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Studies on the Mechanism of the L-3,4-Dihydroxyphenylalanine-Induced Decrease in Tyrosine Hydroxylase Activity

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SUMMARY

The administration of large amounts of L-3,4-dihydroxyphenylalanine (L-dopa) to rats, guinea pigs, and gerbils decreases the measurable tyrosine hydroxylase activity of adrenal tissues. This effect does not appear to be related to the presence of inhibitory substances in the tissues or a change in the kinetic parameters of the enzyme. The concomitant administration of an aromatic L-amino acid decarboxylase inhibitor, N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine (Ro 4-4602), prevents the L-dopa-mediated decrease in tyrosine hydroxylase activity, thus indicating the necessity for decarboxylation of administered L-dopa to catecholamines. The direct involvement of either the endocrine or sympathetic nervous system in this phenomenon is unlikely, since neither hypophysectomy nor adrenal denervation attenuated the L-dopa-induced decrease of adrenal tyrosine hydroxylase activity.

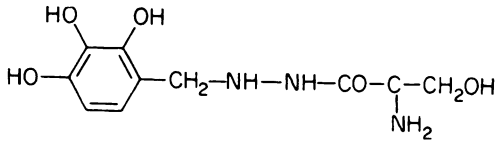
INTRODUCTION

In previous publications it was shown that the administration of L-3,4-dihydroxyphenylalanine (L-dopa) to rats leads to a decrease in the levels of adrenal (1) and mesenteric artery tyrosine hydroxylase activity (2), the rate-limiting enzyme in catecholamine synthesis (3). The present report describes the results of a more extensive investigation of this phenomenon, designed to evaluate the mechanism of this L-dopa effect.

MATERIALS AND METHODS

Male, Hartley strain (500-g) guinea pigs were purchased from Sunrise Farms, Whitehouse Station, N. J. Female Mongolian gerbils (50 g) were obtained from Tumblebrook Farms, Brant Lake, N. Y. Male CF1 mice (20–22 g) were purchased from Carworth Farms, New City, N. Y., as were intact and hypophysectomized, female Sprague-Dawley rats (190–210 g). Unilateral adrenal denervated female Sprague-Dawley rats and sham-operated controls (190–210 g) were obtained from Zivic Miller, Allison Park, Pa. Low-iodine test diet was purchased from Nutritional Biochemicals Corporation. L-3,4-Dihydroxyphenylalanine, N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (Ro 4-4602),[1] and pyridoxal phosphate were supplied by Hoffmann–La Roche, Inc. [3,5-^{3}H] L-Tyrosine (25 Ci/mmole) was purchased from New England Nuclear Corporation. L-Dopa was prepared for administration as a fine suspension, using 0.9% NaCl as the vehicle. Injection volumes of

[1] Ro 4-4602 has the following structure:



0.5 ml were used with rats and guinea pigs, and 0.25 ml with the gerbils and mice.

Adrenal pairs were homogenized in 2 ml of 0.13 M potassium phosphate buffer, pH 7.0. The homogenates were centrifuged at 30,000 × *g* for 10 min, and the supernatant fractions were assayed for tyrosine hydroxylase activity by the method of Nagatsu *et al.* (4). Guinea pig heart tyrosine hydroxylase activity was assayed by the procedure of Levitt *et al.* (5). Liver aromatic L-amino acid decarboxylase activity was assayed as described previously (6). Statistical analysis of the data was performed by Student's *t*-test.

RESULTS

In an earlier report (1) L-dopa administration was shown to decrease tyrosine hydroxylase activity in rat adrenals. It was further demonstrated (1) that the tissue levels of dopa and catecholamine compounds following L-dopa administration were not sufficiently elevated to inhibit tyrosine hydroxylase activity directly (7). This did not rule out the possible presence of other inhibitory substances in the high-speed supernatant fraction of adrenal homogenates following L-dopa treatment. The following experiments were conducted to investigate this point. As shown in Table 1, tyrosine hydroxylase activity in the supernatant fraction from adrenals of L-dopa-treated rats was lower than that of control rats, even when the amounts of tissue extracts were varied over a 6-fold range. When control tissue extracts were mixed with tissue extracts from the L-dopa-treated animals, the measured enzyme activities were additive. The tissue extracts from the drug-treated animals, therefore, did not contain substances which inhibit tyrosine hydroxylase. The administration of L-dopa might have caused a redistribution of tyrosine hydroxylase activity into a sedimentable particulate fraction. This proved not to be the case, in that the dopa-induced decrease of tyrosine hydroxylase activity was demonstrable and occurred to the same extent when assays were carried out with the crude homogenates or supernatant fraction.

