Attenuation of Postganglionic Sympathetic Nerve Activity by L-Dopa

By Thomas L. Whitsett, M.D., Perry V. Halushka, Ph.D., M.D., and Leon I. Goldberg, Ph.D., M.D.

ABSTRACT

In anesthetized dogs and cats, intravenous L-dopa reduced the increase in mean blood pressure in response to bilateral carotid occlusion by 77.5% (P<0.01). In the presence of a dopa decarboxylase inhibitor (Ro 4-4602), this effect was absent. Transmission across the superior cervical sympathetic ganglion in the cat was unaltered by the administration of L-dopa. Carotid artery infusion in the dog of 20% of the effective intravenous dose of L-dopa or dopamine failed to inhibit the response to bilateral carotid occlusion. The pressor response to intravenous tyramine in the dog was potentiated (P<0.05) by L-dopa. In the dog, the increase in femoral vascular resistance to lumbar sympathetic nerve stimulation was attenuated (P<0.05) by L-dopa while the response to intra-arterial norepinephrine was unchanged. Thus, inhibition of postganglionic sympathetic nerve activity is a possible mechanism by which L-dopa impaired the carotid sinus reflex.

ADDITIONAL KEY WORDS
cat  dog  carotid sinus reflex
decarboxylase inhibition  ganglionic transmission  dopamine
norepinephrine  tyramine

Patients with Parkinson’s disease treated with L-dopa have frequently experienced orthostatic hypotension (1-6). Although a number of theories have been proposed to explain this phenomenon, the mechanism of action has not yet been elucidated. In the present study we found that the pressor response to bilateral carotid artery occlusion of anesthetized dogs and cats was markedly impaired by intravenous administration of L-dopa. By systematic elimination of several possible mechanisms, we conclude that impairment of the activity of postganglionic sympathetic nerves is the most likely explanation for the attenuation of the carotid sinus reflex.

Methods

STUDIES IN THE DOG

Mongrel dogs (11 to 17 kg) were anesthetized with pentobarbital (25 mg/kg iv). This was followed by a continuous infusion of the drug at a rate of approximately 5 mg·kg⁻¹·hour⁻¹. Ventilation was provided by a Harvard respirator pump. Esophageal temperature was maintained at 37°C by electric heating pads applied to the surface of the body. After bilateral vagotomy, the common carotid arteries were isolated several centimeters proximal to the carotid sinus and loose cotton ligatures were placed around them. To occlude the arteries, the ligatures were pulled gently through a piece of polyethylene tubing for 30 seconds. The response to two or more carotid occlusion tests was obtained before, during, and after L-dopa administration.

Blood pressure was measured from a femoral artery by a Statham P23-D transducer and electrically integrated mean blood pressure was recorded on a Grass polygraph. A femoral vein was used for intravenous injections and infusions. An initial loading dose (5 mg/kg) of L-dopa was administered, followed by a continuous infusion (0.2 mg·kg⁻¹·min⁻¹). In four animals the infusion rate was increased 25 to 90% to attenuate
the response to carotid occlusion in a shorter period of time. In no experiment was the total dose administered greater than 29 mg/kg, which is comparable to the maximum single oral dose of L-dopa a 70-kg patient would receive in the treatment of Parkinson’s disease (4).

Arterial blood pH was measured before, during, and after the administration of L-dopa in five dogs using a Radiometer-Copenhagen pH meter 27. The arterial blood pH did not change significantly during the experiments.

Procedure 1.—The effects of bilateral carotid artery occlusion were measured before, during, and after the infusion of L-dopa in 16 dogs.

Procedure 2.—The effect of tyramine (50 μg/kg iv) on the arterial pressure was measured before, during, and after the infusion of L-dopa in five dogs.

Procedure 3.—The effect of an infusion of L-dopa on the response to carotid occlusion was determined in five dogs. After the response to carotid occlusion had returned to control values, 30 mg/kg of Ro 4-4602 [N1-(DL-eryl)-N2-(2,3,4-trihydroxybenzyl) hydrazine], a dopa decarboxylase inhibitor (7), was injected intravenously. Thirty minutes later L-dopa was again infused and the response to carotid occlusion repeated. In four additional dogs the response to carotid occlusion was determined before and after two separate infusions of L-dopa without administration of Ro 4-4602.

