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Changes in Tyrosine Hydroxylase and Dopa Decarboxylase Induced by Pharmacological Agents

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RECOGNITION that there are regulatory mechanisms, operating at the enzymatic level, which control the synthesis of the sympathetic neurotransmitter, norepinephrine, has now been well established. In this paper we will discuss evidence for two new types of potential regulatory mechanisms which function at the level of tyrosine hydroxylase and aromatic L-amino acid decarboxylase.

Tyrosine Hydroxylase

Historically, the first catecholamine biosynthetic regulatory mechanism to be described was a modification of the activity of tyrosine hydroxylase in response to relatively short periods of increased nerve activity produced in various ways (1, 17, 23, 42). These changes in tyrosine hydroxylase occur rapidly and without demonstrable increases in tissue levels of the enzyme, and it has been proposed that the tissue levels of catecholamines are regulatory through end-product inhibition (1, 36, 43).

A second type of regulatory mechanism was discovered more recently. In the latter part of 1969, the laboratories of Dr. Axelrod (35) and Dr. Kirshner (51) reported that tissue levels of tyrosine hydroxylase in intact animals were increased after the administration of either 6-hydroxydopamine or insulin. Shortly thereafter, a number of laboratories demonstrated that the induction[1] of these enzymes was dependent on chronically increased sympathetic nerve activity (38, 47, 52).

It has been reported that reserpine induces tyrosine hydroxylase when administered to animals. This effect is apparently due to increased sympathetic nerve activity (47) which may be an attempt to compensate for the depleted stores of the neurotransmitter, norepinephrine (8). It is known that L-dopa can replenish the depleted catecholamine stores of a reserpinized animal (7). It does so because it is the product of tyrosine hydroxylase, the enzyme which catalyzes the rate limiting step in catecholamine biosynthesis (32). It seemed

[1] The term "induction" has been used in the literature to describe the ability of a number of stressful situations to increase the activity of tyrosine hydroxylase and some data has been reported to implicate protein synthesis *de novo*. However, there is as yet no direct evidence for increased levels of enzyme protein. With this reservation we have used the term induction in this review.

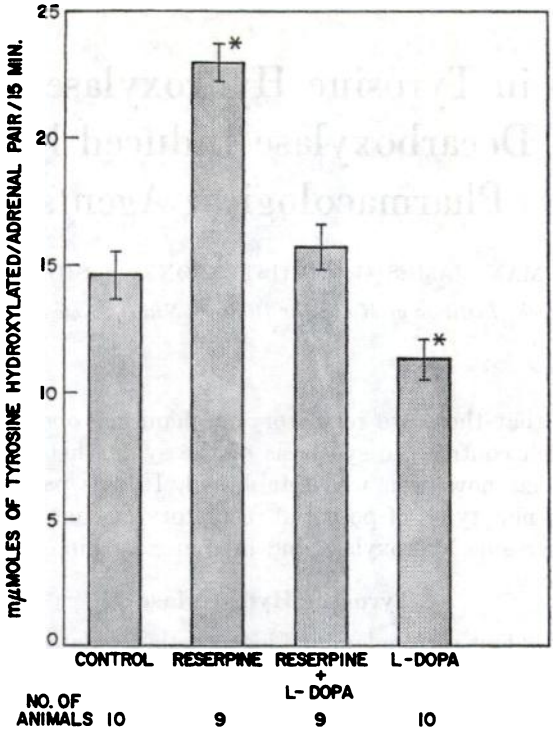

FIG. 1. The effect of L-dopa on the reserpine mediated elevation of rat adrenal tyrosine hydroxylase. Female Sprague-Dawley rats (200 g) were given daily subcutaneous injections of 2.5 mg/kg of reserpine for 2 consecutive days. A second set of animals received reserpine plus the concomitant subcutaneous administration of 1000 mg/kg/day of L-dopa for 2 days. A third group received only L-dopa and a fourth group 0.9% NaCl. The animals were killed 1 day after the last dosing and the tyrosine hydroxylase activity of their adrenals assayed. *, significantly different from control ($P < .02$).

likely, therefore, that administration of L-dopa concomitantly with reserpine might prevent the induction of tyrosine hydroxylase by reserpine. The following experiment was designed to test this hypothesis. One group of animals received only reserpine, a second group received both reserpine and a large amount of L-dopa and two control groups received only L-dopa or the vehicle. The results of this experiment (fig. 1) showed that the administration of L-dopa can prevent the induction of tyrosine hydroxylase by reserpine. However, this experiment also revealed a much more interesting effect. It was noted that the administration of L-dopa alone decreased adrenal tyrosine hydroxylase activity by a small, but significant, amount in comparison to the saline controls. This observation seemed worthy of more intensive study.

The administration of large amounts of L-dopa to rats over a period of 2 to 7 days was found to lead to a progressive decrease in the activity of adrenal tyrosine hydroxylase (fig. 2) as measured *in vitro*. This effect of L-dopa has now been demonstrated in several other species and in other tissues, including the guinea

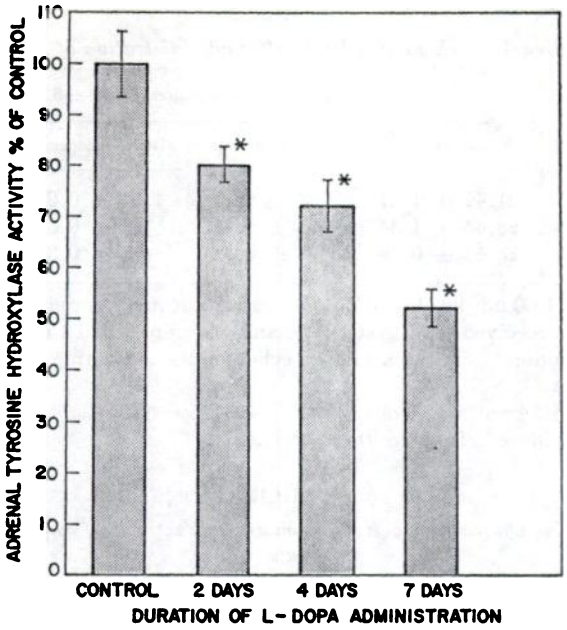

FIG. 2. Lowering of rat adrenal tyrosine hydroxylase activity by administered L-dopa. Rats were administered L-dopa, 1000 mg/kg/day subcutaneously for 2, 4 or 7 days. Controls received 0.9% NaCl. The animals were killed 1 day after the last dose, and the adrenals were assayed for tyrosine hydroxylase activity. Ten animals were used in each group. *, significantly different from control ($P < .02$).

