Effects of L-Dopa on Norepinephrine Metabolism in the Brain

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ABSTRACT Rats received intracisternal [^{3}H]norepinephrine and, after 5 min, intraperitoneal L-dopa (100 mg/kg); they were killed at intervals during the subsequent 24 hr. Their brains were assayed for norepinephrine, L-dopa, S-adenosylmethionine, and [^{3}H]norepinephrine and its metabolites. Rats that received L-dopa had markedly lower brain concentrations of S-adenosylmethionine and of O-methylated metabolites of [^{3}H]norepinephrine than controls. An increase (15–40%) in brain norepinephrine content and accelerated turnover of brain norepinephrine was also observed in the animals receiving L-dopa. These changes were all transient, lasting about 1 hr and coinciding with the period when appreciable amounts of L-dopa were detectable in the brain.

The initial enzymatic reaction in the biosynthesis of brain catecholamines involves the formation of the catechol amino acid L-dihydroxyphenylalanine (L-dopa) from L-tyrosine. Once formed, the L-dopa is immediately decarboxylated to form dopamine, which, in some neurons, is further transformed to norepinephrine (1, 2). Dopa cannot be detected normally in the brain or in the blood (3), and thus it is unlikely that circulating dopa is a physiological precursor for brain catecholamines. However, when exogenous L-dopa is administered to experimental animals, the concentration of dopamine in the brain increases (4, 5). This observation, coupled with the finding that dopamine concentrations measured at autopsy in brains of patients with Parkinson's disease are low (6), suggested that exogenous L-dopa might be useful in the treatment of Parkinsonism. That hypothesis has now been confirmed in numerous clinical studies (7–9).

However, the importance of the conversion of L-dopa to brain dopamine as the basis of its therapeutic action remains unclear. Recent studies in mice have shown that <0.1% of injected labeled L-dopa is detectable as brain catecholamines at any time after administration of the amino acid (10). L-Dopa is also known to influence the fates of other putative brain neurotransmitters in addition to dopamine. Its administration is followed by a reduction of brain serotonin concentration (11, 12), possibly because the dopamine formed *in vivo* competes with serotonin for intraneuronal storage sites (13); moreover, some (5, 14, 15) but not all (12) observers have reported increased concentrations of brain norepinephrine after L-dopa administration.

If experimental animals are given doses of L-dopa similar to those used in the treatment of Parkinson's disease, a major portion of the administered catechol amino acid is rapidly metabolized by O-methylation to form 3-O-methyl-dopa and the 3-O-methylated and deaminated product of dopamine,

homovanillic acid (10). A marked decrease in the brain concentration of the methyl donor S-adenosylmethionine (SAMe) is found during the hour after L-dopa administration (16). This suggests that large amounts of SAMe are utilized in the formation of these methylated compounds. If that is so, L-dopa administration might interfere with a large number of methylation reactions in which SAMe serves as the methyl donor. One such reaction of major physiological significance is the inactivation of tissue catechols by 3-O-methylation (1), a process catalyzed by the enzyme catecholmethyl transferase (S-adenosylmethionine:catechol O-methyltransferase, EC 2.1.1.6) (17). The present studies were undertaken to examine the O-methylation of [^{3}H]norepinephrine in the brains of rats receiving doses of L-dopa equivalent to those used in the treatment of Parkinson's disease.

METHODS

Treatment of animals

Young male Sprague-Dawley rats (150–180 g) (Charles River Laboratories), housed five per cage and given Purina laboratory rat chow and water *ad libitum*, received intraperitoneal injections of L-dopa (100 mg/kg) or its diluent (0.05 N HCl). Brain catecholamine stores were labeled with tritiated norepinephrine injected intracisternally (18). Either [^{3}H]dl-norepinephrine (7–9 Ci/mmol, New England Nuclear Corp.) or [7-^{3}H]l-norepinephrine (2–4 Ci/mmol, Amersham/Searle Corp.) was administered. The [^{3}H]norepinephrine, purified before use by ion-exchange chromatography followed by alumina-adsorption chromatography, was made isotonic with cerebrospinal fluid, adjusted to pH 6.5, and injected in a constant volume of 50 μl.

