Effect of L-Dopa on Sympathetic Nerve Activity and Blood Pressure in the Spontaneously Hypertensive Rat

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SUMMARY We examined the effect of L-dopa, after peripheral L-amino acid decarboxylase inhibition, on sympathetic nerve activity (SNA) and blood pressure in spontaneously hypertensive rats (SHR) and in normotensive control rats. L-Dopa reduced SNA in both groups of animals. The SHRs were significantly more sensitive to the depressor effect of L-dopa than were the control animals, the threshold dose for reduction of SNA being 3 mg/kg in SHR and 15 mg/kg in control rats. Similarly, the magnitude of inhibition of SNA was substantially greater in the SHR than in normotensive rats. The reduction in SNA in the SHR was accompanied by a parallel fall in blood pressure. In contrast, blood pressure in control rats did not change significantly, even though SNA was diminished. Studies of the penetration of L-dopa into the cerebral parenchyma revealed that equivalent amounts of the amino acid entered the brains of the two groups of rats. These results suggest that the SHRs are more sensitive to the SNA-inhibiting effects of L-dopa than are normotensive rats. In addition, they confirm our previous suggestion that excessive SNA plays a causative role in the hypertension of the SHR.

A growing body of evidence indicates that a significantly causative factor in the hypertension of the Okamoto strain of spontaneously hypertensive rat (SHR) is excessive sympathetic nerve activity (SNA). The major observations supporting such a conclusion are that pharmacological or surgical abolition of SNA leads to a reduction in blood pressure in the SHR, and that directly measured SNA is markedly increased. In addition, studies of baroreceptor regulation of blood pressure demonstrate a decreased baroreceptor sensitivity in the SHR compared to normotensive control rats. Although the mechanism underlying the excessive SNA and decreased baroreceptor sensitivity is not known, it seems reasonable to postulate that the abnormality resides in certain vasomotor regulatory centers in the brain. Further, in light of the knowledge that the antihypertensive agents which are thought to act substantially or primarily in the central nervous system (CNS) interact with adrenergic nerves or receptors, it appears possible that a CNS abnormality in the SHR which leads to hypertension involves brain catecholamine metabolism, as previously suggested by other investigators.

One approach to studying CNS adrenergic regulation of physiological responses in intact animals is to use drugs to either inhibit or activate CNS adrenergic systems. Such an approach, using agents such as reserpine, intra-cerebral 6-hydroxydopamine, alpha-methyl dopa, clonidine, intracerebral dopamine, or ergot derivatives, has been applied to the study of depression, extrapyramidal disorders, hypertension, and the regulation of hypothalamic releasing factors. By using L-dopa, an amino acid precursor of catecholamines, we and others have shown that an increase in CNS catecholamine content results in a reduction in efferent SNA that is associated with a decrease in blood pressure in anesthetized cats and dogs.

The present study, in which we have assessed the effects of L-dopa on blood pressure, heart rate, and SNA in the SHR, was performed because of the demonstrated excessive SNA in these rats and the knowledge that L-dopa can reduce SNA. Our results show that L-dopa, when given with an extracerebral inhibitor of L-amino acid decarboxylase, is a potent depressor drug in the SHR. The mechanism for the hypotensive effect of L-dopa in this model of hypertension appears to be a reduction in efferent SNA.

Methods

All experiments were conducted on male SHRs (Okamoto Strain, Cox Laboratory Animal Supply) and male Kyoto-Wistar normotensive rats (Cox Laboratory Animal Supply), the genetic parent of the SHR. All rats tested were between 16 and 18 weeks old, an age when the hypertension is well developed in the SHR. Rats were anesthetized with sodium pentobarbital (20 mg/kg, ip), and anesthesia was maintained with supplemental doses of 2.5 mg/kg, intravenously, as needed.

Arterial blood pressure was measured by using a pressure transducer (Statham P23Dd) connected to a heparinized saline-filled polyethylene tube (PE-60, thin walled, 10 cm long) which was inserted through the right
femoral artery to the terminal aorta. Mean arterial pressure was derived by using an RC integrator with a time constant of 1 second. The right femoral vein was cannulated with a similar polyethylene tube for infusion of drugs. Heart rate was determined by counting the number of arterial pressure pulsations per minute.

