The Effect of Levodopa on the Norepinephrine Stores in Rat Heart

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L-Dihydroxyphenylalanine (L-dopa), effective in the treatment of Parkinson's disease, frequently causes postural hypotension and arrhythmias. The large doses used may alter peripheral adrenergic function. To evolve a rational anesthetic management for the increasing number of parkinsonism patients maintained on L-dopa therapy, central and peripheral catecholamine stores and the turnover of myocardial norepinephrine (NE) as affected by L-dopa were studied in rats. Large doses of L-dopa (100–200 mg/kg, i.p.) increased the concentration of dopamine (DM) in the brain without changing the concentration of NE significantly. DM accumulated in the heart following L-dopa treatment, accompanied by a decrease in NE concentration. After labelling of the myocardial NE store with tracer doses of ^{3}H-NE, treatment with L-dopa caused a more rapid decline of specific activity of myocardial NE. Therefore, DM may displace NE from peripheral sympathetic nerve endings and interfere with adrenergic transmission. (Key words: L-Dopa; Catecholamines; Norepinephrine; Dopamine; Parkinson's disease; Adrenergic transmission.)

THE EFFICACY of L-dihydroxyphenylalanine (L-dopa) in the treatment of Parkinson's disease is now well established.[1–7] Clinical usage of L-dopa for parkinsonism can be expected to increase. The use of L-dopa in the treatment of depression [8] and hypertension [9] is currently being investigated. L-Dopa is the immediate precursor of dopamine (DM). Dopamine has been found to be effective therapeutically in shock and congestive heart failure with shock, in that it has a beta-adrenergic stimulating effect on the heart and causes renal vasodilation and diuresis.[10, 11]

In parkinsonism, daily doses of 2 to 8 g of L-dopa are administered. Side-effects on the cardiovascular system, such as arrhythmias,[1, 6] hypotension,[3, 12] and postural hypotension,[1, 5] occur frequently. These effects suggest that massive doses of L-dopa may alter peripheral adrenergic function. Indeed, Whitsett *et al.*[13] and Whitnack *et al.*[14] have reported that in experimental animals L-dopa interfered with barostatic reflexes and cardiac response to sympathetic stimulation. A possible explanation for these observations is that L-dopa changes the catecholamine stores in peripheral adrenergic tissues. The present study was undertaken to determine the effect of L-dopa on the norepinephrine (NE) stores in the rat heart, an organ rich in sympathetic innervation. Changes in concentrations of NE and DM in the brain after administration of L-dopa were also measured.

Methods

Male Sprague-Dawley rats weighing approximately 200 g were used in all experiments. L-Dopa was dissolved in hydrochloric acid and diluted in an appropriate volume of water; *p*H of the solution was brought to 7.0 with sodium hydroxide. Groups of rats received L-dopa (100–200 mg/kg in 1 ml) intraperitoneally. Control animals were given equal volumes of solvent. L-Dopa-treated rats were killed in groups of five by decapitation one, two, four and six hours after injection. Hearts and brains were immediately removed, rinsed in water, blotted dry and frozen for subsequent analysis of NE and DM concentrations.

Tissues were homogenized in 3–10 volumes of ice-cold 0.4 N perchloric acid. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 g for ten minutes. A 4.7-ml sample of the supernatant was transferred to another tube and 0.3 ml of 10 M potassium

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TABLE 1. Tissue Concentrations of Norepinephrine and Dopamine (μg/g) after L-Dopa, 100 mg/kg, i.p.*

	Control	Hours after L-Dopa			
		1	2	4	6
Brain					
Norepinephrine	0.49 ± 0.02	0.49 ± 0.02	0.49 ± 0.01	0.47 ± 0.03	0.48 ± 0.02
Dopamine	0.97 ± 0.04	2.32 ± 0.14†	1.36 ± 0.07†	1.11 ± 0.02	1.05 ± 0.06
Heart					
Norepinephrine	1.00 ± 0.05	0.55 ± 0.04†	0.60 ± 0.05†	1.04 ± 0.08	0.96 ± 0.12
Dopamine	0.00	2.71 ± 0.08	1.57 ± 0.14	0.33 ± 0.05	0.00

* Values are means for five rats ± SE.
† $P < 0.001$ compared with control.

acetate added to precipitate the excess perchlorate. After centrifugation, 4 ml of the supernatant were passed through a Dowex column (50 X 4, Na^+ form, 20 mm × 20 mm^2, prepared according to Costa *et al.*[15] for the separation of dopa, NE, and DM. After washing with 5 ml each of 0.1 N sodium acetate buffer (*p*H 6) and 0.4 N hydrochloric acid, NE was eluted with 10 ml of 0.4 N hydrochloric acid. Subsequently, DM was eluted with 4 ml of 4 N hydrochloric acid. Amine in the eluate was further extracted with alumina NE and DM were assayed spectrofluorometrieluted with 0.4 N hydrochloric acid (2 ml). (prepared according to Crout[16]) and then cally by the trihydroxyindole method.[17]

The effect of L-dopa on myocardial NE stores was studied in another series of experiments. Groups of rats were given a tracer dose (20 μc/kg in 1 ml of saline solution) of *d,l*-^{3}H-NE (5 c/mM, New England Nuclear Corporation) intravenously. Thirty minutes to two hours later, experimental animals received 200 mg/kg of L-dopa intraperitoneally while control animals received an equal volume of the solvent. Groups of rats were killed at intervals after the injection of L-dopa. NE and DM in the heart were measured as described above. In addition, a sample of the alumina eluate was put in Bray's mixture[18] and placed in a liquid scintillation spectrometer (Intertechnique, model SL40). Specific activity of heart NE was calculated after correction for recovery and expressed as counts per minute (cpm) per μg of NE. The half-life of the myocardial NE store was calculated from the decline of NE specific activity, using best-fitting regression lines.

