End-Product Inhibition of Tyrosine Hydroxylase as a Possible Mechanism for Regulation of Norepinephrine Synthesis

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SUMMARY

The present study was undertaken to evaluate the influence of endogenous norepinephrine content on the rate of norepinephrine synthesis *in vivo*. Tissue levels of norepinephrine were increased in guinea pigs by administration of a monoamine oxidase inhibitor and tyrosine-^{14}C and DOPA-^{3}H were used to measure norepinephrine synthesis. In brain and heart when norepinephrine levels were increased 2- to 3-fold, conversion of tyrosine-^{14}C to norepinephrine was decreased markedly. When tyrosine hydroxylase was bypassed by administering DOPA-^{3}H, conversion to norepinephrine was actually increased. These findings lend support to the hypothesis that norepinephrine synthesis is regulated by a mechanism of end-product inhibition at the tyrosine hydroxylase step.

INTRODUCTION

Increased sympathetic stimulation associated with exercise or exposure to cold leads to increased catecholamine synthesis (1). It appears that the acceleration of norepinephrine biosynthesis under such conditions is a result of increased nerve stimulation since it has been shown that electrical stimulation of the cat adrenal gland (2, 3), guinea pig vas deferens (4, 5), rat salivary gland (6), and rat heart (7) increases norepinephrine synthesis in those organs. Studies with radioactive tyrosine and 3,4-dihydroxyphenylalanine (DOPA) indicate that the increase in norepinephrine synthesis is a result of an increased activity of tyrosine hydroxylase (1, 7).

An important question concerns the mechanism whereby the increased nerve activity increases hydroxylation of tyrosine in sympathetically innervated tissues. Several obvious mechanisms have been considered. The possibility of allosteric activators was investigated by Ikeda *et al.* (8). Increased formation of the enzyme does not appear to occur.[2] A likely possibility is that tyrosine hydroxylase is regulated by a mechanism of end-product inhibition since it has been shown that catechol derivatives are inhibitors of the enzyme (8–10). If this were so, then an increase in tissue levels of norepinephrine should diminish synthesis of the transmitter. One way of increasing tissue levels of norepinephrine is to administer monoamine oxidase (MAO) inhibitors. The following experiments were undertaken to determine whether increased tissue levels of norepinephrine, associated

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[2] Inhibitors of protein synthesis have failed to block increased conversion of tyrosine-^{14}C to norepinephrine brought about by increased sympathetic activity (M. Lipton, R. Gordon and S. Udenfriend, to be published).

with monoamine oxidase inhibition, diminish norepinephrine synthesis *in vivo* and whether the effect is at the rate-limiting step, tyrosine hydroxylase (10a).

MATERIALS AND METHODS

Male, Hartley strain guinea pigs weighing 180–220 g were used in the study. The monoamine oxidase inhibitor *n*-methyl-*n*-benzyl-2-propynalamine (Pargyline) was kindly supplied by Abbott Labs, North Chicago, Illinois. L-Tyrosine-^{14}C (uniformly labeled, 376 μC/μmole) was obtained from New England Nuclear Corporation. L-3,4-Dihydroxyphenylalanine (ring-2,5,6-^{3}H, 28.7 or 37.4 mC/μmole) was obtained from Nuclear Chicago Corporation. One hundred microcuries were diluted with 1 μmole of carrier DOPA prior to injection.

Pargyline (80 mg/kg) was injected intraperitoneally at stated intervals prior to the amino acids. The radioactive compounds, L-tyrosine-^{14}C (25 μC in 0.5 ml of saline) or L-DOPA-^{3}H (100 μC in 0.5 saline) were injected into the saphenous vein. In all experiments animals were killed exactly 1 hr after administration of the appropriate radioactive precursor. Norepinephrine, tyrosine, and DOPA were then isolated from heart and brain, and radioactivity and specific activity were determined as previously described (11). Radioactivity was determined in a Tri-Carb liquid scintillation spectrometer. The efficiency for ^{14}C was 75% and for ^{3}H was 15%.

