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Increased Conversion of Tyrosine to Catecholamines in the Intact Rat Following Elevation of Tissue Tyrosine Hydroxylase Levels by Administered Phenoxybenzamine

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SUMMARY

When phenoxybenzamine (20 mg/kg) was administered to rats for 2 consecutive days, a 2-fold increase in tryosine hydroxylase relative to controls was observed in adrenal and heart homogenates. A 4–6-fold increase in the incorporation of label from ^{14}C-L-tyrosine into catecholamines was observed following 2 days of phenoxybenzamine treatment, in comparison to a 2–3-fold increase following a single dose of the *alpha*-adrenergic blocking agent. The administration of a ganglionic blocking agent 2 hr prior to the administration of ^{14}C-L-tyrosine completely prevented the increased incorporation of label into catecholamines following 1 day of phenoxybenzamine treatment, but caused only a partial reduction in the accelerated rate of conversion of tyrosine to catecholamines observed after 2 days of phenoxybenzamine treatment. These data are consistent with the concept that the increased tyrosine hydroxylase activity measured in tissue homogenates expresses itself physiologically as an acceleration of catecholamine synthesis in the intact animal.

INTRODUCTION

Many laboratories have now demonstrated that procedures which increase sympathetic nervous activity, such as exercise, exposure to cold, *alpha*-receptor blockade, thyroidectomy, and electrical stimulation of sympathetic nerves, result in an almost immediate acceleration in the rate of catecholamine synthesis as a result of increased tyrosine hydroxylase activity (1–11). The increased rate of catecholamine synthesis occurs without an increase in the amount of enzyme, and evidence has been presented that this phenomenon results from release of tyrosine hydroxylase from end-product inhibition (12, 13).

Recently, Mueller *et al.* (14) reported increased tyrosine hydroxylase activity in homogenates of rat adrenal gland following the administration of 6-hydroxydopamine. This agent disrupts the peripheral sympathetic nerve endings but does not have the same effect on the chromaffin cells of the adrenal medulla (15). More recently, Thoenen *et al.* (16) and Mueller *et al.* (17) reported increased amounts of tyrosine hydroxylase activity in homogenates of adrenals and sympathetic ganglia after chronic administration of reserpine or phenoxybenzamine. This elevation of adrenal tyrosine hydroxylase levels could be prevented by severing the splanchnic nerve (16, 18). Thoenen *et al.* (18) proposed that a chronic increase in splanchnic nerve activity results in an increase in the tyrosine hydroxylase content of the adrenal. This would, therefore, comprise a second mechanism for regulation of catecholamine synthesis.

The elevation of tissue tyrosine hydroxylase levels could be the result of an induc-

tive process, an activation of pre-existing enzyme, or a decreased degradation of enzyme. Mueller *et al.* (19) have presented evidence that increased amounts of enzyme protein are responsible for the increased tyrosine hydroxylase levels and that this is the result of an increase in the rate of enzyme synthesis.

The purpose of the present study was to determine whether the increase in tyrosine hydroxylase activity, measured in tissue homogenates, expresses itself physiologically as an acceleration of catecholamine synthesis in the intact animal.