TABLE 1

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

Species*	Tissue	Tyrosine Hydroxylase Activity†	
		Control	L-Dopa
Gerbil	Adrenal	45.73 ± 1.05 (8)	33.9‡ ± 2.6 (9)
		34.2 ± 1.55 (10)	25.3§ ± 1.29 (9)
Guinea pig	Adrenal	172.5 ± 19.07 (7)	99.7‖ ± 5.0 (5)
	Heart	6910 ± 460 (7)	3835§ ± 298 (6)

* Female gerbils (50 g) were given 1000 mg/kg of L-dopa, subcutaneously, daily for 4 consecutive days and killed on the 5th day. Male guinea pigs (500 g) were given L-dopa 1000 mg/kg 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 then killed 1 day later. The numbers in parentheses refer to the number of animals used.

† Adrenal data expressed as nmoles ± (S.E.M.) of tyrosine hydroxylated to L-dopa per adrenal pair per 15 min. Heart data expressed as cpm of tritiated water released from 300,000 cpm of tritiated tyrosine per 100 λ of heart press juice per 15 min.

‡ Significantly different from control ($P < .005$).

§ Significantly different from control ($P < .001$).

‖ Significantly different from control ($P < .01$).

TABLE 2

Adrenal catecholamine levels after administration of L-dopa

Group*	Catecholamine (μg/adrenal pair) ± S.E.M.			
	Epinephrine	Norepinephrine	Dopa + dopamine	Total
Control	21.40 ± 1.31	7.3 ± 0.83	1.91 ± 0.26	30.61 ± 0.93
L-Dopa (4 days)	23.43 ± 1.34	5.5 ± 1.4	4.3 ± 0.52†	33.20 ± 0.97
L-Dopa (7 days)	23.43 ± 0.19	6.4 ± 0.88	4.5 ± 0.35‡	34.33 ± 1.36

* Rats received 1000 mg/kg/day of L-dopa, subcutaneously, for either 4 or 7 consecutive days. The controls received 0.9% NaCl. The animals were killed 1 day after the last dose and the adrenal content of L-dopa and catecholamines determined. Seven animals were used in each group.

† Significantly different from controls ($P < .005$).

‡ Significantly different from controls ($P < .001$).

TABLE 3

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

Enzyme Amount	nmoles of Tyrosine Hydroxylated/15 min		
	Control	L-Dopa	Control + L-Dopa
ml			
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	

* Rats were given L-dopa (1000 mg/kg/day, subcutaneously) 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, pH 7.0, as were the adrenals from five control animals. Various amounts of the high speed supernatants for each group of rats and various combinations of the two crude enzymatic fractions were assayed for tyrosine hydroxylase activity.

pig heart and adrenals, gerbil adrenals (19) and the mesenteric arteries of the rat and rabbit (45) (table 1). However, tyrosine hydroxylase activity in the brain was unchanged by the L-dopa. This is probably a reflection of the relatively small amount of the administered amino acid which ultimately enters the central nervous system (55).

Under the conditions of assay used for tyrosine hydroxylase, catechols can competitively inhibit the enzyme by about 50% at concentrations of about 1×10^{-3} M (50). Therefore, it was important to determine whether the concentrations of L-dopa and the resulting catecholamines were sufficiently elevated after the administration of L-dopa to account for the observed decrease in tyrosine hydroxylase activity *in vitro*. As shown in table 2, the combined level of epinephrine, norepinephrine, dopa and dopamine in the adrenals was not significantly elevated in rats which had received 1000 mg/kg/day of L-dopa for up

to 7 days. The calculated concentration of catechol compounds in the assay incubation was 3.7×10^{-6} M, far below that necessary to exert a measurable inhibitory effect on the enzyme.

The above experiment did not rule out the possibility that L-dopa administration led to the appearance of other types of inhibitory substances or the removal of enzyme activators. To investigate this possibility enzyme extracts from the adrenals of L-dopa-treated and control rats were mixed in varying proportions. As shown in table 3, the tyrosine hydroxylase activities of the extracts in each mixture were additive, indicating that neither extract contained inhibitors or activators.

In all of the above experiments tyrosine hydroxylase activity was assayed in the 30,000 $\times$ *g* supernatant fraction of the initial homogenate. It was possible, therefore, that L-dopa administration might have changed the distribution of the enzyme activity, making some of it sedimentable, and that this might account for the observed decrease of enzyme activity. However, the same decrease in tyrosine hydroxylase activity was observed in crude homogenates as in the supernatant fractions. Furthermore, essentially all of the enzyme activity of rat adrenals is solubilized by simple homogenization before or after L-dopa administration.

The possibility remained that the decreased tyrosine hydroxylase activity *in vitro* might be due to the formation of an altered form of the enzyme in which the K_m values for either tyrosine or the reduced pteridine cofactor were increased. Therefore, adrenal extracts obtained from rats treated for 4 days with 1000 mg/kg/day of L-dopa or saline were subjected to kinetic analysis and the K_m values for both tyrosine and a synthetic pteridine[2] were determined by Lineweaver-Burk analysis with the method of least squares. The K_m values were found to be essentially unchanged:

Controls: tyrosine, 3.7×10^{-5} M; pteridine, 3.3×10^{-4} M;
L-dopa treated: tyrosine, 4.4×10^{-5} M; pteridine, 3.6×10^{-4} M.

From all of the above it would appear that the L-dopa mediated decrease in tissue levels of tyrosine hydroxylase activity represents an actual reduction in enzyme protein. The molecular mechanism for this event could involve either a decreased synthesis *de novo* of tyrosine hydroxylase or a specific increase in the rate of proteolytic degradation or a combination of both events. In the rat, modulation of proteolytic degradation is thought to play a role in the increase in tryptophan oxygenase mediated by tryptophan, apparently through a decrease in the rate of degradation of this enzyme (28, 41). Questions regarding the turnover of tyrosine hydroxylase and the effect of L-dopa on it could be investigated by the use of specific antibodies to the enzyme. This, however, will have to await the purification of tyrosine hydroxylase to homogeneity.

The product of tyrosine hydroxylase activity is L-dopa. Although the latter is a normal intermediate, it is usually not detectable in tissues (2) due to its

[2] The synthetic pteridine used was 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine.