Results

In rats treated with L-dopa (100 mg/kg) brain NE concentrations remained unchanged while DM concentrations increased (table 1, fig. 1). Brain DM concentration rose from a control value of 0.97 ± 0.04 to 2.32 ± 0.14 μg/g (mean ± SE) at one hour, returning to control levels at four hours.

Concentrations of NE and DM in the heart after L-dopa treatment are presented in table 1 and figure 2. In control animals the heart contained 1.00 ± 0.05 μg/g of NE and negligible amounts of DM. L-Dopa treatment produced a sharp rise in DM concentration, peaking to 2.71 ± 0.08 μg/g at one hour; this decreased gradually, approaching zero by six hours. When the heart DM concentration was at maximum, NE concentration fell to 0.55 ± 0.04 μg/g, about 55 per cent of control, gradually returning to control level at four hours.

Heart NE specific activity declined at a faster rate in L-dopa-treated rats (table 2). An hour after administration of L-dopa, NE specific activity was 73 per cent that of control. At four hours the specific activity amounted to only 29 per cent of control values measured after the same interval. Thus, during the four hours after administration of L-dopa, 71 per cent of the original heart NE store was lost and replaced by newly synthesized NE. This amounted to 1.00 × 0.71 over four hours, or 0.18 μg/g/hr. Under normal conditions NE in the heart turns over at a rate of approximately 0.05 μg/g/hr.[19] Therefore, administration of a large dose of L-dopa resulted in an almost fourfold increase in the turnover of NE in the heart.

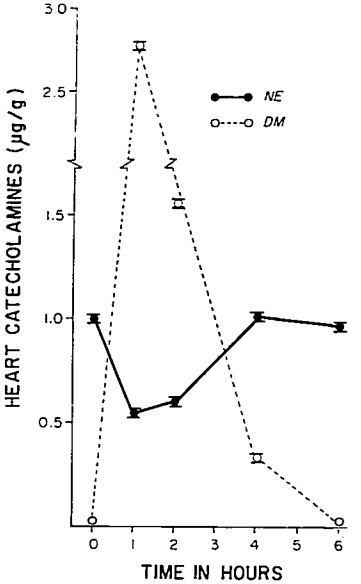

FIG. 1. Changes in norepinephrine and dopamine concentrations in the brain following injections of L-dopa, 100 mg/kg, i.p.

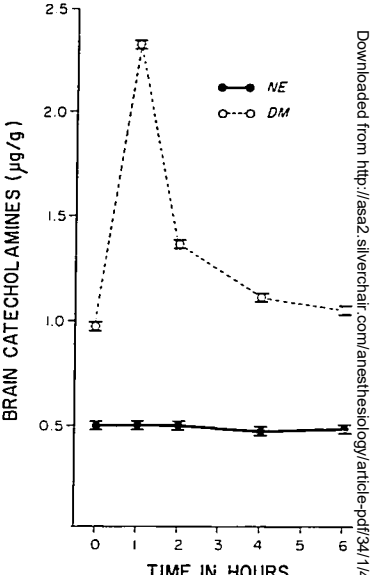

FIG. 2. Changes in norepinephrine and dopamine concentrations in the heart following injection of L-dopa, 100 mg/kg, i.p. Note the absence of dopamine in control rats and six hours after injection of L-dopa. Norepinephrine concentration decreased significantly at one and two hours.

Another way of expressing the rate of loss of the original NE store is to measure the half-life of NE specific activity. This was studied in another experiment. In control animals 4.5 hours were necessary for heart NE specific activity to decline to half that at zero time. In L-dopa-treated animals only 1.7 hours were necessary (fig. 3).

Discussion

Our results indicate that after L-dopa treatment a significant amount of DM accumulated in the rat heart. We have no evidence to rule out the possibility that the conversion of L-dopa to DM could occur extraneuronally. However, the persistence of DM in the heart for as long as four hours and the concurrent decrease in NE concentration suggest that DM is being formed in or transported to adrenergic nerve endings, and that DM is displacing NE from its storage site. Persson and Waldeck[20] injected tracer doses of ^{3}H-dopa (5 μg/kg) intravenously in mice and found ^{3}H-DM in the heart, persisting for an hour, followed by the appearance of ^{3}H-NE.