METHODS

Sprague-Dawley female rats, obtained from Carworth Farms, New City, N. Y., and weighing 160–200 g, were used. ^{14}C-L-Tyrosine (uniformly labeled, 376 μCi/μmole) was obtained from New England Nuclear Corporation. Phenoxybenzamine hydrochloride (20 or 25 mg/kg), pentolinium tartrate (5 mg/kg), or 0.9% sodium chloride was administered intraperitoneally. The animals were routinely fasted for 16 hr before they were killed to eliminate variations due to the intake of dietary tyrosine. Rates of incorporation of isotope into catecholamines from labeled tyrosine were measured as described previously (20). Tracer doses of ^{14}C-L-tyrosine (25 μCi) were administered intravenously 1 hr before the animals were killed. Norepinephrine and epinephrine were isolated from tissues as described previously (21), and tyrosine was isolated by the method of Lewander and Jonsson (22). Norepinephrine and epinephrine were assayed by a modification of the trihydroxyindole procedure (23). Tyrosine was determined according to the method of Waalkes and Udenfriend (24). Adrenal tyrosine hydroxylase activity was determined by the method of Nagatsu *et al.* (25) on adrenal pairs homogenized in 2 ml of 0.13 M potassium phosphate buffer, pH 7.0, and enzymatic activity of the heart was estimated by the method of Levitt *et al.* (26). Tritium and ^{14}C were counted in Bray's solution (27), with an efficiency of 20% for the tritiated and 90% for the ^{14}C-labeled catecholamines. The ^{14}C-tyrosine fractions were made 0.5 N with respect to HCl; 0.5-ml aliquots of this solution in 10 ml of Bray's solution reduced the counting efficiency to 75%.

Statistical analyses were performed using Student's *t*-test. Those values found to be significantly different at the 0.05 probability level or lower are so designated in the tables.

RESULTS

All three agents which Thoenen *et al.* (16, 18) have shown to be capable of increasing tissue tyrosine hydroxylase—i.e., reserpine, 6-hydroxydopamine, and phenoxybenzamine—decrease tissue catecholamine levels. The first two result in almost complete depletion of catecholamines, so that they cannot be used to estimate rates of catecholamine synthesis based on the conversion of

TABLE 1

Effect of 2 days of phenoxybenzamine treatment in the rat on tissue tyrosine hydroxylase activity

Rats were given phenoxybenzamine (20 mg/kg) or 0.9 % NaCl intraperitoneally. Twenty-four hours later the injections were repeated. Twenty hours after the last administration of phenoxybenzamine or 0.9% NaCl, the animals were killed, and the adrenals and hearts were removed and assayed for tyrosine hydroxylase activity.

Tissue	Tyrosine hydroxylase activity[a]	
	Control	Phenoxybenzamine
Adrenals	5.1 ± 0.49 (6)[b]	9.6[c] ± 1.1 (6)
	6.0 ± 0.40 (5)	11.9[c] ± 1.1 (5)
Heart	411 ± 104 (6)	896[c] ± 96 (7)

[a] Adrenal tyrosine hydroxylase activity is reported as nanomoles of tyrosine converted to 3,4-dihydroxyphenylalanine per adrenal pair per 10 min (mean ± standard error). The low enzymatic activity of rat heart does not permit dilution of the radioactive tyrosine with carrier for measurement of activity under conditions of saturating amounts of substrate and with a substrate of known specific activity. The values for the heart hydroxylase activity are therefore relative and are reported as counts per minute of tritiated water liberated from 3,5-^{3}H-L-tyrosine (2×10^5 cpm) per 40 μl of heart press juice in 15 min (mean ± standard error).

[b] The numbers in parentheses refer to the number of animals used.

[c] Significantly different from controls ($p < 0.05$).

radioactive tyrosine to epinephrine and norepinephrine *in vivo*. Phenoxybenzamine can be used for such estimates because it lowers rat adrenal catecholamines less than 30% and heart norepinephine less than 50–60%.

Effect of phenoxybenzamine on tissue tyrosine hydroxylase activity. We have previously shown that a single dose of phenoxybenzamine (25 mg/kg) does not alter the level of tyrosine hydroxylase activity in rat adrenal homogenates 1 day following its administration (2). The administration of two separate doses of phenoxybenzamine 1 day apart resulted in a 2-fold increase in enzyme levels observed 1 day after the last dose of the *alpha*-adrenergic blocking agent. This confirms the findings of Thoenen *et al.* (16, 18). A similar increase in tyrosine hydroxylase activity was observed in the heart press juice of rats dosed with phenoxybenzamine for 2 consecutive days (Table 1).

