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L-3,4-Dihydroxyphenylalanine-induced release of norepinephrine from the rat heart

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LARGE doses of L-dopa have been employed successfully in the symptomatic treatment of patients with Parkinson's disease for the last 3–4 years.[1–3] Some patients treated with L-dopa have experienced considerable interference with peripheral autonomic regulation. Orthostatic hypotension frequently has been reported and, less commonly, cardiac arrhythmias and hypertension have been noted.[1,3] Information is accumulating rapidly about the effects of L-dopa on brain amines.[4–6] Less is known, however, about the changes induced by L-dopa on the sympathetic innervation of organs outside the central nervous system. These studies were undertaken to explore the effects of L-dopa on norepinephrine (NE) metabolism at the sympathetic nerve endings in the heart.

Tracer doses of [^{3}H]NE, administered as an intravenous pulse, were utilized in this study. After intravenous administration [^{3}H]NE mixes with the endogenous NE pool and serves as a valid marker of changes in NE turnover.[7] Evidence is presented in support of the hypothesis that L-dopa, after conversion to dopamine, releases NE from the stores in the sympathetic nerve endings of the heart.

Material and Methods

All experiments were performed on female Sprague–Dawley rats, 150–180 g. The methods used were similar to those described previously.[8] DL-[7-^{3}H]NE (10–13 c/m-moles) was obtained from the New England Nuclear Corp. and purified prior to use by column chromatography with alumina. It was administered (25 μc/kg) to unanesthetized animals via the tail vein. L-Dopa, supplied as Laradopa

by the Hoffman-LaRoche Co. was dissolved in 0·15 N HCl and 0·45% saline. Chlorisondamine was provided as Ecolid by the Ciba Pharmaceutical Co.; α-hydrazinomethyl-β-(3,4-dihydroxyphenyl) propionic acid (MK-485; α-methyl dopa hydrazine) was supplied by Merck, Sharpe & Dohme. It was dissolved in isotonic saline and 0·2 N HCl. Dopamine was supplied by CalBiochem. The details of administration are given in the Table and Figure legends.

The rats were killed by a blow at the base of the skull and the hearts rapidly removed, weighed and homogenized in ice cold 0·4 N $HClO_4$. Norepinephrine was isolated on columns of prepared alumina after titration of the perchlorate extract to pH 8·6,[9] and eluted with 6·0 ml 0·2 N acetic acid. Aliquots of the alumina eluate were counted by liquid scintillation spectrometry at an efficiency for tritium of about 18 per cent. Endogenous norepinephrine was determined spectrofluorometrically on aliquots of the alumina eluate by the trihydroxyindole oxidation technique of von Euler and Lishajko.[10] Values were corrected for a recovery of 85–95 per cent as determined in each experiment.

Results

Effect of L-dopa on the retention of [^{3}H]NE by the rat heart. In experiment 1 (Table 1), the effect of treatment with L-dopa for 24 hr is shown. Rats treated with 100 mg/kg, i.p., every 6 hr retained significantly less [^{3}H]NE than controls. In experiment 2 (Table 1) the acute effect of a single dose of L-dopa on the retention of [^{3}H]NE is shown. Three and one-half hr after the administration of L-dopa there was a significant reduction in the retention of the tracer.

Effect of L-dopa on [^{3}H]NE retention dose–response relationship. The retention of [^{3}H]NE after the administration of different doses of L-dopa is shown in Fig. 1. There was a progressive decline in [^{3}H]NE retention between 25 and 100 mg/kg.