It was necessary to determine that this dose of Ro 4-4602 sufficiently inhibited decarboxylase to prevent production of dopamine from L-dopa in the amounts used in these studies; Ro 4-4602 completely blocked the positive inotropic action of L-dopa in two dogs, indicating that the enzyme was sufficiently inhibited. This in-vivo method for measuring the action of decarboxylase inhibition, based on the fact that the positive inotropic effect of L-dopa is due entirely to inhibition, based on the fact that the positive inotropic effect of L-dopa is due entirely to inhibition, based on the fact that the positive inotropic effect of L-dopa is due entirely to the production of dopamine, was previously found to compare well with an in-vitro determination of decarboxylase inhibition (8).

Procedure 4.—Blood flow in the right femoral artery of dogs was measured by a Statham M-4001 sine-wave electromagnetic flowmeter. Zero flow at the beginning and end of each experiment was determined by occluding the vessel distal to the probe. Care was taken to avoid disturbing the nerves leading to the artery. Atropine sulfate, 1 mg/kg iv, was administered to avoid possible vasodilatation produced by activation of sympathethic cholinergic fibers during nerve stimulation. In six dogs the right lumbar sympathetic chain was exposed by a midline abdominal incision and sectioned at approximately the L3 level. The distal trunk was stimulated for 30 seconds with a Grass S5 stimulator at 5 volts with frequencies of 0.2, 2, and 12 impulses/sec for 0.3-msec duration. Femoral vascular resistance was calculated in peripheral resistance units (PRU), defined as the ratio of mean blood pressure in mm Hg to femoral blood flow in ml/min. L-dopa was then administered as described above and lumbar sympathetic stimulation was repeated when the response to carotid occlusion was impaired. When the response to carotid occlusion had returned to control values after discontinuing L-dopa, the sympatheic nerves were stimulated again. In five dogs (including two of the above), the response of femoral vascular resistance to intra-arterial injections of graded doses of norepinephrine (0.125, 0.25, and 0.50 μg in 0.2 ml) was determined before, during, and after administration of L-dopa. The femoral artery injections were made through a 25-gauge hypodermic needle in which saline was being infused by a Holter pump (RD 045) at a rate of 0.5 ml/min.

Procedure 5.—The left carotid sinus and body were denervated by dissecting them free of all visible nerves. Thoroughness of denervation was determined by visual inspection and their failure to participate in the carotid sinus reflex. Injections were made into the right common carotid artery proximal to the carotid sinus, which was not denervated. The effect of occlusion of the right carotid artery in mean blood pressure was determined before, during, and after intravenous administration of L-dopa and dopamine. The latter drug was infused at a sufficient rate to attenuate the carotid sinus reflex. After return to the control response, 20% of the effective intravenous dose was then given intra-arterially and the response to right carotid artery occlusion was again determined at the same time intervals used after the intravenous injections.

STUDIES IN THE CAT

Cats (1.5 to 5 kg) were anesthetized with intraperitoneal injections of chloralose (40 mg/kg) and urethane (600 mg/kg). Mean arterial blood pressure recordings, intravenous injections, carotid artery occlusions, and tracheostomies were performed as in the dog experiments. Unlike the dog studies, the vagi were left intact and respiration was unassisted. The pre- and postganglionic fibers of the left superior cervical ganglion were isolated and severed; care was taken to separate the vagus from the sympathetic trunk. The preganglionic fibers were stimulated with bipolar silver electrodes by a Grass S5 stimulator at 2 impulses/sec, at supramaximal voltage with a stimulus duration of 0.3 msec. A cervical well was made with muscle and skin tissue and the nerve and ganglion were kept submerged in mineral oil. Action potentials were recorded from the fibers of the preganglionic
neuron by the use of bipolar platinum electrodes. The potentials were amplified (200 μV/cm) on a 502 dual-beam Tektronix oscilloscope and recorded with a Polaroid camera. The pressor response to carotid occlusion and the height of the evoked action potentials were measured at 5, 15, 30, 45, and 60 minutes after intravenous injection of L-dopa (40 mg/kg over 5 minutes). This dose was chosen on the basis of preliminary experiments which indicated that a dose of 20 mg/kg produced inconsistent and short-lasting attenuation of the pressor response to carotid occlusion.

