We express our thanks to the workers of the Isotope Laboratory of this Institute for radioactivity counts.

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# Increased Synthesis of Norepinephrine in the Rat Heart on Electrical Stimulation of the Stellate Ganglia

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(Received September 23, 1966, and in revised form October 6, 1966)

SUMMARY

Electrical stimulation of the stellate ganglia results in increased formation of radioactive norepinephrine (NE) in the rat heart from L-tyrosine-$^{14}$C but not from L-dopa-$^{3}$H. These findings represent additional evidence that the activity of tyrosine hydroxylase, the rate-limiting step in NE synthesis, is increased by sympathetic nerve activity.

## INTRODUCTION

The increased sympathetic stimulation associated with exercise or exposure to cold results in accelerated synthesis of norepinephrine (NE) and epinephrine in sympathetically innervated tissues of the intact

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rat (1). The increased synthesis *in vivo* has been shown to be due to greater activity at the tyrosine hydroxylase step, and not at a subsequent stage in synthesis (2). The following experiments were undertaken to determine whether the rise in the rate of synthesis of NE in a tissue could result directly from increased stimulation of the nerve to that tissue.

## METHODS

Male Sprague-Dawley rats, weighing 350–400 g, were used in the study. Following anesthesia with pentobarbital sodium (40–60 mg/kg), the anterior chest wall was cut away and the animal was maintained by artificial respiration. Both stellate ganglia were isolated and placed on platinum electrodes. Bilateral stellate ganglia stimulation was at 30 per sec, 2 msec duration and of supramaximal voltage, using 10–15 volts. Control preparations were produced in the same way, but no stimulus was applied. Recordings of cardiac rate and contractile force were made via a force-displacement FT 10 transducer connected to a Grass Model 5A polygraph. Those animals which did not show a proper response (see below) during an initial 5-min period of stimulation were not used for the experiments. L-Tyrosine-$^{14}$C, uniformly labeled, 376 $\mu$C/$\mu$mole, was obtained from New England Nuclear Corporation. Thirty microcuries in carrier free form, was injected per animal. L-3,4-Dihydroxyphenylalanine [ring-2,5,6-$^{3}$H] 28.7 mC/$\mu$mole, was obtained from Nuclear Chicago Corporation. One hundred microcuries was diluted with 1 $\mu$mole of carrier dopa for injection into each animal. Injection in all cases was over a period of 20 sec into the femoral vein. Tissues were assayed for radioactivity and catecholamines essentially as described by Udenfriend and Zaltzman-Nirenberg (3).

The exposed hearts were subjected to electrical stimulation through the nerves for a period of 5 min; under these conditions the heart rate was increased up to 90% above control levels of about 150 per min and the amplitude by 50–200%. The electrical stimulus was discontinued, and when the cardiac activity returned to control values (within 1 min) tyrosine-$^{14}$C or dopa-$^{3}$H was injected. Electrical stimulation was started again and continued for 60 min. At the end of this time the animals were killed and heart, brain, and spleen were removed; catecholamines were isolated and their specific activities were measured.

## RESULTS

As shown in Table 1, stimulation of the stellate ganglia increased the incorporation of tyrosine-$^{14}$C into NE at least 2-fold. Because of the release of NE during electrical stimulation the increase may have been greater than this. Stimulation of the stellate ganglia did not increase significantly the incorporation of tyrosine-$^{14}$C into the NE in the tissues other than the heart (Table 2). Uptake of tyrosine-$^{14}$C into the heart and other tissues was not affected by the electrical stimulation, a result showing that the increased radioactivity in the NE is due to increased conversion of tyrosine to catecholamines. When dopa-$^{3}$H was administered electrical stimulation of the stellate ganglia did not result in increased incorporation into heart NE (Table 3) or into catecholamines of other tissues.

TABLE 1

*Effect of electrical stimulation of stellate ganglia on conversion of tyrosine-$^{14}$C to norepinephrine in rat heart*

Tyrosine-$^{14}$C was administered intravenously, and the animals were killed 1 hr later. The figures represent the results of two separate experiments.

| Treatment | Norepinephrine concentration ($\mu$g/g) | Radioactivity in norepinephrine | |
|---|---|---|---|
| | | Cpm/g heart | Cpm/$\mu$g NE |
| Expt. 1 | | | |
| Control | 0.81 | 247 | 305 |
| Control | 0.68 | 247 | 362 |
| Stimulated | 0.55 | 346 | 629 |
| Stimulated | 0.62 | 516 | 832 |
| Expt. 2 | | | |
| Control | 1.04 | 278 | 267 |
| Control | 1.01 | 278 | 275 |
| Stimulated | 0.42 | 573 | 1364 |
| Stimulated | 0.57 | 511 | 896 |

TABLE 2
*Incorporation of tyrosine-$^{14}C$ into norepinephrine in heart, brain, and spleen*

| Tissue | Radioactivity in norepinephrine[a] | |
|---|---|---|
| | Control | Stimulated |
| Heart | 262 ± 8 | 487 ± 42 |
| Brain | 181 ± 12 | 202 ± 11 |
| Spleen | 77 ± 8 | 99 ± 14 |

[a] The figures represent the mean ± S.E. stated as counts per minute per gram of tissue. There were four animals in each group.