Effect of ganglionic blockade on the [^{3}H]NE response to L-dopa. In the experiment depicted in Fig. 2, after the administration of [^{3}H]NE, half the animals were pretreated with chlorisondamine (a ganglionic-blocking agent) while the other half received an equal volume of isotonic saline. L-Dopa was administered to half the animals in each pretreatment group. The effectiveness of ganglionic blockade is demonstrated by the significant increase in [^{3}H]NE retention in the control group pretreated with chlorisondamine as compared with the saline-pretreated controls. Ganglionic blockade did not, however, reverse the effect of L-dopa on [^{3}H]NE retention. In the animals receiving L-dopa, [^{3}H]NE retention did not differ significantly in the chlorisondamine-pretreatment group as compared

TABLE 1. EFFECT OF L-DOPA ON THE RETENTION OF [^{3}H]NE BY THE RAT HEART*

Expt. No.	Rats	[^{3}H]NE (mμc/g)	Specific activity (mμc/μg)
1. L-dopa treatment for 24 hr	Control	22·5 ±2·8	29·0 ±1·9
	L-dopa	5·1† ±1·1	6·7† ±1·0
2. Single dose L-dopa	Control	99·1 ±15·9	156·0 ±29·2
	L-dopa	44·5‡ ±6·9	53·5‡ ±4·7

* Six to seven animals per group were used (means ± S.E.M.). Heart weights and endogenous NE did not differ significantly in any group. All animals received 25 μc/kg of [^{3}H]NE, i.v., and 100 mg/kg of L-dopa, i.p. In experiment 1, L-dopa was administered beginning 5 min after the tracer [^{3}H]NE was injected. The last dose was 6 hr before the animals were killed. In experiment 2, a single dose of L-dopa was administered 2·5 hr after the [^{3}H]NE and the rats were killed 3·5 hr later.

† $P < 0.001$ as compared with controls.

‡ $P < 0.01$.

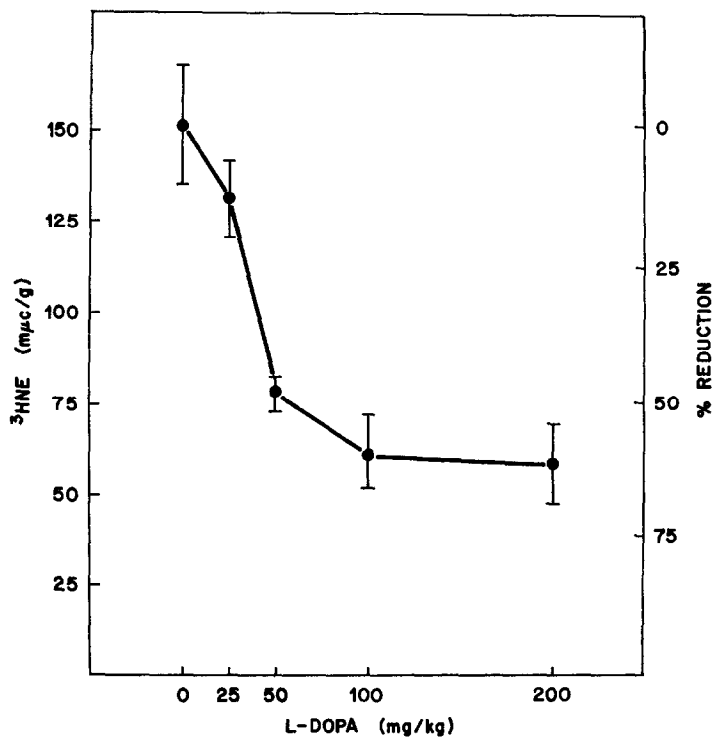

FIG. 1. Effect of L-dopa on [^{3}H]NE retention (dose–response curve). Six to seven animals per group were used (means ± S.E.M.). L-Dopa was administered 2·5 hr after [^{3}H]NE and the rats were killed 3 hr after the L-dopa. Heart weights and endogenous NE were not significantly different from those of the controls in any group. Values at 50, 100, 200 mg/kg differed significantly from controls ($P < 0{\cdot}005$).

with the saline-pretreated group. In both pretreatment groups the reduction in [^{3}H]NE retention after L-dopa was significant.