ANALYSIS OF DATA

The response produced to carotid occlusion and intravenous injection of tyramine is presented as percent increase in mean blood pressure. The vasoconstriction produced by lumbar sympathetic stimulation and intra-arterial injections of norepinephrine was calculated as percent increase in femoral vascular resistance. The response to carotid occlusion was evaluated by two different techniques in the cat: (1) the percent increase in mean blood pressure, and (2) [(observed change in mean blood pressure)/(mean blood pressure-60)]×100 (9). The latter formula was employed since L-dopa decreases the mean blood pressure in cats. Prochnik et al. (9) demonstrated that the change in mean blood pressure during carotid occlusion is directly proportional to mean BP-60 and that application of the above formula eliminates the variations in the pressor response to carotid occlusion when the initial pressure is between 70 to 160 mm Hg. All results are expressed as the mean ± SE. The paired "t"-test was used to determine statistical significance between treatment and control periods.

MATERIALS

The following drugs were used: L-dopa HCl (Calbiochem and Hoffman-La Roche); dopamine HCl (Calbiochem); tyramine HCl (Calbiochem); 1-norepinephrine bitartrate (Winthrop); pento-barbital sodium (Merck); atropine sulfate (Merck); and Ro 4-4602 (Hoffmann-La Roche). Doses of drugs were calculated as the salt, except for 1-norepinephrine which was calculated as the base.

Results

STUDIES IN THE DOG

1. Effect of L-dopa on the Carotid Sinus Reflex.—Figure 1 illustrates the effect of intravenous infusion of L-dopa on the carotid occlusion response in 16 dogs. The average response to carotid occlusion during L-dopa was significantly less (P < 0.001) than those obtained before and after infusion of L-dopa. In all experiments, the response to carotid occlusion was maximally depressed 60 minutes or less after the start of the infusion of L-dopa. Full recovery occurred between 15 and 60 minutes after L-dopa was discontinued. In most experiments, there was a transient increase in mean blood pressure after the initial 5 mg/kg dose of L-dopa. However, when the response to carotid occlusion was maximally depressed, the average mean blood pressure (mm Hg) was not significantly different from control values (before L-dopa, 105.8 ± 3.6; during L-dopa, 106.1 ± 5.1; after L-dopa, 106.7 ±3.9).

2. Effect of L-dopa on the response to Tyramine.—In five dogs the average percent increase in mean blood pressure in response to tyramine was 20 ±3.3 during the period before L-dopa, 51 ±8.2 during the L-dopa infusion, and 16 ±2.4 in the period after L-dopa. The response to tyramine was significantly greater (P < 0.025) during the L-dopa administration when compared to periods both before and after L-dopa.

3. Effect of L-dopa after Administration of Ro 4-4602.—In five dogs L-dopa produced typical reductions of the response to carotid occlusion before administration of Ro 4-4602.
After administration of this dopa decarboxylase inhibitor, the same infusion of L-dopa failed to impair the response to carotid occlusion. This lack of effect was not due to tachyphylaxis, since in four additional dogs the first and second infusions of L-dopa produced equivalent reductions of the pressor response to carotid occlusion (Fig. 2). In these experiments L-dopa did not significantly alter mean blood pressure, either before or after Ro 4-4602.

4. Effect of L-dopa on Femoral Vascular Resistance during Lumbar Sympathetic Stimulation and after Intra-arterial Norepinephrine.—In six dogs when L-dopa had impaired the response to carotid occlusion, the percent increase in femoral vascular resistance produced by lumbar sympathetic stimulation was significantly decreased (P < 0.01) as shown in Figure 3. The response to carotid occlusion and sympathetic stimulation returned to control values simultaneously following discontinuation of L-dopa.

