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Methylation of exogenous 3,4-dihydroxyphenylalanine (L-dopa)—Effects on methyl group metabolism*

WHEN ANIMALS are given 3,4-dihydroxyphenylalanine (L-dopa) in doses (100 mg/kg, i.p.) similar to those used to treat patients with Parkinson's disease, a major portion of the administered catechol amino acid is *O*-methylated to form 3-*O*-methyldopa (OMD) and other methylated products.[1] In the process, the concentrations of the methyl donor, *S*-adenosylmethionine (SAM), in brain and adrenal fall markedly.[2] Single doses of SAM do not affect its concentration in liver. However, after chronic L-dopa treatment, hepatic SAM levels are acutely lowered by further doses.[3]

The possibility that the methylation of large amounts of exogenous L-dopa might ultimately deplete the body of free methionine has been suggested.[1,4] If dietary methionine provided the only source of methyl groups for SAM synthesis, a 70-kg human taking 6–10 g L-dopa per day and consuming proteins containing 1–2 g methionine should become methionine deficient. However, even after prolonged L-dopa administration, the percentage of the drug that is excreted in the urine as methylated product remains very high.[5]

This paper presents evidence that methionine concentrations in serum and tissue do not fall in animals treated chronically with sufficient L-dopa to depress hepatic SAM. Hence, sources of methyl groups other than dietary methionine must be available to the body during L-dopa therapy.

Sprague–Dawley male rats (Charles River Laboratories, Wilmington, Mass.) weighing 150, 200 or 300 g were housed five per cage and exposed to light (Vita-Lite, Duro-Test Corp., North Bergen, N.J.) between 9 a.m. and 9 p.m. daily. They had free access to Purina laboratory rat chow and water. The animals received intraperitoneal injections of L-dopa (100 mg/kg) or its diluent alone (0.05 N HCl) and were decapitated at the times described. Serum and the various tissues were frozen on dry ice until assayed; the muscle specimen was taken from the thigh.

All chemicals were of the highest purity commercially available. L-Dopa was a gift of the Hoffmann-La Roche Co. (Nutley, N.J.); yeast *t*-RNA and methyl-^{14}C-L-methionine (50 μCi/m-mole) were purchased from Schwarz–Mann Co. (Orangeburg, N.Y.).

SAM concentrations were assayed according to the method of Baldessarini and Kopin,[6] and the results expressed as percentages of the control values.

A modification of the *t*-RNA loading assays of Holley and Chambers was used for the methionine assay.[7] Limiting amounts of crude yeast *t*-RNA were incubated with saturating amounts of ^{14}C-methionine (100 pmoles) and the unlabeled methionine present in 10 μl of the sample to be analyzed.

Tissues were prepared for analysis by homogenization in 5 vol. of 0·4 N perchloric acid; the 34,000 g supernatant fluid was brought to neutrality by precipitating the perchloric acid with potassium hydroxide. To start the reaction, we added an excess of *Escherichia coli* amino acid-activating enzyme purified to the DEAE-cellulose step;[8] the mixture was then incubated at 37° for 15 min. At the end of the incubation period, 0·100 ml of the 0·110-ml final incubation volume was transferred onto Whatman 3 MM filter paper. After washing and drying, the radioactivity remaining on the filter paper was counted in a liquid scintillation counter. The amounts of unlabeled methionine present in the sample were calculated using the isotope dilution formula

$$[methionine] = 100 \text{ pmols} \times \left(\frac{\text{counts per minute of control}}{\text{counts per minute of sample}} - 1 \right).$$

Standard curves using 5–1000 pmoles L-methionine were run with the samples to be analyzed. When the reaction mixture was incubated for more than 15 min, the apparent incorporation of free methionine into methionyl t-RNA decreased, probably due to RNAse activity contaminating the amino acid-activating enzyme preparation. (We have been unable to eliminate contaminating RNase activity in human serum even after perchloric acid treatment. However, RNase activity does not appear to contaminate the rat tissue samples.) The methionine assay was unaffected by the presence of 100 or 1000 pmoles L-dopa, serine, tryptophan, homocystine, cystathionine, OMD or melatonin.