It was conceivable that L-dopa administration might result in an altered form of tyrosine hydroxylase which could appear less active under our assay conditions. Therefore, kinetic analysis with respect to both tyrosine and 2 - amino - 6,7 - dimethyl - 5,6,7,8-tetrahydropteridine was performed with the supernatant fraction of adrenal homogenates obtained from rats that had received four daily subcutaneous injections of either 0.9% sodium chloride or 1000 mg/kg of L-dopa. The animals were killed 1 day later. The K_m values determined from a Lineweaver-Burk analysis, using the method of least squares, were as follows: controls—tyrosine, 3.7×10^{-5}; pteridine, 3.3×10^{-4}; L-dopa-treated animals— tyrosine, 4.4×10^{-5}; pteridine, 3.6×10^{-4}. The reduction in the computed V_{max} values seen after L-dopa treatment was in good agreement with the decline in enzyme activity seen under standard assay conditions.

The ability of L-dopa to alter the level of adrenal tyrosine hydroxylase activity in species other than the rat was also investigated. As shown in Table 2, administration of L-dopa led to decreased levels of tyrosine hydroxylase activity in both gerbils and guinea pigs. Similar reductions in enzyme activity have also been demonstrated in the mesenteric arteries of rats and rabbits by

TABLE 1

Additivity of tyrosine hydroxylase activity from adrenal extracts of control and L-dopa-treated rats

Rats were given L-dopa subcutaneously, 1000 mg/kg/day, for 4 consecutive days. The controls received 0.9% NaCl. One day later the animals were killed. The adrenals from five L-dopa-treated rats were pooled and homogenized in 10 ml of 0.13 M potassium phosphate buffer, as were the adrenals from five control animals. Various amounts of the high-speed supernatant fraction for each group of rats and various combinations of the two crude enzymatic fractions were assayed for tyrosine hydroxylase activity.

Enzyme	Tyrosine hydroxylated		
	Control	L-Dopa	Control + L-dopa
ml	*nmoles/15 min*		
0.05	0.32	0.20	0.53
0.10	0.74	0.39	1.10
0.20	1.44	0.84	
0.30	1.92	1.09	

TABLE 2

Tyrosine hydroxylase activity of tissues from gerbil and guinea pig following administration of L-dopa

Female gerbils (50 g) were given 1000 mg/kg of L-dopa subcutaneously daily for 4 consecutive days and killed on the fifth day. Male guinea pigs (500 g) were given 1000 mg/kg of L-dopa subcutaneously daily for 5 consecutive days, and then no drug for 2 days, followed by 2 additional days of L-dopa administration (1000 mg/kg/day). The animals were killed 1 day later. The numbers in parentheses refers to the numbers of animals used.

Species	Tissue	Tyrosine hydroxylase activity[a]	
		Control	L-Dopa
Gerbil	Adrenal	45.73 ± 1.05 (8)	33.9 ± 2.6[b] (9)
		34.2 ± 1.55 (10)	25.3 ± 1.29[c] (9)
Guinea pig	Adrenal	172.5 ± 19.07 (7)	99.7 ± 5.0[d] (5)
	Heart	6910 ± 460 (7)	3835 ± 298[c] (6)

[a] Adrenal data are expressed as nanomoles (± standard error of the mean) of tyrosine hydroxylated to L-dopa per adrenal pair per 15 min. Heart data are expressed as counts per minute of tritiated water released from 300,000 cpm of tritiated tyrosine per 100 μl of heart press juice per 15 min.

[b] Significantly different from control ($p < 0.005$).

[c] Significantly different from control ($p < 0.001$).