To determine whether this effect was due to a blockade of norepinephrine at the alpha receptor or to some other effect of L-dopa on the arterioles, norepinephrine was injected intra-arterially in two of the above experiments and in three additional dogs. The mean response to intra-arterial injections of norepinephrine was unchanged by L-dopa (Fig. 4). Furthermore, the vasoconstriction produced by norepinephrine was unaltered during the time period that the response to sympathetic nerve stimulation was depressed in the two dogs in which both procedures were performed.

In these nine dogs, the average femoral vascular resistance increased during L-dopa infusions from 1.27 ± 0.18 to 1.8 ± 0.15 PRU (P < 0.05), probably due to an action of dopamine on alpha receptors. This increase in femoral vascular resistance, however, could not be responsible for the reduction of vasoconstriction following sympathetic nerve stimulation.
stimulation since this reduction was also observed in two dogs in which femoral vascular resistance was slightly reduced.

5. Effect of L-dopa and Dopamine Infusions into an Innervated Carotid Artery.—As in the previous procedures, intravenous infusions of L-dopa (five dogs) significantly impaired the response to carotid occlusion (Table 1). When 20% of the intravenous dose was infused into the innervated right carotid artery of the same animal, the carotid sinus reflex was unaltered (Table 1).

The rate of intravenous infusion of dopamine required to significantly attenuate the carotid sinus reflex varied between 10 and 15 $\mu$g•kg$^{-1}$•min$^{-1}$. The average time required for the appearance of this effect was 50 minutes. By approximately 15 minutes after discontinuation of the infusion, the response to carotid occlusion had returned to control values. As with L-dopa, when 20% of the intravenous dose was infused into the right carotid artery, the response to carotid occlusion was unchanged.

**STUDIES IN THE CAT**

1. Effect of L-dopa on the Carotid Sinus Reflex.—In eight cats, the response to carotid occlusion was significantly depressed ($P < 0.05$) at 5, 15, and 30 minutes after the injection of L-dopa (40 mg/kg iv). By 45 minutes, the response was not significantly different from control values. During the period when the pressor response was impaired, the rate of rise of the mean blood pressure was decreased. The mean blood pressure was significantly decreased ($P < 0.05$) within 5 minutes after L-dopa was injected and did not return to control levels by 60 minutes (Fig. 5). This was in contrast to the response to carotid occlusion which had returned to control values by 45 minutes. The response to carotid occlusion was still significantly depressed even when it was adjusted for the lowered mean blood pressure (see analysis of data).

2. Effect of L-dopa on the Sympathetic Ganglion.—L-dopa did not significantly alter

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**TABLE 1**

*Effects of Intravenous and Intracarotid Infusions of L-dopa and Dopamine on the Response to Carotid Occlusion in the Anesthetized Dog*

<table>
<thead>
<tr>
<th></th>
<th>Intravenous injections</th>
<th>Intracarotid injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBP (mm Hg) before CO</td>
<td>% increase in MBP during CO</td>
</tr>
<tr>
<td>Before L-dopa</td>
<td>111 ± 8.8</td>
<td>32 ± 6.7</td>
</tr>
<tr>
<td>During L-dopa*</td>
<td>102 ± 5.2</td>
<td>5 ± 1.4†</td>
</tr>
<tr>
<td>Before dopamine</td>
<td>119 ± 7.8</td>
<td>33 ± 3.8</td>
</tr>
<tr>
<td>During dopamine†</td>
<td>123 ± 9.7</td>
<td>13 ± 4.5‡</td>
</tr>
</tbody>
</table>

The dogs had their left carotid sinus denervated and drugs infused into right carotid artery. Values are mean ± se.

*Initial dose (5 mg/kg) followed by an infusion of 0.2 mg·kg$^{-1}$·min$^{-1}$ (n = 5); †Average infusion rate 13 $\mu$g·kg$^{-1}$·min$^{-1}$ (n = 4); ‡P < 0.05; §20% of intravenous dose. MBP = mean blood pressure; CO = carotid occlusion.

