(*10*). This might be explained by scatter into the surround, which would be more effective in producing inhibition at those wavelengths. These two units are in rough agreement with the findings of Gouras (*11*), who studied the intact monkey retina. However, Gouras used chromatic adaptation of the surround and thereby was able to eliminate the inhibitory effect.

Our findings suggest that, at the ganglion cell level, the visual system of man is not unlike that of other higher animals. This would lend support to the results of workers who attempt to correlate electrophysiological experiments with psychophysical data.

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12. Supported by grant EY00369 from the National Eye Institute. We thank Dr. John Dowling for his advice.
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1 September 1970; revised 16 November 1970

Decrease in Adrenal Tyrosine Hydroxylase and Increase in Norepinephrine Synthesis in Rats Given L-Dopa

Abstract. *When large doses of* L-*dopa* (*1000 milligrams per kilogram, given subcutaneously*) *were administered to rats, the rate of catecholamine synthesis was increased in both the heart and adrenal gland. It is likely that increases also occur in other sympathetically innervated tissues. When the same dose of* L-*dopa was given daily for 4 or 7 days, the levels of tyrosine hydroxylase in the adrenal were lowered in comparison to controls. These findings may be a further indication of the existence of a regulatory mechanism which modifies endogenous levels of tyrosine hydroxylase in response to changes in the biosynthetic demand for norepinephrine.*

L-Dihydroxyphenylalanine (L-dopa) is an intermediate in the biosynthetic pathway leading to the formation of the sympathetic neurotransmitter norepinephrine and the sympathetic hormone epinephrine. Studies on the effects of administered L-dopa on the enzymes involved in catecholamine biosynthesis were initiated because large doses of L-dopa are now used in the treatment of Parkinsonism (*1*). We now present data that administration of L-dopa to rats increases norepinephrine synthesis and brings about significant reduction of adrenal tyrosine hydroxylase activity.

Female Sprague-Dawley rats (Carworth Farms, New City, New York) weighing between 175 and 220 g were used. L-Dopa was administered subcutaneously as a fine suspension or in saline. Control animals received subcutaneous injections of 0.9 percent NaCl. The volume of all injections was 0.5 ml. The animals were killed 1 day after the final injection of either an L-dopa suspension or saline and were routinely fasted 16 hours before they were killed. Adrenal glands were removed and homogenized in 2.0 ml of 0.13*M* potassium phosphate buffer, *p*H 7.0. Portions (0.5 ml) of the homogenates were immediately added to 9.5 ml of 5 percent trichloroacetic acid, and the catecholamines were isolated by alumina adsorption (*2*, *3*). Norepinephrine and epinephrine were determined by the method of Crout (*3*) and dopa and dopamine were determined by a modification of the method of Drujan *et al.* (*4*). Portions of the adrenal homogenates and the supernatant fractions of brain homogenates were assayed for tyrosine hydroxylase activity by the method of Nagatsu *et al.* (*5*).

In the studies involving heart norepinephrine and adrenal catecholamine turnover, rats received either 10 μc of DL-[7-^{3}H]norepinephrine (10 c/mmole; New England Nuclear) or 200 μc of generally labeled L-3,4-[^{3}H]dihydroxyphenylalanine (9.9 c/mmole; New England Nuclear) intravenously, and the decline in the specific activity of heart norepinephrine or adrenal catecholamines was followed with time. The slope of the decline K was determined, and the half-life $T_{1/2}$ was calculated from the equation $T_{1/2} = 0.693/K$ (*6*). Tritiated L-dopa was used to label the adrenal catecholamine stores since this cannot be reliably accomplished with radioactive norepinephrine (*7*). The data were statistically analyzed by the Student *t*-test.

One day after the administration of L-dopa (1000 mg/kg) to rats for two consecutive days there was a small but significant and reproducible decrease in the level of adrenal tyrosine hydroxylase, from 23.6 ± 0.8 in controls to 18.4 ± 1.6 μmole per pair per 15 minutes (five to six animals in each group; $P < .025$). The questions of whether additional administration of L-dopa would result in a more pronounced decrease in adrenal tyrosine hydroxylase and whether similar changes occurred in other tissues were then studied. When L-dopa was administered for longer periods a progressive reduction of adrenal tyrosine hydroxylase occurred. After 1 week on L-dopa, the level of tyrosine hydroxylase in the adrenal gland was reduced to about 50 percent of that in the controls, while that in the brain remained unaltered (Table 1).