[d] Significantly different from control ($p < 0.01$).

Tarvar *et al.* (2). However, L-dopa, when administered to mice in doses up to 1000 mg/kg/day for 4 consecutive days, failed to alter adrenal tyrosine hydroxylase activity.

It was of interest to determine whether L-dopa itself is responsible for the reduction of tissue tyrosine hydroxylase activity or whether decarboxylation of this substance to catecholamines is a necessary prerequisite. To investigate this point, an aromatic L-amino acid decarboxylase inhibitor, Ro 4-4602 (8), was used. If L-dopa itself were the primary agent, the concomitant administration of both L-dopa and Ro 4-4602 should not attenuate the decrease in adrenal tyrosine hydroxylase activity. In fact, potentiation might be expected from this combination, since inhibition of decarboxylase activity should elevate tissue levels of L-dopa (9). Conversely, if decarboxylation to catecholamines were a requirement, the administration of Ro 4-4602 would be expected to antagonize this L-dopa effect. As shown in Table 3, administration of the decarboxylase inhibitor completely prevented the L-dopa-induced lowering of adrenal tyrosine hydroxylase activity. Ro 4-4602 alone was without effect on the tyrosine hydroxylase activity. However, Ro 4-4602, when given as described in Table 3, resulted in inhibition of more than 85% of liver decarboxylase activity *in vitro* at the termination of the experiment. Thus the administered L-dopa must undergo decarboxylation if adrenal tyrosine hydroxylase activity is to be reduced. It therefore seems likely that the catecholamines dopamine and/or norepinephrine or their metabolites, which are formed from the administered L-dopa, are in some manner responsible for the decrease in adrenal tyrosine hydroxylase activity.

Attempts were made to determine the time required for the adrenal tyrosine hydroxylase activity to return to control levels following the discontunuation of L-dopa administration. Initial results, shown in Table 4, indicated that approximately 3 days were required. However, the duration of this recovery period proved to be spurious, since the subcutaneous administration of the L-dopa suspension led to the formation of depots from which the drug was gradually released for several days following the termination of dosing. To overcome this problem, a decarboxylase inhibitor, Ro 4-4602, was given following the discontinuation of L-dopa. This was aimed at preventing the formation of catecholamines from the depots of L-dopa during the interval in which the recovery of enzyme activity was to be measured. Under these conditions adrenal tyrosine hydrox-

TABLE 3

Prevention of L-dopa-induced lowering of adrenal tyrosine hydroxylase activity by concomitant administration of a decarboxylase inhibitor, Ro 4-4602

Rats were given the following compounds, contained in 0.5 ml of 0.9% NaCl, for 4 consecutive days: L-dopa, 1000 mg/kg subcutaneously (once daily), plus 0.9% NaCl intraperitoneally (twice daily); or L-dopa, 1000 mg/kg subcutaneously (once daily), plus Ro 4-4602, 50 mg/kg intraperitoneally (twice daily); or Ro 4-4602, 50 mg/kg intraperitoneally (twice daily), plus 0.9% NaCl subcutaneously (once daily). Controls received 0.9% NaCl subcutaneously (once daily) and intraperitoneally (twice daily). One day following the last dose the animals were killed and the adrenals were assayed for tyrosine hydroxylase activity. Eight animals were used in each group.

Treatment	Adrenal tyrosine hydroxylase activity (±SEM)
	nmoles tyrosine hydroxylated/15 min/adrenal pr.
Control	7.67 ± 0.89
Ro 4-4602	6.67 ± 0.30
L-Dopa	3.87 ± 0.35[a]
L-Dopa + Ro 4-4602	8.08 ± 1.15

[a] Significantly different from all other groups ($p < .005$).

ylase returned to control levels within 24 hr (Table 5).

The effect of L-dopa on adrenal tyrosine hydroxylase activity was unimpaired in rats hypophysectomized either 1 or 3 weeks prior to the initiation of L-dopa administration. Hypophysectomy itself caused a significant decrease in adrenal tyrosine hydroxylase activity (Table 6), as others (10, 11) have found. It should be noted that the combination of hypophysectomy and L-dopa caused a greater decrease in tyrosine hydroxylase than either treatment alone.

