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EFFECTS OF SYNAPTIC AND ANTIDROMIC STIMULATION ON TYROSINE HYDROXYLASE ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION

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SUMMARY

1. The effects of orthodromic and antidromic stimulation of the rat superior cervical ganglion on the specific activity of the enzyme tyrosine hydroxylase have been studied.

2. Orthodromic stimulation of the ganglion via the cervical sympathetic trunk produced an increase in the activity of tyrosine hydroxylase when measured 3 days later while causing no change in the protein content of the ganglion. This increase in the specific activity of tyrosine hydroxylase was blocked by administration of the nicotinic antagonist, hexamethonium.

3. Antidromic stimulation of the superior cervical ganglion by stimulating the internal carotid nerve, the external carotid nerve or both nerves simultaneously produced no change in the specific activity of tyrosine hydroxylase.

4. Parallel increases in tyrosine hydroxylase activity and protein content per ganglion were seen when the internal carotid nerve was stimulated but similar changes were seen in 'sham-stimulated' animals. These 'non-specific' changes were apparently produced by the trauma involved in the extensive dissection necessary to position electrodes on this nerve trunk.

5. We conclude that an increased frequency of firing in post-ganglionic neurones is not a sufficient stimulus to elevate the specific activity of tyrosine hydroxylase. Rather some other aspect of nicotinic receptor stimulation seems to be required.

INTRODUCTION

Excitation of the cervical sympathetic trunk by electrical stimulation has been shown to produce an increase in the activity of the enzyme tyrosine hydroxylase (TH) (tyrosine 3-monooxygenase, E.C. 1.14.16.2), in the superior cervical ganglion (s.c.g.) of the rat. This enzyme is localized in adrenergic neurones and catalyzes the rate-limiting step in the synthesis of noradrenaline. The increase in TH activity reaches a maximum between 48 and 96 hr and occurs in the absence of any significant change in the total protein content of the ganglion (Zigmond & Ben Ari, 1977; Zigmond & Chalazonitis, 1979). The magnitude of the increase depends both on the frequency and on the duration of preganglionic nerve stimulation.

In the present study, the mechanism by which preganglionic nerve activity alters

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the activity of an enzyme present in post-ganglionic neurones has been examined. First, it will be shown that the increase in the specific activity of TH can be prevented by blocking nicotinic transmission in the ganglion using hexamethonium, a nicotinic antagonist. Secondly, it will be shown that antidromic stimulation of the ganglion does not mimic the effects of orthodromic stimulation. These results suggest that some consequence of nicotinic receptor stimulation other than, or in addition to, the triggering of action potentials is necessary for the increase in the specific activity of TH to occur. A preliminary report of this work was presented to the Society for Neuroscience (Chalazonitis & Zigmond, 1977).

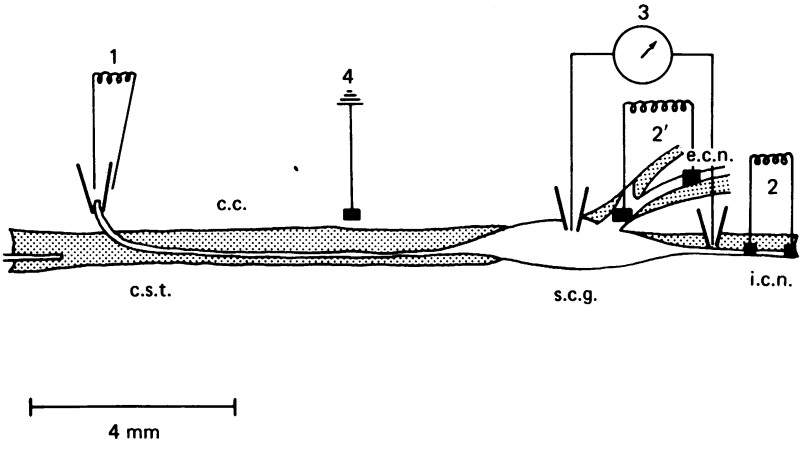

Fig. 1. The arrangement of stimulating and recording electrodes used in the present study. 1, a suction electrode is used for orthodromic stimulation of the distal cut end of the cervical sympathetic trunk (c.s.t.); 2 and 2′, bipolar electrodes filled with 0·9% NaCl agar gel are placed, respectively, on the intact internal carotid nerve (i.c.n.) and on the external carotid nerve (e.c.n.) and used for antidromic stimulation; 3, a double lumened suction electrode placed on the surface of the superior cervical ganglion (s.c.g.) is used to record differentially the elicited compound ganglion action potentials; 4, earthing electrode; c.c., common carotid artery.