Effect of decarboxylase inhibition on the [^{3}H]NE response to L-dopa and dopamine. In the experiment shown in Fig. 3, after the NE stores had been labeled, half the animals were pretreated with α-methyl dopa hydrazine (αMDH; MK-485), an inhibitor of 1-aromatic acid decarboxylase. L-Dopa, dopamine or diluent was administered to animals with and without αMDH pretreatment. As shown in Fig. 3, dopa and dopamine produced the anticipated decrease in [^{3}H]NE retention in animals without αMDH pretreatment. In the group pretreated with decarboxylase inhibitor, dopamine but not L-dopa significantly reduced the retention of tracer [^{3}H]NE.

Discussion

These experiments show that treatment with L-dopa reduces the retention of [^{3}H]NE by the rat heart. The decrease in [^{3}H]NE retention is demonstrable after a single dose of L-dopa and is dose-related (Fig. 1) up to 100 mg/kg (an amount comparable to the daily dosage of patients being treated for Parkinson's disease). The fact that L-dopa decreased [^{3}H]NE retention in the presence of significant ganglionic blockade (Fig. 2) is evidence that centrally mediated sympathetic activity is not involved and indicates that, in these experiments, the peripheral sympathetic nerve is the site of action of L-dopa. Furthermore, decarboxylation of L-dopa to dopamine appears to be essential for decreased retention of [^{3}H]NE (Fig. 3). L-Dopa administered after the decarboxylase inhibitor (αMDH) does not reduce the retention of the labeled compound; the effectiveness of dopamine administered after the inhibitor, however, is unimpaired.

During the preparation of this report, Dairman and Udenfriend[11] presented similar observations on the effect of a large dose (1000 mg/kg) of L-dopa on cardiac NE turnover. The results described here