In an acute experiment, animals were lightly anesthetized with ether, and 12 μCi of [^{3}H]dl-norepinephrine was injected intracisternally. After 5 min, animals were given L-dopa or its diluent (0.05 N HCl) intraperitoneally, and 50 min later they were killed. Another group of rats was killed 5 min after the intracisternal injection, without receiving L-dopa or the diluent, so that we could monitor the uptake of labeled amine and have an initial reference or "zero time" point for the disappearance of tritiated material. Whole brains were removed immediately after death, weighed, homogenized, and assayed for dopa, norepinephrine, and [^{3}H]norepinephrine and its metabolites.

In a chronic experiment, animals were given L-dopa or the acid vehicle daily for 10 days. 24 hr after the tenth injection they were given 8 μCi of [^{3}H]l-norepinephrine intracisternally, followed 5 min later by intraperitoneal injection of L-dopa or its diluent as before. Animals were killed at 50 min, or 3, 6, or 12 hr after the intraperitoneal injection.

Abbreviation: SAMe, S-adenosylmethionine.

"Zero time" groups of rats (not given an intraperitoneal injection for 24 hr) were again obtained 5 min after the intracisternal injection. Brains were immediately removed and dissected over ice into left and right halves; these were weighed and randomly allocated for the assay of SAMe or of L-dopa, norepinephrine, and [³H]norepinephrine and its metabolites. In another type of chronic experiment, designed to determine whether L-dopa exerted different effects on the fate of *dl-* and of *l*-norepinephrine, the animals received 10 μCi of racemic [³H]*dl*-norepinephrine intracisternally and were killed 50 min after the intraperitoneal injection of L-dopa or its vehicle. Essentially the same results were obtained whether *l*-norepinephrine or the racemic mixture was used. Whole brains were used for the assay of L-dopa, norepinephrine, and tritiated compounds. Other animals received no [³H]norepinephrine but were killed 50 min or 24 hr after the last intraperitoneal injection; their brains and livers were used for the determination of SAMe concentration.

Estimation of [³H]norepinephrine and metabolites

Brains were homogenized in 5 volumes of ice-cold 0.4 N perchloric acid and centrifuged at $17,000 \times g$ for 10 min. An aliquot of the supernatant fluid was used to measure the total tritium present, and the remainder was passed over alumina columns at pH 8.6 (19). The radioactivity of unchanged [³H]norepinephrine in the acetic acid eluate of these columns was measured (20), and tritiated catechol-deaminated metabolites in these eluates were extracted into ethyl acetate and assayed by scintillation spectrometry (21). The effluent and the first 5 ml of acetate-buffered wash solution from the alumina columns were combined and passed over an ion-exchange column (Dowex 50W-X4, 200–400 mesh, [H+] form). Tritiated, O-methylated, deaminated metabolites were collected in the effluents from these columns, and [³H]normetanephrine was then eluted from the columns with 3 N NH₄OH (21).

All data thus obtained were corrected for column recoveries, determined by using [³H]norepinephrine and [³H]normetanephrine, or by utilizing fluorescence of authentic reference standards of 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylglycol, vanillylmandelic acid, and 3-methoxy,4-hydroxyphenylglycol(22). The radioactivity in the four fractions of metabolites assayed accounted for 90–105% of the mean total tritium present in the initial brain extracts. These four fractions and their major constituents were: (*i*) unchanged [³H]norepinephrine; (*ii*) tritiated, catechol-deaminated metabolites (3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylglycol; (*iii*) tritiated O-methylated amines (normetanephrine); and (*iv*) tritiated O-methylated, deaminated compounds (vanillylmandelic acid and 3-methoxy,4-hydroxyphenylglycol).

