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In vitro synthesis of noradrenaline in ganglia and salivary glands after *in vivo* preganglionic stimulation*

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The extent of synthesis of [^{14}C]noradrenaline from [^{14}C]tyrosine and from [^{14}C]dopamine was assessed in slices of superior cervical ganglia, representing cell bodies, and in submaxillary salivary glands, representing terminals of noradrenergic neurons of the cat. Immediately and 6 h after preganglionic stimulation for 3 h the rate of synthesis of noradrenaline from tyrosine and dopamine was not altered in ganglia. In salivary glands, however, synthesis of noradrenaline from both tyrosine and dopamine was increased at both times. These results suggest that acute periods of increased neural activity results in the acceleration of noradrenaline synthesis in the terminals but not in the cell bodies of noradrenergic neurons.

Until recently, there has been little effort to determine what factors control the synthesis of noradrenaline in various parts of the noradrenergic neuron, but it is now apparent that these factors are different in the cell body, axon and terminal (Roth, Stjärne & von Euler, 1967; Bhatnagar & Moore, 1971a,b,c). Many of the studies on the regulation of noradrenaline synthesis have been made in the adrenal medulla (Thoenen, Mueller & Axelrod, 1969; Weiner & Mosiman, 1970) or in tissues that contain a mixture of both cell bodies and terminals of noradrenergic neurons such as the brain (Nybäck, Borzecki & Sedvall, 1968; Goldstein, Ohi & Backstrom, 1970) and vas deferens (Weiner & Rabadjija, 1968a,b; Boadle-Biber, Hughes & Roth, 1970). We have examined the synthesis of noradrenaline in cell bodies and terminals of the same noradrenergic neurons after acute periods of increased activity.

METHODS

Cats of either sex, 2–4 kg, were anaesthetized with an injection of Dial-Urethane (i.p.) (sodium diallylbarbiturate, 70 mg/kg; urethane 280 mg/kg; monoethylurea, 280 mg/kg) and prepared surgically for stimulation of the sympathetic chain according to Bhatnagar & Moore (1971c). Briefly, the cervical sympathetic trunks on both sides were dissected free from the vagus nerves, placed on platinum electrodes, and immersed in mineral oil. The preganglionic fibres on one side were stimulated intermittently with square wave pulses (15 V, 0·2 ms) at 10 Hz. Stimuli were applied for 30 s of each minute for 3 h. The preganglionic fibres on the contralateral side were prepared in an identical manner but were not stimulated. Immediately or 6 h after the 3 h period of stimulation the superior cervical ganglia and submaxillary glands on both sides were dissected out and placed in an ice-cold incubating solution.

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Each litre contained (g): NaCl,6·89; KCl, 0·324; $CaCl_2$, 0·282; $MgCl_2$, 0·11; $NaHCO_3$, 2·1; NaH_2PO_4, 0·14; glucose, 2·0; Na_2EDTA, 0·015; L-ascorbic acid, 0·2. Slices of submaxillary glands were prepared with a razor blade and a frosted glass template, and approximately 250 mg of these slices were placed in 20 ml beakers containing 4 ml of the incubating solution. Each superior cervical ganglion was weighed (approximately 10 mg), sliced into 5-8 longitudinal strips, and placed into 20 ml beakers containing 4 ml of incubating solution. Dissection of the tissue from each cat and the preparation of the slices took approximately 1 h; the tissues were kept at 2–4° during this period.

Incubations of tissue slices were performed in essentially the same manner as reported by Goldstein & others (1970). Briefly, the slices were incubated in a Dubnoff shaker at 37° and gassed with 5% carbon dioxide in oxygen. After a preincubation period of 10 min, either L-[^{14}C]tyrosine (2 μCi, 2×10^{-8} mol, for salivary gland slices and 0·5 μCi, 5×10^{-9} mol, for ganglia slices) or [^{14}C]dopamine (50 nCi, $8{\cdot}1 \times 10^{-9}$ mol) was added to 4 ml incubating solution in each beaker and the mixture was incubated for 30 min. The incubation was stopped by transferring the slices into 15 ml of ice-cold, substrate-free incubating solution. After 15 min the slices were removed, rinsed with 5 ml of cold incubating solution, blotted with filter paper, and homogenized in 4 ml cold 0·4N perchloric acid. The perchloric acid extracts were stored at −27° until analysed for [^{14}C]catecholamine according to Bhatnagar & Moore (1971c, 1972). Radioactivity was determined with a Beckman Model LS 100 Spectrometer. The counting efficiency, determined with an external standard, was approximately 87% and the results were corrected for counting efficiency but not for recovery from isolation procedures.