RESULTS

Effect of Monoamine Oxidase Inhibition on Endogenous Norepinephrine Levels and on Conversion of Tyrosine-^{14}C and DOPA-^{3}H to Norepinephrine

From previous experiments (12) it is known that 24 hr after administration of the monoamine oxidase inhibitor Pargyline (80 mg/kg), the norepinephrine levels in guinea pig heart and brain are almost doubled. To test the effect of increased norepinephrine levels in tissues on overall norepinephrine biosynthesis, tyrosine-^{14}C was administered to guinea pigs treated 24 hr earlier with Pargyline. As shown in Table 1, administration of the monoamine oxidase inhibitor increased the endogenous levels of norepinephrine almost 2-fold in the heart. Concomitant with this, there was more than a 50% diminution in conversion of L-tyrosine-^{14}C to norepinephrine. It should be noted that the specific activities of tyrosine in the tissues of control and monoamine oxidase-inhibited groups were the same, indicating that Pargyline did not influence the uptake or excretion of the radioactive precursor.

TABLE 1

Effect of MAO inhibition on norepinephrine synthesis from tyrosine-^{14}C in heart

Animals were injected with 80 mg of Pargyline per kilogram intraperitoneally and 24 hr later were given L-tyrosine-^{14}C (25 μC) intravenously. Guinea pigs were killed 1 hr after the administration of the radioactive precursor. Tissues were assayed for tyrosine and norepinephrine concentration, reported as micrograms per gram of tissue (μg/g), and specific activity (cpm/μg). Radioactivity in norepinephrine is reported as cpm per gram of tissue (cpm/g). Figures represent individual experiments.

	Tyrosine		Norepinephrine	
Treatment	μg/g	cpm/μg	μg/g	cpm/g
Control	14.8	1510	1.50	1247
Control	15.7	1630	1.55	1233
Control	15.8	1670	1.55	1628
MAO-I	15.3	1590	3.02	487
MAO-I	15.2	1670	2.63	445
MAO-I	14.9	1630	2.63	921

Table 2 shows similar results in the brain stem. Inhibition of monoamine oxidase increased norepinephrine levels in the brain stem over 2-fold while the formation of radioactive norepinephrine from tyrosine-^{14}C was greatly diminished. Again Pargyline did not change the endogenous content or specific activity of tyrosine in the tissue.

Since pulse labeling *in vivo* would be influenced by changes in metabolism or distribution of the precursor by the experimental drug, it was necessary to study the effects of Pargyline on the disposition of tyrosine-^{14}C in the early stages of the ex-

TABLE 2

Effect of MAO inhibition on norepinephrine synthesis from tyrosine-^{14}C in brain stem

Experimental conditions were the same as in Table 1.

Treatment	Tyrosine		Norepinephrine	
	μg/g	cpm/μg	μg/g	cpm/g
Control	18.9	1693	0.33	506
Control	16.5	1563	0.34	264
Control	17.7	1551	0.34	431
MAO-I	18.1	1655	0.82	167
MAO-I	16.4	1554	0.61	160
MAO-I	17.0	1590	0.86	213

periment. As shown in Fig. 1, tyrosine-^{14}C levels in plasma were relatively unaffected by Pargyline treatment. Table 3 also indicates that the specific activity of tyrosine and tyrosine levels in brain and heart were unaffected by the inhibitor.