Estimation of nonradioactive norepinephrine and L-dopa

Portions of the acetic acid eluate of the alumina columns were used to estimate nonradioactive norepinephrine or dopa by the method of von Euler and Lishajko (23). This method utilizes ferricyanide oxidation to produce strongly fluorescent derivatives of norepinephrine and dopa, with different fluorescence spectra, having activation/fluorescence maxima at 395/505 and 365/495 nm, respectively (24–26). At 395/505 nm, the relative intensity of the fluorescence produced by dopa was only 9% of that produced by norepinephrine, in good agreement with the 12% reported by Anton and Sayre

(19). Norepinephrine and dopa present were differentially estimated by measurement of the fluorescence of all samples at both sets of wavelengths (24, 26). Dopamine produces a weakly fluorescent product upon ferricyanide oxidation. Although its activation and fluorescence maxima are the same as those of norepinephrine, its relative fluorescence intensity is only 1.5–2.0% of that produced by norepinephrine. Thus dopamine should not interfere with measurements of brain norepinephrine at the tissue concentrations encountered (19, 24).

Estimation of S-adenosylmethionine

SAMe concentrations were measured in two ways. Usually, with tissues not contaminated with tritium (as [³H]norepinephrine or its metabolites), it was possible to use the enzymatic double-isotope-derivative, isotope-dilution assay previously reported (27). When tissues were contaminated by tritium, a single-label modification of the usual double-label assay was used. Mean brain SAMe concentration by the single-label method was 16.6 μg/g, in agreement with values obtained by the double-label technique in this study and previously (27). When catechols were added to incubation mixtures in amounts more than twice those encountered in tissues of rats treated with L-dopa, the estimate of tissue SAMe was found to be decreased by 6–17%. This control experiment suggests that the present assay methods are valid if the tissue concentrations of potential methyl acceptors (catechols) undergo limited increases.

RESULTS

Acute L-dopa administration

Brain norepinephrine concentration (424 ± 26 ng/g brain) was significantly elevated ($P < 0.05$) in L-dopa-treated animals, when compared with "zero time" animals (298 ± 9 ng/g) or with control rats killed 50 min after the intraperitoneal injection of 0.05 N HCl (296 ± 8 ng/g). In L-dopa-treated animals, the brain L-dopa concentration at this time was 1427 ng/g, or about 3.5 times that of norepinephrine; total brain L-dopa represented about 0.01–0.02% of the injected dose. L-Dopa was undetectable in brains of control rats.

The amount of unmetabolized [³H]norepinephrine remaining in brain 50 min after the injection of L-dopa was significantly lower than in the control group, whether the data are expressed as concentration (nCi/g tissue, Table 1), or as specific radioactivity (nCi/ng norepinephrine). The pattern of [³H]norepinephrine metabolites present in the brain at 50 min was also changed significantly by L-dopa treatment. The concentration of O-methylated metabolites was decreased, while that of the catechol-deaminated metabolites was raised (Table 1).

Chronic L-dopa administration

Brain norepinephrine content was raised by 41% ($P < 0.01$) 50 min after the tenth daily dose of L-dopa, i.e., from 351 to 496 ng/g. Brain L-dopa concentration at this time was 2302 ng/g, or about 4.5 times that of norepinephrine. L-Dopa was not detectable at later times, and there were no significant differences in the brain norepinephrine contents of treated or control animals at any other time. 50 min after injection of L-dopa, there were decreases of approximately 30% ($P < 0.01$) in the concentration and specific activity

(Fig. 1) of unchanged brain [³H]norepinephrine. This differ-
ence, too, disappeared by 3 hr.

The pattern of tritiated metabolites found in the brains
was markedly altered by L-dopa. 50 min after the final dose
of L-dopa on the tenth day, profound changes were evident;
they were qualitatively similar to those seen in the acute
experiment but much more striking. In general, concentrations
of methylated products were decreased and deaminated
metabolites increased (Fig. 1; Table 1). The brain concentra-
tion of [³H]O-methylated amine metabolites ([³H]normeta-
nephrine) was 37% lower ($P < 0.005$) and [³H]O-methylated,
deaminated metabolites 47% lower ($P < 0.001$) in the L-dopa-
treated animals. There was, simultaneously, an 87% increase
($P < 0.001$) in [³H]catechol-deaminated metabolites in the
brains of treated animals (Fig. 1). By 3 hr after the final
L-dopa injection, the metabolite pattern in the L-dopa-treated
animals had nearly reverted to that present in the correspond-
ing control group. The only change that tended to persist
was the decrease in [³H]O-methylated, deaminated metabo-
lites: concentrations of these compounds tended to remain
lower then controls for 12 hr after the last injection and the