After L-dopa was administered for three consecutive days, the amounts of this amino acid in the tissues remained elevated for at least 1 day after the last dose. For example, in the heart 2, 5.5, and 18 hours after the last dose (subcutaneous), the amounts of L-dopa were 4.2, 1.6, and 0.7 μg/g, as compared to undetectable amounts in hearts from control animals. There was the possibility, therefore, that L-dopa or catecholamines or both might remain at a sufficient concentration in the adrenal to result in inhibition of adrenal tyrosine hydroxylase when measured in vitro (*8*). For this reason, catecholamines were assayed in the same adrenal homogenates used for the assay of tyrosine hydroxylase. One day after the administration of L-dopa (1000 mg/kg per day) for 4 or 7 days, small but significant elevations in adrenal dopa and dopamine were observed. However, total catechols were elevated only about 5 percent over control values (Table 2). This small increase could not account for the reduction in tyrosine hydroxylase activity as measured in the in vitro assay.

The presence of L-dopa in the tissues should result in an increased synthesis of norepinephrine by bypassing the rate-limiting step, tyrosine hydroxylase (*9*). This could be readily determined by following the decline in specific activity of prelabeled stores of heart norepinephrine after the administration of L-dopa. For the first 8 hours after the administration of L-dopa the turnover of heart norepinephrine was 5.5 times faster than that of the control animals (Fig. 1). Increased turnover continued for some time beyond 8 hours, as is shown by the slope between the 8- and 20-hour points.

In order to investigate turnover in the adrenal gland, [^{3}H]dopa was administered intravenously to rats, and the rate of decline in the specific activity of the adrenal catecholamines was followed with time. The animals were killed 2, 7, 25, and 48 hours later. Some of the rats received nonradioactive L-dopa (1000 mg/kg) subcutaneously 2 or 25 hours (or both) after the administration of the radioactive, trace dose. The remaining animals received saline and served as the control group. The $T_{1/2}$ values calculated from the rate of decline in the specific activity of the adrenal epinephrine was 107 hours for the controls as compared to 65 hours for those animals given L-dopa, an increase of approximately 70 percent in synthesis in the adrenal compared to a fivefold increase in the heart. The actual increment in catecholamine synthesis after administration of L-dopa is probably of the same order of magnitude, since adrenal catecholamine levels are approximately 25 μg per pair in comparison to norepinephrine levels of 0.6 μg per heart. If these values are used as a base, after L-dopa administration, the adrenals synthesized an additional 0.09 μg of epinephrine per pair per hour in comparison to an additional 0.10 μg/hour for the heart. It is likely that the increased catecholamine synthesis in the heart and adrenal gland after L-dopa administration is indicative of increased synthesis in other sympathetically innervated tissues.

Tyrosine hydroxylase is subject to regulation by at least two different types of mechanisms. The first of these mechanisms involves control of tyrosine hydroxylase activity through a mechanism considered to involve end-product inhibition by catecholamines (*10*). This mechanism is capable of rapidly adjusting the rate of catecholamine synthesis by altering the activity of tyrosine hydroxylase without changing the actual level of the enzyme (*11*). It probably accounts for the bulk of regulation necessary to maintain homeostasis.

A second mechanism which results in an elevation in the level of tyrosine hydroxylase has been demonstrated (*12*). These increased enzyme levels have been observed mainly in the adrenal gland several days after the administration of large doses of 6-hydroxydopamine, phenoxybenzamine, or reserpine (*13*). Similar increases have also been observed after prolonged periods of restraint (*14*). Each of the above conditions can be expected to result in a sustained, long-term increase in sympathetic nerve activity in an attempt by the animal to maintain homeostasis. The experiments of Thoenen *et al.* (*13*) suggest that this sustained increase in adrenergic nerve activity in some manner mediates an induction of tyrosine hydroxylase. The increase in tissue enzyme produced by the above experimental procedures manifests itself in vivo by an increased synthesis of catecholamines from tyrosine in the intact animal (*15*).