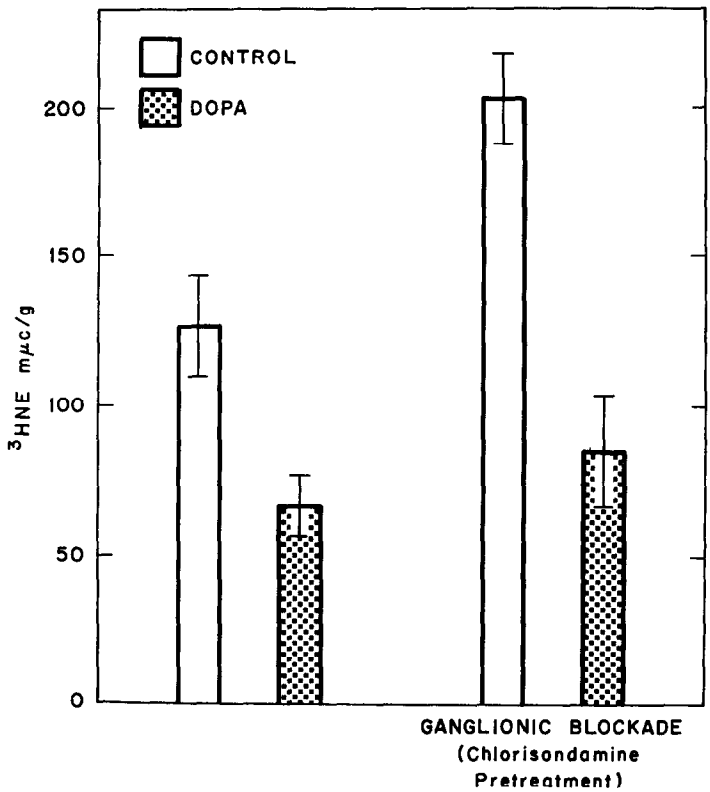

FIG. 2. Effect of L-dopa on [^{3}H]NE retention after ganglionic blockade. Six to seven animals per group were used (means ± S.E.M.). [^{3}H]NE (25 μc/kg) was administered to all the animals; one-half the animals received chlorisondamine 15 mg/kg, i.p., in 1/2 ml isotonic saline. The chlorisondamine was administered 5 min after the [^{3}H]NE and again after 3 hr; controls received saline, i.p., at the same intervals. L-Dopa was administered (100 mg/kg, i.p.) to one-half the rats in each group, as a single dose 2 hr after the [^{3}H]NE, and these animals were killed 3 hr after the L-dopa. Heart weights and endogenous NE were not significantly different in any of the groups. Control animals differed significantly from the chlorisondamine-treated animals ($P < 0.01$) and from the L-dopa-treated animals ($P < 0.02$). The chlorisondamine-treated animals differed significantly from the group that received chlorisondamine and L-dopa ($P < 0.001$). There was no significant difference between the two groups of dopa-treated animals.

agree with their findings. In interpreting these results, they emphasized the effect of L-dopa on NE synthesis. They attributed the decreased retention of [^{3}H]NE noted in their study to increased synthesis of NE resulting from the provision of precursor (L-dopa) distal to the rate-limiting biosynthetic step (hydroxylation of tyrosine). Clearly, decreased [^{3}H]NE retention with an unchanged level of endogenous NE in L-dopa-treated rats indicates an increase in cardiac NE turnover and synthesis. It seems likely, however, from the results described here as well as from the literature that release of NE by dopamine is the primary event after L-dopa administration. The following lines of evidence support this viewpoint: (1) L-dopa administration is not associated with increased tissue levels of NE. Increased synthesis, therefore, must be accompanied by either increased release of active NE or increased neuronal metabolism, either of which would result in decreased [^{3}H]NE retention. (2) L-dopa administration causes a rise in blood pressure in the rat,[12] as well as other sympathomimetic effects.[13–15] This suggests the release of active NE rather than increased intraneuronal metabolism. (3) L-dopa[12] and dopamine[16] administration are associated initially (within 30 min) with a fall in endogenous NE concentration. This supports the contention that release of [^{3}H]NE is the primary event.