Effect of phenoxybenzamine on conversion of radioactivity from ^{14}C-L-tyrosine to catecholamines. It has been demonstrated that within 3–24 hr following a single dose of phenoxybenzamine the incorporation of radioactivity from ^{14}C-L-tyrosine into catecholamines is markedly accelerated in the intact rat (2, 28). It has been proposed that the mechanism for this increased synthesis involves an increase in reflex sympathetic nervous activity and release of tyrosine hydroxylase from end-product inhibition (2, 29). If dosage with phenoxybenzamine for 2 consecutive days results in the formation of additional enzyme, one should observe even greater incorporation of label from ^{14}C-tyrosine into catecholamines than is seen 1 day following a single dose. Furthermore, the increased catecholamine synthesis observed 1 day following a single dose of phenoxybenzamine, being entirely dependent on increased nerve activity, should be sensitive to the effects of ganglionic blocking agents, in contrast to that component of catecholamine synthesis due to increased enzyme, which is seen after 2 days of phenoxybenzamine treatment. As shown in Table 2, 1 day of phenoxybenzamine treatment resulted in a 2–3-fold stimulation of incorporation of label from ^{14}C-tyrosine into the catecholamines of adrenals and hearts, while 2 days of treatment resulted in levels of incorporation

TABLE 2

Effect of ganglionic blockade on conversion of tyrosine to catecholamines in rat adrenals and heart following single and multiple injections of phenoxybenzamine

At zero time, 10 rats were dosed with phenoxybenzamine (20 mg/kg), and 16 rats, with 0.9% NaCl, all intraperitoneally. Twenty-four hours later, 10 of the 0.9% NaCl-treated group (1 day) received phenoxybenzamine (20 mg/kg) intraperitoneally. Those rats originally given phenoxybenzamine received a second injection of this agent (2 days) (20 mg/kg), and the remaining six animals of the 0.9% NaCl-treated group received 0.9% NaCl a second time. Twenty hours later, five rats from each phenoxybenzamine-treated group were given pentolinium tartrate (10 mg/kg) intraperitoneally. Two hours later, all animals received 25 μCi of ^{14}C-L-tyrosine intravenously and were killed after 1 hr. The data are reported as means ± standard errors.

Treatment	Duration	Adrenal epinephrine		Heart norepinephrine	
		Specific activity	Total radioactivity	Specific activity	Total radioactivity
	days	*cpm/μg*	*cpm/adrenal pair*	*cmp/μg*	*cpm/g*
Control		80 ± 7 (6)[a]	1705 ± 87 (6)	709 ± 75 (6)	340 ± 25 (6)
Phenoxybenzamine	1	261 ± 25[b] (5)	4722 ± 309[b] (5)	1896 ± 214[b] (4)	970 ± 43[b] (4)
Phenoxybenzamine + pentolinium	1	119 ± 23 (4)	2352 ± 276 (4)	607 ± 33 (5)	367 ± 36 (5)
Penoxybenzamine	2	456 ± 31[b] (5)	6220 ± 331[b] (5)	3053 ± 212[b] (5)	875 ± 43[b] (5)
Phenoxybenzamine + pentolinium	2	351 ± 41[b] (5)	5421 ± 451[b] (5)	1663 ± 251[b] (5)	567 ± 75[b] (5)

[a] Numbers in parentheses refer to the number of animals used.

[b] Significantly different from controls ($p < 0.05$).

TABLE 3

Specific activity of tyrosine in heart and adrenals following ganglionic blockade after single and multiple injections of phenoxybenzamine

Conditions were the same as described for Table 2. Data are reported as the means ± standard errors.

Treatment	Duration	Specific activity of tyrosine		
		Heart	Adrenal	Plasma
	days	*cpm/μg*	*cpm/μg*	*cpm/μg*
Control		370 ± 30 (6)[a]	272 ± 17 (6)	941 ± 52 (6)
Phenoxybenzamine	1	326 ± 15 (5)	305 ± 15 (5)	842 ± 42 (5)
Phenoxybenzamine + pentolinium	1	306 ± 25 (5)	313 ± 7 (4)	815 ± 54 (5)
Phenoxybenzamine	2	345 ± 15 (5)	279 ± 18 (5)	802 ± 42 (5)
Phenoxybenzamine + pentolinium	2	389 ± 26 (5)	335 ± 26 (5)	900 ± 62 (5)

[a] Numbers in parentheses refer to the number of animals used.