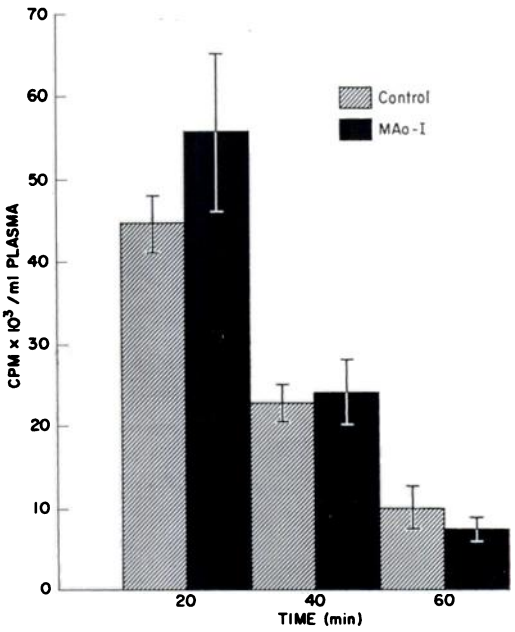


FIG. 1. *Effects of Pargyline treatment on plasma levels of tyrosine-^{14}C*

Tyrosine-^{14}C (15 μc, 13 μg) was injected intravenously via the saphenous vein. Animals were killed at specified times and free plasma tyrosine was isolated. Each time represents the mean of 6 animals ± SE. Monoamine oxidase was inhibited by administering Pargyline 16 hr prior to tyrosine.

TABLE 3

Levels and specific activity of tyrosine-^{14}C in heart and brain stem following MAO inhibition

Guinea pigs were injected with 80 mg of Pargyline per kilogram intraperitoneally, and the animals were killed 1 hr after the intravenous administration of L-tyrosine-^{14}C (25 μC). Tissues were assayed for tyrosine concentration represented as micrograms per gram of tissue (μg/g), counts per minute per gram of tissue (cpm/g), and specific activity (cpm/μg).

Tissue	Treatment	Tyrosine		
		μg/g tissue	cpm/g tissue	cpm/μg
Heart	Control	17.1	32421	1901
	Control	15.9	28714	1804
	Control	15.0	26913	1790
	Control	17.1	31888	1863
	MAO-I	14.5	25516	1763
	MAO-I	15.4	27473	1781
	MAO-I	14.1	26429	1877
	MAO-I	15.3	29431	1921
Brain stem	Control	17.4	34283	1970
	Control	17.2	21919	1856
	Control	18.1	36773	2032
	Control	16.8	33371	1986
	MAO-I	16.5	31866	1931
	MAO-I	18.4	37418	2034
	MAO-I	18.7	40271	2154
	MAO-I	19.1	37966	1988

L-DOPA-^{3}H was also used as precursor for norepinephrine biosynthesis. The data in Table 4 show that along with a rise in endogenous levels of norepinephrine there was an increase of over 2-fold in the incorporation of radioactivity from DOPA-^{3}H into norepinephrine. This is in contrast to the decrease in labeling from tyrosine-^{14}C under comparable conditions. As shown in Table 5, similar results were obtained with DOPA-^{3}H in the brain stem. This effect on DOPA-^{3}H incorporation is exactly the same as that seen when α-methyltyrosine was used to inhibit tyrosine hydroxylase (13).

Time Course of Conversion of Tyrosine-^{14}C and DOPA-^{3}H to Norepinephrine after Monoamine Oxidase Inhibition

The experiments presented above indicate that monoamine oxidase inhibition de-

TABLE 4

Effect of MAO inhibition on norepinephrine synthesis from DOPA-^{3}H in heart

Guinea pigs were injected with 80 mg of Pargyline per kilogram intraperitoneally and 24 hr later were given L-DOPA-^{3}H (100 μC) intravenously. The animals were killed 1 hr after the administration of the radioactive precursor. Tissues were assayed for radioactive DOPA reported as counts per minute per gram of tissue (cpm/g) and norepinephrine concentration as micrograms per gram of tissue (μg/g) and counts per minute per gram of tissue (cpm/g). Figures represent individual experiments.