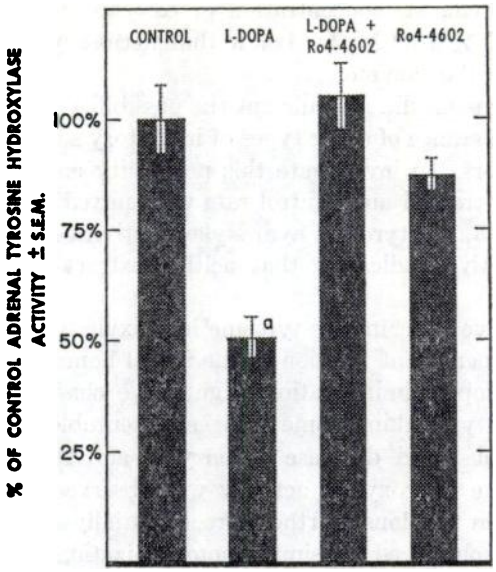

FIG. 3. Prevention of L-dopa induced decrease of adrenal tyrosine hydroxylase by decarboxylase inhibition. Rats were administered the following compounds for four consecutive days: L-dopa 1000 mg/kg subcutaneously (once daily) plus 0.9% NaCl intraperitoneally (twice daily); or L-dopa 1000 mg/kg subcutaneously (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). The animals were killed 1 day after the last dose and the adrenals assayed for tyrosine hydroxylase activity. Eight animals were used in each group. [a] Significantly different from all other groups ($P < .005$).

rapid decarboxylation by the enzyme, aromatic L-amino acid decarboxylase (32). Following the administration of large amounts of L-dopa, significant tissue levels of this amino acid are observed (18). These abnormally high amounts of L-dopa in the tissues could, in themselves, be sufficient for triggering the molecular mechanisms involved in the reduction of tyrosine hydroxylase levels. An alternative possibility is that the increased catecholamines formed from the administered L-dopa are the causative agents. In order to distinguish between these two possibilities, L-dopa was given together with an inhibitor of aromatic L-amino acid decarboxylase, RO 4-4602[8] (6). The concomitant administration of the decarboxylase inhibitor RO 4-4602 with L-dopa completely prevented the L-dopa mediated decrease in rat adrenal tyrosine hydroxylase (fig. 3). The administration of RO 4-4602 alone was without effect on tyrosine hydroxylase activity but resulted in greater than 85% inhibition of *in vitro* liver decarboxylase activity. At the dose of RO 4-4602 used in these studies there is no inhibition of dopamine-β-hydroxylase, dopa and tyrosine transaminases, catechol-O-methyl transferase or monoamine oxidase (6), enzymes which are also involved in the metabolism of L-dopa and catecholamines. Furthermore, decarboxylase inhibition

[8] N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine.

elevates and sustains tissue levels of L-dopa after L-dopa administration (4). Therefore, it seems clear that neither L-dopa, 3-methyldopa (4, 29) or a transamination product are the agents responsible for the decrease in tyrosine hydroxylase. It seems likely, then, that dopamine and/or norepinephrine or their metabolites, which are formed in increased amounts from the administered L-dopa (18), are the agents responsible for the decrease in tyrosine hydroxylase activity. Physiologically, this decrease in tyrosine hydroxylase activity may be viewed as an attempt by the animal to compensate for the overproduction of catecholamines.

Hypophysectomy has been reported to decrease tyrosine hydroxylase activity (30, 34). It was therefore plausible that the decrease of tyrosine hydroxylase activity seen after L-dopa administration might be mediated through an interaction with the endocrine system. In confirmation of the finding of previous investigations (30, 34), we observed that the adrenal tyrosine hydroxylase activity of rats hypophysectomized for 12 days was decreased by approximately 40% in comparison to sham-operated controls. However, the ability of L-dopa to decrease tyrosine hydroxylase was not impaired in the hypophysectomized animals (fig. 4). Similar results were obtained in rats hypophysectomized 3 weeks prior to the initiation of L-dopa administration. It thus appears unlikely that endocrine

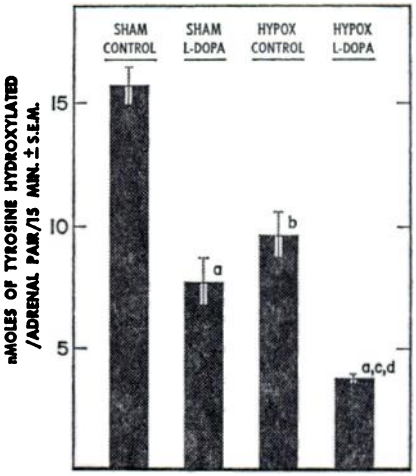

FIG. 4. Effect of L-dopa on adrenal tyrosine hydroxylase in the hypophysectomized rat. Hypophysectomized and sham-operated rats were prepared 8 days prior to the initiation of dosing. These animals received 1000 mg/kg/day of L-dopa subcutaneously or 0.9 NaCl for 2 consecutive days and then 500 mg/kg of L-dopa subcutaneously or 0.9% NaCl for an additional day. The animals were killed 1 day after the last dose and the adrenals assayed for tyrosine hydroxylase activity. Five animals were used in each group except the hypophysectomized saline group in which 10 were used. All the animals were kept from the time of the surgical manipulation until the termination of the experiment in a 30°C room and maintained on a low iodine test diet and drinking water containing 5% sucrose. [a] Significantly different from control ($P < .001$); [b] significantly different from control ($P < .005$); [c] significantly different from sham-L-dopa ($P < .005$); [d] significantly different from hypox-saline ($P < .001$).

factors under the control of the pituitary are involved in the decrease of tyrosine hydroxylase mediated by L-dopa.

There have been a number of studies correlating chronically increased sympathetic nerve activity with increased levels of tyrosine hydroxylase (38, 47, 52). After L-dopa administration, the increased formation of catecholamines might be expected reflexly to decrease sympathetic nerve activity. Some evidence to this effect has been obtained by Whitsett *et al.* (54). If a chronically increased sympathetic nerve activity leads to an elevation of tyrosine hydroxylase, perhaps a decreased level of neuronal activity would have the opposite effect. Investigations with a strain of spontaneously hypertensive rats have provided some support for this hypothesis. Louis *et al.* (33) have suggested that these animals have a diminished sympathetic nerve activity which may be an attempt to compensate for the hypertension. Tarver *et al.* (45) have demonstrated a decreased tyrosine hydroxylase activity in the blood vessels of these animals coincident with the development of the hypertension. However, a number of laboratories have demonstrated that splanchnic nerve transection, which should abolish all sympathetic nerve activity to the adrenal, does not decrease the activity of adrenal tyrosine hydroxylase, even after periods of up to 2 weeks (38, 47, 52). It seemed unlikely, therefore, that the catecholamines formed from L-dopa exerted their effect on tyrosine hydroxylase *via* decreased sympathetic nerve activity. It was possible to test this experimentally in the following way. Unilateral adrenal denervation was carried out in rats by splanchnic nerve transection. Five days after surgery, L-dopa administration was initiated. The results of these experiments (fig. 5) demonstrated that L-dopa administration