METHODS

Long-Evans male rats (Charles River Breeding Laboratories) 150–175 g were housed in individual cages on a 12 hr/12 hr light/dark cycle for a week prior to all experiments. The animals were given food and water *ad libitum*. The rats were anaesthetized with chloral hydrate (Sigma) dissolved in isotonic NaCl at a dose of 450 mg/kg, I.P. In some experiments the animals were re-injected with chloral hydrate periodically to maintain anaesthesia for up to 3 hr.

To stimulate the ganglion orthodromically, the preganglionic cervical sympathetic trunks were cut on both sides of the animal about 7 mm before they enter the ganglion. The distal end of one of the trunks was drawn into a glass suction electrode containing chloridized silver wires (arrangement 1 in Fig. 1). In experiments in which the s.c.g. was stimulated antidromically, both cervical sympathetic trunks were cut and the s.c.g. and a few mm of its two major post-ganglionic trunks (the internal carotid nerve and the external carotid nerve) were exposed. Both of these post-ganglionic nerves remained uncut and one or both were stimulated on one side of the animal via bipolar glass electrodes filled with agar gel in isotonic saline and containing chloridized silver wires (arrangements 2 and 2′ in Fig. 1). The stimulating cathode was proximal to the ganglion. In both the orthodromic and the antidromic experiments, the same biochemical results were found whether the right or the left s.c.g. was stimulated.

The dissection required to reach the post-ganglionic trunks of the s.c.g. in the rat is quite extensive. In most experiments these trunks were exposed and stimulated only on one side of the animal. The TH activity in the s.c.g. on that side was subsequently compared both to that in the contralateral ganglion and to that in an s.c.g. from a second animal in which electrodes were put in position, but current was not turned on ('sham stimulation'). In a few experiments, antidromic stimulation of the internal carotid nerve was performed in ganglia that had been decentralized 9–11 days previously.

Pre- and post-ganglionic stimulation was performed using 0·5 msec pulses and current intensities ranging from about 250 to 500 μA (for orthodromic stimulation) and 500 to 1000 μA (for antidromic stimulation). The intensity was set at a level 50 % higher than that required to produce a maximal compound ganglionic response, recorded as described below. Stimuli were either delivered at 10 Hz continuously or in 40 Hz trains. In the latter case, each train lasted 250 msec and was followed by a 500 msec pause. The total duration of stimulation was 30, 60 or 90 min. Excitation of the ganglionic neurones was monitored with a suction electrode (Fig. 1). The signals were fed into an a.c. coupled differential amplifier (band width 0·1–1·0 kHz), and displayed on a storage oscilloscope. During the period of stimulation the body temperature of the rat was maintained at 37 °C using an electric blanket.

Hexamethonium and chlorisondamine were administered to block nicotinic, ganglionic transmission. Hexamethonium chloride (ICN Pharmaceuticals Inc.) was infused at a dose of 40–60 mg/kg via a cannula inserted into the saphenous vein. Chlorisondamine (Ciba-Geigy) was injected s.c. at 15 mg/kg. Both drugs were dissolved in isotonic saline.

At the end of the stimulation, the dissected area was closed and the animal returned to its cage. In all experiments the rats were sacrificed 72 hr later with ether vapour and both s.c.g. were removed and stored at −80 °C. Ganglia were also removed from unoperated animals received in the same shipment as the experimental animals. In one experiment where the s.c.g. was bisected, a cut was made across the short axis of the ganglion at the level of the external carotid nerve dividing the ganglion into a rostral part, whose neurones have been shown to project primarily out the internal carotid nerve, and a caudal part, whose neurones have been shown to project primarily out the external carotid nerve (see Fig. 5 in Bowers & Zigmond, 1979).

TH activity was assayed by measuring the production of [^{3}H]DOPA from [^{3}H]tyrosine as previously described (Zigmond & Chalazonitis, 1979). Tetrahydrobiopterin (0·7 mM) was used as the cofactor. Ganglion protein content was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as the standard. Student's paired t test (two-tailed) was used to compare enzyme activity in stimulated and contralateral control ganglia. Comparisons between stimulated and 'sham-stimulated' ganglia were made using the t test for two means (two-tailed). All data are expressed as mean values ± S.E. of the mean. When a percentage change is given, the value is the mean percentage difference between the stimulated and contralateral ganglia

$$\left(\frac{\text{stimulated ganglion}}{\text{contralateral ganglion}} \times 100\right).$$

RESULTS

I. *Effects of a nicotinic, ganglionic-blocking drug on the increase in TH activity obtained by orthodromic stimulation of the s.c.g.*

To assess the role of nicotinic transmission in the s.c.g. in the increase of TH activity produced by orthodromic stimulation, the preganglionic trunk was stimulated after administration of hexamethonium. Slow intravenous infusion of the nicotinic antagonist at a dose of 15–20 mg/kg, 15 min prior to the beginning of the stimulation, blocked the ganglion compound action potentials elicited by 40 Hz trains of pulses (Fig. 2). However, in order to maintain this blockade for 90 min, animals had to be infused with lower doses of hexamethonium (5 mg/kg) every

