5.2 ± 0.35* (12)
L-Phenylalanine	
500	3.4 ± 0.25 (3)
1000	2.8 ± 0.27‡ (4)
L-Tyrosine	
500	6.0 ± 0.26‡ (2)
1000	4.8 ± 0.35† (4)

* Mean value differs from control value of 3.7, with $P < .05$. † Mean value differs from control value of 3.7, with $P < .01$. ‡ Mean value differs from control value of 3.7, with $P < .001$.

Since polysome aggregation in liver (*11*) and in rat brain after treatment with phenylalanine (*12*) had been shown to vary with the tryptophan content of the tissues, amounts of tryptophan in brain were also measured in 7- to 9-day-old rats given a single dose of L-dopa (*15*). The amounts of free tryptophan were found to change in response to treatment with L-dopa, but in the opposite direction from that anticipated (Table 1). All doses of L-dopa that disaggregated polysomes caused the tryptophan content of brain to increase significantly over that in controls. However, the time course of the increase in tryptophan differed from the effect of L-dopa on the polysome profile. The tryptophan content of brain was greatly increased at all times examined 20 to 120 minutes after administration of L-dopa. There was also a dissociation between the behavior of tryptophan and that of polysome aggregation when the brains of older animals treated with L-dopa were analyzed. In the case of the 45- to 55-g rats described earlier whose polysomes were not disaggregated by a dose of 200 mg/kg, the amount of tryptophan in brain nevertheless increased after administration of L-dopa (control, 7.3 ± 1.26 μg per gram of brain; treated, 17.0 ± 1.64 μg/g). In addition, free tryptophan was measured in the brains of 100- to 120-g rats given L-dopa in doses varying from 100 to 500 mg/kg. All doses raised the tryptophan level significantly, although only the 500 mg/kg dose caused polysome disaggregation (Table 2). Samples of brain supernatants from animals with increased amounts of tryptophan were examined in an amino acid analyzer to determine whether any other amino acids were affected by a single dose of L-dopa (*16*). No other amino acids showed increased concentrations; two showed significant declines (alanine, 14 percent; methionine, 15 percent; $P < .05$).

To confirm the differential effect of L-phenylalanine and L-dopa on brain tryptophan content, we measured the tryptophan in brains from 100- to 120-g rats 60 minutes after they had received intraperitoneal injections of L-phenylalanine in 0.42 percent NaCl, L-tyrosine in 0.05*M* HCl, or L-dopa in 0.05*M* HCl. Both L-dopa and L-tyrosine caused increases (Table 2); in contrast, phenylalanine caused a significant decline. The biochemical mechanism responsible for this difference between the effects of L-phenyl-

alanine and its hydroxylated derivatives is unknown.

These data suggest that a single administration of L-dopa causes transient but extensive changes in the mechanisms responsible for brain protein synthesis. Our data do not distinguish between possible effects on glia and on neurons. Since the neurons contain an abundance of free polysomes compared to membrane-attached polysomes, whereas the glial cells have more of the latter, it is possible that specific changes in neuronal polysomes would be indicated if the disaggregation is confined to the free polysomes. The unexpected increase in the amount of free tryptophan in brain that follows a single dose of L-dopa indicates that the mechanism by which L-dopa disaggregates brain polysomes probably differs from the mechanism of disaggregation by L-phenylalanine which is accompanied by a fall in free tryptophan levels (*12*). It is possible that L-dopa acts on polysomes by limiting the availability of other amino acids, for example methionine (*9*).

In studies to determine the mechanism for the increased brain tryptophan we have found thus far that administration of L-dopa (500 mg/kg) to 100- to 120-g rats results in a rise in plasma tryptophan associated with the rise in brain tryptophan. The increase in tryptophan in plasma after administration of L-dopa was proportionately greater than that in the brain, resulting in a decrease in the ratio of tryptophan in brain to that in plasma. The concurrent increase in tryptophan in plasma and brain is not surprising, because physiological variations in the tryptophan content of brain have been reported to be associated with the amount of tryptophan in plasma, as exemplified by their daily rhythms (*17*).

It is not known whether the changes in polysome profile reported here are associated with parallel changes in brain protein synthesis. Such changes could participate in either the therapeutic or the toxic actions of the drug.

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Polysome pellets were prepared by layering 6 ml of the postmitochondrial supernatant on a discontinuous sucrose gradient with 3 ml each of 2*M* sucrose in TKM buffer and 0.5*M* sucrose in TKM buffer. Gradients were spun in the L2 Spinco ultracentrifuge at 40,000 rev/min (105,000*g*) for 4 hours in a Ti50 rotor. Pellets were washed with 5 ml of cold TKM buffer, carefully suspended in 0.3 ml of the same buffer, and frozen at —40°C until they were used in 1 or 2 days.

The thawed ribosome preparations were incubated in a 37°C water bath for 2 minutes before they were applied to the linear 10 to 40 percent continuous sucrose gradients. The gradients, done in duplicate, were spun in an SW50 rotor at 38,000 rev/min for 70 minutes, in the L2 Spinco ultracentrifuge. The extinction profiles at 260 nm were recorded automatically with a Gilford model 2000 spectrophotometer, as the gradient was displaced upward through a flow cell.
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Identification of the Germination Self-Inhibitor from Wheat Stem Rust Uredospores

Abstract. *Two germination inhibitors from wheat rust uredospores were identified as the* cis *and* trans *isomers of methyl 4-hydroxy-3-methoxycinnamate (methyl ferulate). They are the self-inhibitors from these spores described previously.*

Rust fungi are among the most important disease agents of plants. In nature, these fungi are obligately dependent upon their hosts and are highly selective in regard to host compatibility. Uredospores of the rust fungi fail to germinate if floated in dense populations on water because endogenous self-inhibitors released from the spores prevent initiation of germ tubes (*1*). The ecological function of the self-inhibitors apparently is to minimize spore germination where survival would be poor, especially within the fructification structure.

Self-inhibitors of spore germination were first described for the rust fungi by Allen (*1*), when he found that crowding of wheat stem rust uredospores reduced their germination. Washing the spores with water reduced the self-inhibition, but the solutions on which the uredospores were floated contained a substance which was highly active in preventing germination. We report here the isolation and identification of this inhibitor.

Inhibitors were extracted from uredospores of the wheat stem rust fungus (*Puccinia graminis* Pers. var. *tritici* Eriks. & E. Henn., race 56) essentially as described previously (*2*). Uredospores were stirred in water (1 g/50 ml), and the inhibitor was re-