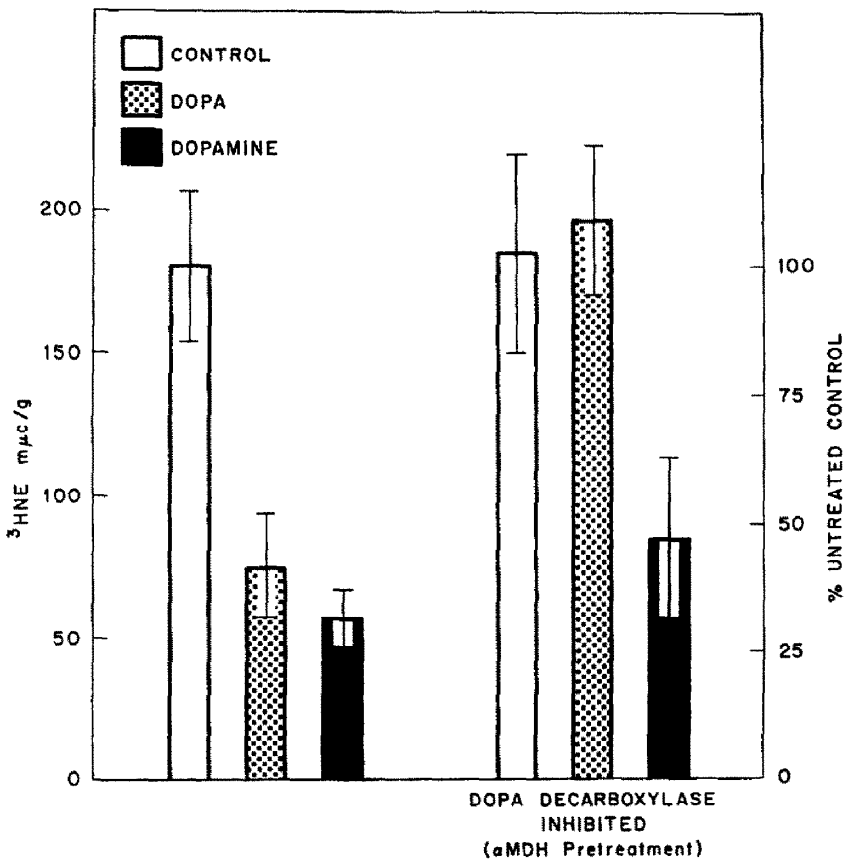

FIG. 3. Effect of L-dopa and dopamine on [^{3}H]NE retention after decarboxylase inhibition. Six to seven animals per group were used (means ± S.E.M.). All the animals were injected with [^{3}H]NE (25 μc/kg). After 2·5 hr, one-half the animals received MK-485 (αMDH) 100 mg/kg, i.p. Control animals received an equal volume of diluent (1·0 cc 0·2 N HCl in isotonic saline). One-half hour later either L-dopa (100 mg/kg), dopamine (50 mg/kg) or diluent (0·45% saline in 0·15 N HCl) was administered to equal numbers of animals in each pretreatment group. The animals were killed 3 hr later. Control animals differed significantly from those treated with L-dopa ($P < 0·01$), dopamine ($P < 0·005$) and αMDH + dopamine ($P < 0·05$), but not from those treated with αMDH alone or αMDH + L-dopa. Animals treated with αMDH did not differ from the controls or those treated with αMDH + L-dopa, but did differ significantly from those treated with αMDH + dopamine ($P < 0·05$). Heart weights and endogenous NE did not differ significantly in any of the groups except for the αMDH + L-dopa-treated animals in which an 80–90 per cent increase in endogenous NE was noted (1·8 ± 0·23 μg/g). This presumably reflects the accumulation of unmetabolized L-dopa in the hearts of this group, since the product of dopa oxidation (but not dopamine) fluoresces at the same wavelength as NE in the trihydroxyindole method used here.[9]

Of further interest in this regard is the fact that NE concentrations do not exceed normal levels despite excess precursor which bypasses the rate-limiting step. It has been suggested that when substrate levels of dopamine are present in the nerve ending, vesicular uptake of dopamine may become rate limiting.[17,18] Thus, the provision of precursor L-dopa may not increase synthesis appreciably in and of itself, but rather may depend on release of stored NE with the subsequent opening of vesicular sites where NE synthesis can proceed.

The dual role of dopamine as sympathomimetic amine[19] and precursor of NE synthesis has been emphasized in 1963 by Harrison *et al.*[16] These experiments support their conclusion that the pharmacology of dopamine (and L-dopa) depends in part on both these properties. Unlike other sympatho-

mimetic amines which release NE, dopamine is not associated with depletion of NE stores. Presumably, this reflects rapid resynthesis of NE from dopamine.

It is noteworthy that the same dose of MK-485 that prevented NE release in these experiments has been reported to prevent the increase in blood pressure associated with the administration of L-dopa.[1,2] Norepinephrine release from the peripheral sympathetic nerves may thus be an important factor in the pharmacology of L-dopa. Although it does not provide an explanation for the occurrence of orthostatic hypotension which commonly accompanies the clinical use of L-dopa, NE release may be related to the cardiac arrhythmias[1,3] or hypertensive reactions[1] occasionally seen in patients receiving this drug.

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Liver *N*-demethylating activity—temperature effect and phenobarbital induction in different species

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DIFFERENCES in the activities of drug-metabolizing enzymes in the livers of various species have been reported.[1,2] The view that the drug-metabolizing microsomal system has developed as one feature of the adaptation from life in an aqueous medium to that of terrestrial life has been suggested by Brodie *et al.*[2] Induction of microsomal drug-metabolizing enzymes has been widely reported by many authors, mainly in the rat.[3,4]