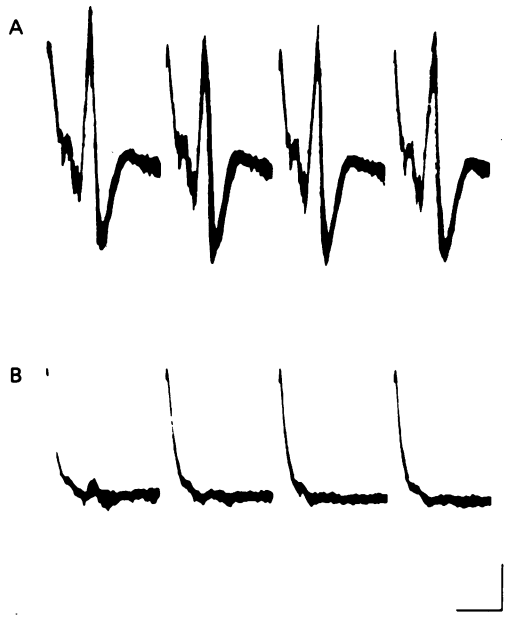

Fig. 2. Blockade by hexamethonium of transmission in the s.c.g. during orthodromic stimulation. *A*, control ganglionic response to orthodromic stimulation (40 Hz trains). The responses to the first four pulses of the train are shown. *B*, ganglion response following an intravenous injection of hexamethonium (25 mg/kg). Note that the action potentials are blocked except following the first stimulus of the train where a weak response persists. The four stimulus artifacts can be seen. By re-injecting hexamethonium the s.c.g. responses could be blocked for 90 min. Traces *A* and *B* are obtained from five superimposed oscilloscope sweeps. Calibrations: 100 μV, 10 msec.

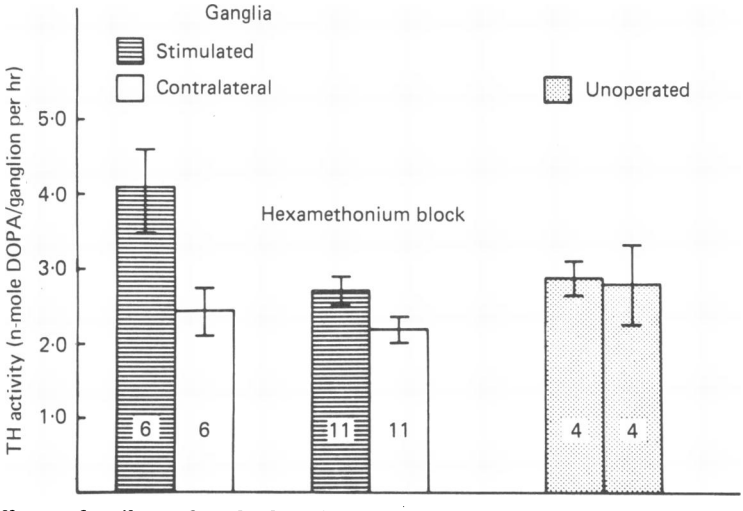

Fig. 3. Effects of unilateral orthodromic stimulation (40 Hz trains for 90 min) on the ganglionic TH activity (in n-mole DOPA/ganglion per hr ± S.E. of mean) in control and in hexamethonium-treated rats. In uninjected animals TH activity was on average 72% higher in stimulated than in contralateral control ganglia. In hexamethonium-treated animals, TH activity in stimulated ganglia was only 30% higher than in contralateral control ganglia. There was no significant difference in TH activity between right and left ganglia in unoperated animals. The number of animals in each group is indicated at the bottom of each bar.

10 min. The average total dose of hexamethonium administered ranged from 40–60 mg/kg. In some animals, it was impossible to block completely the ganglion response to the first stimulus of each train without using doses of hexamethonium that lead to the death of the animals during the subsequent 72 hr. However, even when the blockade was not total, only an extremely small ganglionic response remained (Fig. 2*B*), accompanied by a slight retraction of the eyelid on the ipsilateral side.

TABLE 1. Blockade of the increase in the specific activity of TH in the s.c.g. after preganglionic nerve stimulation

Drug treatment	Ganglia	*n*	TH Activity (n-mole DOPA per mg protein per hr)
None	Stimulated	5	14·35 ± 0·69*
	Contralateral	5	10·04 ± 0·80
Hexamethonium	Stimulated	6	10·63 ± 0·53
	Contralateral	6	9·18 ± 0·51

The cervical sympathetic trunk was stimulated unilaterally at 10 Hz for 60 min and 72 hr later the animals were sacrificed. TH activity and total protein content were measured in the synaptically stimulated and contralateral control ganglia. One group of animals was injected with hexamethonium at a mean dose of 42 ± 8 mg/kg. The data are expressed as the mean values ± S.E. of mean.

