Biochemical Pharmacology, Vol. 21, pp. 2345–2350. Pergamon Press, 1972. Printed in Great Britain.

EFFECT OF L-DIHYDROXYPHENYLALANINE ON METHYLATION OF ^{3}H-NOREPINEPHRINE AND ^{3}H-HISTAMINE

NGUYEN B. THOA, VIRGINIA K. WEISE and IRWIN J. KOPIN
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Md. 20014, U.S.A.

(*Received* 23 *December* 1971; *accepted* 27 *March* 1972)

Abstract—Acute administration of L-dihydroxyphenylalanine (L-dopa) to mice produced a dose-dependent decrease in *O*-methylation of ^{3}H-norepinephrine. It was maximal 35 min after L-dopa and was still apparent for up to 2 hr. It was not reversed by concomitant administration of methionine and presumably is a consequence of competitive inhibition of *O*-methylation of norepinephrine by the administered L-dopa. Formation of *N*-methylhistamine from histamine was also partly inhibited by L-dopa. This effect was short in duration; it terminated within 1 hr. It was partially reversed by methionine but not by leucine administration. The unchanged ^{3}H-norepinephrine was the same in both control and L-dopa-treated mice. ^{3}H-histamine retention was significantly higher in the L-dopa-treated animals than in control mice. The results are discussed in terms of specific and nonspecific effects of L-dopa on methylation processes.

O-METHYLATION is a major route for the metabolism of L-dihydroxyphenylalanine L-dopa)[1,2] as well as for a wide variety of other catechols which are substrates for catechol-*O*-methyltransferase (COMT).[3] When large doses of L-dopa are administered, a major portion of the dietary methionine may be required to form the methyl donor, *S*-adenosylmethionine (SAME), which in turn is required for the formation of 3-*O*-methyldopa; thus levels of SAME may transiently decrease in some tissues.[4]

Since *O*-methylation is dependent on SAME,[5] administration of L-dopa might interfere with methylation of catechols and other compounds by depleting the methyl donor. L-Dopa could also interfere with *O*-methylation of catechols by competing for COMT. The extent and time course of inhibition of norepinephrine *O*-methylation was therefore determined after L-dopa administration. To assess the relative roles of methyl-donor depletion and competitive enzyme inhibition in the interference with *O*-methylation of norepinephrine, *N*-methylation of histamine and the effects of pre treatment with large doses of methionine were also ascertained.

MATERIALS AND METHODS

Male mice (NIH strain) weighing 20–30 g received saline alone or saline containing varying doses of L-dopa (10–500 mg/kg) by intraperitoneal injection. Thirty min after injection of the amino acid, ^{3}H-norepinephrine (15 μc, 6·88 nmoles) was administered intravenously.

Five min after injection of the labeled amine, the mice were stunned, their tails were cut off and their bodies were homogenized in a Waring blender containing 100 ml of cold 0·4 N perchloric acid. The homogenates were frozen overnight, thawed the next

morning and centrifuged at 10,000 rpm for 15 min at 4°. The supernatants were assayed for ^{3}H-normetanephrine (^{3}H-NME) and for ^{3}H-norepinephrine (^{3}H-NE) as described by Whitby *et al.* [6] and by Anton and Sayre[7], respectively.

In another series of experiments, mice received 100 mg/kg of L-dopa intraperitoneally and ^{3}H-NE intravenously at various intervals up to 12 hr after the amino acid had been administered. The animals were killed, and the methylated amines were assayed as above.

In the experiments in which the effects of L-dopa on the methylation of ^{3}H-histamine were examined, the labeled amine (5·7 μc, 0·69 nmoles) was given intravenously 15 min to 2 hr after L-dopa, and the animals were sacrificed 10 min after injection of ^{3}H-histamine. DL-Methionine (100 and 500 mg/kg) or DL-leucine (100 and 500 mg/kg) was administered intraperitoneally immediately prior to the injection of L-dopa, and their effects were examined. ^{3}H-NE was separated from the supernatant solution by adsorption on alumina[7] and eluted with 7 ml of 0·2 N acetic acid. Tritium content of aliquots of the eluate was determined by liquid scintillation spectrometry.

^{3}H-NME was extracted from aliquots of the homogenate after addition of 0·5 vol. of borate–sodium hydroxide. The pH of the aqueous phase was 9·3.[6] The *O*-methylated amine was extracted into isoamyl alcohol and an aliquot of the organic phase was assayed for radioactivity in a liquid scintillation spectrometer.