A possible role of sympathetic nerve in the mediation of the L-dopa effect was investigated in rats in which one adrenal gland was denervated by means of splanchnic nerve transection 5 days prior to initiation of the treatment schedule, as shown in Table 7. The results of these experiments clearly demonstrate that L-dopa reduces tyrosine hydroxylase activity in the denervated adrenal. It should also be noted that denervation of one adrenal significantly increases the tyrosine hydroxylase activity of the innervated gland. Nevertheless, L-dopa treatment decreases the tyrosine hydroxylase activity of the innervated gland, as would be expected from the foregoing results.

TABLE 4

Recovery of adrenal tyrosine hydroxylase activity following discontinuation of L-dopa administration to rats

Rats were given L-dopa (1000 mg/kg) or 0.9% NaCl subcutaneously daily for 7 consecutive days. One, two, three, and six days later animals from each group were killed and the adrenals were assayed for tyrosine hydroxylase activity. Numbers in parentheses refer to the numbers of animals used.

Days following last dose	Tyrosine hydroxylase activity (±SEM)		
	Control	L-Dopa	Percentage of control
	nmoles tyrosine hydroxylated/15 min/adrenal pr.		
1	14.39 ± 0.72 (6)	8.25 ± 0.76[a] (6)	57
2	10.28 ± 0.99 (5)	6.69 ± 0.63[b] (6)	65
3	11.32 ± 2.24 (6)	9.22 ± 1.03 (5)	81
6	10.74 ± 0.59 (6)	13.43 ± 2.0 (6)	125

[a] Significantly different from control ($p < 0.001$).

[b] Significantly different from control ($p < 0.025$).

DISCUSSION

We have previously ruled out the possibility that the observed diminution of adrenal tyrosine hydroxylase activity was due to inhibition of the enzyme by accumulated catechols in the tissues of dopa-treated animals (1). The mixing experiments presented in Table 1 of this report confirm this observation for the enzyme in the adrenals, and also rule out any other type of inhibitory substances. A redistribution of the enzyme seems unlikely, in that the effect of L-dopa administration can be observed with both the homogenate and supernatant fraction. In addition, the K_m for both tyrosine and 2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8,-

TABLE 5

Recovery of adrenal tyrosine hydroxylase levels following termination of L-dopa decarboxylation by a decarboxylase inhibitor, Ro 4-4602

Two groups of rats (Nos. 2 and 3) received 1000 mg/kg daily of L-dopa subcutaneously suspended in 0.9% NaCl. Two other groups (Nos. 1 and 4) received daily subcutaneous injections of 0.9% NaCl. These dosage regimens were maintained for 4 consecutive days. On the morning of the fifth day groups 1 and 2 were killed. Group 4 was then divided into two subgroups, 4a and 4b. Animals in groups 3 and 4a, starting on the morning of the fifth day, received 50 mg/kg of the decarboxylase inhibitor, Ro 4-4602, intraperitoneally at 8:30 a.m. and 5:30 p.m., while group 4b was given 0.9% NaCl intraperitoneally at these times. On the morning of the sixth day the animals from each of these groups were killed. Numbers in parentheses refer to the numbers of animals used.

Treatment	Group	Day killed	Tyrosine hydroxylase activity (±SEM)
			nmoles tyrosine hydroxylated/15 min/adrenal pr.
0.9% NaCl	1	5th	20.79 ± 1.05 (10)
L-Dopa	2	5th	9.64 ± 0.25[a] (10)
L-Dopa +Ro 4-4602	3	6th	19.56 ± 0.93 (5)
0.9% NaCl + Ro 4-4602	4a	6th	22.65 ± 1.51 (10)
0.9% NaCl	4b	6th	19.2 ± 1.50 (10)

[a] Significantly different from control ($p < 0.001$).