* Signifies that the difference between enzyme activity in stimulated and in contralateral control ganglia was significant ($P < 0·05$).

Administration of hexamethonium using the protocol described above reduced the effect of preganglionic nerve stimulation on TH activity. In control animals stimulation of the cervical sympathetic trunk by 40 Hz trains for 90 min led to an average increase in TH activity in stimulated ganglia, compared to contralateral control ganglia, of 72 %. In animals injected with hexamethonium a smaller (30 %) though significant increase in TH activity was seen (Fig. 3). Furthermore, the absolute activity of the enzyme in stimulated ganglia was significantly higher in the control animals than in those treated with hexamethonium ($P < 0·01$). In contrast, the TH activity in the contralateral control ganglia in these two groups of animals was not significantly different, suggesting that hexamethonium by itself had no significant effect on the enzyme activity (Fig. 3).

In a second experiment hexamethonium completely blocked the increase in TH activity produced by preganglionic nerve stimulation. In this case hexamethonium was administered as described above; however, only animals that displayed no eye response during stimulation were included in the experiment. The cervical sympathetic trunk was stimulated at 10 Hz continuously for 60 min, and 72 hr later both the TH activity and the protein content of the ganglia were measured. Under these conditions, the specific activity of TH in the stimulated ganglia increased by 40 % over that in the contralateral ganglia in the animals which had not been injected. However, there was no significant increase in the specific activity of the enzyme in hexamethonium-treated rats (Table 1).

In other groups of animals (not shown here) it was found that neither decentralization by itself nor decentralization plus hexamethonium altered the specific activity of TH.

II. *Effects of antidromic stimulation of the s.c.g. on the activity of TH*

1. Recordings of the compound ganglionic responses elicited by antidromic stimulation of the internal and external carotid nerves

Compound action potentials could be recorded from the surface of the s.c.g. following stimulation of either the internal or external carotid nerves (Fig. 4). The amplitude of these potentials varied depending on the exact position of the recording electrode on the ganglion. For instance, the compound potential elicited by stimula-

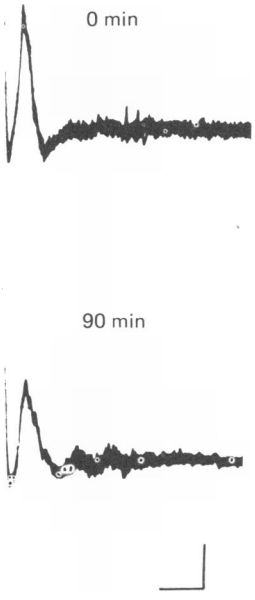

Fig. 4. Compound ganglion action potentials elicited by antidromic stimulation of the internal carotid nerve. The internal carotid nerve was stimulated at 10 Hz for 90 min with 0·5 msec pulses using 750 μA current. The top traces show the first and the bottom traces, the last compound action potentials recorded from the surface of the ganglion. The area under the latter potential is 90 % of that under the former. The traces are obtained from five superimposed oscilloscope sweeps. Calibrations: 40 μV, 10 msec.

tion of the external carotid nerve was larger if the electrode was placed on the caudal portion of the s.c.g. than if it was placed on the rostral portion. The opposite was found for stimulation of the internal carotid nerve. These results are in agreement with the recent anatomical findings of Bowers & Zigmond (1979) that the cell bodies of neurones projecting into the internal carotid nerve are localized mainly in the rostral part of the ganglion and the neurones projecting into the external carotid nerve are mainly localized in the caudal part. Administration of hexamethonium (32 mg/kg, I.V.) or chlorisondamine (15 mg/kg, S.C.) at concentrations which abolished the compound ganglionic potential elicited by stimulation of the cervical sympathetic trunk had no detectable effect on the amplitude of the compound action potential elicited by stimulation of either the internal carotid nerve or the external carotid nerve.

To ensure that the responses of the ganglion cells to antidromic stimulation could be maintained for the entire duration of stimulation used in these studies, recordings obtained at the beginning and at the end of the stimulation periods were compared. The size of the compound action potential was determined by measuring the area included between the ganglion potential base line and the negative peak of the triphasic response. No diminution in the size of the compound action potential was seen after stimulation of the internal carotid nerve for up to 90 min or of the external carotid nerve for up to 60 min (Table 2 and Fig. 4).

TABLE 2. Stability of the size of the compound ganglion action potential elicited by antidromic stimulation of the s.c.g.

Post-ganglionic trunk stimulated	Parameters of Stimulation			Final ganglion potential as % of initial response
	Frequency	Mode	Duration	
Internal carotid	10 Hz	Continuous	30 min	105 ± 9 (5)
Internal carotid	10 Hz	Continuous	60 min	98 ± 4 (3)
External carotid	10 Hz	Continuous	60 min	118 ± 13 (4)
Internal carotid	40 Hz	Trains	90 min	103 ± 8 (5)

The internal carotid and external carotid nerves were stimulated as described above and the elicited compound action potentials recorded from the s.c.g. The size of the final ganglion potential is expressed as a percentage of the first response ± S.E. of mean. The number of animals studied is shown in parentheses.