SNA was recorded, as previously described, from a postganglionic renal nerve bundle coursing to the left kidney. The nerves were exposed via a left lateral retroperitoneal incision, stripped of their connective tissue sheaths, and placed over a pair of stainless steel recording electrodes where they were bathed in a pool of paraffin oil to prevent tissue drying. The efferent signals were recorded by means of an A.C. differential preamplifier (Grass Instruments, model P-15) with a time constant of 3 msec. The amplified nerve spikes were displayed on an oscilloscope (Tektronix, model R103N) for visualization and photographing (Tektronix camera, model C-12). The signals were further amplified, then rectified with a full-wave rectifier circuit and integrated continuously with an R.C. integrator (time constant 20 msec). The integrated signals were averaged, using an R.C. network with a time constant of 1 second. The nerve data presented in this paper are expressed as mean nerve activity and are calibrated in microvolts above noise level. The noise level (3-7 μV) was determined by shorting out the input electrodes and these values for each experiment were subtracted from the electroneurogram to obtain actual mean SNA. All analog and electronically averaged signals were recorded on a Beckman type R dynograph. To summarize for graphical presentation, average mean SNA was determined by measuring the area under the electronically derived curve over a 20- to 30-second period. This was done at 2.5- to 5-minute intervals, and the data were plotted with simultaneously measured mean blood pressure and heart rate.

Both L-dopa and L-a-hydrazino-a-methyl-β-(3,4-dihydroxyphenyl) propionic acid (carbidopa) were dissolved in isotonic saline acidified to a pH of 1.5 with concentrated hydrochloric acid. After the drug was dissolved, the pH was titrated to 7.0 with sodium hydroxide. Ascorbic acid (10 μg/ml) was added to both drug solutions to prevent oxidation of the drugs at neutral pH. Carbidopa was given in a dose of 10 mg/kg to all rats that received this treatment. Twenty-five to 30 minutes after administration of carbidopa, L-dopa (0.03 to 30 mg/kg) was infused over a 2- to 3-minute period.

In some experiments in which L-dopa was given alone, blood pressure was clamped to a constant level by draining arterial blood into a reservoir as blood pressure began to increase in response to L-dopa.

Tritiated L-dopa (specific activity, 11.9 Ci/mmol; generally labeled) was purchased from New England Nuclear and was purified before each experiment by adsorption and elution from alumina. A total of 50 mCi of 3H-L-dopa was administered to each rat with sufficient unlabeled L-dopa to give a total dose of 30 mg/kg. The brain was removed rapidly, chilled in ice-cold saline, and all major vessels and the meninges removed. The brain was then dissected according to the method of Gloewinski et al., except that the hypothalamus was removed initially by first cutting perpendicular to the base of the brain at the hypothalamic sulcus and then parallel to the base of the brain at a depth demarcated by the intersection of the anterior commissure with the initial incision. Brain tissue was then weighed and homogenized in approximately 10 volumes (wt/vol) of 0.1 N ice cold perchloric acid. After centrifugation for 10 minutes at 7000 g, a sample was obtained and quantified by liquid scintillation spectrometry. Data were calculated to represent total L-dopa and metabolite per milligram of wet weight.

Results

Effect of L-Dopa on Sympathetic Nerve Activity, Blood Pressure, and Heart Rate in the Spontaneously Hypertensive Rat

L-Dopa Alone

When given alone, L-dopa (30 mg/kg) produced a marked pressor response in the SHR, just as it does in other species, including cats and dogs (Fig. 1). This pressor response presumably was mediated by catecholamines (dopamine and/or norepinephrine) which were formed from L-dopa in sympathetic nerves or other tissues. Associated with the pressor response, there was a concomitant reduction in SNA and heart rate, the latter presumably resulting from a reflex withdrawal of SNA as well as an augmentation of vagal activity. That the reduction in SNA was only partially due to reflex inhibition of activity was suggested by the pressure clamp experiments. Even though blood pressure was held constant, there still was a substantial reduction in SNA and a smaller diminution in heart rate (Fig. 1).

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Effect of L-dopa (30 mg/kg) on blood pressure, heart rate, and mean renal sympathetic nerve activity in spontaneously hypertensive rats. After rats were surgically prepared, they were allowed to stabilize for about 30 minutes. Recordings of physiologial parameters were then begun (at zero time) and continuously recorded throughout the remainder of the experiment. Values represent means ± SEM of five animals. L-dopa was given by intravenous infusion over 1–2 minutes at the 30-minute time point. In the pressure clamp experiments, changes in pressure were prevented by removing arterial blood into a reservoir.
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L-Dopa Combined with a Peripheral Decarboxylase Inhibitor

When L-dopa was given after pretreatment with the peripheral inhibitor of decarboxylase, carbidopa, the amino acid became a potent depressor agent (Fig. 2). Blood pressure with L-dopa, 30 mg/kg, fell from 160 ± 5 mm Hg \( (n = 8) \) to 96 ± 7 mm Hg at the time of maximal L-dopa effect. Concomitant with this fall in blood pressure, there was a reduction in SNA which fell to 38% below control values at the time of peak L-dopa effect. Heart rate also decreased in these rats, and the decrease in both heart rate and blood pressure appeared to occur simultaneously with the diminution in SNA. The maximum reduction in SNA occurred about 10 minutes after administration of L-dopa, and then SNA began to gradually return toward control levels. Blood pressure and heart rate also returned to control levels with time, and the rate of recovery of these cardiovascular parameters was similar to that for SNA.