^{3}H-histamine determination. Total radioactivity present in homogenates of animals which had received ^{3}H-histamine was determined as above. ^{3}H-histamine and ^{3}H-*N*-methylhistamine were determined by the double-extraction method of Snyder and Axelrod.[8] Aliquots of the supernatant were extracted at basic pH with chloroform or butanol–chloroform (3:2). Known amounts of ^{3}H-histamine and ^{3}H-*N*-methylhistamine were carried through the above extractions, and the partition coefficients were used to calculate the amounts of radioactive amine present.

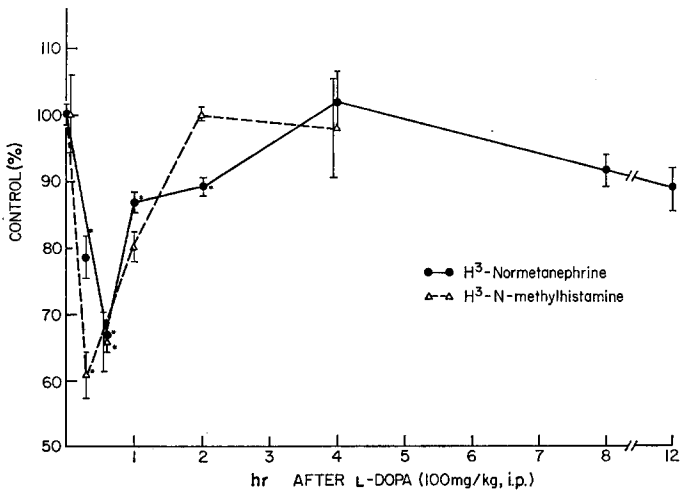

FIG. 1. Effect of L-dopa on the *O*-methylation of ^{3}H-norepinephrine and the *N*-methylation of ^{3}H-histamine in the whole mouse. Each point represents the mean ±S.E.M. computed from a group of six mice. 100% *O*-methylnorepinephrine = 24·58 ± 0·47 per cent of injected dose of ^{3}H-norepinephrine; 100% *N*-methylhistamine = 27·38 ± 1·64 per cent of total dose of ^{3}H-histamine injected. The asterisk indicates that $P < 0·01$ (L-dopa-treated group vs. control groups).

Drugs and materials. 7-^{3}H-DL-NE (2·18 c/m-mole) and ^{3}H-(G)-histamine (1 mc/0·0362 mg) were obtained from the New England Nuclear Corp. (Boston, Mass.). ^{3}H-*N*-methylhistamine was purified from perchloric acid supernatant of mice injected with ^{3}H-histamine. Repeated extractions with chloroform at pH 9·3 were performed until a single radioactive peak corresponding to *N*-methylhistamine was obtained in two solvent systems, ethyl acetate–butanol–acetic acid–water (1:1:1:1) and ethanol–0·1 N hydrochloric acid (95:5).[6] L-Dopa (L-dihydroxyphenylalanine) was purchased from CalBiochem (Los Angeles, Calif.), and DL-methionine and DL-leucine were ordered from the Nutritional Biochemical Corp. (Cleveland, Ohio). L-Dopa was dissolved in 0·9% saline containing 0·01 M hydrochloric acid and 50 mg ascorbic acid. The pH of the solution was then buffered to pH 6·2–6·4 with sodium hydroxide. The other drugs were dissolved in saline prior to use.

^{3}H-NE was purified on an alumina column immediately before use and was stabilized by the addition of 0·002% ascorbic acid and 0·004% EDTA. ^{3}H-histamine was evaporated to dryness under nitrogen before use to remove any tritiated water. Both of the tritiated amines were injected into the tail veins of mice as solutions in 0·3 ml isotonic sodium chloride at pH 6–7.

RESULTS

Effect of L-*dopa on methylation of* ^{3}*H-NE and* ^{3}*H-histamine.* After a single injection of L-dopa (100 mg/kg), there was a significant decrease in formation of ^{3}H-NME from ^{3}H-NE administered intravenously to mice. The maximum reduction (to two-thirds of control) was seen 35 min after injection of L-dopa and was still apparent at 120 min (Fig. 1). Four hr after L-dopa injection, ^{3}H-NME formation was normal.