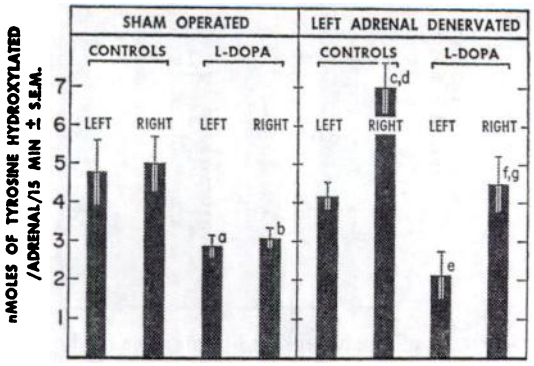

FIG. 5. Effect of L-dopa on tyrosine hydroxylase activity in denervated rat adrenals. Sham-denervated rats and animals with the left adrenal denervated received subcutaneous injections daily of either L-dopa 1000 mg/kg or saline for 4 consecutive days. The animals were killed on the 5th day and each adrenal assayed for tyrosine hydroxylase activity. Surgery was performed 5 days prior to initiation of dosing. From 7 to 10 animals were used for group. [a] Statistically different from sham saline, left adrenal ($P < .05$); [b] statistically different from sham saline, right adrenal ($P < .02$); [c] statistically different from denervated saline, left adrenal ($P < .01$); [d] statistically different from sham saline, right adrenal ($P < .05$); [e] statistically different from denervated saline, left adrenal ($P < .01$); [f] statistically different from innervated saline, right adrenal ($P < .025$); [g] statistically different from denervated dopa, left adrenal ($P < .05$).

TABLE 4

Uptake of ^{3}H-norepinephrine by the heart after 4 days of L-dopa administration

	Heart Norepinephrine	
	dpm/g Heart ± S.E.M.	dpm/μg NE
Control	365,515 ± 63,600	406,312 ± 59,000
L-Dopa	465,707 ± 56,300	505,910 ± 62,000

Female Sprague-Dawley rats (200 g) were dosed with L-dopa (1000 mg/kg/day, subcutaneously) or 0.9% NaCl for 4 consecutive days. Twenty-four hours after the last dosing, the animals were administered 10 μCi of ^{3}H-DL-norepinephrine (5 Ci/mmole) intravenously and killed 5 min later. Five animals were used per group.

reduced the tyrosine hydroxylase activity of both the denervated and contralateral intact gland, thus clearly ruling out any influence of sympathetic nerve activity on this process. It should be noted that the tyrosine hydroxylase activity of the denervated gland of the saline control animals was not reduced in comparison to the sham control. However, in the unilaterally denervated animal there was an increase in the tyrosine hydroxylase activity of the contralateral gland. This effect is most likely mediated through a compensatory increase in nerve activity to the intact gland, such as is observed after 6-hydroxydopamine, reserpine, or phenoxybenzamine administration (47).

All of our experimental data indicate that the increased formation of catecholamines after the administration of L-dopa can reduce the tissue level of tyrosine hydroxylase independently of changes in sympathetic neuronal activity or of endocrine events under the control of the pituitary. Our data do not exclude the elaboration of substances which are independent of sympathetic or pituitary influences. One factor which needs consideration would be the formation of 6-hydroxydopa which might arise from the autoxidation of L-dopa. If this substance were formed, it could be decarboxylated to 6-hydroxydopamine (37), a compound capable of destroying sympathetic nerve endings (48), in this manner decreasing tissue levels of tyrosine hydroxylase (35). Of the sympathetically innervated tissues studied, the heart is the most susceptible to the destruction of sympathetic nerve endings by both 6-hydroxydopa and 6-hydroxydopamine. This is readily shown by depletion of catecholamine stores and destruction of the uptake mechanism for circulating catecholamines (5, 44, 48). Thus, if either of these compounds were formed *in vivo* after administration of L-dopa, one would expect to observe their effects in the heart. As shown in table 4, the ability of the heart to take up radioactive norepinephrine was not impaired in rats which had been dosed with large amounts of L-dopa for several days. It is thus unlikely that L-dopa decreases tyrosine hydroxylase activity through the formation of these compounds.

In an attempt to evaluate the relative importance of the formation of dopamine and norepinephrine in the L-dopa-mediated decrease of tyrosine hydroxylase, a dopamine-β-hydroxylase inhibitor, U14-624[4] (26), was used. This compound was

[4] 1-phenyl-3-(2-thiazolyl)-2-thiourea.

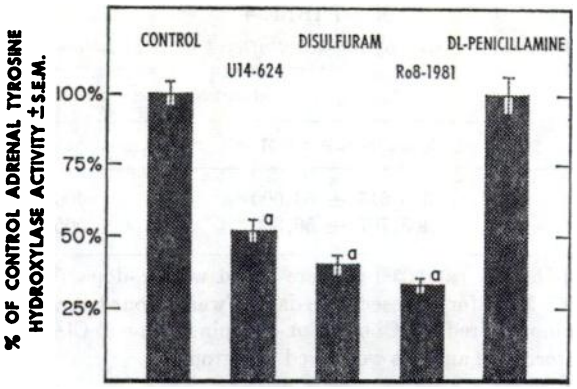

FIG. 6. Decrease of adrenal tyrosine hydroxylase by dopamine-β-hydroxylase inhibitors. Rats were administered intraperitoneally the following compounds for 4 consecutive days: Disulfuram, 100 mg/kg/day; RO 8-1981, 100 mg/kg for the 1st day and thereafter 50 mg/kg/day; DL-penicillamine, 100 mg/kg/day; U14-624, 35 mg/kg twice daily; or 0.9% NaCl. The animals were killed 1 day after the last dose and the adrenals assayed for tyrosine hydroxylase activity. From 7 to 10 animals were used in each group. [a] Significantly different from control ($P < .001$).

administered concomitantly with L-dopa, with the hope that the inhibition of dopamine-β-hydroxylase would block the increased formation of norepinephrine without interfering with the formation of dopamine from the administered L-dopa. The results of this experiment were rather unexpected in that the administration of U14-624 for 4 days produced, by itself, a decrease in adrenal tyrosine hydroxylase activity. The magnitude of this reduction was of the same order as that observed with the combination of L-dopa plus U14-624 or with L-dopa alone. Thus, one could not differentiate between the relative effects of dopamine and norepinephrine formation. Several other dopamine-β-hydroxylase inhibitors were investigated with the hope of finding a compound which would not decrease tyrosine hydroxylase activity when administered to rats for several days. As shown in figure 6, the ability of U14-624 to decrease adrenal tyrosine hydroxylase was a property also shared by the dopamine-β-hydroxylase inhibitors disulfuram and RO 8-1981.[5] It should be pointed out that each of these compounds is a chelator of copper. For this reason, another copper chelator, DL-penicillamine, was administered to the animals. This compound failed to decrease tyrosine hydroxylase activity (fig. 6). The dopamine-β-hydroxylase inhibitors do not inhibit tyrosine hydroxylase directly even at concentrations as high as 1×10^{-4} M. When given in a single dose of 200 mg/kg, U14-624 did not decrease adrenal enzyme activity up to 7 hr after its administration. When extracts of control adrenals and those obtained from rats treated with U14-624 for 4 days (which exhibited a 50% decrease in tyrosine hydroxylase activity) were mixed together, the activities were additive.