Effect of L-Dopa on Sympathetic Nerve Activity, Blood Pressure, and Heart Rate in the Normotensive Kyoto-Wistar Rat

L-Dopa Alone.

The mean control blood pressure of the Kyoto-Wistar rats was substantially lower than that of the SHR (122 ± 5 vs. 160 ± 6; \( n = 5 \) for both groups). Likewise, mean SNA in the Kyoto-Wistar rats was only 28.5 /μV compared to 65.3 /μV in the SHR \( (n = 5 \) for both groups). As was seen with the SHRs, L-dopa (30 mg/kg) alone produced a marked increase in blood pressure in the Kyoto-Wistar rats (Fig. 3). Associated with the increased pressure there was a reflex inhibition of SNA and a reduction in heart rate. When the blood pressure was clamped at the control level, SNA still decreased as did heart rate, although the reduction in both of these parameters was less than that seen during L-dopa-induced hypertension.

L-Dopa Combined with a Peripheral Decarboxylase Inhibitor

Peripheral inhibition of L-amino acid decarboxylase with carbidopa abolished the pressor effect of L-dopa in the Kyoto-Wistar rats just as it did in the SHRs (Fig. 4). Also, as seen in the SHR, SNA and heart rate were reduced by L-dopa in carbidopa pretreated rats. Unlike the SHR, however, the Kyoto-Wistar rat did not respond to L-dopa combined with carbidopa with a decrease in blood pressure (Fig. 4).

Responses of Spontaneously Hypertensive Rats and Kyoto-Wistar Rats to Varying Doses of L-Dopa after Peripheral Decarboxylase Inhibition

In all the previously described experiments, L-dopa was given in a dose of 30 mg/kg. Because it appeared that the SHR was more sensitive to the depressor effect of L-dopa
than the Kyoto-Wistar rats, a series of dose-response experiments were performed. When assessed in terms of changes in SNA or blood pressure, the SHR clearly was more sensitive to L-dopa than the Kyoto-Wistar rat. The threshold dose for reduction in SNA and blood pressure in the SHR was about 3 mg/kg, whereas for the Kyoto-Wistar rats the threshold was between 10 and 15 mg/kg (Fig. 5). Furthermore, the magnitude of reduction of both SNA and blood pressure with higher doses of L-dopa was greater in the SHR than in the control rats. In contrast, the magnitude of reduction in heart rate with incremental doses of L-dopa was similar in the two groups.

Cerebral Accumulation of L-Dopa in the Spontaneously Hypertensive and Kyoto-Wistar Rats

The dose-response experiments suggested that SHRs were more sensitive to the SNA-reducing and depressor effects of L-dopa than the normotensive control rats. However, another explanation could be that more L-dopa penetrated into the cerebral parenchyma in SHRs than in control rats, perhaps because of differences in the blood-brain barrier. Experiments therefore were performed to evaluate this possibility. After administration of tritiated L-dopa (30 mg/kg) following carbidopa pretreatment, the brains of SHR and Kyoto-Wistar rats (five rats in each group) were divided into 10 regions and the tissue content of L-dopa and metabolites between SHR and control rats (Table 1). In rats that were not pretreated with carbidopa, the cerebral content of L-dopa was about one-half that found in pretreated rats and the content was the same in the hypertensive and normotensive groups (data not shown).

**TABLE 1 Content of L-Dopa and Metabolites in Various Brain Regions of Spontaneously Hypertensive Rats and Normotensive Kyoto-Wistar Rats**

<table>
<thead>
<tr>
<th>Brain region</th>
<th>SHR</th>
<th>Kyoto-Wistar rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>10.2 ± 0.77</td>
<td>10.6 ± 0.41</td>
</tr>
<tr>
<td>Septum</td>
<td>7.7 ± 2.06</td>
<td>8.0 ± 0.49</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>10.7 ± 0.73</td>
<td>10.5 ± 0.53</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>11.2 ± 0.73</td>
<td>10.5 ± 0.53</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>12.1 ± 1.11</td>
<td>11.1 ± 0.24</td>
</tr>
<tr>
<td>Anterior medulla</td>
<td>9.6 ± 0.33</td>
<td>9.0 ± 0.20</td>
</tr>
<tr>
<td>Posterior medulla</td>
<td>9.3 ± 0.65</td>
<td>8.5 ± 0.25</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>10.6 ± 0.52</td>
<td>9.9 ± 0.29</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>10.6 ± 0.50</td>
<td>10.0 ± 0.42</td>
</tr>
<tr>
<td>Amygdala</td>
<td>9.5 ± 0.86</td>
<td>9.1 ± 0.38</td>
</tr>
</tbody>
</table>

* Values (means ± SEM) from brains of five rats in each group are expressed as ng of tritiated L-dopa and metabolites per mg of tissue wet weight. A total dose of 30 mg/kg L-dopa was administered after carbidopa treatment.