2. Effect of stimulation of the internal carotid nerve on TH activity in the s.c.g.

In the guinea-pig, Perri, Sacchi & Casella (1970) have shown that antidromic stimulation of the internal carotid nerve produces a synaptic response in the s.c.g. which can be blocked by a nicotinic antagonist. While the surface recordings referred to above obtained in the presence and in the absence of ganglionic blocking drugs provide no evidence that a substantial amount of nicotinic transmission occurs in the rat upon stimulation of the internal carotid nerve, we sought to ensure that any change in TH activity seen after stimulation of that trunk would be due to antidromic stimulation rather than to synaptic stimulation via such axon collaterals. Therefore, our initial experiments were performed on animals whose s.c.g. had been previously decentralized.

Antidromic stimulation of the internal carotid nerve was performed at 10 Hz for 30 min in a series of rats whose s.c.g. had been decentralized bilaterally 9–11 days previously. Under these conditions, the TH activity per ganglion was significantly higher in stimulated ganglia than in contralateral control ganglia or in ganglia from decentralized or unoperated control animals (Table 3). However, when the TH activity was expressed per mg protein, the values for all four groups were similar (Table 3).

Since stimulation of the internal carotid nerve at 10 Hz for 30 min produced no change in the specific activity of TH, an experiment was performed in which the internal carotid nerve was stimulated for twice as long. In this experiment, the animals were not decentralized prior to the day of the experiment. In addition 'sham-stimulated' animals were included (see Methods). Both the TH activity and

TABLE 3. Effects of antidromic stimulation of the internal carotid nerve on TH activity in previously decentralized s.c.g.

Ganglia	n	TH activity: n-mole DOPA/ganglion per hr	TH activity: n-mole DOPA/mg protein per hr
Stimulated	7	2·12 ± 0·30*	13·1 ± 1·8
Contralateral control	7	1·49 ± 0·14	13·8 ± 1·8
Decentralized control	4	1·32 ± 0·14	12·0 ± 1·7
Unoperated control	4	1·47 ± 0·17	15·4 ± 1·2

Seven rats were decentralized 9–11 days prior to the day of stimulation. The s.c.g. was stimulated antidromically at 10 Hz for 30 min via the internal carotid nerve. Three days later the animals were sacrified, and stimulated and contralateral control ganglia were assayed for TH activity and protein content. Ganglia from four animals decentralized 14 days before sacrifice and from four unoperated animals were also examined. The data are expressed as mean values ± S.E. of mean. n = the number of ganglia of each type.

* Indicates a significant difference between the TH activity of stimulated ganglia and that of their contralateral controls ($P < 0{\cdot}01$).

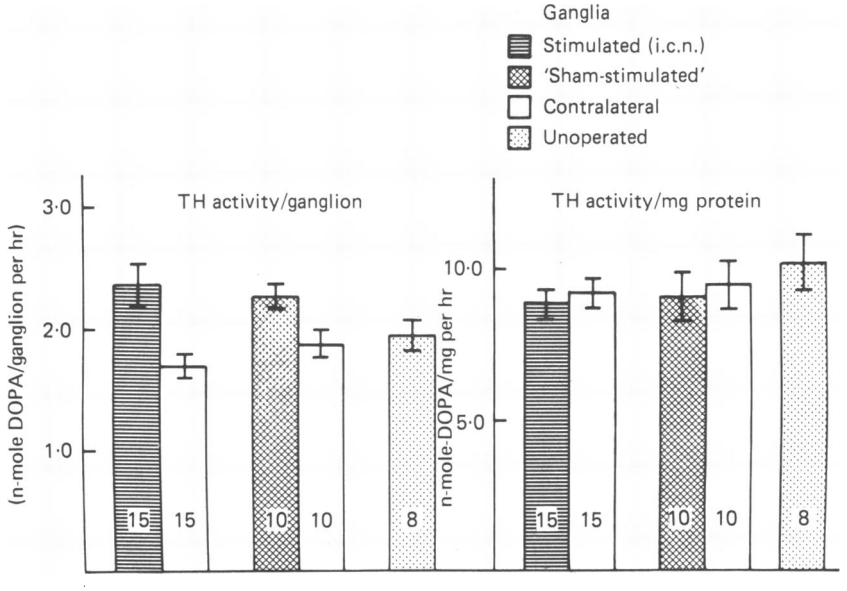

Fig. 5. Effect of antidromic stimulation of the internal carotid nerve (i.c.n.) on the specific activity of TH in the s.c.g. The internal carotid nerve was stimulated unilaterally at 10 Hz for 60 min. In a separate group of animals an electrode was placed on the internal carotid nerve unilaterally but no current was applied ('sham-stimulation'). Seventy-two hr later TH activity was higher in the stimulated and in the 'sham-stimulated' ganglia than in their respective contralateral control ganglia or in ganglia from unoperated control animals. The data are expressed as the means ± S.E. of mean. The number of ganglia in each group is shown for each bar.

the protein content were higher in the antidromically stimulated ganglia than in their contralateral control ganglia (45 % and 46 % increase respectively) (Fig. 5). Similarly the 'sham-stimulated' ganglia had a higher TH activity (28 %) and protein content (36 %) than their contralateral controls. As in the previous experiment, no difference was found in the specific activity of TH in the different experimental groups (Fig. 5).