Rats received one, two, three or four injections of L-dopa at 45-min intervals, and were killed 45 min after the last dose. SAM concentrations were assayed in brain, liver, kidney and muscle. Control rats either received no injections or were killed 45 min after the last of four injections of the L-dopa vehicle alone. Since the tissue SAM concentrations of the two control groups were similar, we pooled these data. SAM concentrations expressed in micrograms per g ±S.E. for tissues from control animals were: brain, 10·0 ± 0·8; liver, 38·7 ± 3·1; kidney, 18·7 ± 1·9; muscle, 52·2 ± 5·6.
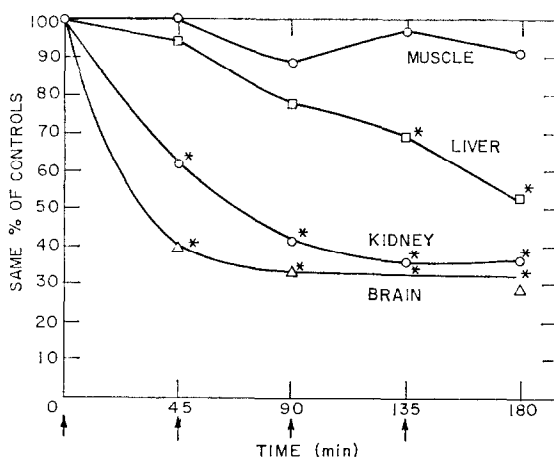

FIG. 1. Tissue S-adenosylmethionine concentrations after L-dopa administration. Rats received one, two, three or four L-dopa injections at 45-min intervals (indicated by arrows) and were killed 45 min after the last injection. Each point represents the mean of data from six animals. Data are expressed as per cents of control values for each tissue. * P < 0·001 differs from controls.

A single L-dopa injection markedly decreased SAM concentrations of the brain and kidneys, but not of liver or muscle (Fig. 1). Additional L-dopa doses did not further decrease brain SAM; the second dose did further deplete kidney SAM concentrations, but subsequent doses were without effect on this organ. Hepatic SAM fell significantly (to 70 per cent of control levels) only after three L-dopa injections. It declined further (to 53 per cent of control values) after four injections.

There was no change in the SAM concentration of muscle at any of the times studied; after four doses of L-dopa, muscle SAM was still 91 per cent of the amount present in tissue from control animals. This failure of muscle SAM to decline after repeated L-dopa doses is consistent with the known lack of COMT activity (i.e. by assay *in vitro*) in this tissue.[9]

Brain methionine levels were not significantly changed by the acute injection of L-dopa (35·6 ± 1·7 and 37·1 ± 2·8 nmoles/g at 1 and 3 hr after a single dose of L-dopa, as compared with 35·6 ±1·1 nmoles/g in control animals), or by single daily doses of the amino acid for 20 days (91–116 per cent

of control values in brains sampled at 1, 3, 6 or 24 hr after the last injection). When L-dopa was repeatedly administered at 45-min intervals, brain methionine began to fall after the third injection (Fig. 2). The methionine concentration 45 min after the fourth injection was only 69 per cent of that found in brains of control animals.
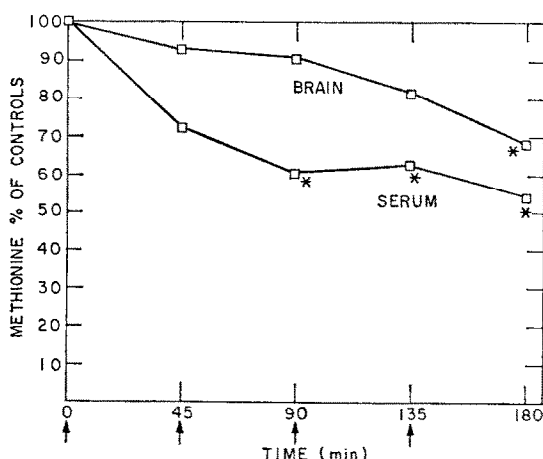

FIG. 2. Brain and serum methionine concentrations after L-dopa administration. Rats received one, two, three or four L-dopa injections at 45-min intervals (indicated by arrows) and were killed 45 min after the last injection. Each point represents the mean of data from six animals. Data are expressed as per cents of control values for each tissue. * P < 0·001 differs from controls.