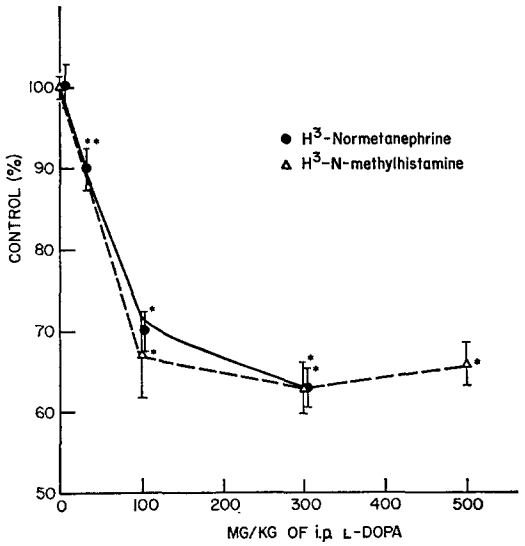

FIG. 2. Dose–response curve showing the effects of L-dopa on the *O*-methylation of ^{3}H-norepinephrine and the *N*-methylation of ^{3}H-histamine. L-Dopa was given 30 min prior to ^{3}H-NE and 15 min prior to ^{3}H-histamine. Animals were sacrificed 5 min after ^{3}H-NE and 10 min after ^{3}H-histamine respectively. Each point represents the mean ±S.E.M. of a group of six mice. The single asterisk indicates that $P < 0·05$; the double asterisk, $P < 0·01$.

Formation of ^{3}H-*N*-methylhistamine from ^{3}H-histamine in mice was also depressed (by 39 per cent) 25 min after L-dopa administration. Recovery of methylation of histamine was faster than methylation of ^{3}H-NE and was normal 120 min after administration of L-dopa (Fig. 1). With increasing doses of L-dopa up to 100 mg/kg, there was a progressive decrease in ^{3}H-NME formation from ^{3}H-NE. Increasing the dose to 300 mg/kg produced no further effect (Fig. 2). L-Dopa up to 30 mg/kg did not alter the *N*-methylation of ^{3}H-histamine. At 100 mg/kg, a 33 per cent decrease was observed, but a dose of 500 mg/kg did not cause an additional effect (Fig. 2).

Effect of L-*dopa on the overall metabolism of* ^{3}*H-NE and* ^{3}*H-histamine.* Five min after ^{3}H-NE injection, 47 per cent of the administered amine was not metabolized and 22 per cent of the radioactivity was ^{3}H-NME. L-Dopa treatment lowered the amount of ^{3}H-NME found, but it did not appear to affect significantly the rate of disappearance of ^{3}H-NE. The total tritium present as amine was unchanged (Table 1). Ten min after its administration, approximately 75 per cent of ^{3}H-histamine was metabolized. ^{3}H-*N*-methylhistamine accounted for 27 per cent of the total radioactivity injected. L-Dopa significantly decreased the concentration of ^{3}H-*N*-methylhistamine and more unchanged ^{3}H-histamine was retained in the whole mouse (Table 1).

TABLE 1. EFFECT OF L-DOPA ON METHYLATION OF ^{3}H-NOREPINEPHRINE AND ^{3}H-HISTAMINE*

Treatment	Total radioactivity (nc/2 ml supernatant)	Total radioactivity (%)		
		^{3}H-norepinephrine	^{3}H-normetanephrine	Total amine recovery
Control	94·45 ± 4·08	46·9 ± 2·5	22·6 ± 0·8	69·5
L-dopa	92·53 ± 3·97	50·7 ± 2·1	14·8 ± 1·4†	65·5
		^{3}H-histamine	^{3}H-*N*-methylhistamine	
Control	93·38 ± 2·73	25·3 ± 2·2	26·9 ± 0·5	52·1
L-dopa	93·87 ± 3·31	43·5 ± 1·9†	17·0 ± 0·8†	60·0

* Six animals were studied in each group.
† $P < 0{\cdot}001$ (L-dopa-treated group vs. control group).

Effects of methionine and leucine on methylation of ^{3}*H-NE and* ^{3}*H-histamine.* The reduction in ^{3}H-NME formation from ^{3}H-NE produced by adminsistration of L-dopa was not alleviated by prior injection of methionine or leucine. The reduction in ^{3}H-*N*-methylhistamine formation produced by L-dopa, however, appeared to be reversed by methionine but not by leucine (Fig. 3). Neither methionine nor leucine altered the levels of the methylated amines in the absence of L-dopa (unpublished observations).