_Circulation Research, Vol. XXVII, October 1970_
the average evoked action potentials recorded from the postganglionic neuron of the superior cervical ganglia of seven cats (Fig. 6). During the 5, 15, and 30 minute periods after administration of L-dopa, when the response to carotid occlusion was maximally depressed (Fig. 5B), the evoked action potential was increased in three experiments and decreased in four. In two experiments in which the evoked action potential and the response to carotid occlusion were simultaneously measured, there was no temporal relationship between changes in the evoked action potential and changes in the pressor response to carotid occlusion.

Discussion
The impaired carotid sinus reflex associated with L-dopa administration in the present study could have been caused by several mechanisms. Because this effect of L-dopa was transient, we were able to investigate potential sites of inhibition to determine whether the changes observed correlated temporally with the impairment of the reflex.

Pronounced reduction of blood pressure can decrease the response to carotid occlusion (9, 11). Blood pressure was not changed at the time the response was decreased in the dog experiments. Blood pressure was lowered in the cat by the dose of L-dopa required to decrease the response in that species. The hypotensive response, however, persisted much longer than the decreased response to carotid occlusion and was of insufficient magnitude to explain the phenomenon when
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THE data were subjected to analysis by the formula suggested by Prochnik et al. (9). Thus the effect of L-dopa on the carotid occlusion was not due to the decrease in mean blood pressure.

In order to determine whether L-dopa or dopamine acted directly on the carotid baroreceptors, we infused L-dopa and dopamine into the innervated carotid artery. This procedure did not result in decrease of mean blood pressure or its response to carotid occlusion. Thus, it appears unlikely that an effect on the carotid sinus was responsible for the impairment of the response to carotid occlusion. However, these results do not prove that L-dopa or dopamine do not affect the carotid sinus for to rule out such an action, other measurements would be required, such as phasic blood pressure within the carotid sinus during occlusion of the common carotids. Jacobs and Comroe (12) reported that intracarotid administrations of 1 to 10 μg of dopamine or 10 mg of L-dopa in the dog caused stimulation of the carotid chemoreceptors; they did not report any action on baroreceptors.

We next considered the possibility that L-dopa altered the response to carotid occlusion by blockade of sympathetic ganglia. Kadziela et al. (13) reported that close intra-arterial injections of dopamine could block the evoked action potential of the superior cervical ganglion of the cat, and Bogaert and DeSchaepdryver (14) concluded that intravenous infusions of dopamine reduced vasoconstriction in the splanchnic bed of the cat by ganglionic blockade. Since L-dopa is converted to dopamine, ganglionic blockade was a distinct possibility in the cat. However, we found that the dose of L-dopa which inhibited the response to carotid occlusion in the cat had no consistent effect on transmission across the superior cervical ganglion, suggesting that ganglionic blockade was not the responsible mechanism.

The possibility of alpha-receptor blockade produced by L-dopa was suggested by Godwin-Austin et al. (15), who found that the mydriatic action of phenylephrine was reduced in patients treated with L-dopa. Alpha-receptor blockade could not explain the reduced carotid sinus reflex in our study since L-dopa did not affect the vasoconstriction produced by administration of l-norepinephrine (NE) into the femoral artery. Furthermore, the augmented vasopressor response to tyramine could not have occurred in the presence of alpha-receptor blockade.
To further define the mechanism of the attenuated response to carotid occlusion, L-dopa was administered before and after a decarboxylase inhibitor. Ro 4-4602 has been reported to be a peripheral dopa decarboxylase inhibitor in rats (7); however, whether it crosses the blood-brain barrier in dogs at this dose has not been established. The dose of Ro 4-4602 used was effective in preventing the positive inotropic effect of L-dopa, indicating significant inhibition of dopa decarboxylase peripherally (8). Since Ro 4-4602 prevented the inhibitory effect of L-dopa on the carotid sinus reflex, it is probable that a decarboxylated product of L-dopa, dopamine or one of its metabolites, was responsible for this effect. This is further substantiated since an infusion of dopamine decreased the carotid sinus reflex.