TABLE 6

Effect of L-dopa on adrenal tyrosine hydroxylase activity in hypophysectomized rats

Hypophysectomized and sham-operated control rats were prepared 8 days prior to experimentation. The animals received 1000 mg/kg of L-dopa suspended in 0.5 ml of 0.9% NaCl or vehicle daily for 2 days, and then 500 mg/kg of L-dopa or vehicle for 1 additional day. The animals were killed 1 day later. All animals were kept from the time of the surgical manipulation until the termination of the experiment in a 30° room and maintained on a low-iodine test diet and drinking water containing 5% sucrose. Numbers in parentheses refer to the numbers of animals used.

Treatment	Tyrosine hydroxylase activity (±SEM)
	nmoles typr tyr tyrosine hydroxylated/15 min/adrenal pr.
Sham-operated, 0.9% NaCl	15.71 ± 0.76 (5)
Sham-operated, L-dopa	7.76 ± 0.98[a] (5)
Hypophysectomized, 0.9% NaCl	9.66 ± 0.92[b] (10)
Hypophysectomized, L-dopa	3.77 ± 0.10[a,c,d] (5)

[a] Significantly different from control ($p < 0.001$).

[b] Significantly different from control ($p < 0.005$).

[c] Significantly different from sham-operated, L-dopa-treated rats ($p < 0.005$).

[d] Significantly different from hypophysectomized, NaCl-treated rats ($p < 0.001$).

tetrahydropteridine was unaltered following L-dopa treatment. Thus the decrease in enzyme activity probably reflects a reduction in actual enzyme content. However, experiments with antibodies to tyrosine hydroxylase are needed to establish this point. In addition, the successful production of a specific antibody to tyrosine hydroxylase will make it possible to determine whether a decreased rate of enzyme synthesis *de novo* or an increased rate of degradation is involved.

The product of tyrosine hydroxylase activity is L-dopa. Although this compound is normally produced *in vivo*, its tissue levels are not detectable because of its rapid decarboxylation (3, 12, 13). The administration of large amounts of L-dopa results in significant tissue levels of this amino acid (1). Since it is well known that many enzyme systems can be repressed by their products, it would not have been surprising to find that the tissue levels of L-dopa itself had reduced tyrosine hydroxylase activity. This proved not to be the case, since the concomitant administration of an aromatic L-amino acid decarboxylase inhibitor completely prevented the L-dopa-induced lowering of adrenal tyrosine hydroxylase activity. The decarboxylase inhibitor, Ro 4-4602, in the doses employed in our experiments, has been reported not to inhibit dopa and tyrosine transaminase, catechol *O*-methyltransferase, dopamine β-hydroxylase, and monoamine oxidase (14), enzymes which are involved in the metab-

TABLE 7

Effect of L-dopa on tyrosine hydroxylase activity in denervated adrenals

Sham-denervated rats and animals with the left adrenal denervated received subcutaneous injections of either L-dopa, 1000 mg/kg, or 0.9% NaCl daily for 4 consecutive days. Surgery was performed 5 days prior to initiation of dosing. The animals were killed on the fifth day, and each adrenal was assayed for tyrosine hydroxylase activity. Numbers in parentheses refer to the numbers of animals used.

Surgery	Drug	Adrenal assayed	Tyrosine hydroxylase activity (±SEM)
			nmoles tyrosine hydroxylated/ 15 min/adrenal
Sham	0.9% NaCl	Left	4.76 ± 0.86 (7)
		Right	4.98 ± 0.74 (7)
	L-Dopa	Left	2.87 ± 0.30[a] (10)
		Right	3.07 ± 0.24[b] (10)
Left adrenal denervation	0.9% NaCl	Left	4.15 ± 0.38 (9)
		Right	6.96 ± 0.67[c, d] (9)
	L-Dopa	Left	2.13 ± 0.61[e] (7)
		Right	4.49 ± 0.73[f, g] (9)

[a] Statistically different from NaCl-treated, left adrenal ($p < 0.05$).
[b] Statistically different from sham-operated, NaCl-treated, right adrenal ($p < 0.02$).
[c] Statistically different from denervated, NaCl-treated, left adrenal ($p < 0.01$).
[d] Statistically different from sham-operated, NaCl-treated, right adrenal ($p < 0.05$).
[e] Statistically different from denervated, NaCl-treated, left adrenal ($p < 0.01$).
[f] Statistically different from innervated, NaCl-treated, right adrenal ($p < 0.025$).
[g] Statistically different from denervated, L-dopa-treated, left adrenal ($p < 0.05$).