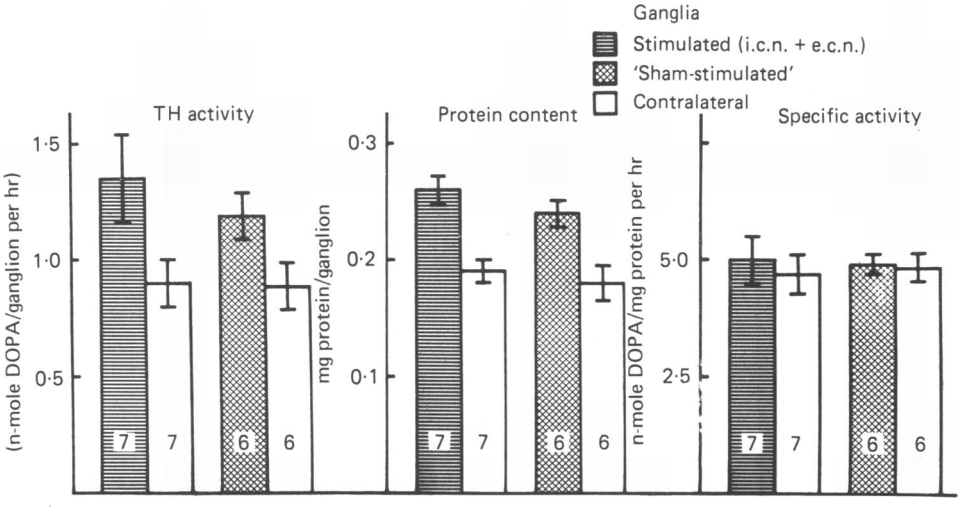

Fig. 6. Effects of antidromically stimulating the s.c.g. via both the internal and external carotid nerves (i.c.n. and e.c.n.) on the specific activity of TH. The two major post-ganglionic nerves of the s.c.g. were stimulated at 10 Hz for 60 min in the presence of the nicotinic ganglionic antagonist, chlorisondamine. In a second group of chlorisondamine-treated animals, the nerves were 'sham-stimulated'. Seventy-two hr later similar increases in TH activity and protein content per ganglion were found in stimulated and sham stimulated ganglia when they were compared to their respective contralateral controls. No difference was found in the specific activity of TH between the four groups of ganglia.

3. Effect of stimulation of both the internal and external carotid nerves on TH activity in the s.c.g.

Since the internal carotid nerve is only one of the post-ganglionic trunks of the superior cervical ganglion, a smaller proportion of neurones would be expected to be excited by stimulation of this trunk than by orthodromic stimulation via the cervical sympathetic trunk. Therefore experiments were performed in which both major post-ganglionic trunks of the s.c.g., the internal carotid nerve and the external carotid nerve, were stimulated synchronously. To prevent any possible synaptic activation of the neurones which might occur upon antidromic stimulation of these post-ganglionic nerves, the nicotinic antagonist, chlorisondamine, was injected subcutaneously 60 min prior to stimulation, at a dose (15 mg/kg) known to block ganglionic transmission but not antidromic responses (see Results II, 1). Under these conditions, ganglia were stimulated via both post-ganglionic trunks at 10 Hz for 60 min. As in the previous experiments, an increase in both TH and protein content

was seen in both antidromically and 'sham-stimulated' ganglia (Fig. 6). However, here again no increase in specific activity of TH was seen. A second experiment in which the internal and external carotid nerves were stimulated simultaneously with 40 Hz trains for 90 min produced similar results.

Since 'sham-stimulation' of the post-ganglionic trunks of the s.c.g. produced increases in both TH activity and protein content similar to those seen after antidromic stimulation, we concluded that these 'non-specific' changes were due to the trauma caused by the extensive dissection necessary to reach the post-ganglionic trunks.

TABLE 4. Effects of antidromic stimulation of the external carotid nerve on TH activity in the rostral and caudal portions of the s.c.g.