The finding that L-dopa decreases the change of femoral vascular resistance to lumbar sympathetic stimulation during a time when the vasoconstriction produced by norepinephrine was not reduced, suggests that postganglionic sympathetic nerve function was impaired. The temporal relationship of this phenomenon with the impaired response to carotid occlusion makes it the most likely explanation of the action of L-dopa on the carotid sinus reflex. In this regard, Farmer (16) reported that L-dopa and dopamine attenuated the contraction of the cat nictitating membrane produced by postganglionic nerve stimulation. This effect, however, was observed only when L-dopa was injected after administration of a MAO inhibitor. Farmer's failure to observe impairment of the response to postganglionic stimulation with L-dopa alone may have been related to the fact that attenuation of the response to carotid occlusion persists for less than 45 minutes, whereas he was forced to wait 15 hours after L-dopa administration because of contraction of the nictitating membrane. A similar problem was encountered in the study of Whitnack et al. (17). These investigators reported that intravenous injections of 100 mg/kg of L-dopa in anesthetized dogs decreased the chronotropic response to cardio-accelerator nerve stimulation without affecting the chronotropic response to norepinephrine. This effect was not tested until 4 to 6 hours after the injection of L-dopa because of tachycardia. Although these data also suggest that L-dopa inhibits postganglionic sympathetic nerve activity, the mechanism may be different from that described in the present investigation because of the much larger dose used and the delayed recognition of the inhibition.

Attenuation of postganglionic sympathetic nerve function by L-dopa could have been produced by release and subsequent depletion of endogenous norepinephrine. However, Harrison et al. (18) and Potter and Axelrod (19) demonstrated that, although administration of dopamine causes release of norepinephrine, depletion does not occur. The latter investigators speculated that dopamine does not cause norepinephrine depletion because the increased availability of dopamine as a precursor enhanced the synthesis of norepinephrine in the nerve. Evidence against norepinephrine depletion was obtained in our experiments, i.e., the pressor effects of tyramine were potentiated rather than attenuated. This phenomenon was also observed by Leon et al. (20) in patients receiving L-dopa for the treatment of Parkinson's disease.

The concept of different pools (21) of functional norepinephrine in the adrenergic nerve ending could explain the potentiation of the pressor effects of tyramine, and inhibition of the effects of postganglionic nerve stimulation. Harrison et al. (22) demonstrated that during the 1-hour period after reserpine administration in the dog, the cardiac effects of tyramine administration were potentiated at the same time as the effects of stimulation of the postganglionic nerve were decreased. Trendelenburg (23) demonstrated that after reserpine pretreatment, the action of tyramine on the guinea pig atrial pacemaker returned before responses to stimulation of the cardiac accelerator nerve. The present results could be explained if L-dopa or dopamine significantly increased the size of the tyramine releasable pool while at the same time reduced the amount of norepinephrine released by nerve.

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Stimulation. Dopamine could act as a false neurotransmitter by replacing norepinephrine in the latter pool.

This hypothesis is reasonable since dopamine is a weaker vasoconstrictor than norepinephrine (24). In addition, dopamine can be taken up and stored by sympathetic nerves during the time course of our experiments and is releasable by nerve stimulation (23-27). However, further investigations are necessary before it can be established that attenuation of sympathetic nerve function was due to production of a false neurotransmitter or some other action of dopamine on the postganglionic nerve.

Although the present results suggest a possible basis for the postural hypotension produced by the administration of L-dopa, several other mechanisms have to be considered. Barbeau et al. (28) reported that L-dopa decreases plasma renin activity to undetectable levels in Parkinsonian patients and postulated that this causes orthostatic hypotension. It is also possible that the vasodilation produced by dopamine in the renal and mesenteric vascular beds (24, 29, 30) may be of sufficient degree to diminish the reflex vasoconstriction produced by postural changes. Finally, dopamine increases sodium excretion (29). Sodium depletion could lower blood pressure and contribute to the hypotension produced by the other postulated mechanisms.

Acknowledgment

We would like to thank Dr. Neil Moran and Dr. Sheldon Skinner for their helpful suggestions; also Mr. Homer Ryan and Mr. Cary Mungroove for their technical assistance and Dr. William B. Abrams and Dr. John J. Burns of Hoffmann-La Roche, Inc., for providing L-dopa and Ro 4-4602.

References

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Circ Res. 1970;27:561-570
doi: 10.1161/01.RES.27.4.561
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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