olism of L-dopa. It would thus appear that Ro 4-4602 prevents the L-dopa-induced decrease of tyrosine hydroxylase activity via its ability to inhibit aromatic L-amino acid decarboxylase, the enzyme responsible for the decarboxylation of L-dopa to dopamine. Thus nondecarboxylated metabolites of L-dopa, such as 3-methoxydopa (15), or transamination products are also ruled out as active intermediates, since these compounds would also be expected to accumulate under conditions of decarboxylase inhibition. The decarboxylation products, dopamine, or norepinephrine or their metabolites, appear to be responsible in some indirect way for the decrease in enzyme activity.

Hypophysectomy has been shown to decrease adrenal tyrosine hydroxylase activity (10, 11). In the present report this was confirmed. It is of interest, however, that L-dopa lowered adrenal tyrosine hydroxylase further and that the combination of hypophysectomy and L-dopa caused a greater decrease than either treatment alone.

A number of studies have demonstrated that chronic increased sympathetic nervous activity to the adrenal leads to elevation of adrenal tyrosine hydroxylase activity (16–18). Following the administration of L-dopa the resulting catecholamine formation would be expected to decrease reflexly, sympathetic nerve activity, and some evidence has been provided for this by Whitsett *et al.* (19). If an increase in sympathetic nerve activity results in an elevation of tyrosine hydroxylase activity, perhaps a decrease in sympathetic nerve activity would have the opposite effect. However, such a mechanism for the action of L-dopa is not supported by the denervation experiments reported in Table 7. In addition, a number of other laboratories have measured the tyrosine hydroxylase activity of acutely denervated adrenals and have reported no decrease in enzymatic activity (16–18).

It appears that the L-dopa-mediated decrease in tyrosine hydroxylase activity is a localized phenomenon or involves the elaboration of a circulating factor which is not dependent on either the pituitary or sympa-

thetic nervous system. Since increased catecholamine synthesis has been shown to occur in both the heart and adrenal in response to L-dopa administration (1), it seems likely that the locally produced catecholamines can in some manner reduce the neuronal level of tyrosine hydroxylase. On this basis, it is conceivable that L-dopa would exert a similar effect on tyrosine hydroxylase in a neuroblastoma tissue culture system or in adrenal organ culture.

Although tyrosine hydroxylase activity was not altered in denervated adrenals, the intact contralateral gland manifested an increased tyrosine hydroxylase activity with respect to its denervated control and the adrenals of sham-operated, NaCl-treated controls. A number of other investigators did not observe increased enzyme activity in the contralateral control glands after unilateral adrena denervation (16, 18). However, Weiner and Mosimann (17), using unilateral adrenal denervated cats, reported changes of the type presented here. The effects of unilateral adrenal denervation on the contralateral gland are entirely plausible. It is not unreasonable to expect some degree of compensation on the part of the contralateral innervated gland. The signal of this increase is probably mediated through an increased splanchnic nerve activity, as is the case following treatment with reserpine, 6-hydroxydopamine (16), and insulin (17, 18).

The other enzymatic activities involved with norepinephrine formation, aromatic L-amino acid decarboxylase (6) and adrenal dopamine β-hydroxylase,[2] are also lowered following L-dopa administration. The former is unchanged in tissues such as kidney, heart, adrenal, and brain. However, decreases in liver aromatic L-amino acid decarboxylase (approximately 50%) have been demonstrated in the rat (6) and the mouse (20). In addition, L-dopa administration in the rat has been demonstrated to increase the monoamine oxidase activity of mesenteric artery and heart (2), whereas the erythrocyte catechol *O*-methyltransferase activity of Parkinsonian patients on L-dopa therapy has been reported to be decreased (21). These and the present results make it apparent that animals respond to the administration of L-dopa by altering the activities of enzymes involved in both the synthesis and degradation of catecholamines.

[2] B. Hartman and W. Dairman, unpublished observations.

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