L-Dopa-induced Modification of DNA, RNA and Protein Synthesis[1] (36419)

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The theoretical basis for use of L-dopa in parkinsonism rests upon the observation that brains of such patients showed striking diminution of cerebral dopamine concentration (1). L-Dopa raises brain dopamine levels in experimental animals (2) and markedly depletes brain *S*-adenosylmethionine (3) possibly resulting from the *O*-methylation of L-dopa itself. The speculation that the therapeutic action of L-dopa in parkinsonism is mediated through increasing neurotransmitter substances in part by stimulation of DNA → RNA → protein (4) inspired the present study on the effects of L-dopa on DNA, RNA, and protein synthesis. The adrenergic effects of L-dopa and isoproterenol and the existing evidence that isoproterenol specifically stimulates DNA synthesis as well as modifies RNA and protein synthesis in the salivary glands of the mouse (5), led us to use isoproterenol as a nonspecific control, inasmuch as it has no effect in parkinsonism, for our studies on the effects of L-dopa.

Materials and Methods. Chemicals and radioisotopes. L-Dopa (3,4-dihydroxyphenylalanine) was obtained from Nutritional Biochemicals. Isoproterenol [2-(3,4-dihydroxyphenyl)2-isopropylaminoethanol HCl] was a gift from Winthrop Laboratories, Rensselaer, NY; 2-^{14}C-thymidine from New England Nuclear, specific activity ~50 mCi/mmole; 5-^{3}H-uridine from Schwarz BioResearch, specific activity ~20 Ci/mmole; L-^{3}H-valine from Schwarz, sp act 6.5 Ci/mmole. All isotopes were made to 100 μCi/ml in water for injection; 10 μCi was injected sc into each mouse.

DNA extraction and estimation. Three times washed homogenate was heated in 10 ml of 0.5 *N* $HClO_4$ at 90° for 30 min, cooled in ice, centrifuged, neutralized with 0.5 ml of 10 *N* KOH, cooled, centrifuged and an aliquot of supernatant assayed for radioactivity. DNA in tissue was estimated by ultraviolet spectrophotometry with a Cary 15 spectrophotometer. Results are expressed in cpm/mg DNA.

RNA extraction and estimation. Three times homogenate was washed with 10 ml EtOH–Et_2O, then 10 ml Et_2O and suspended in 10 ml 0.5 *N* KOH, incubated at 37° for 18 hr, left in ice for 20 min, neutralized with 0.4 ml 70% $HClO_4$, left in ice for about 1 hr, centrifuged to remove $KClO_4$ and an aliquot of supernatant counted. The amount of RNA in tissue was measured by ultraviolet spectrophotometry as for DNA.

Protein extraction and estimation. Three times washed homogenate was heated in 10 ml 0.5 *N* $HClO_4$ at 90° for 15 min, cooled in ice, centrifuged, washed with 10 ml of cold EtOH–Et_2O (1:2), then 10 ml Et_2O. Each sample was dissolved in 5.0 ml of concentrated formic acid and an aliquot counted. Protein in tissue was estimated by uv spectrophotometry. A standard curve of crystalline bovine albumin dissolved in formic acid served as a standard. Results are expressed in cpm/mg protein.

Assay of radioactivity. Aliquots were added to a scintillation liquid (5 g Packard PreMix "P" in 1 liter of toluene and 250 ml of ethanol or Aquasol, New England Nuclear). Radioactivity was measured in a Beckman LS 233 scintillation spectrometer. The sensitivity was adjusted for each run. Results were not corrected for quenching as

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TABLE I. Incorporation of ^{14}C-thymidine into DNA.[a]

Treatment	No. of mice	Sp act in salivary glands	Sp act in brain	Sp act in liver
Control	5	1724 ± 151		
Isoproterenol	4	9535 ± 1508		
L-Dopa	6	987 ± 86		
Control	4		288 ± 25	370 ± 37
L-Dopa	5		258 ± 26	285 ± 27[b]
Control	4	2585 ± 328	406 ± 21	
L-Dopa (chronic)	6	1797 ± 205	386 ± 27	

[a] All animals given L-dopa or isoproterenol were sacrificed 26.5 hr later, 30 min after ^{14}C-thymidine administration. The last group represents chronic administration of L-dopa. Results are given in cpm/mg DNA with the SE.

[b] Seven mice.

shifting was minimal.

Single-dose administration of L-dopa and isoproterenol. Swiss female albino mice of the Hale–Stoner strain were used when about 5 weeks old and weighing 20 g. Animals were starved for 2 hr before injection of isotope. Mice were injected ip with either L-dopa 0.3 mg/g dissolved in water for injection with 2% gum tragacanth or isoproterenol 1.0 μmole/g dissolved in water for injection. Controls for L-dopa received gum tragacanth. The isotopes were given 26 hr after drug administration. Thirty minutes later, the animals were killed by cervical dislocation and one of the following organs removed. Salivary glands were dissected from adhering fat and lymph nodes. The three major salivary glands (submaxillary, sublingual, and parotid) were pooled and homogenized in water. Brains without the cerebellum and a portion of liver weighing about 300 mg were dissected and weighed. Tissues were homogenized in water by means of a teflon pestle in a Potter homogenizer. Brains were homogenized with all-glass hand homogenizers.