FIG. 1.   [³H]norepinephrine metabolism and SAMe concentra-
tion after chronic L-dopa administration. *Top panel:* disappear-
ance of [³H]norepinephrine. *Middle panel:* concentration of
tritiated metabolites of [³H]norepinephrine in animals given
L-dopa, expressed as a percentage of the concentrations in the cor-
responding control group. The "O-methylated products" include
the O-methylated amine metabolites and the O-methylated,
deaminated compounds shown in Table 1. *Bottom panel:* brain
SAMe concentration in L-dopa-treated rats expressed as a per-
centage of the levels found in the corresponding control group.
The "zero time" animals had not received L-dopa for 24 hr.
Statistically significant changes are indicated by an asterisk; *P*
values are given in Table 1.

difference was significant at 6 hr ($P < 0.05$). No other differ-
ences in [³H]norepinephrine metabolites were noted in animals
killed more than 50 min after the last L-dopa injection.

Brain SAMe concentrations (Fig. 1) were markedly de-
creased (by 83%) 50 min after the last dose of L-dopa, in
agreement with previously reported findings (16). At 3 hr,
however, brain SAMe concentrations no longer differed sig-
nificantly from those of control animals. 6 hr after L-dopa,
brain SAMe concentrations were elevated. Increases of 30–
35% persisted to 12 hr ($P < 0.01$), and SAMe was still
15% above control at the "zero time" point, or 24 hr after
the tenth and final dose of L-dopa ($P < 0.05$). Thus, brain
SAMe concentrations apparently showed a biphasic response
to the administration of L-dopa: a marked initial decrease
while L-dopa was detectable in the brain and a prolonged
small increase after L-dopa was no longer detectable.

Hepatic SAMe levels examined in these chronically treated
animals were decreased by $21.2 \pm 6.1\%$ ($P < 0.02$) 50 min
after the final dose of L-dopa and $16.8 \pm 3.4\%$ ($P < 0.03$)
after 24 hr.

DISCUSSION

Administration of L-dopa systemically to rats appears to
modify brain norepinephrine metabolism in at least three
ways: (*i*) there is a transient increase in brain norepinephrine
concentration, probably because some of the L-dopa that
enters the brain is converted to norepinephrine. (*ii*) The
metabolism of the norepinephrine present in the brain is
altered. The methyl donor SAMe is depleted (Fig. 1) (16),
the O-methylation of norepinephrine is inhibited, and the
accumulation of its O-methylated metabolites is reduced
(Table 1, Fig. 1). (*iii*) There is an apparent brief acceleration
in the turnover of brain norepinephrine (Fig. 1). These
modifications are all profound but transient, persisting for
the most part during the hour or so after L-dopa administra-
tion, when large amounts of this amino acid can still be de-
tected in the brain.

In the present experiments, brain concentrations of non-
radioactive norepinephrine were elevated by 15–41% 50
min after the administration of L-dopa, but had returned
to normal at 3 hr. The time of the elevation coincided with
the times when L-dopa could be detected in the brain and
when the reduction in brain SAMe was maximal (Fig. 1).
There is some disagreement in the literature concerning
the extent to which L-dopa treatment elevates the con-
centration of brain norepinephrine. One study found a 70%
increase in rat cortex 1 hr after the intraperitoneal administra-
tion of 75 mg/kg of L-dopa (5). A 36% increase in cat spinal-
cord norepinephrine was found 30 min after a dose of 20
mg/kg (14), but a 117% increase was found in the cat brain
stem 90 min after the same dose of L-dopa (15). In contrast,
another study (12) reported no changes in whole brain
norepinephrine concentration in mice 30 min after a very
large intraperitoneal dose of L-dopa (450 mg/kg). Some of
these variations may be due to differences in dosage, timing,
species, and brain regions studied.