It is tempting to relate the inhibition of dopamine-β-hydroxylase to the decrease in tyrosine hydroxylase activity. However, the dosage regimen of U14-624

[5] RO 8-1981: N-(2-thiazolyl)-1-piperidinecarbothioamide.

and disulfuram which resulted in a decreased adrenal tyrosine hydroxylase activity were judged to be ineffective in inhibiting norepinephrine formation *in vivo*, in that they failed to lower the content of the heart norepinephrine. Regardless of the actual mechanism by which the dopamine β-hydroxylase inhibitors act to decrease tyrosine hydroxylase activity, one should use caution in interpreting results when these compounds are used chronically to block norepinephrine formation, since they also diminish the rate limiting step, tyrosine hydroxylase.

Aromatic L-Amino Acid Decarboxylase

A number of observations made in parkinsonian patients treated with L-dopa suggested that the continued administration of this agent might induce alterations in its own metabolism. It had been reported that the efficacy of the drug increased during treatment and that some of the peripheral side effects decreased with continued administration (13, 14, 22). It was also known that the administration of pyridoxine, the vitamin analogue of the cofactor for aromatic L-amino acid decarboxylase, could antagonize the therapeutic effectiveness of L-dopa in Parkinson's disease (11, 20, 25). For these reasons, and in the light of the fact that L-dopa administration decreased tyrosine hydroxylase activity, it was logical to investigate the relationship between L-dopa administration and the next enzyme in the catecholamine biosynthetic pathway, aromatic L-amino acid decarboxylase.

When rats were given varying amounts of L-dopa ranging from 100 mg/kg to 1000 mg/kg per day for a period of 4 consecutive days and killed 24 hr after the final dose, a dose-dependent decrease in the activity of liver aromatic L-amino acid decarboxylase was observed (fig. 7). The lowest dose used in this experiment

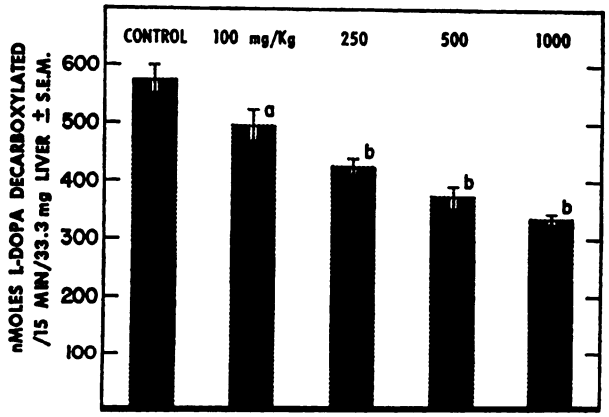

FIG. 7. Dose response for the L-dopa mediated decrease in rat liver decarboxylase. Rats were given 100, 250, 500 or 1000 mg/kg per day of L-dopa, subcutaneously, for 4 consecutive days. Controls received daily injections of 0.9% NaCl. The animals were killed 1 day after the last dose and livers assayed for decarboxylase activity. Six animals were used per group. [a] Significantly different from control ($P < .05$); [b] significantly different from control ($P < .001$).

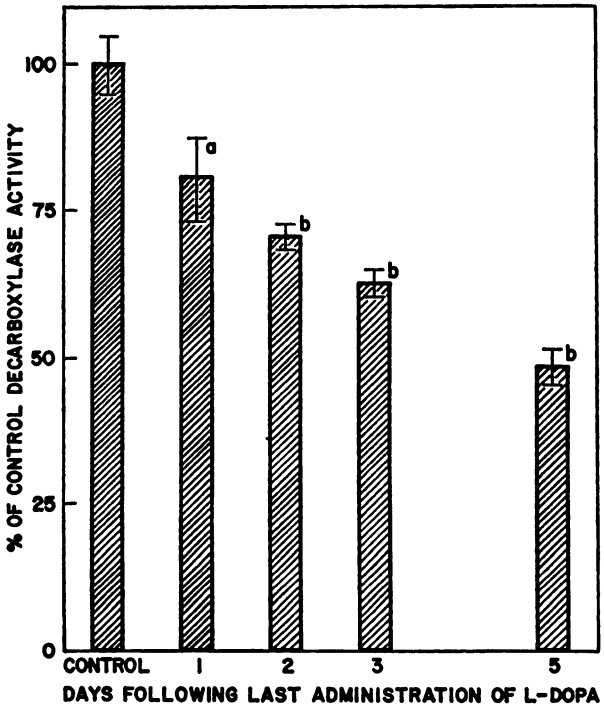

FIG. 8. Decrease in liver decarboxylase activity with time after discontinuation of L-dopa administration. Rats were given 500 mg/kg per day of L-dopa subcutaneously or 0.9% NaCl for 4 consecutive days and were killed at the times indicated after the last dose. Livers were assayed for decarboxylase activity. Six animals were used per group and data are presented ± S.E.M. [a] Significantly different from control ($P < .02$); [b] significantly different from control ($P < .001$).

(100 mg/kg), which produced a significant reduction in liver decarboxylase, is comparable to that used clinically for the treatment of parkinsonism. It was of interest to determine how long the depression of liver decarboxylase activity would persist after discontinuation of L-dopa. Animals were given daily subcutaneous injections of 500 mg/kg of L-dopa for 4 consecutive days and then killed either 1, 2, 3 or 5 days later. The liver decarboxylase activity showed a progressive decrease, with the greatest reduction being observed 5 days after the last dose (fig. 8). This result is not particularly surprising when one takes into account the physical properties of L-dopa and the route of administration. The compound is given subcutaneously as a suspension in 0.9% sodium chloride, in which it has limited solubility. Under these conditions, the compound forms a depot from which it is slowly released for several days. This is consistent with the finding that a 2-fold increase in the rate of norepinephrine turnover in the heart can be demonstrated to occur for at least 3 days after the discontinuation of L-dopa administration. The ability of L-dopa to lower liver decarboxylase activity appears to be a time-dependent phenomenon, since the administration

of a large amount of L-dopa (1000 mg/kg/day) for 2 consecutive days failed to decrease enzyme activity.

It was important to determine whether the oral administration of L-dopa was capable of decreasing liver decarboxylase, since parkinsonian patients take the drug in this fashion. The result of such a study is shown in table 5. It can be seen that orally administered L-dopa, at a dose of 100 mg/kg/day, which is comparable to that administered to parkinsonian patients, effectively lowered decarboxylase activity after 1 week.