		TH activity (n-mole DOPA per portion per hour)	
Ganglia	n	Rostral	Caudal
Stimulated	12	$1{\cdot}94 \pm 0{\cdot}22$	$4{\cdot}31 \pm 0{\cdot}40$
Contralateral	12	$1{\cdot}54 \pm 0{\cdot}19$	$3{\cdot}64 \pm 0{\cdot}31$
Sham Stimulated	6	$1{\cdot}92 \pm 0{\cdot}26$	$3{\cdot}24 \pm 0{\cdot}54$
Contralateral	6	$1{\cdot}84 \pm 0{\cdot}32$	$2{\cdot}61 \pm 0{\cdot}45$

The s.c.g. was stimulated antidromically at 10 Hz for 60 min via the external carotid nerve on one side of the animal. Three days later the animals were sacrificed and the ganglia removed and bisected as described in the Methods. TH activity was measured in both the rostral and caudal portions of the s.c.g. (In this experiment 6-methyl-5,6,7,8-tetrahydropterin (3·2 mM) was used as the cofactor in the TH assay.) A second group of animals was treated identically except that no current was passed through the stimulating electrode. No significant differences in TH activity were seen between stimulated and contralateral ganglia in either group for either portion of the s.c.g. The data are expressed as means ± S.E. of mean.

Unfortunately, such non-specific changes might mask a specific change produced by antidromic stimulation. Two experiments were performed to control for this possibility. The first experiment was designed to determine whether the changes produced by the dissection necessary for antidromic stimulation would prevent the increase in the specific activity of TH normally seen after orthodromic stimulation. After exposing the internal and external carotid nerves as in the experiments described above, the cervical sympathetic trunk was stimulated at 10 Hz for 60 min. Seventy-two hr later there was a large increase in TH activity ($178 \pm 15\%$) and in protein content ($141 \pm 10\%$) in the stimulated ganglia compared to their contralateral controls ($n = 7$). However, there was also a highly significant increase in the *specific activity* of TH on the stimulated side ($127 \pm 6\%$, $P < 0{\cdot}01$ by the paired t test), indicating that a specific increase in TH activity could be seen in the presence of a large increase in total ganglion protein.

In a second experiment, the s.c.g. was stimulated antidromically (10 Hz for 60 min) via the external carotid nerve alone. Since this trunk is more accessible than the internal carotid nerve, it seemed that this procedure might cause less trauma to the ganglion and perhaps less increase in protein content. At the end of the experiment, when the ganglia were removed, they were bisected into a rostral and a caudal portion as neurones projecting out the external carotid nerve are known to be located predominantly in the caudal part of the s.c.g. (Bowers & Zigmond, 1979). No significant change in either TH activity or protein content was found in either

the caudal or the rostral portion of the s.c.g. in either the antidromically stimulated or 'sham-stimulated' animals (Table 4). Thus even under conditions in which there was no change in ganglion protein content, no change in the specific activity of TH could be seen after antidromic stimulation.

DISCUSSION

In previous publications we have shown that electrical stimulation of the preganglionic input of the s.c.g. leads to an increase in the specific activity of TH in this ganglion and that the magnitude of the increase depends on the frequency and duration of stimulation (Zigmond & Ben Ari, 1977; Zigmond & Chalazonitis, 1979). The experiments reported here with hexamethonium demonstrate that ganglionic nicotinic receptors play a vital role in this trans-synaptic regulation of TH. When the cervical sympathetic trunk was stimulated at 10 Hz continuously for 60 min, hexamethonium blocked the increase in TH activity. In another experiment, in which the cervical sympathetic trunk was stimulated with 40 Hz trains for 90 min, hexamethonium reduced, though it did not completely prevent, the increase. In the latter case, the residual increase might have resulted from muscarinic stimulation of the post-ganglionic neurones which occurs most prominently at high frequencies (Haefely, 1974; McIsaac, 1977). Alternatively, as discussed below, the increase might have resulted from incomplete blockade of the nicotinic synaptic potentials produced by preganglionic stimulation.

One consequence of increased stimulation of nicotinic receptors in the s.c.g. is to increase the firing rates of post-ganglionic neurones. To determine whether an increase in action potential frequency *per se* was capable of mimicking the effects of synaptic stimulation, the effects of orthodromic and antidromic stimulation were compared. In contrast to what was found with orthodromic stimulation, antidromic stimulation produced no change in the specific activity of TH. Furthermore, under conditions in which antidromically stimulated ganglia showed parallel increases in TH activity and protein content, similar increases were seen in 'sham-stimulated' ganglia.

In these experiments it is obviously crucial that the current intensities used are adequate to stimulate the predominantly non-myelinated post-ganglionic fibres (Eccles, 1937; Dunant, 1967). Since only short portions of the post-ganglionic trunks of the s.c.g. in the rat are experimentally accessible, it is difficult to study this question directly. However, studies done on the cervical sympathetic trunk which is also made up almost entirely of small, non-myelinated fibres showed that a maximum compound action potential was obtained from this trunk at intensities below those used in the antidromic experiments described in the present paper.

