

DISAGGREGATION OF BRAIN POLYSOMES BY L-5-HYDROXYTRYPTOPHAN:
MEDIATION BY SEROTONIN

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Summary

In rats, intraperitoneal administration of L-5-hydroxytryptophan (200 mg/kg) causes extensive disaggregation of whole brain polysomes after one hour. Polysome disaggregation is prevented if the conversion of L-5-hydroxytryptophan to serotonin is blocked by pretreatment with an aromatic L-amino acid decarboxylase inhibitor; disaggregation is potentiated by pretreatment with a monoamine oxidase inhibitor. The brain polysome disaggregation induced by L-phenylalanine administration (1 g/kg) is not blocked by decarboxylase inhibition.

Introduction

The stability of brain polysomes is altered in rats receiving L-dihydroxyphenylalanine (L-dopa) (1) [an aromatic amino acid that is not normally present in the circulation (2) and is not incorporated into proteins (3)], as well as by large doses of phenylalanine (4) or other circulating amino acids (5). The resulting polysome disaggregation by L-dopa is mediated by its conversion to the catecholamine dopamine (6) within the majority of brain cells (7-9). Like L-dopa, the amino acid L-5-hydroxytryptophan (L-5-HTP), which is not found in protein, is also taken up within most brain cells (9, 10) and is an excellent substrate for the enzyme aromatic L-amino acid decarboxylase (11), which catalyzes its conversion to a biogenic monamine, i.e., serotonin. We now show that L-5-HTP

administration also disaggregates brain polysomes, and that this effect also requires formation of its decarboxylation product. In contrast, the brain polysome disaggregation caused by phenylalanine administration does not necessitate formation of the corresponding amine.

Materials and methods

Sprague-Dawley rats (Charles River CD, Wilmington, Mass.) were exposed to light from 9 a.m. to 9 p.m. daily and were given access to food and water *ad libitum*. Rats were administered all drugs intraperitoneally and were decapitated at 1 p.m.

Male rats, weighing 50-75 g and housed six per cage, received various doses of L-5-HTP (Nutritional Biochemicals Corp., Cleveland, Ohio) dissolved in 0.05 N HCL or the diluent alone and were killed after 1 hour, the time after L-dopa administration at which maximal polysome disaggregation is observed (1). Whole brain polysome profiles were prepared as described before (1,6), using pools of two brains.

In experiments where rats received L-5-HTP after receiving an inhibitor, they were either pretreated with 800 mg/kg RO4-4602 (Hoffmann-LaRoche, Inc., Nutley, N.J.; dissolved in water), the aromatic L-amino acid decarboxylase inhibitor (AAADI), 30 min before receiving 500 mg/kg L-5-HTP or pretreated with 10 mg/kg pargyline (Abbott Labs, N. Chicago, Ill.; dissolved in water), the monoamine oxidase inhibitor (MAOI), 120 min before the L-5-HTP dose. Alternatively, rats received each drug alone; and were killed 90 min after RO4-4602, 180 min after pargyline. Control rats were killed 60 min after a single dose of 0.05 N HCL.

In studies with L-phenylalanine, lactating mothers with 10-pup litters were placed in individual cages 5 days after the pups

were born. At 7 days, rats received 1 g/kg L-phenylalanine (Nutritional Biochemicals Corp., Cleveland, Ohio) dissolved in 0.42% NaCl or 0.85% NaCl; animals were killed an hour later, and polysome profiles were prepared using pools of five brains.

Results

As little as 200 mg/kg of L-5-HTP caused significant disaggregation of rat brain polysomes (Table I); the effects of larger doses were correspondingly greater, so that only 30% of brain ribosomal RNA was polysomal among rats receiving 500 mg/kg, as compared with 71% in control animals ($P < 0.01$).

TABLE I
Dose Response of L-5-Hydroxytryptophan on Brain Polysomes

Dose (mg/kg)	Polysomes (% of profile)
0	71 ± 0.9 (4)
50	70 ± 1.5 (3)
150	73 ± 0.9 (3)
200	61 ± 3.6 (3)*
350	41 ± 1.2 (3)**
500	30 ± 9.9 (3)***

Data are presented as mean ± standard error and were evaluated statistically by Student's *t*-test. The number of determinations in a group is given in parentheses.

* $P < 0.05$ differs from control group.

** $P < 0.001$ differs from control group.

*** $P < 0.01$ differs from control group.

When serotonin synthesis was prevented in rats by pretreatment with a large dose of the aromatic L-amino acid decarboxylase

inhibitor RO4-4602, polysomes were not disaggregated by L-5-HTP (Table II). In contrast, inhibition of serotonin metabolism through monoamine oxidase by pretreatment with pargyline potentiated brain polysome disaggregation by L-5-HTP (Table II). Neither enzyme inhibitor by itself affected polysome aggregation.

TABLE II

Brain Polysome Profiles in Rats Treated with L-5-Hydroxytryptophan: Effect of Pretreatment with a Decarboxylase Inhibitor or a Monoamine Oxidase Inhibitor

L-5-HTP	AAADI (RO4-4602)	MAOI (Pargyline)	Polysomes (% of profile)
-	-	-	71 ± 2.2 (5)
+	-	-	40 ± 2.8 (5)*
-	+	-	63 ± 3.6 (3)
+	+	-	63 ± 1.5 (3)
-	-	+	63 (63, 53)
+	-	+	29 (39, 19)**

Data are presented as mean ± standard error and were analyzed statistically by Student's *t*-test. The number of determinations in a group is given in parentheses. If two determinations were made for a group, the two values are given in parentheses.

**P*<0.001 differs from control group.

***P*<0.01 differs from control group.

Treatment with the amino acid phenylalanine significantly decreased the percent of brain ribosomal RNA present as polysomes (53% *vs.* 73% in control animals; *P*<0.001); pretreatment with RO4-4602 had no effect by itself on polysome aggregation (77%) and also did not block the disaggregation caused by phenylalanine (56% aggregated).

These observations indicate that L-dopa and L-5-HTP, two hydroxylated aromatic amino acids that are excellent substrates for aromatic L-amino acid decarboxylase, disaggregate brain polysomes by being converted to their monoamine products, dopamine and serotonin, respectively. In contrast, the disaggregation induced by phenylalanine, an amino acid normally present in tissue proteins and in the circulation, is not mediated by a decarboxylated derivative. It has previously been shown that phenylalanine administration lowers brain tryptophan concentrations in rats, and that its effect on polysome aggregation can be blocked by concurrent treatment with tryptophan (4); L-dopa administration actually elevates brain tryptophan content in young rats (1). Hence, it seems likely that the mechanism of phenylalanine-induced polysome disaggregation involves a disturbance in the amino acid pattern within brain cells, probably secondary to the competition between phenylalanine and other neutral amino acids for uptake into the brain (12, 13). The mechanism by which dopamine and serotonin, formed intracellularly from their exogenous amino acid precursors, disaggregate brain polysomes and suppress brain protein synthesis (14) awaits analysis. On the basis of our past and present observations, it can be hypothesized that intracellular amines may normally participate in the control of brain protein synthesis.

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