The effect of L-dopa administration on the decarboxylase activity of tissues other than the liver was investigated. As shown in table 6, the subcutaneous administration of L-dopa, in dosage schedules which were capable of decreasing decarboxylase activity in the liver, failed to alter this activity in heart, kidney,

TABLE 5

Decrease of liver decarboxylase activity after oral administration of L-Dopa

	Liver Decarboxylase Activity (nmoles of L-dopa decarboxylated/33.3 mg/15 min ± S.E.M.)
Control L-Dopa (mg/kg)	711 ± 28.9
100	479* ± 26.2
500	516* ± 11.3
750	477* ± 10.6
1000	489* ± 20.6

Rats were given 0.9% NaCl or L-dopa orally with a stomach tube. The daily dosage listed in the table was given in divided doses three times a day. This regimen was maintained for 7 consecutive days; the rats were killed 1 day after the last dose. Liver supernatants were assayed for decarboxylase activity. Six rats were used in each group. [From Dairman *et al.* (16).]

* Significantly different from control ($P < .001$).

TABLE 6

Decarboxylase activity in rat tissues after subcutaneous administration of L-dopa

Tissue	Decarboxylase Activity (nmoles of L-dopa decarboxylated/15 min ± S.E.M.)	
	Control	L-Dopa treated
Adrenals (pairs)*	185 ± 8.3 (9)‡	169 ± 7.3 (9)
Brain (41.7 mg)*	26.3 ± 1.3 (9)	27.3 ± 0.74 (9)
Heart (125 mg)†	71.8 ± 2.4 (6)	72.8 ± 8.3 (6)
Kidneys (41.7 mg)†	585 ± 79 (6)	590 ± 28.7 (6)

* Rats were given daily injections of either L-dopa (1000 mg/kg) or saline for 7 consecutive days, and killed 1 day after the last dose.

† These animals received daily injections of either L-dopa (500 mg/kg) or 0.9% NaCl for 4 consecutive days. Hearts and kidneys were taken from animals killed 1 and 5 days, respectively, after the last dose.

‡ Numbers in parentheses refer to the numbers of rats used. [From Dairman *et al.* (16).]

adrenal and brain. Thus, of the tissues investigated, the effect is specific for the liver. If the administration of L-dopa to patients caused a similar phenomenon—that is, a decreased liver decarboxylase activity without a change in the level of the enzyme in the brain—this could account for the increased efficacy of the drug and decreased peripheral side effects which are observed with continued administration (13, 14, 22).

The rationale for using L-dopa in parkinsonism is based on the observation that patients with this disease have a deficiency of brain dopamine (21). However, the administration of dopamine itself is not effective since it does not penetrate the central nervous system. Thus, L-dopa is used, since it can enter the brain and be decarboxylated to dopamine within this organ. However, once L-dopa is converted to dopamine in the periphery, it is not therapeutically useful, but it can exert dopaminergic and sympathetic side effects. Decarboxylation is a major route of L-dopa metabolism and the liver comprises most of the decarboxylase activity present in the peripheral tissues of the rat. A decrease in the activity of liver decarboxylase should spare L-dopa in the periphery and thus make more of it available for entry into the brain for decarboxylation to dopamine. In addition, a decreased peripheral decarboxylase activity should diminish peripheral side effects associated with excess catecholamines. It is of interest that several compounds have been developed which selectively inhibit aromatic L-amino acid decarboxylase peripherally (6, 49). The clinical use of these inhibitors illustrates the importance of the peripheral levels of decarboxylase activity, since when these compounds are administered to parkinsonian patients, the therapeutic dose of L-dopa can be markedly reduced (3, 12).

A report has appeared in the literature claiming that human red blood cells contain aromatic L-amino acid decarboxylase and that this activity decreases in parkinsonian patients taking L-dopa (46). These results at first appeared to confirm our speculation with respect to the effect of chronic L-dopa administration on the peripheral levels of decarboxylase. However, recent investigations have revealed that the ability of human erythrocytes to liberate CO_2 from L-dopa is not associated with aromatic L-amino acid decarboxylase (15). This is based on the following observations. The activity is not stimulated by pyridoxal phosphate nor is it inhibited by high concentrations (10^{-4} M) of Brocresine, a compound capable of inhibiting aromatic L-amino acid decarboxylase at concentrations of 1×10^{-7} M (39). The erythrocyte activity decarboxylates both the D and L isomers of dopa with equal efficiency, in contrast to aromatic L-amino acid decarboxylase which is specific for the L-isomer. Finally, a monospecific antiserum to hog kidney aromatic L-amino acid decarboxylase which is capable of precipitating the human liver enzyme, has no effect on the erythrocyte activity. The activity in erythrocytes may not be associated with any type of decarboxylase, but may be the result of oxidative attack by some red cell component on dopa. With carboxyl-labeled dopa, $^{14}CO_2$ could thus appear without formation of dopamine. Studies with the activity contained in human erythrocytes cannot be used to correlate the effects of L-dopa administration with the peripheral levels of aromatic L-amino acid decarboxylase. It is conceivable, however, that destruc-

tion of circulating L-dopa by red cells may play a significant role in the inactivation of administered drug.

It is well documented that administration of pyridoxine reverses the therapeutic effect of L-dopa in parkinsonian patients (11, 20, 25). Therefore, it was logical to determine whether the ability of L-dopa administration to decrease rat liver decarboxylase activity was in any way related to its cofactor, pyridoxal phosphate. It was found that added pyridoxal phosphate stimulated the decarboxylase activity of rat liver extracts from animals treated with L-dopa or saline to the same extent (fig. 9). In this particular experiment, the decarboxylase activity of the L-dopa-treated rats was 50% lower than controls and, as can be deduced from the data presented in figure 9, this decreased activity was maintained at all concentrations of pyridoxal phosphate. When pyridoxine HCl (5 mg/kg/day, intraperitoneally) was administered concomitantly with L-dopa (500 mg/kg/day, subcutaneously), the L-dopa mediated decrease in liver decarboxylase was not attenuated. It appears, therefore, that the reduction of enzyme activity is in no way related to a vitamin deficiency or defect in cofactor binding.

The question still remained as to whether L-dopa treatment led to a modification of the enzyme or to an actual decrease in enzyme protein. The availability of a specific antiserum to the hog kidney decarboxylase which was capable of precipitating the rat liver enzyme, made it possible to investigate this question (16). As shown in table 7, titration of a given amount of antiserum with liver supernatant, obtained from animals treated with either L-dopa or saline, showed that the L-dopa treated group had approximately one-half the amount of antigen