One very likely explanation for the observed increase
in brain norepinephrine concentration after L-dopa administra-
tion is that this amino acid, which lies beyond the rate-limiting
step in norepinephrine synthesis (i.e., tyrosine hydroxylation),
is converted to brain norepinephrine as long as it is available
to central noradrenergic neurons. Only a minute fraction
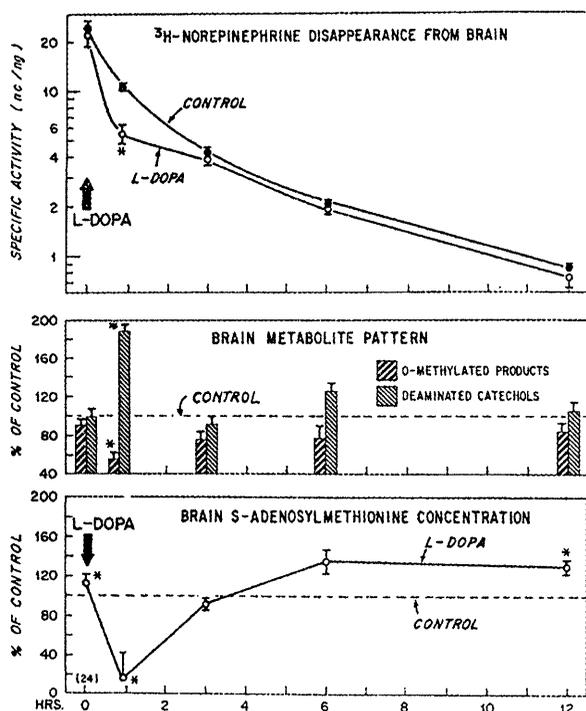of administered unlabeled L-dopa or of [¹⁴C]L-dopa (28)

TABLE 1. *Effects of L-dopa on the metabolism of intracisternal [^3H]norepinephrine*

	Total tritium	[^3H]norepinephrine	Tritiated O-methylated amine metabolites	Tritiated O-methylated deaminated metabolites	Tritiated catechol-deaminated metabolites
A. *After single injection of L-dopa*					
"Zero time" control group	1920 ± 123	1220 ± 82.7	502 ± 48.3	470 ± 10.5	56.3 ± 5.7
50-min control group	1780 ± 90.2	891 ± 45.7	174 ± 16.8	515 ± 29.9	62.2 ± 3.5
50-min L-dopa group	1480 ± 113 ($P < 0.05$)	758 ± 61.1 ($P < 0.05$)	146 ± 11.1	337 ± 27.4 ($P < 0.001$)	89.3 ± 6.1 ($P < 0.05$)
B. *After tenth daily injection of L-dopa*					
50-min control group	1950 ± 122	774 ± 37.1	196 ± 25.0	509 ± 42.4	52.1 ± 5.1
50-min L-dopa group	1360 ± 47.4 ($P < 0.005$)	556 ± 21.5 ($P < 0.001$)	103 ± 4.5 ($P < 0.005$)	315 ± 10.4 ($P < 0.001$)	81.0 ± 5.0 ($P < 0.005$)

Values are nanocuries per gram of brain, mean ± SE. P values by Student's t test. Comparisons are made with the corresponding 50-min control group.

is changed to brain norepinephrine, probably because most of the amino acid is destroyed outside the brain by O-methylation, decarboxylation, and deamination (10, 28), and because few brain cells contain the enzymes necessary for norepinephrine biosynthesis (1, 2). Thus, the production of new norepinephrine molecules by administration of L-dopa is very inefficient. In addition to serving as a precursor for catecholamines, administered L-dopa may also tend to spare dopamine and norepinephrine by competitively inhibiting their inactivation by O-methylation.