Treatment	DOPA cpm/g	Norepinephrine μg/g	Norepinephrine cpm/g
Control	7211	1.70	1590
Control	5109	1.50	2229
Control	3514	1.75	2365
MAO-I	3322	2.70	4787
MAO-I	3704	2.75	5122
MAO-I	4116	2.80	3830

creases incorporation of tyrosine-^{14}C into norepinephrine and conversely increases incorporation of DOPA-^{3}H. The question remains whether the effects on incorporation of precursors are related to the increased

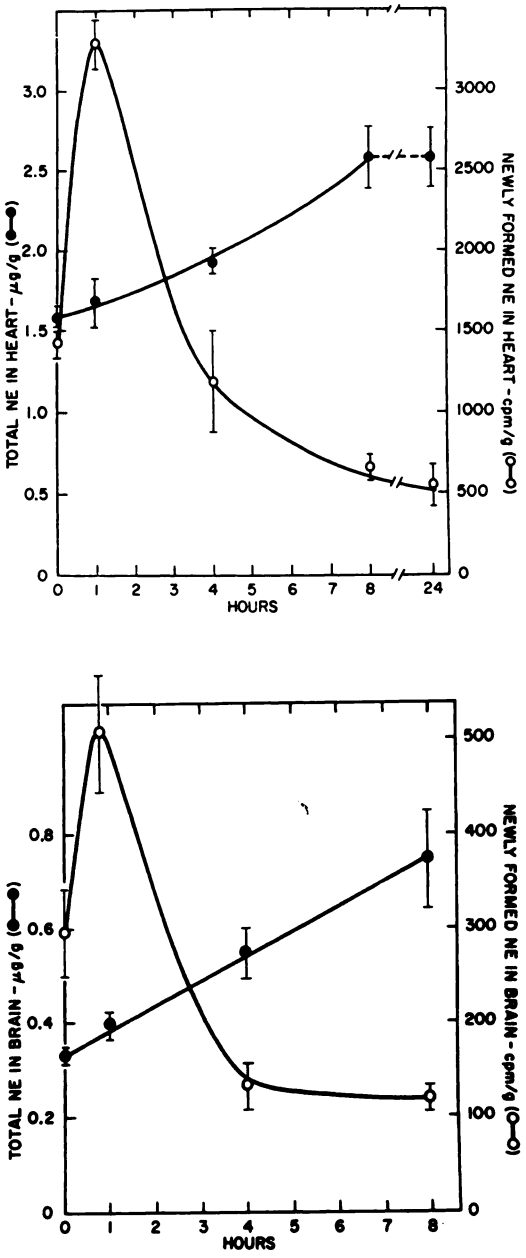
TABLE 5

Effect of MAO inhibition on norepinephrine synthesis from DOPA-^{3}H in brain stem

Experimental conditions were the same as in Table 4.

Treatment	DOPA cpm/g	Norepinephrine μg/g	Norepinephrine cpm/g
Control	3375	0.34	283
Control	3230	0.32	226
Control	2571	0.31	183
MAO-I	2153	0.72	895
MAO-I	3005	0.80	719
MAO-I	2957	0.80	798

concentrations of norepinephrine in the tissues or result from some other action of the inhibitor, Pargyline. To differentiate these actions, incorporation of tyrosine-^{14}C into norepinephrine was examined at various time intervals after administration of the drug. At the dosage of Pargyline employed, inhibition of monoamine oxidase



FIGS. 2 and 3. *Effects of Pargyline treatment on norepinephrine levels in heart and brain*

Guinea pigs were injected with Pargyline, 80 mg/kg, intraperitoneally to inhibit monoamine oxidase. At specified intervals animals were killed. One hour prior to sacrifice each animal was given L-tyrosine-^{14}C (25 μC) by intravenous route. Tissues were assayed for the concentration and radioactivity of norepinephrine. The data represent means ± SE of at least 3 animals.

is maximal and almost complete within 30 min (12). This degree of inhibition is maintained for over 24 hr. By contrast, norepinephrine levels rise slowly and do not reach maximal values until 8 hr after Pargyline administration. This is shown in Figs. 2 and 3 for guinea pig heart and brain. It should be noted, that the initial effect of Pargyline, when there was still little change in tissue norepinephrine levels, was to increase incorporation of tyrosine-^{14}C into norepinephrine. DOPA-^{3}H incorporation was also increased at this time (Table 6). However, whereas incorporation of tyrosine-^{14}C into norepinephrine fell far below control levels when the tissue levels of norepinephrine became significantly elevated, incorporation of DOPA-^{3}H remained above control levels throughout the experiment.

