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Electrical stimulation of preganglionic nerve increases tyrosine hydroxylase activity in sympathetic ganglia

(tyrosine 3-monooxygenase/superior cervical ganglion)

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ABSTRACT **The effect of synaptic stimulation on tyrosine hydroxylase [tyrosine 3-monooxygenase; L-tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] activity in the rat superior cervical ganglion was studied. The preganglionic cervical sympathetic trunk was stimulated unilaterally at 10 Hz for 30 min. Forty-eight hours later tyrosine hydroxylase activity was 33% higher on the stimulated than on the control side. The enzyme activity remained elevated in the stimulated ganglia for 2 days. No change was observed in total ganglion protein. Comparable increases in tyrosine hydroxylase activity were observed in anesthetized and conscious animals.**

The activity of the enzyme tyrosine hydroxylase [tyrosine 3-monooxygenase; L-tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2], which catalyzes the rate-limiting step in norepinephrine synthesis, has been shown to increase in the rat superior cervical ganglion 1–2 days after a systemic injection of reserpine (1). This increase in enzyme activity can be prevented by cutting the preganglionic cervical sympathetic trunk (2) or by injection of nicotinic receptor-blocking drugs such as chlorisondamine or pempidine (3) prior to reserpine administration.

These results have been repeatedly interpreted to indicate a causal relationship between the firing rate of the preganglionic cholinergic nerves and the activity of tyrosine hydroxylase in the ganglion cell bodies (e.g., ref. 4). This claim is based on the hypothesis that reserpine increases the firing rate in the preganglionic nerves either as a reflex resulting from the loss of peripheral sympathetic tone or as a result of an action of reserpine in the central nervous system. However, there is no direct evidence that an increase in firing rate occurs in the preganglionic cervical sympathetic trunk after reserpine treatment. In fact, Iggo and Vogt (5), who are often said to have shown such an effect in the cat, did not come to this conclusion, but instead stated that the "preganglionic sympathetic discharge both spontaneous and in response to stimuli was not fundamentally altered by doses of reserpine which abolished all peripheral sympathetic activity and caused severe depletion of the stores of noradrenaline and 5-hydroxytryptamine in the brain." One alternative explanation of the decentralization and ganglionic blockade experiments is that there is no change in synaptic stimulation of the ganglion after reserpine but that a tonic cholinergic input to the ganglion cell bodies plays a permissive role allowing the cells to respond to other (e.g., humoral) factors.

To investigate directly whether an increase in synaptic stimulation of the superior cervical ganglion alters ganglionic tyrosine hydroxylase activity, we have studied the effects of electrically stimulating the preganglionic trunk. The results demonstrate that a brief period of electrical stimulation produces a delayed increase in tyrosine hydroxylase activity. A preliminary report of this work was presented to the Physiological Society (6).

METHODS

Male Long–Evans rats (200–275 g) were anesthetized with Equithesin (4 ml/kg, intraperitoneally, Jensen–Salsbury Laboratories, Kansas City, MO). The preganglionic cervical sympathetic trunks on both sides of the animal were exposed, separated from the common carotid artery and the vagus nerve, and cut 6–8 mm from the ganglion. The distal portion of the trunk on one side was then stimulated at 10 Hz with pulses of 2 msec duration and approximately 100 μA current with a suction electrode. The effectiveness of the stimulation was monitored by observing the response of the animal's eye. Stimulation was only considered adequate if the animals exhibited exopthalamos, pupillary dilation, and retraction of the eyelid on the stimulated side. The nerve trunk was stimulated for 30 min, and then the incision was sewn up and the animal was returned to its cage. Animals were killed in groups of seven or more at various intervals after the stimulation, and both superior cervical ganglia were removed and stored at −20°. To assay tyrosine hydroxylase activity, individual ganglia were homogenized in ground glass homogenizers in 40 μl of 5 mM Tris·HCl buffer at pH 6.0 containing 0.1% (vol/vol) Triton X-100, and enzyme activity was measured in 10-μl aliquots using L-[*side chain* 2,3-^{3}H]tyrosine as substrate (8 μM) and 6,7-dimethyl-5,6,7,8-tetrahydropterin as cofactor (1 mM) (7). Ganglia from unoperated animals were included in each assay as controls. For the 72-hr group, the total protein content of the ganglion homogenates was determined by the method of Lowry *et al.* with bovine serum albumin as the standard (8).

RESULTS

Control unoperated animals showed no significant differences in tyrosine hydroxylase activity between the right and the left superior cervical ganglia (Fig. 1). Similarly, there was no significant difference in enzyme activity between the two ganglia 24 or 36 hr after unilateral stimulation of the preganglionic cervical sympathetic trunk. However, 48 hr after a 30-min stimulation, tyrosine hydroxylase activity was 33% higher on the stimulated side. The enzyme activity remained significantly higher on the stimulated side up to 96 hr after stimulation, after which no significant difference could be detected between the two sides. Because the tyrosine hydroxylase activity reached

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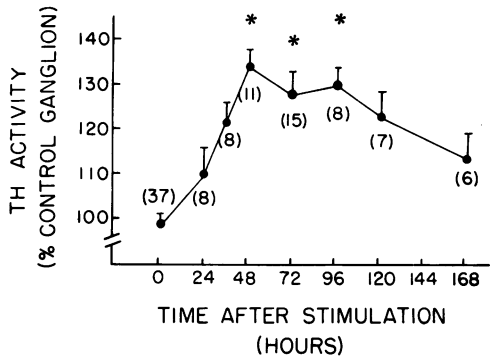