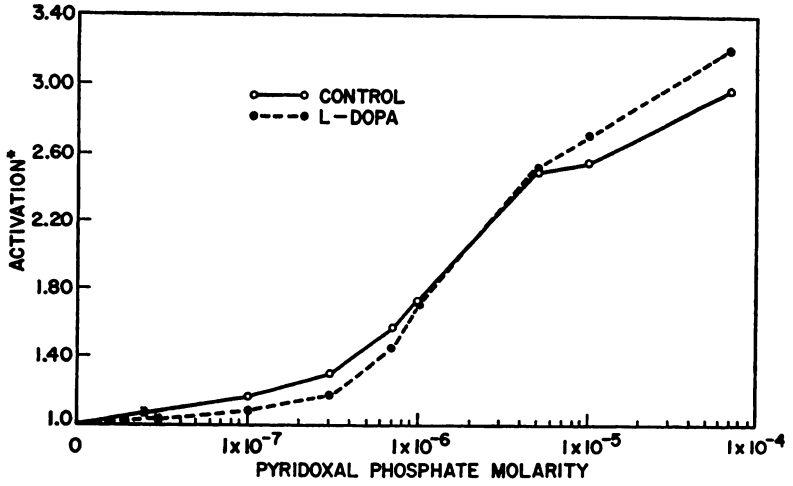

FIG. 9. Activation by pyridoxal phosphate of decarboxylase from livers of control and L-dopa treated rats. Aliquots of enzyme extracts obtained from rats administered 1000 mg/kg/day of L-dopa, subcutaneously 4 consecutive days or 0.9% NaCl were pooled and assayed for decarboxylase activity in the presence of varying amounts of added pyridoxal phosphate. [From Dairman *et al.* (16).] * Activation represents the ratio of the activities in the presence and absence of the indicated amounts of pyridoxal phosphate.

TABLE 7

*Immunological assay of liver decarboxylase in control rats and rats treated with L-dopa**

Decarboxylase Specific Activity		Decarboxylase Activity Added to Antiserum		Decarboxylase Activity Remaining after Treatment with Antiserum		
Control	L-Dopa treated	Control	L-Dopa treated	Control	L-Dopa treated	Ratio of L-dopa/control
units†/g liver		units		units†/g liver		
		39	40	639	321	0.50
		26	27	557	273	0.49
789	402	13	13	424	216	0.51
		7	7	379	228	0.60

* Livers from control rats and rats treated with L-dopa (1000 mg/kg, subcutaneously, for 4 days, killed on the 5th day) were homogenized in three volumes of 5 mM sodium phosphate buffer (pH 7.2). The homogenates were centrifuged at 20,000 × *g* for 15 min. The supernatants were assayed and found to contain 263 and 134 enzyme units/ml, respectively. The control supernatant was diluted with an equal volume of buffer and aliquots of the diluted control and L-dopa-treated supernatants were incubated with 5 μl of antiserum for 1 hr at room temperature. The incubation mixtures contained 0.9% NaCl; 5 mM sodium phosphate buffer (pH 7.2); 5 μl of antiserum; 0.05, 0.1, 0.2, or 0.3 ml of enzyme; and sufficient bovine-serum albumin to maintain a roughly constant total protein concentration, all in a total volume of 1 ml. Control mixtures contained 5 μl of serum from a goat immunized against another enzyme. After incubation, the mixtures were centrifuged at 100,000 × *g* for 60 min and 0.4 ml aliquots of the supernatants were assayed in duplicate. Decarboxylase activity remaining after treatment with antiserum has been corrected for the small amount of activity that was lost in the presence of the control (goat) serum. [From Dairman *et al.* (16).]

† One unit of enzyme activity is defined as that amount of enzyme that yields 1 nmol of CO_2/min under the conditions of the standard assay.

as the controls. This is in excellent agreement with the reduction of enzyme activity observed in these animals after L-dopa administration. One must conclude, therefore, that L-dopa administration decreases liver aromatic L-amino acid decarboxylase activity through an actual reduction of enzyme protein. Studies are now in progress to determine whether this effect is due to decreased synthesis *de novo*, an increased rate of degradation, or to a combination of both factors.

It can be asked whether L-dopa or the catecholamines formed from it are the causative agents in reducing liver aromatic L-amino acid decarboxylase. A somewhat different approach had to be taken in this case than was used in the studies involving tyrosine hydroxylase, where a decarboxylase inhibitor could be used. In this instance, it was reasoned that by using a monoamine oxidase inhibitor, one should be able to differentiate between a reduction resulting directly from L-dopa or from the catecholamines formed from the amino acid. Monoamine oxidase oxidizes dopamine or norepinephrine to their corresponding aldehydes, but does not attack L-dopa. If the catecholamines were involved in decreasing the liver decarboxylase levels, then inhibition of monoamine oxidase should potentiate their effects and, conversely, if L-dopa itself were responsible,

monoamine oxidase inhibition should have no effect. Rats were given a small amount of L-dopa (10 mg/kg/day) in conjunction with pargyline, a monoamine oxidase inhibitor. As shown in figure 10, the administration of L-dopa alone at this small dose had no effect. The combination of L-dopa and pargyline decreased liver decarboxylase to a level which was significantly lower than all the other groups. Again, it would appear that the catecholamines or their non-deaminated metabolites are the agents which evoke the decrease in liver decarboxylase levels. In support of this hypothesis, we have now been able to demonstrate a decrease in liver decarboxylase after the administration of small amounts of dopamine, when given in conjunction with a monoamine oxidase inhibitor (fig. 11).

Aromatic L-amino acid decarboxylase is the sole enzyme responsible for the decarboxylation of L-5-hydroxytryptophan to serotonin, as well as for the decarboxylation of L-dopa (10). Therefore, it seemed possible that the serotonin formed after the administration of L-5-hydroxytryptophan might cause a decrease in the enzyme, as do the catecholamines. The administration of L-5-hydroxytryptophan (500 mg/kg/day, 4 days, subcutaneously) indeed was found to be effective in lowering liver decarboxylase. To determine whether the amino acid or serotonin was the causative agent, low doses of L-5-hydroxytryptophan (5 mg/kg/day, subcutaneously) were administered concomitantly with the monoamine oxidase inhibitor, pargyline (50 mg/kg/day, intraperitoneally). Under this regimen, L-5-hydroxytryptophan alone did not significantly decrease the decarboxylase, but L-5-hydroxytryptophan plus pargyline led to a decrease in the liver enzyme which was significantly lower than both the saline controls and the controls which had received pargyline alone. Thus, it appears that

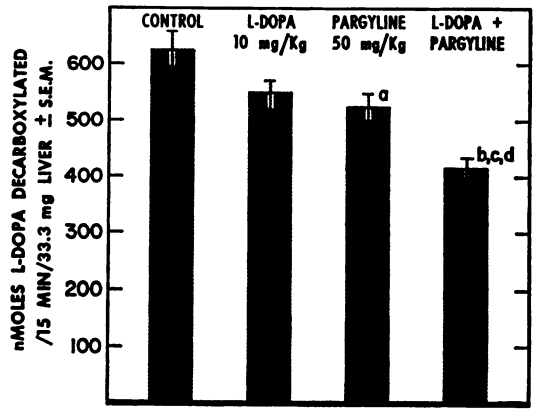

FIG. 10. Decrease of rat liver decarboxylase by L-dopa and monoamine oxidase inhibitors. Rats received the following compounds for 5 consecutive days. L-dopa, 10 mg/kg/day, subcutaneously; or pargyline, 50 mg/kg/day, intraperitoneally; or L-dopa, 10 mg/kg/day, subcutaneously, plus pargyline, 50 mg/kg/day, intraperitoneally. Controls received 0.9% NaCl. Six hours after the last dose the animals were killed and the livers assayed for decarboxylase activity. Eight animals were used in each group. [a] Significantly different from control ($P < .025$); [b] significantly different from pargylline ($P < .005$); [c] significantly different from L-dopa ($P < .001$); [d] significantly different from control ($P < .001$).