[^{14}C]Dopamine HBr (0·05 mCi/1·9 mg) and L-[^{14}C]tyrosine (uniformly labelled, 1·0 mCi/1·83 mg) were obtained from New England Nuclear Corp. The purity of [^{14}C]dopamine was checked by thin layer chromatography (Carr & Moore, 1969) and [^{14}C]tyrosine was purified as described by Lewander & Jonsson (1968).

Statistical analysis was carried out with a paired comparison Student's *t*-test (Goldstein, 1964).

RESULTS

The formation of [^{14}C]noradrenaline (^{14}C-NA) and [^{14}C]dopamine from [^{14}C]tyrosine in slices of the glands and superior cervical ganglia obtained immediately after and 6 h after a 3 h period of preganglionic stimulation is summarized in Table 1. There was no significant difference between the total radioactivity or the contents of [^{14}C]dopamine or ^{14}C-NA in stimulated and nonstimulated ganglia at zero time and at 6 h. The formation of ^{14}C-NA and [^{14}C]dopamine in stimulated salivary glands, however, was increased 4·6 and 1·9 fold respectively at zero time. 6 h after termination of stimulation the formation of ^{14}C-NA but not of [^{14}C]dopamine was still elevated. At no time was the content of [^{14}C]tyrosine in stimulated salivary glands different from that in nonstimulated glands, so that the increased formation of catecholamines was not the consequence of an increased uptake of [^{14}C]tyrosine during the post-stimulation period. The tissue/medium ratio of total radioactivity (primarily [^{14}C]tyrosine) was 3·3 $\pm$ 0·2 for ganglia and 1·8 $\pm$ 0·2 for salivary glands (d/min per g tissue÷d/min per ml media; mean $\pm$ s.e.; n = 6).

The relatively greater concentration of ^{14}C-NA than [^{14}C]dopamine in salivary gland slices could result from any one of a number of factors, one being that during

the post-stimulation period the conversion of dopamine to noradrenaline was enhanced. This was tested directly by comparing the ability of stimulated and non-stimulated ganglia and salivary gland slices to form ^{14}C-NA from [^{14}C]dopamine (Table 2). Preganglionic stimulation did not alter the rate of noradrenaline synthesis in ganglia but in the salivary glands there was an accelerated conversion of dopamine to noradrenaline immediately after (0 time) and 6 h after the termination of the stimulation period. This increase was not the result of increased uptake of [^{14}C]-dopamine in stimulated tissues since the total radioactivity in stimulated and non-stimulated tissues was the same. The tissue/medium ratio of total radioactivity (primarily [^{14}C]dopamine) was 7·2 ± 0·6 for ganglia and 4·8 ± 0·2 for salivary

Table 1. *Formation of [^{14}C]dopamine and [^{14}C]noradrenaline from [^{14}C]tyrosine in slices of submaxillary salivary glands and superior cervical ganglia.*

			Radioactivity		
			Total	^{14}C-DA	^{14}C-NA
Ganglia (d/min per 10 mg)	0 h	NS	8600 ± 1300	170 ± 9	1100 ± 80
		S	11 100 ± 670	180 ± 5	1200 ± 140
		S/NS	1·3 ± 0·1	1·1 ± 0·04	1·1 ± 0·19
	6 h	NS	8200 ± 1200	190 ± 30	860 ± 100
		S	8300 ± 750	190 ± 30	820 ± 50
		S/NS	1·0 ± 0·1	1·0 ± 0·13	1·0 ± 0·05
Glands (d/min per g) ..	0 h	NS	723 700 ± 27 300	1400 ± 50	2100 ± 300
		S	699 600 ± 27 000	2650 ± 220*	9200 ± 400*
		S/NS	1·0 ± 0·03	1·9 ± 0·23	4·6 ± 0·9
	6 h	NS	713 000 ± 98 500	1300 ± 144	1800 ± 470
		S	775 000 ± 85 000	1100 ± 73	3400 ± 530*
		S/NS	1·1 ± 0·04	0·9 ± 0·04	2·0 ± 0·26

The preganglionic sympathetic fibres were stimulated intermittently (15 V, 0·2 ms square wave pulses, 10 Hz for 30 s/min) for 3 h. Immediately after (0 h) or 6 h after (6 h) the termination of stimulation the superior cervical ganglia (Ganglia) and submaxillary salivary glands (Glands) from the stimulated (S) and nonstimulated (NS) sides were dissected out, sliced, and incubated with [^{14}C]tyrosine as described in Methods. Values are reported as the mean ± s.e. as determined from 3 separate experiments.

* Stimulated values are significantly different from nonstimulated values (P <0·05).