TABLE 6

Conversion of DOPA-^{3}H to norepinephrine in heart and brain stem following MAO inhibition

Guinea pigs were injected with 80 mg of Pargyline per kilogram intraperitoneally; the animals were killed 1 and 8 hr after the MAO inhibitor. The animals were given L-DOPA-^{3}H (100 μC) intravenously and killed 1 hr after the administration of the radioactive precursor. The 1 hr time after MAO inhibition was obtained by administering the precursor immediately after the inhibitor was given; for the 8 hr figure, the precursor was given 7 hr after the inhibitor. Tissues were analyzed for DOPA and norepinephrine concentrations. Figures represent individual experiments.

Tissue	Treatment	Time (hr)	DOPA	Norepinephrine	
			cpm/g	μg/g	cpm/g
Heart					
	Control	—	10,175	1.53	2147
			8,752	1.38	1565
	MAO-I	1	10,887	1.49	4629
			10,904	1.72	4002
	MAO-I	8	12,142	2.15	2683
			10,901	2.27	3430
Brain stem					
	Control	—	7,630	0.35	1069
			4,876	0.35	683
	MAO-I	1	5,651	0.35	1962
			6,467	0.35	1477
	MAO-I	8	6,671	0.64	1633
			6,435	0.65	2019

DISCUSSION

The use of tyrosine-^{14}C in this type of study may be considered to be as a pulse label. It serves as an indicator of overall norepinephrine synthesis in the intact animal under the defined conditions of the experiment. The DOPA-^{3}H is also used as a pulse label indicator of norepinephrine synthesis. However, it serves as a measure of norepinephrine synthesis subsequent to the tyrosine hydroxylase step. The validity of this use of tyrosine-^{14}C and DOPA-^{3}H to detect and localize inhibition of norepinephrine synthesis *in vivo* was demonstrated in studies with the tyrosine hydroxylase inhibitor, α-methyltyrosine (13). When sufficient α-methyltyrosine was administered to produce a tissue concentration of the drug 10–35 times higher than its K_I the *in vivo* conversion of tyrosine-^{14}C to norepinephrine was completely inhibited (14). When DOPA-^{3}H was administered to bypass tyrosine hydroxylase, incorporation into norepinephrine was actually increased (13). Increased incorporation of DOPA-^{3}H into norepinephrine is a direct consequence of tyrosine hydroxylase blockade since under such conditions the DOPA-^{3}H would not be diluted as much by endogenously formed DOPA. It follows that inhibition of tyrosine hydroxylase by any other means should lead to both decreased incorporation of tyrosine-^{14}C and increased incorporation of DOPA.

In the present studies DOPA-^{3}H served a dual function. In addition to being a precursor which permitted bypassing the tyrosine hydroxylase step it also served as an excellent control for other factors which could influence norepinephrine synthesis and metabolism. For example its incorporation into norepinephrine still requires one of the two hydroxylation steps. Many conditions which could affect one enzyme would modify the activity of the other in a similar manner. Therefore changes in oxygen tension would be expected to produce comparable effects with both substrates. DOPA-^{3}H

is also an excellent control for changes in norepinephrine degradation. It can be seen, however, (Fig. 2) that after 8–24 hr when norepinephrine had risen to maximal levels DOPA-^{3}H incorporation was increased above control values while tyrosine-^{14}C incorporation was decreased. These findings are similar to those obtained with α-methyltyrosine and suggest not only that the increased amount of norepinephrine in tissues inhibits norepinephrine synthesis but that the inhibition is at the tyrosine hydroxylase step.