TABLE 4

Effect of various conditions which increase sympathetic nervous activity on rat and guinea pig adrenal tyrosine hydroxylase

Phentolamine (5 mg/kg) or 0.9% NaCl was administered intraperitoneally to rats, and the animals were killed 2 hr later. Rats were exercised in a large, motor-driven, rotating drum divided into individual cages (1). The rate of rotation was 7 rpm. Rats and 300-g female Hartley guinea pigs were kept at 5° in individual metal cages having wire fronts and bottoms.

Treatment	Duration	Tyrosine hydroxylase activity[a]	
		Control	Experimental
	hr	*mμmoles/adrenal pair/10 min*	
Rat			
Phentolamine, 5 mg/kg	2	7.7 ± 0.82 (4)[b]	8.0 ± 0.53 (4)
Exercise	3	13.4 ± 1.96 (3)	10.1 ± 0.71 (3)
Exposure to 5°	23	11.8 ± 0.91 (4)	11.1 ± 0.59 (4)
Guinea pig			
Exposure to 5°	53	17.2 ± 1.11 (5)	21.9 ± 3.08 (5)
Exposure to 5°	192		
Then shaved and exposed to 5°	48	25.2 ± 2.5 (7)	20.4 ± 0.87 (7)

[a] The results are expressed as nanomoles of tyrosine converted to 3,4-dihydroxyphenylalanine (means ± standard errors) per adrenal pair in 10 min.

[b] Numbers in parentheses refer to the number of animals used.

of radioactivity which were 4–6 times the control values. Administration of the ganglionic blocking agent pentolinium tartrate prior to the administration of the ^{14}C-tyrosine completely prevented the increased incorporation of label seen after 1 day of phenoxybenzamine treatment. However, after 2 days of phenoxybenzamine treatment, when the level of enzyme had increased, ganglionic blockade resulted in only a partial reduction in the incorporation of label from ^{4}C-L-tyrosine into the catecholamines. We have previously demonstrated that the administration of pentolinium alone does not reduce the relative rate of catecholamine synthesis in hearts or adrenals of control animals when measured by the techniques used in this study (29).

In order to establish that the observations presented in Table 2 were not due to alter-

ations in tissue levels of the precursor, ^{14}C-L-tyrosine, its specific activity was determined. The results presented in Table 3 show that treatment with phenoxybenzamine and/or pentolinium tartrate did not significantly affect the specific activity of ^{14}C-L-tyrosine in either heart, adrenals or plasma; tissue levels of tyrosine were not altered by these experimental procedures.

Effect of phentolamine, exercise, and cold exposure on adrenal tyrosine hydroxylase. It was of interest to determine whether other conditions under which sympathetic nerve activity increases and catecholamine synthesis is accelerated would lead to increased tissue levels of tyrosine hydroxylase. The effects on adrenal tyrosine hydroxylase of acute *alpha*-adrenergic blockade (phentolamine), exercise, and cold exposure were studied in the rat, and the effects of acute and chronic cold exposure were investigated in the guinea pig. None of these conditions led to any significant increase in adrenal tyrosine hydroxylase activity of adrenal homogenates (Table 4).

DISCUSSION

The procedure for comparing relative rates of norepinephrine synthesis from ^{14}C-tyrosine has been used for several years (1, 12, 20). While the use of a single injection of ^{14}C-tyrosine does not permit the calculation of rates of catecholamine synthesis, it does allow changes in the rate of catecholamine synthesis to be estimated if the procedure is used as originally proposed (20). The conclusion that phenoxybenzamine increases the rate of catecholamine synthesis, originally reported in this laboratory (1), was confirmed by Reid *et al.* (30), who followed the decline in the specific activity of heart norepinephrine after the administration of radioactive norepinephrine, and also by Bigelow *et al.* (28), who demonstrated an increase in the urinary excretion of norepinephrine and metabolites after phenoxybenzamine administration.