When L-dopa is supplied, the rate-limiting step in the NE synthesis pathway is bypassed. Decarboxylation of L-dopa to DM proceeds rapidly. ^{14}C-DM has been isolated from the rat heart perfused for only two minutes with solution containing ^{14}C-L-dopa.[21] DM is further converted to NE by beta-hydroxylation. When a large dose of L-dopa, such as that used in these experiments, was administered, the kinetics of NE synthesis and storage appear to have been disrupted. Our results from the studies using ^{3}H-NE indicate that the rapid recovery of heart NE stores, after the initial decrease, was affected through the conversion of L-dopa to DM and to NE.

The possible physiologic significance of displacement of NE from the peripheral adrenergic stores by DM is considered. The rapid loss or release of NE, about four times the rate of control, could explain the occurrence of arrhythmias soon after the administration of L-dopa. If DM is transiently stored in peripheral adrenergic nerve terminals in the place of NE it may interfere with sympathetic transmission and cardiovascular response to sympathetic activation. The potency of DM in eliciting cardiovascular responses is much less than that of NE. Katz *et al.*[22] found that the threshold doses of DM for eliciting arrhythmias, compared with NE, were 100 times greater in five of six cats studied. Collins and West[23] showed that ^{3}H-dopa is taken up by the rabbit ileum preparation *in vitro*. Electrical stimulation of mesenteric nerve released ^{3}H-DM. It is likely that during L-dopa treatment the accumulation of DM in the peripheral adrenergic tissue may act as a less efficient "false" transmitter.

Recent studies of Whitsett *et al.*[12] demonstrated that L-dopa treatment obtunded barostatic reflexes in the dog and cat. During L-dopa infusion the pressor response to bilateral common carotid occlusion was significantly reduced. By a systematic process of elimination they concluded that the action of L-dopa is most likely to occur at postganglionic sites. In the cat, ganglionic transmission remained normal during L-dopa infusion. Concurrent administration of a peripheral decarboxylase inhibitor, RO 4-4602, prevented the reduction of pressor response to bilateral carotid occlusion. Decarboxylase inhibition prevented the conversion of L-dopa to DM in the periphery but not in the central nervous system. Furthermore, the vasoconstrictor response in the femoral vascular bed to lumbar sympathetic

TABLE 2. Specific Activity (cpm/μg) of Norepinephrine in the Heart in Control and L-Dopa-treated Rats*

	Hours after Treatment	
	1	4
Control	6,581 ± 242	6,186 ± 577
L-Dopa-treated	4,797 ± 389	1,794 ± 140
Per cent control	72.9	29.0

* Values are means for five rats ± SE. L-Dopa, 200 mg/kg, i.p. administered two hours after i.v. injection of *dl*-^{3}H-NE (20 μc/kg).

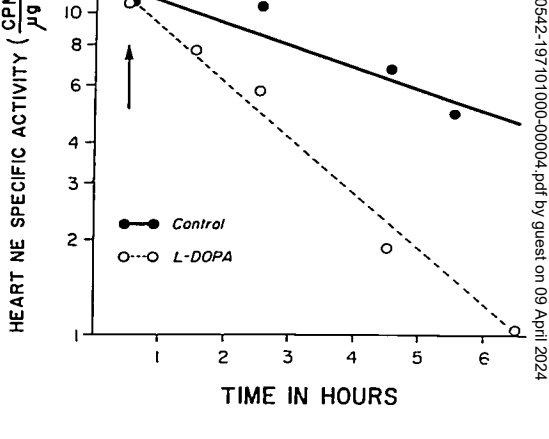

FIG. 3. Declines in specific activity of norepinephrine in the hearts of control rats and those treated with L-dopa, 200 mg/kg, i.p., 30 minutes after intravenous injection of *d,l*-^{3}H-norepinephrine, 20 μc/kg.

nerve stimulation was diminished during administration of L-dopa. Similarly, Whitnack *et al.*[14] observed in anesthetized dogs that the chronotropic responses to stimulation of pre- and postganglionic sympathetic nerves were diminished after L-dopa treatment.

It would appear that the accumulation of DM and displacement of NE from the peripheral adrenergic store could explain some of the cardiovascular effects of L-dopa observed clinically. Interference with barostatic reflexes would account for the postural hypotension. Also, the initial release of NE could be responsible for the hypertensive response[1, 7] and arrhythmias.[1, 5-7]

We have had experience with patients being treated with L-dopa for parkinsonism who were anesthetized for a variety of surgical procedures. Initially, fearing cardiovascular complications, we discontinued L-dopa therapy for one to two weeks before operation. It soon became apparent that continued therapy with L-dopa up to the night prior to anesthesia should not pose an anesthetic hazard. The half-life of L-dopa and its immediate metabolite, DM, is of the order of a few hours. Peaston and Bianchine[4] administered ^{14}C-L-dopa by mouth to patients with Parkinson's disease and found a peak serum level of L-dopa one to two hours after ingestion, with two-thirds of the administered dose excreted as metabolites in the urine by eight hours. Therefore, normal adrenergic function may be expected during anesthesia when the last dose of L-dopa is administered the night before operation. We did not encounter unexpected cardiovascular complications during anesthesia in our patients on L-dopa therapy.

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