Table 2. *Formation of [^{14}C]noradrenaline from [^{14}C]dopamine in slices of superior cervical ganglia and submaxillary salivary glands.*

			Radioactivity	
			Total	^{14}C-NA
Ganglia (d/min per 10 mg)	0 h	NS	2400 ± 320	300 ± 30
		S	2500 ± 370	300 ± 30
		S/NS	1·1 ± 0·03	1·0 ± 0·04
	6 h	NS	2300 ± 520	350 ± 80
		S	2100 ± 560	256 ± 25
		S/NS	0·9 ± 0·05	0·8 ± 0·1
Glands (d/min per g) ..	0 h	NS	99 600 ± 2600	5800 ± 1150
		S	102 000 ± 3700	8810 ± 1060*
		S/NS	1·0 ± 0·02	1·7 ± 0·16
	6 h	NS	119 800 ± 7000	4500 ± 360
		S	124 100 ± 7400	8700 ± 1500*
		S/NS	1·0 ± 0·02	1·9 ± 0·23

These experiments were made in the same manner as those described in legend to Table 1 except that the slices were incubated with ^{14}C-DA rather than with [^{14}C]tyrosine; see Methods for details. Values are reported as means ± s.e. as determined from 3–4 experiments.

* Stimulated values are significantly different from nonstimulated values (P <0·05).

glands (d/min per g tissue ÷ d/min per ml medium; mean ± 1 s.e.; n = 9); these ratios were the same in stimulated and in nonstimulated slices.

DISCUSSION

Increased activity of catecholaminergic neurons is accompanied by an accelerated rate of noradrenaline synthesis (Alousi & Weiner, 1966; Gordon, Reid & others, 1966; Sedvall, Weise & Kopin, 1968; Kupferman, Gillis & Roth, 1970; Gewirtz & Kopin, 1970). In those studies, radioactive precursors were added to the tissues *in vitro* or *in vivo* during the actual period of stimulation. In our study, the synthesis of noradrenaline was determined *in vitro* after stimulation of the tissues *in vivo*. Despite the different procedure, our results are consistent with the previous reports.

Effects of nerve stimulation on noradrenaline synthesis have been examined almost exclusively in tissues that contain either terminals (heart, spleen, salivary glands) or mixtures of terminals and cell bodies (vas deferens, brain). We examined these two parts of the neuron separately and our results essentially confirm the previous findings that increased neuronal activity results in accelerated synthesis of noradrenaline in terminals but not in the soma (Bhatnagar & Moore, 1971a,b,c). In cell bodies, noradrenaline synthesis proceeds rapidly and independently of afferent input; the same appears to be true in the axon (Roth, & others, 1967).

Previously we found that where the parameters of stimulation were identical to those used in the present experiments, 3 h of stimulation reduced the content of noradrenaline in the salivary gland and increased the rate of its synthesis from tyrosine when measured *in vivo* (Bhatnagar & Moore, 1971c). An unexpected finding in that *in vivo* study was an increased formation of noradrenaline from dopamine, an effect also noted in the present *in vitro* study. The mechanism of the accelerated conversion is not clear. It would not appear to result from an increased uptake of dopamine into the neurons since the tissue/medium ratios for dopamine in stimulated and nonstimulated slices were the same. Furthermore, it seems unlikely to result from an increased amount of dopamine-β-hydroxylase since the amount of this enzyme increases only after more prolonged increased neuronal activity than we used (Kvetňanský, Gewirtz & others, 1971). In addition, it is generally believed that enzymes involved in the biosynthesis of noradrenaline are themselves synthesized in the cell body and then slowly transported down the axon to the terminals (Thoenen & others, 1970). Accordingly, if the synthesis of dopamine-β-hydroxylase did increase after the brief period of nerve stimulation it should be detected first in the cell bodies; stimulation, however, did not increase the conversion of dopamine to noradrenaline in the ganglia.

It has been reported that neuronal activity increases the synthesis of labelled noradrenaline from tyrosine, but not from low concentrations of dopa (Gordon & others, 1966; Roth & others, 1967; Sedvall & others, 1968). These findings emphasized the importance of tyrosine hydroxylase in controlling noradrenaline synthesis. Under certain circumstances, however, regulation of noradrenaline synthesis occurs at a step other than that controlled by tyrosine hydroxylase. For example, in the presence of high concentrations of dopa, nerve stimulation increases noradrenaline formation (Austin, Livitt & Chubb, 1967). Weiner & Rabadjija (1968a) found that the formation of noradrenaline, but not dopamine, was increased in stimulated vas deferens preparations if the tissue had been incubated with dopa during the post-stimulation

period, suggesting an accelerated conversion of dopamine to noradrenaline. Thierry, Blanc & Glowinski (1971) have reported that, under certain conditions, the conversion can play a rate limiting role in the synthesis of noradrenaline in the brain. Our results also indicate that acute periods of intense neuronal activity enhances the formation of noradrenaline from dopamine.

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