Chronic administration of L-dopa. For injection, L-dopa was suspended in water by sonication. Animals were injected ip twice a day (9 AM and 5 PM) with 0.3 mg/g for 7–11 days. Twenty-six hours after the last injection, the isotopes were administered. Mice were starved for 2 hr before injection of isotope. Liver, brain and salivary glands were taken and weighed as described previously.

Results. Incorporation of ^{14}C-thymidine into DNA (Table I) in the salivary glands was significantly inhibited ($p < .001$) 26 hr after a single injection of L-dopa. Inhibition

TABLE II. Incorporation of ^{3}H-uridine into RNA.[a]

Treatment	No. of mice	Sp act in salivary glands	Sp act in brain	Sp act in liver
Control	3	2742 ± 139		
Isoproterenol	5	2818 ± 468		
Control	4	1755 ± 253		
L-Dopa	6	644 ± 89		
Control	4		517 ± 39	2075 ± 150
L-Dopa	4		498 ± 9	1723 ± 72[b]
L-Dopa (chronic)	5		450 ± 21	1752 ± 99

[a] All animals given L-dopa or isoproterenol were sacrificed 26.5 hr later, 30 min after ^{3}H-uridine administration. The last group represents chronic administration of L-dopa. Results are given in cpm/mg RNA with the SE.

[b] Three mice.

TABLE III. Incorporation of ^{3}H-valine into protein.[a]

Treatment	No. of mice	Sp act in salivary glands	Sp act in brain	Sp act in liver
Control	4	1538 ± 237		
Isoproterenol	3	1847 ± 222		
L-Dopa	4	1079 ± 155		
Control	5		127 ± 8	
L-Dopa	4		108 ± 10	
Control	3			397 ± 16
L-Dopa (chronic)	6		121 ± 11	381 ± 23

[a] All animals given L-dopa or isoproterenol were sacrificed 26.5 hr later, 30 min after ^{3}H-valine administration. The last group represents chronic administration of L-dopa. Results are given in cpm/mg protein with the SE.

was not significant after chronic administration of L-dopa. The inhibition in the brain and liver was of the order of 5–23% and was not statistically significant. Incorporation of ^{14}C-thymidine was markedly increased 26 hr after a single dose of 1.0 μmole of isoproterenol.

Incorporation of ^{3}H-uridine into RNA (Table II) was inhibited by about 60% ($p > .001$) in the salivary glands, 26 hr after a single injection of 0.3 mg/g of L-dopa. Inhibition was much less in the brain and liver after acute or chronic administration of L-dopa. One micromole of isoproterenol did not modify RNA synthesis.

Incorporation of ^{3}H-valine (Table III) into protein was not significantly inhibited. L-dopa induced a 30% inhibition in the salivary glands. Inhibition was less in the brain and liver in the acute as well as in the chronic group. Isoproterenol slightly increased incorporation of ^{3}H-valine.

Discussion. Isoproterenol-induced increased DNA synthesis in the salivary glands of the mouse has been extensively studied. Increased DNA synthesis peaks in ~ 26 hr after isoproterenol administration (5). Dopamine inhibits DNA synthesis (6); more recently, L-dopa was shown to disaggregate brain polysomes; L-dopa may thus modify brain protein metabolism (7).

In our experiments, L-dopa was given in a dose (0.3 mg/g) which induced known pharmacological effects, *e.g.*, hyperactivity, piloerection and hypersalivation, but was nontoxic as judged by a zero mortality. Our results are based on incorporation studies done 26 hr after L-dopa—a time when it is safe to assume that the generated dopamine had no more effect on the cardiovascular system of the mouse, thus not affecting distribution of isotope. This is supported by the differential response of different precursors.

These data indicate that L-dopa does not cause gross changes in the mechanisms of nucleic acid and protein synthesis. Degree of inhibition in the different organs examined may be attributable to local factors affecting the metabolism of L-dopa. If, as has been speculated, L-dopa is an avid scavenger of labile methyl groups (4), it could be acting therapeutically by demethylating brain nucleic acids, thus modifying the structure of brain nucleic acids without specifically affecting their rate of synthesis. A higher resolution of this system may help ascertain whether these effects are relevant to therapeutic or toxic effects of L-dopa.

Summary. The effect of L-dopa and isoproterenol in DNA, RNA, and protein synthesis was measured in the salivary glands, brain, and liver of the mouse. These data indicate that L-dopa does not cause gross changes in nucleic acid and protein synthesis.

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