It should be noted that 1 hr after administering the monoamine oxidase inhibitor, incorporation of both tyrosine-^{14}C and DOPA-^{3}H were increased. Whatever the reason for this increased incorporation of both precursors, it probably is not related to increased synthesis. If it were, it would have to be subsequent to the tyrosine hydroxylase step. The latter possibility is unlikely since tyrosine hydroxylase is the rate-limiting step. A more likely explanation of the initial enhanced incorporation of both precursors is that the monoamine oxidase inhibitor protects the newly synthesized catecholamines (dopamine and norepinephrine) from being metabolized. Under such conditions, the same amount of radioactive precursor used in the pulse labeling would yield more radioactivity in the norepinephrine in tissues. Rutschmann *et al.* (15) have also demonstrated increased incorporation of DOPA-^{3}H into norepinephrine 1–2 hr after administration of monoamine oxidase inhibitors. These observations imply that the incorporation of precursors at 8 and 24 hr should be compared to the incorporation obtained 1 hr subsequent to monoamine oxidase inhibition rather than incorporation in untreated controls. If this is done, then the degree of inhibition of tyrosine-^{14}C incorporation produced by norepinephrine accumulation may be as much as 80–85%.

The increased labeling seen in the early stages of monoamine oxidase inhibition also suggests that normally a substantial proportion of newly synthesized norepinephrine may be rapidly released and metabolized by monoamine oxidase. It is even conceivable that an appreciable proportion of newly synthesized norepinephrine is released and utilized for sympathetic transmission without pooling with the endogenous stores of norepinephrine.

In more recent studies it has been possible to raise norepinephrine levels in peripheral tissues by injecting large doses of norepinephrine (5 mg/kg) into rats pretreated with adrenergic blocking agents, as described by Crout *et al.* (16), and to demonstrate even more marked reduction of conversion of tyrosine-^{14}C to norepinephrine.

The present findings are consistent with the concept that the rate of synthesis of norepinephrine is controlled at the tyrosine hydroxylase step and that endogenous levels of norepinephrine inhibit this reaction. These studies support the hypothesis, which has been advanced in previous publications from these laboratories (1, 7, 17) and also by Neff and Costa (18), that regulation of norepinephrine synthesis involves end-product inhibition. The present studies localize the inhibition at the tyrosine hydroxylase step. Since monoamine oxidase inhibitors elevate the endogenous content of catecholamines, it is interesting to speculate that their hypotensive action may very well be related to a diminished synthesis of norepinephrine in areas which normally impinge upon specific receptors involved in pressure regulation.

Norepinephrine and related catechol compounds have been shown to be inhibitors of tyrosine hydroxylase *in vitro* (8, 9). Interestingly, they do not inhibit by competition with substrate, but with the reduced tetrahydropteridine cofactor. In *in vitro* studies with relatively large amounts (10^{-4} M) of a synthetic cofactor, about 10^{-3} M norepinephrine is required to produce 50% inhibition of bovine adrenal medulla tyrosine hydroxylase. The normal cofactor, tetrahydrobiopterin, is present in concentrations much lower than 10^{-4}M in tissues (19). Under such conditions norepinephrine would be expected to be even more effective as an inhibitor. Furthermore, the catecholamines in the adrenal gland, sympathetic nerves, and brain are not randomly distributed, but are concentrated in granules or organelles

(20–23). It has been suggested that a substantial amount of the norepinephrine in tissues is found in association with all three enzymes involved in norepinephrine synthesis (17, 24). Such a localization would certainly lead to concentrations of norepinephrine at the enzyme site sufficiently high to produce end-product inhibition.

Based on our present knowledge, a good working hypothesis is that tyrosine hydroxylase in sympathetic nerves, brain, and adrenal gland is always partially inhibited by norepinephrine or epinephrine. Stimulation of sympathetic nerves releases the norepinephrine thereby freeing the enzyme from end-product inhibition and resulting in increased synthesis of norepinephrine. It remains to be seen how this hypothesis fits in with the rest of our knowledge of the operation of the sympathetic nervous system.

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