For some reason the release of endogenous NE with stimulation was not as great in these experiments as in the ones with tyrosine-$^{14}C$. However, it may be noted that incorporation of dopa-$^{3}H$ into NE was lowered as a result of the stimulation. This would be expected if stimulation increased the conversion of endogenous (nonradioactive) tyrosine to dopa. The latter would dilute the injected radioactive material. The effects of dilution of administered dopa-$^{3}H$ by endogenously formed dopa were reported in a previous communication (4).

TABLE 3
*Effect of electrical stimulation on conversion of Dopa-$^{3}H$ to norepinephrine in rat heart*

Dopa-$^{3}H$ was administered intravenously, and the animals were killed 1 hr later. The figures represent the results of two separate experiments.

| Treatment | Norepinephrine concentration (μg/g heart) | Radioactivity in norepinephrine | |
|---|---|---|---|
| | | Cpm/g heart | Cpm/μg NE |
| Expt. 1 | | | |
| Control | 0.86 | 5417 | 6299 |
| Control | 0.96 | 5617 | 5851 |
| Stimulated | 0.81 | 5112 | 6311 |
| Stimulated | 0.87 | 4129 | 4746 |
| Expt. 2 | | | |
| Control | 0.89 | 4980 | 5596 |
| Control | 0.95 | 4615 | 4858 |
| Stimulated | 0.80 | 4262 | 5328 |
| Stimulated | 0.82 | 4365 | 5323 |

## DISCUSSION

The present experiments demonstrate that electrical stimulation of the sympathetic nerves to the heart increases the rate of synthesis of NE in the heart. Since there was no significant increase in the radioactivity of catecholamines in the other organs, it must be concluded that even though the stimulation was carried out in the exposed heart *in situ* the effect was a direct one leading from the stellate ganglia to the heart. Since electrical stimulation influenced incorporation of radioactivity from radioactive tyrosine but not from radioactive dopa, it must also be concluded that the stimulation acts at the tyrosine hydroxylase step. Thus, whether by direct nerve stimulation or indirectly by exercise or exposure to cold, increased sympathetic activity induces increased NE synthesis by accelerating the rate-limiting step in NE synthesis, tyrosine hydroxylase (1).

That electrical stimulation can increase epinephrine synthesis in the adrenal gland was first shown in experiments by Bygdeman and Euler (5) and Holland and Schumann (6). Subsequently, studies in our laboratories showed that increased synthesis of NE occurred in sympathetically innervated organs when animals were subjected to conditions which produce marked sympathetic stimulation (1, 7, 8). More recently, experiments by Weiner (9) and Euler and associates (10) showed that electrical stimulation increases incorporation of radioactive tyrosine into the NE of the isolated guinea pig vas deferens. Sedvall and Kopin (11) have found that in the salivary gland perfused *in situ* electrical stimulation of the nerve results in increased synthesis of NE. As in the present experiments incorporation from radioactive tyrosine was increased by the electrical stimulation whereas incorporation from radioactive dopa was not. The findings in all these different laboratories indicate that stimulation of the sympathetic innervation to an organ induces increased synthesis of NE in that organ. Our experiments and those of Sedvall and Kopin (11) show further that it is the tyrosine hydroxylase ac-

tivity which is increased by the nerve stimulation.

It is well known that an intact sympathetic innervation is necessary for continued NE synthesis. It was recently shown that several days after stellate ganglionectomy in the rat, tyrosine hydroxylase activity as well as NE concentration, fall to negligible levels.[3] However, the mechanism underlying the effects of nerve stimulation is not yet apparent. Stimulation does not increase the amount of enzyme present in the heart or other organs as shown by direct assay for the enzyme in homogenates of the stimulated tissues (2). Furthermore, inhibitors of protein synthesis, cycloheximide etc., do not block the increased synthesis produced by exercise or exposure to cold (2). The possibility of an allosteric activator produced by nerve stimulation has been considered. However, the purified enzyme is not stimulated by the obvious substances involved in nerve stimulation including acetylcholine, $K^+$, $Na^+$, $Ca^{++}$, $Mg^{++}$, ATP, ADP, and DPN (12).

A mechanism which must be considered seriously involves end-product inhibition. This has been discussed in previous publications from these laboratories (1, 13) and by Neff and Costa (14). We have shown that NE and other catecholamines inhibit purified tyrosine hydroxylase (15). Conceivably NE in the nerve is present in association with tyrosine hydroxylase. Stimulation of the nerve releases the inhibitor and might thus result in an increased rate of synthesis of NE. When stimulation stops, the NE again accumulates and the rate of synthesis returns to control levels. Whatever the mechanism, it is clear that any model of the sympathetic nerve ending must include consideration of variations in rates of synthesis of the neurotransmitter.

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