We have now shown that levels of tyrosine hydroxylase in the adrenal gland can also be decreased. Tarver *et al.* (*16*) have shown that tyrosine hydroxylase levels in rabbit and rat arteries falls severalfold as a result of long-term administration of large amounts of L-dopa. We have also demonstrated that in intact animals, as in perfused organs (*9*), administration of L-dopa, in more than trace amounts, results in an increased synthesis of norepinephrine by bypassing tyrosine hydroxylase, the rate-limiting step. It may be that the decrease in tyrosine hydroxylase levels reflects an attempted compensation for the increase in catecholamine synthesis. L-Dopa or one of its subsequent products may repress synthesis of tyrosine hydroxylase or in some way lead to an increased rate of enzyme degradation. It is also conceivable that the increased norepinephrine synthesis brought about by L-dopa diminishes sympathetic nerve activity. Evidence for a decrease in post-

Table 1. The effect of long-term administration of L-dopa on tissue levels of tyrosine hydroxylase. Rats were given a suspension of L-dopa (1000 mg/kg per day) or saline subcutaneously for four or seven consecutive days. One day after the last dose the animals were killed; tissues were removed, homogenized, and assayed for tyrosine hydroxylase activity. Adrenals were treated as described in the text. Brains were homogenized in equal volumes of 0.13*M* potassium phosphate buffer, *p*H 7.0, and centrifuged at 30,000*g* for 10 minutes before samples (0.2 ml) were taken for assay. Brain was assayed with $2 \times 10^{-5}M$ tyrosine rather than $1 \times 10^{-4}M$, which is used for adrenal assay. Tyrosine hydroxylase activity is reported as nanomoles of L-tyrosine converted to 3,4-dihydroxyphenylalanine in 15 minutes (mean ± S.E.M.). Adrenal values are for adrenal pairs; seven animals were used for each group.

Treatment	Tyrosine hydroxylase activity	
	Adrenals	Brains
Control	32.5 ± 0.8	0.61 ± 0.04
L-Dopa (4 days)	23.4 ± 0.6*	
L-Dopa (7 days)	17.2 ± 0.5†	0.70 ± 0.05

* Significantly different from control ($P < .01$).
† Significantly different from control ($P < .001$), and significantly different from the 4-day L-dopa group ($P < .01$).

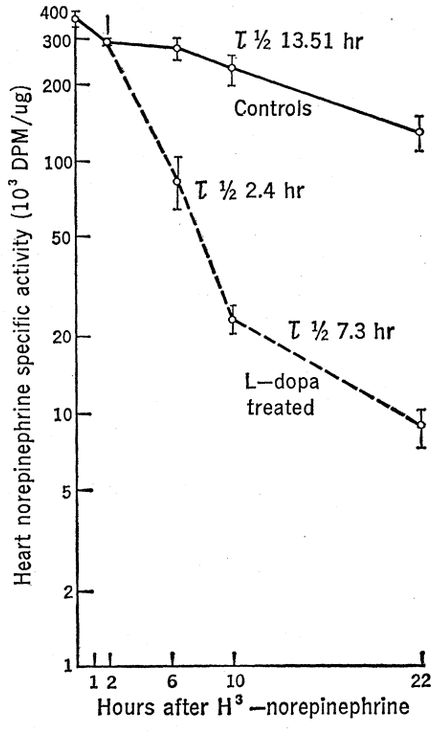

Fig. 1. Turnover of heart norepinephrine after the administration of L-dopa. Control animals received 10 μc of DL-[^{3}H]norepinephrine intravenously. Some of these were killed after 10 minutes. Two other groups of rats received L-dopa (1000 mg/kg) or saline subcutaneously 2 hours after the administration of DL-[^{3}H]norepinephrine and were killed 6.5, 10, and 22 hours after the injection of [^{3}H]norepinephrine. Each point represents the mean ± S.E.M. for five animals. The slope for control rats is 0.0513; the slope for the rats treated with L-dopa at the 2- to 10-hour interval is 0.293, and 0.096 for the 10- to 22-hour interval. The solid line represents control animals and the broken line represents animals treated with L-dopa. The arrow indicates the time at which L-dopa was administered. The concentrations of norepinephrine in the rats treated with L-dopa were not significantly different from those in the controls at any of the time intervals examined.