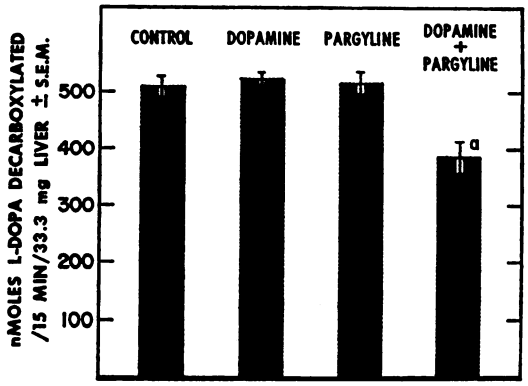

FIG. 11. Decrease of rat liver decarboxylase by dopamine and monoamine oxidase inhibitors. Rats were dosed with either dopamine or pargyline or a combination of both compounds for 5 consecutive days. On day 1, the animals received 50 mg/kg of pargyline intraperitoneally and/or 10 mg/kg of dopamine subcutaneously or vehicle. On day 2, this schedule was repeated except that the dose of dopamine was diminished to 5 mg/kg. On days 3 and 4 the pargyline dosage remained the same except the dopamine in two daily doses of 2.5 mg/kg each 8 hr apart. On the morning of the 5th day the rats received 50 mg/kg of pargyline intraperitoneally and/or 2.5 mg/kg of dopamine subcutaneously or vehicle. The animals were then killed 5 hr later. The dopamine was suspended in 0.1 ml of sesame oil and the pargyline dissolved in 0.5 ml of 0.9% NaCl. Seven animals were used in each group. [a] Significantly different from all other groups ($P < .005$).

serotonin, or an amine metabolite, is the agent which evokes the decrease in liver decarboxylase levels after 5-hydroxytryptophan, just as was the case with catecholamines after administration of L-dopa.

The level of liver enzymes, such as tyrosine transaminase (31), tryptophan oxygenase (27), and glutamate-alanine transaminase (40) can all be changed in response to glucocorticoids. It was found, however, that L-dopa administration was fully capable of reducing the liver decarboxylase activity in hypophysectomized rats thus indicating that the endocrine system is not involved in this phenomenon.

The ability of L-dopa administration to produce this decrease does not appear to be due to a general toxic effect on this organ. The activity of liver monoamine oxidase was found to be unaltered after L-dopa administration. Pentobarbital sleep time, which is a measure of liver microsomal drug-metabolizing activity, was also unchanged.

In addition to the changes in tyrosine hydroxylase and aromatic L-amino acid decarboxylase, several other enzymes involved in catecholamine synthesis and degradation have been reported to be altered as a consequence of L-dopa administration. Dr. Boyd Hartman, in collaboration with our laboratory, has observed a decrease in the adrenal activity of dopamine-β-hydroxylase (24). Tarver *et al.* (45) have reported that the monoamine oxidase activity of heart and mesenteric artery, but not of liver, was increased in rats following L-dopa administration. Weiss *et al.* (53) have observed time-dependent decreases in the

activity of erythrocyte catechol-O-methyl transferase in parkinsonian patients taking L-dopa. The changes in tyrosine hydroxylase, aromatic L-amino acid decarboxylase, dopamine-β-hydroxylase, and monoamine oxidase can all be considered as an attempt by the animal to compensate, at the enzymatic level, for the formation of excess catecholamines arising from the administered L-dopa.

Summary and Conclusions

The administration of L-dopa for several days has been shown to result in decreased activity *in vitro* of the catecholamine biosynthetic enzymes in peripheral tissues, but not in the brain. Decreased tyrosine hydroxylase activity has been demonstrated to occur in adrenals, heart and the vasculature, while the decrease in aromatic L-amino acid decarboxylase seems to be confined to the liver. In both cases, the decreased enzyme activity appears to be due to diminished levels of enzyme protein. In the case of tyrosine hydroxylase, no evidence could be found for the presence of inhibitors or the removal of activators after L-dopa administration nor were the kinetic properties of the enzyme altered. Immunological titration of the liver decarboxylase with a monospecific antiserum to aromatic L-amino acid decarboxylase indicated an actual decrease in enzyme protein, which corresponded to the decrease in enzyme activity observed after L-dopa administration.

The specific decrease in liver decarboxylase without a similar effect in the brain may explain the increased efficacy of L-dopa in the treatment of Parkinson's disease, seen with continued administration of this amino acid. A decreased level of peripheral decarboxylation should make more of the administered L-dopa available for entry into the brain where it may be decarboxylated to dopamine.

The decrease in both tyrosine hydroxylase and aromatic L-amino acid decarboxylase are independent of endocrine factors under the control of the pituitary, since decreases in both these enzymes were observed following L-dopa administration to hypophysectomized animals. The decrease in adrenal tyrosine hydroxylase was not dependent on sympathetic nerve activity, since L-dopa administration diminished enzyme activity in the denervated adrenal gland.

L-Dopa itself does not mediate the decreased levels of tyrosine hydroxylase and aromatic L-amino acid decarboxylase, but rather the catecholamines formed from it appear to be the causative agents. The excess formation of serotonin from administered L-5-hydroxytryptophan also appears to be the causative factor in reducing the liver decarboxylase. The enzyme aromatic L-amino acid decarboxylase is common to both the noradrenergic and serotonergic pathways and is also capable of forming other biologically active amines, such as tryptamine, phenylethylamine, *etc.* (9). It is possible that the presence of any of these amines in excess may have a regulatory effect on liver aromatic L-amino acid decarboxylase similar to that produced by the catecholamines and serotonin.

The effects on tyrosine hydroxylase and aromatic L-amino acid decarboxylase, as well as those on dopamine-β-hydroxylase (24) and monoamine oxidase (45), may be viewed as adaptive mechanisms in response to the overproduction of

biogenic amines. It is possible that the elaboration of large amounts of these substances, such as occurs in patients with pheochromocytoma or carcinoid, may profoundly influence the tissue levels of their biosynthetic and degradative enzymes.

A number of amino acids capable of forming biologically active amines are now in use as therapeutic agents. Examples would include L-dopa in Parkinson's disease, α-methyldopa in hypertension and 5-hydroxytryptophan for the experimental treatment of depression. These compounds are usually given chronically. Physicians who treat their patients with these drugs should be aware of the possibility of inducing changes in the enzyme systems involved in the catecholamine and serotonin pathways.

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