An appreciable fraction of both endogenously synthesized norepinephrine and intrathecally administered [^3H]norepinephrine is normally metabolized by O-methylation alone, or by O-methylation and oxidative deamination (29) (Table 1). In our studies, the administration of L-dopa caused a 40–50% reduction in the amount of O-methylated [^3H]-norepinephrine derivatives present in the brain; concurrently, the proportion of injected [^3H]norepinephrine present in brain as deaminated catechols (not O-methylated) was doubled. This suppressive effect on the methylation of brain norepinephrine was transient, and paralleled the presence of L-dopa in brain and the simultaneous decline in SAMe concentrations. In animals treated for 10 days with L-dopa, the only modifications in brain [^3H]norepinephrine metabolism that persisted for more than 50 min were small decreases in the concentration of [^3H]O-methylated deaminated metabolites (Fig. 1). Although the changes observed 50 min after L-dopa were quantitatively larger in chronically treated animals, their time courses suggest that they primarily reflected acute responses to the most recent dose of L-dopa (Fig. 1). The competition for tissue SAMe that occurs after L-dopa administration may be of sufficient magnitude to interfere with the methylation of other compounds that are substrates for methylating enzymes; these include adrenal norepinephrine, other catechols, pineal N-acetylserotonin, fatty acids, proteins, nucleic acids, and precursors of certain phospholipids and methylated nucleic acids.

The administration of L-dopa produced a transient acceleration in the disappearance of intrathecally administered [^3H]-norepinephrine (Fig. 1), followed by a slow phase of efflux at a rate similar to controls. The increase in initial disappearance rate, which is similar to that recently observed by Gershon *et al.* (30), was evident only while L-dopa was

detectable in the brain; it was not caused by a decrease in initial uptake of [^3H]norepinephrine from the cerebrospinal fluid since the labeled amine was given before the L-dopa and since brain concentrations of [^3H]norepinephrine, 5 min after intracisternal injection, were similar in control rats and in animals chronically treated with L-dopa. Since there is evidence that [^3H]norepinephrine is a valid label of norepinephrine in central noradrenergic neurons (1), it is likely that the increased disappearance of tracer and simultaneously increased synthesis of endogenous amine represent increased turnover of brain norepinephrine. L-Dopa could accelerate the disappearance of brain [^3H]norepinephrine by displacement of readily releasable amine from storage sites or by acting elsewhere to modify the presynaptic input to neurons containing the labeled amine. There is some evidence that L-dopa (31, 32) or, more potently, its metabolites, dopamine and 3-O-methyldopamine (31), can interfere with the retention of [^3H]norepinephrine at storage sites in the brain.

The changes in rat brain SAMe concentration that follow chronic L-dopa administration (Fig. 1) are similar to those reported after a single dose of the amino acid (16). These decreases were observed only when the brain also contained measurable quantities of L-dopa. The subsequent overshoot in brain SAMe levels is unexplained but may reflect the operation of a compensatory mechanism designed to maintain brain SAMe levels.

The effects of chronic L-dopa administration of hepatic SAMe differed from those in the brain. Whereas acute doses did not depress SAMe significantly, chronic administration of L-dopa not only lowered hepatic SAMe levels, but also produced changes lasting 24 hr. These decreases in the liver methyl donor are not readily explained, although we have evidence that the activity of the enzyme responsible for SAMe synthesis is decreased in liver after chronic L-dopa treatment. Thus it is possible that liver SAMe synthesis is unable to keep up with demands imposed by the rapid O-methylation required by large doses of L-dopa; this interpretation can be reconciled with the observation that liver has a much larger methylating capacity than brain (27), since brain appears to be relatively better protected than liver from demands of a large intake of the methyl acceptor. The decrease in hepatic, but not brain, SAMe after chronic

L-dopa injection may reflect other toxic effects of this treatment.

Since the intake of methionine in normal human diets is rather limited (about 30 mg/kg per day), the chronic use of L-dopa could well impose excessive demands on human methylation systems, leading to the depletion of tissue methionine stores, particularly in the liver, as it attempts to keep pace with demands for SAMe synthesis. SAMe concentration has also been determined in the blood of Parkinsonian patients during the administration of L-dopa (S. Matthysse *et al.*, to be published). Doses of L-dopa appear to produce transient decreases in SAMe concentrations, paralleling the changes observed in rat brains. Thus, it is possible that a number of important metabolic pathways utilizing SAMe as a methyl donor may also be suppressed in patients given large doses of the methyl acceptor L-dopa.

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