Further evidence that the post-ganglionic fibres were stimulated in our experiments was the observation of exophthalmus, retraction of the eyelid, and pupillary dilation in the ipsilateral eye. However, fibres controlling these responses may be atypical since, at least in the cat, the post-ganglionic fibres innervating the eye have a lower threshold than the majority of post-ganglionic fibres projecting out of the s.c.g. (Bishop & Heinbecker, 1932). Therefore, we studied the pineal gland as another target tissue innervated by neurones of the s.c.g. Recent work in this laboratory has

established that stimulation of the cut cervical sympathetic trunk with suction electrodes leads to a dramatic increase in the enzyme serotonin: *N*-acetyltransferase in the pineal gland (Bowers & Zigmond, 1978). Stimulation of the internal carotid nerve using the identical procedures used in the present studies produced a comparable increase in pineal serotonin: *N*-acetyltransferase activity (A. Chalazonitis, C. W. Bowers & R. E. Zigmond, unpublished). Finally stimulation of the external carotid nerve led to the accumulation of saliva in the animal's mouth, indicating that sympathetic nerves innervating the salivary glands were being activated.

Since C fibres in the post-ganglionic trunks are effectively excited in these experiments, the question arises as to whether this excitation leads to an antidromic invasion of the ganglion cell bodies. While this question cannot be answered by the surface recordings presented here, intracellular studies in a number of species, including the rat, have shown the elicitation of 'all-or-nothing' spikes by antidromic stimulation of the post-ganglionic nerves of the s.c.g. (Erulkar & Woodward, 1968; Blackman & Purves, 1969; Perri *et al.* 1970; P. Yarowsky, personal communication).

It has been shown recently that at least as many neurones in the s.c.g. project into the external carotid nerve as project into the internal carotid nerve (Bowers & Zigmond, 1979). Therefore, experiments were performed in which both the internal and external carotid nerves were stimulated. However, even under these conditions no change occurred in the specific activity of TH.

Another factor which could hinder the detection of an increase in the specific activity of TH produced by antidromic stimulation was the significant increase in both TH and in total ganglion protein content apparently resulting from the extensive dissection necessary for performing these experiments. However, the experiment in which *orthodromic* stimulation was shown to be capable of increasing the specific activity of TH even after a dissection of the internal carotid nerve and external carotid nerve comparable to that used in the antidromic experiments makes this hypothesis unlikely. Furthermore when the external carotid nerve was stimulated alone no significant increase in protein content or in TH activity were seen in the 'sham-stimulated' ganglia. If the specific activity of TH had increased in the antidromically stimulated ganglia it should have been detected when assayed in the caudal portion of the ganglion since the majority of the neurones in this region project into the external carotid nerve.

In summary, therefore, the results presented here do not support the hypothesis that an increase in the firing rate of sympathetic neurones *per se* is adequate to produce a long-term increase in the specific activity of TH. In addition, two other hypotheses which could have explained the increase in TH activity after orthodromic stimulation have been disproved by our results. One is that orthodromic stimulation causes an increase in the uptake and retrograde transport of nerve growth factor and that nerve growth factor increases TH activity. This hypothesis is plausible based on the finding of Paravicini, Stoeckel & Thoenen (1975) that TH activity increases in the ipsilateral s.c.g. after injection of nerve growth factor in the eye. A second possibility, originally proposed by Silberstein, Lemberger, Klein, Axelrod & Kopin (1972) is that increased release of noradrenaline from sympathetic nerve terminals causes an increase in the accumulation of TH molecules by the neurones. However, if either of these hypotheses could account for the effects of

orthodromic stimulation then antidromic stimulation should have increased TH activity also.

A major difference between orthodromic and antidromic activation of the neurones is that the former involves stimulation of nicotinic receptors. Our results are consistent with the hypothesis that some event resulting from the interaction of ACh with these receptors, other than the triggering of action potentials is responsible for the increase in TH activity. Perhaps this involves the opening of the cation channels responsible for the e.p.s.p.s in the post-ganglionic neurones. Perhaps it involves synthesis of a second messenger, as has been proposed for the regulation of TH activity in the adrenal medulla (Costa, Guidotti & Hanbauer, 1974). From this point of view, in one experiment described above (Fig. 3), an inability to complete block with hexamethonium the increase in TH produced by preganglionic nerve stimulation may be due to subthreshold stimulation of the ganglion. A similar nicotinic mechanism may account for the increase in TH activity produced by continuous exposure of the s.c.g. to carbamylcholine (10^{-4} M) in organ culture for a minimum of 4 hr (Otten & Thoenen, 1976). Since under these experimental conditions the action potential mechanism must rapidly inactivate, these results suggest that increased nerve firing is not a necessary condition for an increase in TH activity to occur. Our antidromic experiments demonstrate that an increase in action potential frequency is not a sufficient condition to produce such a change in enzyme activity.

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