Serum methionine concentrations were unaffected 1 hr after a single dose of L-dopa, or after the last of ten daily doses. These concentrations were 79·7 ± 5·0 nmoles/ml for control rats, and 77·0 ± 4·6 or 64·4 ± 5·8 nmoles/ml for animals receiving L-dopa acutely or chronically. Repeated injections of dopa at 45-min intervals did deplete serum methionine by 47 per cent (Fig. 2).

These observations show that repeated frequent L-dopa injections ultimately reduce the methionine concentrations in brain and serum. However, when rats receive 1, 10 or 20 daily doses of L-dopa comparable to those given to human patients, methionine concentrations are not depressed.

Brains and adrenals of rats given single doses of L-dopa have been shown to exhibit transient decreases in SAM concentrations.[2] These decreases lasted as long as large amounts of dopa were detectable in the tissues, and were not observed in the liver.[2] Our present observations confirm the differences in the responses of brain and liver SAM to L-dopa; they also demonstrate that this treatment reduces the SAM concentration of the kidney. Repeated L-dopa injections given at 45-min intervals do eventually reduce hepatic SAM. The relative capacities of mammalian tissues to maintain normal SAM concentrations while having to transmethylate large amounts of exogenous L-dopa decrease in the sequence: liver > kidney > brain. Muscle SAM is not depleted by L-dopa; however, this tissue apparently lacks COMT,[9] and thus cannot be compared with the other tissues studied.

Brain SAM, brain methionine and serum methionine do not exhibit parallel responses to exogenous L-dopa. A single dose of L-dopa depresses brain SAM (Fig. 1), but does not reduce brain or serum methionine concentrations (Fig. 2). Serum methionine content does fall significantly after two or three L-dopa doses given at 45-min intervals (Fig. 2); however, brain methionine does not fall until the animal has received four such injections.

The disparity between the marked changes in brain SAM and the absence of change in brain methionine after a single dose of L-dopa could indicate that only a relatively small fraction of the brain methionine is used for SAM synthesis. The resistance of brain methionine to depletion by exogenous L-dopa could also be explained by the operation of a mechanism for regulating the methionine level in the brain. This hypothesis is supported by the observation that brain methionine levels remain within their normal range even after L-dopa treatment has lowered serum methionine (Fig. 2).

The failure of even repeated, large daily doses of L-dopa to depress the methionine concentrations of serum or brain is surprising in view of the unusually great demand imposed on methyl groups by L-dopa metabolism. Our rats received 15–30 mg/day of L-dopa; the methionine ingested with their food was only 20–35 mg/day. Since almost all of the dopa administered to rodents is ultimately methylated,[1] a major fraction of the homocysteine formed after dopa methylation must be remeth-

ylated to form methionine, in order for methionine also to be available for other methylations and for protein synthesis.

Though single L-dopa doses did not affect hepatic SAM, the last of ten daily doses, or repeated L-dopa injections at 45-min intervals, caused a significant decline in hepatic SAM. This decline cannot be ascribed to inadequate methionine-activating enzyme levels or to a uniquely great demand for methyl groups, inasmuch as the dose which ultimately depletes (i.e. the last 100 mg/kg injected) has no effect when given singly. We did not measure hepatic methionine levels; however, neither serum nor brain methionine was depressed after the chronic daily administration of L-dopa. The possibility that ATP concentrations can limit hepatic SAM synthesis is suggested by evidence that hepatic ATP concentrations fall when SAM synthesis is stimulated (i.e. by methionine administration).[10]

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Convulsions induced in 10-day-old rats by intraperitonial injection of monosodium glutamate and related excitant amino acids

There has been considerable controversy concerning the safety of monosodium glutamate as a food additive, due largely to the findings of degenerative lesions in the retina and hypothalamus of infant mice following subcutaneous injection of this acidic amino acid salt.[1] L-Glutamate, like many other acidic amino acids, directly excites mammalian central neurones when applied by microelectrophoresis.[2] The acidic amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP), is a more powerful excitant of central neurones than is L-glutamate;[3] ODAP occurs in the seeds of *Lathyrus sativus* and has been implicated as the agent responsible for the crippling disease, neurolathyrism, which sometimes results from the consumption of these seeds.[4] Several excitant amino acids, including