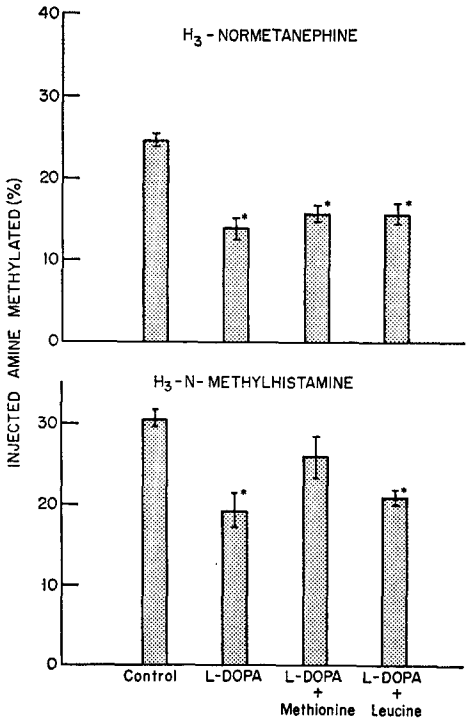

FIG. 3. Effects of various agents on the *O*-methylation of ^{3}H-norepinephrine and the *N*-methylation of ^{3}H-histamine. Each bar represents the mean ±S.E.M. of a group of eight mice. The asterisk indicates that $P < 0.01$ (treated group vs. control group).

DISCUSSION

Shortly after administration of a large dose of L-dopa, there was a decrease in the rate of *N*-methylation of histamine as well as of *O*-methylation of norepinephrine. Since L-dopa is not an inhibitor of histamine-*N*-methyltransferase,[9] it is likely that the utilization of SAME for the methylation of administered L-dopa was responsible for the decrease in the rate of methylation of administered histamine. Pretreatment with methionine reversed in part the inhibition by L-dopa of histamine methylation. Such reversal was not apparent after administration of L-leucine.

Inhibition by L-dopa of norepinephrine methylation was not reversed by pretreatment with methionine or leucine. Long-term treatment with L-dopa diminishes levels of COMT in the red blood cell.[9] Under conditions of the assay for red blood cell COMT, saturating levels of norepinephrine (3×10^{-4} M) were used and L-dopa (equivalent to 20 mg/kg) was not found to be an inhibitor of COMT. In the present experiments, large doses of L-dopa (up to 500 mg/kg) were administered with minute doses of physiologically active labeled norepinephrine.

Since L-dopa is a good substrate for COMT,[3] it is likely that competitive inhibition of methylation of norepinephrine by the administered catecholamino acid is responsible for the diminished role of normetanephrine formation. The dose dependency and the time course of inhibition agree with the findings in brain by Bartholini and

Pletscher[1] and support the view that both methyl-group depletion and competition for COMT are responsible for the inhibition of *O*-methylation of norepinephrine.

Although L-dopa decreased the formation of ^{3}H-NME from ^{3}H-NE, it did not appear to affect the overall metabolism of the catecholamine; therefore, approximately 50 per cent of unchanged catecholamine was retained at sacrifice time in both control and drug-treated groups (Table 1). When the *O*-methylation is decreased, it is possible that there is an increase in other metabolic pathways.

The increased retention of ^{3}H-histamine in L-dopa-treated animals suggests that no compensatory pathways for histamine metabolism developed when *N*-methylation was impaired. Thus, the use of a substrate for methylation reactions may affect methylation of other substances. L-Dopa, for example, affects methylation of ^{3}H-NE, ^{3}H-histamine and probably a number of other naturally occurring amines.

REFERENCES

1. G. BARTHOLINI and A. PLETSCHER, *J. Pharmac. exp. Ther.* **161,** 14 (1968).
2. I. KURUMA, G. BARTHOLINI and A. PLETSCHER, *Eur. J. Pharmac.* **10,** 189 (1970).
3. J. AXELROD, *Science,* **126,** 400 (1957).
4. R. J. WURTMAN, C. M. ROSE, S. MATTHUYSE, J. STEPHENSON and R. BALDESSARINI, *Science,* **169,** 395 (1970).
5. J. AXELROD, In *Transmethylation and Methionine Biosynthesis* (Eds. S. K. SHAPIRO and F. SCHLENK) p. 71. University of Chicago Press, Chicago (1965).
6. L. G. WHITBY, J. AXELROD and H. WEIL-MALHERBE, *J. Pharmac. exp. Ther.* **132,** 193 (1961).
7. A. H. ANTON and D. F. SAYRE, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
8. S. H. SNYDER and J. AXELROD, *Biochem. Pharmac.* **13,** 536 (1964).
9. J. L. WEISS, C. K. COHN and T. N. CHASE, *Nature, Lond.* **234,** 218 (1971).