The finding that after 2 days of phenoxybenzamine treatment the incorporation of radioactivity into heart norepinephrine was increased over the 1-day value, on a specific activity basis, but not when the data are calculated per gram of tissue (Table 2), reflects the ability of phenoxybenzamine to lower endogenous levels of heart norepinephrine (31, 32). This probably results in a decrease in the retention of ^{14}C-norepinephrine. Thus, in terms of specific activity as well as ^{14}C-norepinephrine per gram of heart, ganglionic blockade only partially reduced the phenoxybenzamine-mediated increase in incorporation of label from ^{14}C-tyrosine after 2 days of treatment, but completely abolished the increased synthesis observed following 1 day of treatment with the *alpha*-adrenergic blocking agent. Because it can be completely prevented by the administration of a ganglionic blocking agent, the increased incorporation of label from ^{14}C-tyrosine into catecholamines in heart and adrenals seen after 1 day of phenoxybenzamine treatment appears to be entirely dependent on accelerated sympathetic nervous activity. Since the additional incorporation observed after 2 days of phenoxybenzamine administration is only partially reduced following ganglionic blockade,[1] it would appear that factors other than nerve activity come into play at this time, most probably the elevations in tissue tyrosine hydroxylase levels.

The present findings represent the first demonstration that the elevation in adrenal and heart tyrosine hydroxylase produced by the repeated administration of phenoxybenzamine manifests itself *in vivo* by increasing the rate of catecholamine synthesis in the intact animal. It is probable that the increased tyrosine hydroxylase activities observed in tissue homogenates following the administration of 6-hydroxydopamine or reserpine (14, 16–18) also play a role in increasing the synthesis of catecholamines *in vivo*.

There now appear to be two mechanisms for regulating catecholamine synthesis, one involving end-product inhibition, and the

[1] There is the possibility that in animals treated for the longer period of time with phenoxybenzamine, pentolinium does not evoke complete ganglionic blockade. This was considered, but no simple experiment could be devised to rule it out. However, the dose of pentolinium tartrate employed in these studies is twice that which has been reported to produce maximal ganglionic blockade in the normal rat (33).

other, an apparent enzyme induction. Both these mechanisms exert their effects on the rate-limiting step in catecholamine synthesis, tyrosine hydroxylase (34). Regulation through end-product inhibition is a rapidly occurring event, and the synthesis rate should be capable of an almost instantaneous adjustment in response to altered physiological demands for catecholamines. Under most environmental conditions, this type of mechanism probably accounts for the bulk of the regulation necessary to maintain physiological balance with respect to catecholamines in man and animals.

Elevation of tissue levels of tyrosine hydroxylase is a relatively slower process, which so far has been evoked only by rather drastic procedures. Even conditions as severe as acute *alpha*-adrenergic blockade, exercise, or cold exposure, which markedly accelerate catecholamine synthesis *in vivo* (1, 2), do not increase the tyrosine hydroxylase levels in adrenal homogenates. The procedures which result in an apparent induction of tyrosine hydroxylase are a peripheral chemical sympathectomy with 6-hydroxydopamine (14), repeated injections of large (toxic) doses of reserpine (16, 17), or repeated administration of high doses of phenoxybenzamine. In addition, prolonged exposure to these severe stresses is required before an induction of tyrosine hydroxylase is clearly demonstrable. If one considers the relationship of the two mechanisms of end-product inhibition and enzyme induction to the function of the sympathetic nervous system, one must conclude that the former is undoubtedly in continuous operation while the latter rarely comes into play except under unusually severe conditions. It is conceivable that the prolonged and severe exercise of an athlete in training could produce the degree of sympathetic nervous activity which appears to be necessary for elevating tissue tyrosine hydroxylase levels.

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