FIG. 1. Effect of increased synaptic stimulation on tyrosine hydroxylase (TH) activity in the rat superior cervical ganglion. The preganglionic trunk was stimulated unilaterally for 30 min at 10 Hz, and tyrosine hydroxylase activity was assayed at the times indicated. The enzyme activity is expressed as the ratio of the activity in the stimulated ganglion to that in the control ganglion × 100 for each animal. Each point represents the mean ± SEM for the number of animals shown in parentheses. * indicates that there is a significant difference ($P < 0.05$ by Student's two-tailed t test) between stimulated and control ganglia. The zero time control group represents unoperated animals for which the enzyme activity is expressed as a ratio of the right to left ganglion. The mean enzyme activity in these control animals was 68.2 ± 6.0 pmol of dopa formed/ganglion per hr.

a plateau about 72 hr after stimulation (see Fig. 1), all subsequent experiments were done at this time.

As would be expected from previous studies, there was no significant decrease in enzyme activity in the unstimulated decentralized ganglia during the relatively short period of this experiment (i.e., a maximum of 7 days after decentralization) (9). Thus, the change in enzyme activity in the synaptically stimulated ganglion represents an increase in activity over that found in control (unoperated) animals. Because in this experiment the concentration of tyrosine was below the K_m for the enzyme, we assayed a group of stimulated and control ganglia using a higher concentration of tyrosine (80 μM) and found a similar 30% increase on the stimulated side. This change in tyrosine hydroxylase is not acompanied by an increase in total ganglion protein content.

All the stimulation experiments described so far were performed in decentralized animals to avoid changes in sympathetic activity resulting from the anesthesia and surgery or from the possible central effects of the stimulation. In order to see whether an increase in tyrosine hydroxylase activity could also be demonstrated in animals that had not been decentralized, a group of five rats were stimulated unilaterally with silver wire electrodes placed on the intact preganglionic trunk. When tyrosine hydroxylase activity was measured 72 hr later, the enzyme activity was 38% higher on the stimulated than on the control side (Table 1).

In a further group of rats, preganglionic stimulation was performed in freely moving animals. For this purpose, rats were anesthetized, their preganglionic cervical sympathetic trunks were cut, and silver wire stimulating electrodes were wound around one of the preganglionic trunks, several millimeters proximal to the superior cervical ganglion, and then connected to a terminal block that was fixed by dental cement to the animal's skull. Two hours later when the animals had recovered from anesthesia and were moving about their cages, the preganglionic trunk was stimulated at 50 Hz. As in the anesthetized animals, the responses of the eye were used to check that the ganglion was being stimulated. Seventy-two hours after the stimulation, tyrosine hydroxylase activity was increased in the stimulated ganglia by an average of 64% (Table 1).

Table 1. Effect of preganglionic nerve stimulation under various conditions on tyrosine hydroxylase activity in the rat superior cervical ganglion

Experimental conditions	TH activity, (stimulated/control) × 100
1. Controls	99 ± 4 (37)
2. Stimulation in intact, anesthetized animals	138 ± 12 (5)
3. Stimulation in decentralized, conscious animals	164 ± 13 (6)

The tyrosine hydroxylase (TH) activity in the stimulated ganglia is expressed as a percentage of that found in the contralateral control. Mean percentages ± SEM for the number of animals shown in parentheses are given. In control animals the right ganglion is compared to the left to show there is no inherent asymmetry. Intact preganglionic nerves were stimulated at 10 Hz for 30 min with pulses of 2 msec duration. In the experiments on conscious rats, the preganglionic trunk was stimulated at 50 Hz for 30 min with pulses of 2 msec duration.

DISCUSSION

These results show that electrical stimulation of the preganglionic trunk for a short period in animals either under anesthesia or moving freely in their cages results in a delayed increase in tyrosine hydroxylase activity in the superior cervical ganglion and that the enzyme activity remains elevated for at least 2 days. The time course of this effect is clearly different from the short-term effects of nerve stimulation on tyrosine hydroxylase activity observed in the guinea pig hypogastric nerve–vas deferens preparation (10, 11) and in certain adrenergic neurons in the brain (12, 13). In these studies nerve stimulation produces an increase in tyrosine hydroxylase activity within minutes of the onset of stimulation. Furthermore, in one of the studies, it was shown that the enzyme activity rapidly returned to normal when the stimulation was terminated (13). The increase in tyrosine hydroxylase activity we have described is first seen more than 24 hr after nerve stimulation and persists for at least 48 hr. It is quite likely that different biochemical mechanisms underlie these two effects of nerve stimulation on tyrosine hydroxylase activity—one perhaps involving activation of existing enzyme molecules, the other involving an increase in the number of enzyme molecules.

Increases in ganglionic tyrosine hydroxylase activity following time courses similar to those reported here have been found after cold stress and reserpine treatment (4, 14, 15), and recent studies using immunochemical techniques suggest that these latter changes result from an increase in the number of tyrosine hydroxylase molecules (16). However, whether the effects of cold stress and reserpine treatment are mediated by increased neural firing remains to be determined. In the present study using electrical stimulation we have been able to demonstrate directly that an increase in synaptic activity produces a delayed increase in tyrosine hydroxylase activity. This system should prove useful for further studies of the mechanisms involved in the long-term regulation of this enzyme.

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