Table 2. Adrenal catecholamine levels after administration of L-dopa. These were the same animals as used in Table 1.

Group	Catecholamine (micrograms per adrenal pair)			
	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

* Significantly different from controls ($P < .005$). † Significantly different from control ($P < .001$).

ganglionic sympathetic nerve activity after L-dopa administration has been reported (*17*). This effect may be more directly responsible for lowering tyrosine hydroxylase. Thus prolonged changes in the intensity of nerve activity may directly influence the level of tyrosine hydroxylase in the tissues. Some support for this mechanism has been provided by work with genetically hypertensive rats. Louis *et al.* (*18*) have suggested that these animals have diminished sympathetic nerve activity which may represent an attempt to compensate for their hypertension. Tarver and Spector (*19*) have demonstrated that in these animals the level of tyrosine hydroxylase in blood vessels is decreased. On the other hand splanchnic nerve transection in the rat does not lower tyrosine hydroxylase in the denervated adrenal gland (*13*), a finding that is admittedly difficult to reconcile with the above hypothesis. Thus the mechanism of L-dopa's action on this key enzyme in norepinephrine synthesis remains in doubt.

In addition to that of tyrosine hydroxylase, activities of other enzymes taking part in catecholamine biosynthesis and degradation may be altered by L-dopa administration. Tarver and Spector (*19*) have reported that monoamine oxidase in the tissues is increased in animals given L-dopa. We have also examined aromatic L-amino acid decarboxylase in several tissues of rats given L-dopa for 7 days. The decarboxylase levels were unaltered in adrenals and brain, but were reduced by 30 percent in the kidney. Changes in dopamine-β-hydroxylase and catechol-*O*-methyltransferase have not yet been measured in animals on prolonged L-dopa treatment. The effects of tyrosine hydroxylase may be of greatest significance because of the key regulatory role of this enzyme in catecholamine biosynthesis.

The possibility that L-dopa produces a general inhibition of protein synthesis seems unlikely. The activities of brain tyrosine hydroxylase and of aromatic L-amino acid decarboxylase in several tissues, including the adrenal gland, were unaltered at a time when adrenal tyrosine hydroxylase was diminished. In addition, the activity of a third enzyme, monoamine oxidase, increases after similar treatment with L-dopa (*19*).

Our results may have significance in dealing with Parkinsonian patients on L-dopa therapy. If L-dopa lowers tyrosine hydroxylase in the peripheral tissues of these patients, sudden withdrawal of the drug may leave the patients with a diminished capacity for synthesizing norepinephrine until enzyme levels return to normal.

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4 September 1970; revised 13 November 1970 ■

Scanning Electron Microscopic Observations of Surface Structure of Isolated Human Chromosomes

Abstract. *Isolated human chromosomes dried by the critical-point method have been assumed to retain their original three-dimensional shape when viewed under a transmission electron microscope. Our scanning electron microscopic study confirms this interpretation and reveals an appearance like that of a skein of yarn. The existence of fiber bridges between chromatid pairs and among chromosomes is demonstrated.*

The critical-point drying method (*1*) permits the removal of water from sensitive (fragile) objects without the passage of an air-liquid interphase through it. In drying, the object is not affected by surface tension. Also eukaryotic chromosomes dried by such a technique and examined by transmission electron microscopy appear to be preserved in their original three-dimensional extension (*2, 3*). This belief was supported by quantitative evaluation of contrast (*3*).

The great depth of focus of scanning electron microscopy has allowed us to examine in a direct manner the three-dimensional aspect of chromosomes dried by the critical-point method. Human chromosomes appeared as a skein of chromatin fibers, which confirmed previous concepts of chromosome conformation.

Peripheral lymphocytes from human male donors were cultured according to standard techniques. Cells were harvested by centrifugation, treated with hypotonic Hanks solution for 10 minutes, and applied to the surface of distilled water in a Langmuir trough. Surface tension disrupted the cells causing the release of their chromosomes, which we picked up by touching Formvar-coated aluminum disks to the surface of the water. Chromosomes adhere better to Formvar-coated disks than to uncoated disks. The wet chromosomes were quickly submersed in 30 percent ethanol, dehydrated in