**FIGURE 5** Dose-response curves relating changes in blood pressure, heart rate and mean renal nerve activity with various doses of L-dopa in carbidopa (10 mg/kg) pretreated rats. Recorded changes are the peak effects of the drugs. Values represent means ± SD of five to eight rats.

**Discussion**

The results of this study demonstrate that L-dopa, when given with a peripheral inhibitor of L-amino acid decarboxylase, is a potent inhibitor of SNA in both the SHR and normotensive Kyoto-Wistar rats. This effect of L-dopa is thus similar to that previously found in normotensive, anesthetized cats and dogs. In the SHR, the reduction in SNA was accompanied by a decrease in blood pressure and heart rate indicating, as we have previously suggested, that the excessive SNA in the SHR plays a causative role in the hypertension. SNA also decreased in the Kyoto-Wistar rats, but in them this reduction was accompanied by a change in heart rate without a significant change in blood pressure. The mechanism by which L-dopa, acting in the CNS, inhibits SNA and thereby reduces blood pressure in the SHR and heart rate in both groups of rats is unknown. From previous studies in other species, it is likely that L-dopa must be converted into catecholamines (dopamine, norepinephrine, or possibly epinephrine) in the brain to produce the hypotensive effect. It has been shown for both normotensive and hypertensive rats and dogs that if cerebral as well as peripheral L-amino acid decarboxylase is inhibited, the depressor effect of L-dopa is abolished.13, 15, 26 The precise area of the brain on which the formed catecholamines are acting also is unknown. Similarly, it is unclear whether the formed catecholamines are activating inhibitory centers or inhibiting excitatory centers to result in the diminution of SNA. Whatever the precise mechanism, L-dopa, presumably by increasing CNS catecholamine content, restores the abnormally high SNA in the SHR to lower levels and thus lowers blood pressure in these animals.

When L-dopa was given alone to either the SHR or Kyoto-Wistar rats, there was a marked pressor response, presumably due to the rapid accumulation of catechol-
amines (dopamine and/or norepinephrine) in peripheral tissues. These results are also consistent with those previously obtained for normotensive cats and dogs. The extracerebral pressor effects of L-dopa masked the central inhibitory effects of the amino acid. Thus, when the arterial pressure was clamped to prevent the development of hypertension, it was found both groups of rats that SNA was inhibited even when L-dopa was given alone. It therefore appears that sufficient quantities of L-dopa enter the cerebral parenchyma to inhibit SNA even when the L-dopa is given without a peripheral decarboxylase inhibitor. The marked inhibition of SNA seen when L-dopa was given alone was presumably due to both baroreceptor reflex inhibition and CNS inhibition of activity.

The dose-response experiments revealed that the SHR is more sensitive to SNA inhibition by L-dopa than is the Kyoto-Wistar rat. This increase in apparent sensitivity could not be attributed to a difference in the amount of drug reaching the cerebral parenchyma. Of further interest, the depressor response of the SHR was much greater than that of the Kyoto-Wistar rat. In the SHR, therefore, it appears that the excessive SNA is the major and perhaps single cause of the hypertension. In contrast, the normotensive Kyoto-Wistar rat, which has normal SNA, responded to L-dopa with only a trivial fall in blood pressure, suggesting that factors other than SNA were operative in maintaining blood pressure. Similar small reductions in blood pressure in normotensive rats in response to large doses of L-dopa, combined with a peripheral decarboxylase inhibitor, have been reported previously. Heart rate in both groups of rats appeared to fall coincidentally with the diminution of SNA.

Although in the present study only renal SNA was measured, it is likely that the inhibition of SNA produced by L-dopa was generalized to most if not all sympathetic nerves. Otherwise, the close correspondence of blood pressure changes, which reflect SNA to many vascular beds, with renal SNA would not be expected. Moreover, in a previous study we showed that the increased SNA in the SHR was generalized to the cervical sympathetic, splanchnic, renal, and splenic nerves. It seems likely that the reduction in renal SNA is the mechanism by which L-dopa inhibits renin output from the kidney.

The present study confirms previous work from our laboratory as well as results from many other groups which strongly suggest that SNA is increased in the SHR and that this excessive activity is a major cause of the hypertension. Our results also support the suggestion previously made by other investigators that an abnormality in CNS catecholamine metabolism may play a role in the hypertension of the SHR. Other centrally active antihypertensive agents such as alpha-methyl dopa and clonidine also might be expected to diminish SNA and reduce blood pressure in these rats. These agents currently are being investigated.

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