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^{3}H-Noradrenaline-accumulating ability of cholinergic nerve terminals in cat sympathetic ganglia

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Summary

1. After incubation of pieces of cat posterior mesenteric ganglion in [^{3}H]-noradrenaline solution, the localization of the isotope was assessed by electron-autoradiography.

2. Significantly higher concentrations of [^{3}H]-noradrenaline were found over acetylcholinesterase-positive axons terminal on ganglion cells than in adjacent background areas.

3. [^{3}H]-Noradrenaline was not accumulated by ganglion cells under these circumstances.

4. The significance of these findings in relation to previous physiological and pharmacological investigation is discussed.

Introduction

Marrazzi (1939) and Bulbring (1944), in pharmacological and physiological studies on the cat superior cervical ganglion, have shown that preganglionic transmission may be influenced by catecholamines. Eccles & Libet (1961) attributed preganglionic inhibition to the presence of chromaffin cells and the intraganglionic diffusion from them of catecholamines.

Using an enzyme histochemical technique for the demonstration of acetylcholinesterase combined with autoradiography, the presence of [^{3}H]-noradrenaline has been demonstrated in sympathetic postganglionic axons in several situations (Esterhuizen, Graham, Lever & Spriggs, 1968; Lever, Spriggs & Graham, 1968; McQuiston, Ivens, Lever, Spriggs & Graham, 1971). The object of this investigation was to ascertain if, by the same means, the presence of noradrenaline could be detected in relation to preganglionic terminals in sympathetic ganglia.

Methods

Specimens from cat posterior mesenteric ganglia were reduced to 1 mm cubes (approx.) and incubated at 37° C for 30 min in Krebs solution (composition NaCl, 118 mM; KCl, 4·69 mM; $CaCl_2$, 2·52 mM; $MgCl_2$, 1·15 mM; NaH_2PO_4, 1·17mM; $NaHCO_2$, 24·4 mM; dextrose, 11·1 mM) gassed with 5% CO_2 in O_2 and containing 81·3 μCi/ml [H^3]-DL-noradrenaline ($1{\cdot}2 \times 10^{-5}$ g/ml). Excess noradrenaline was removed by successive rinses with Krebs solution over 30 minutes. After fixation for 4 h in 2% glutaraldehyde (in cacodylate buffer pH 7·4) samples were processed for the demonstration of acetylcholinesterase activity in the presence of a specific pseudocholinesterase inhibitor (ethopropazine, 2×10^{-4}M) according to the method

of Lewis & Shute (1966). Specimens were postfixed in osmium tetroxide (1%, buffered to pH 7·5 with veronal acetate), embedded in Araldite and fine (silver-gold interference colours) sections were stained with lead citrate (Reynolds, 1963) and examined in a Siemens Elmiskop I electron microscope.

Autoradiographic assessment was made by comparison of silver grain counts over outlined and planimetrically measured acetylcholinesterase-positive terminal and near terminal axons, with those over adjacent background areas including extracellular space and connective tissues by a method previously reported (McQuiston, Ivens, Lever, Spriggs & Graham, 1971). For each nerve terminal situation estimates of silver grains per unit area of axon and per unit area of background were obtained by dividing grain numbers over relevant micrograph areas by the measured figures for those areas. Comparisons were then made by the Student's *t* test (Table 1). Axons were considered terminal if they showed synaptic relation with, or close apposition to, dendritic processes, without intervention of Schwann cell elements.

Drugs

DL-Noradrenaline-7-[^{3}H]-hydrochloride (1·4 Ci/mM), Radiochemical Centre, Amersham.

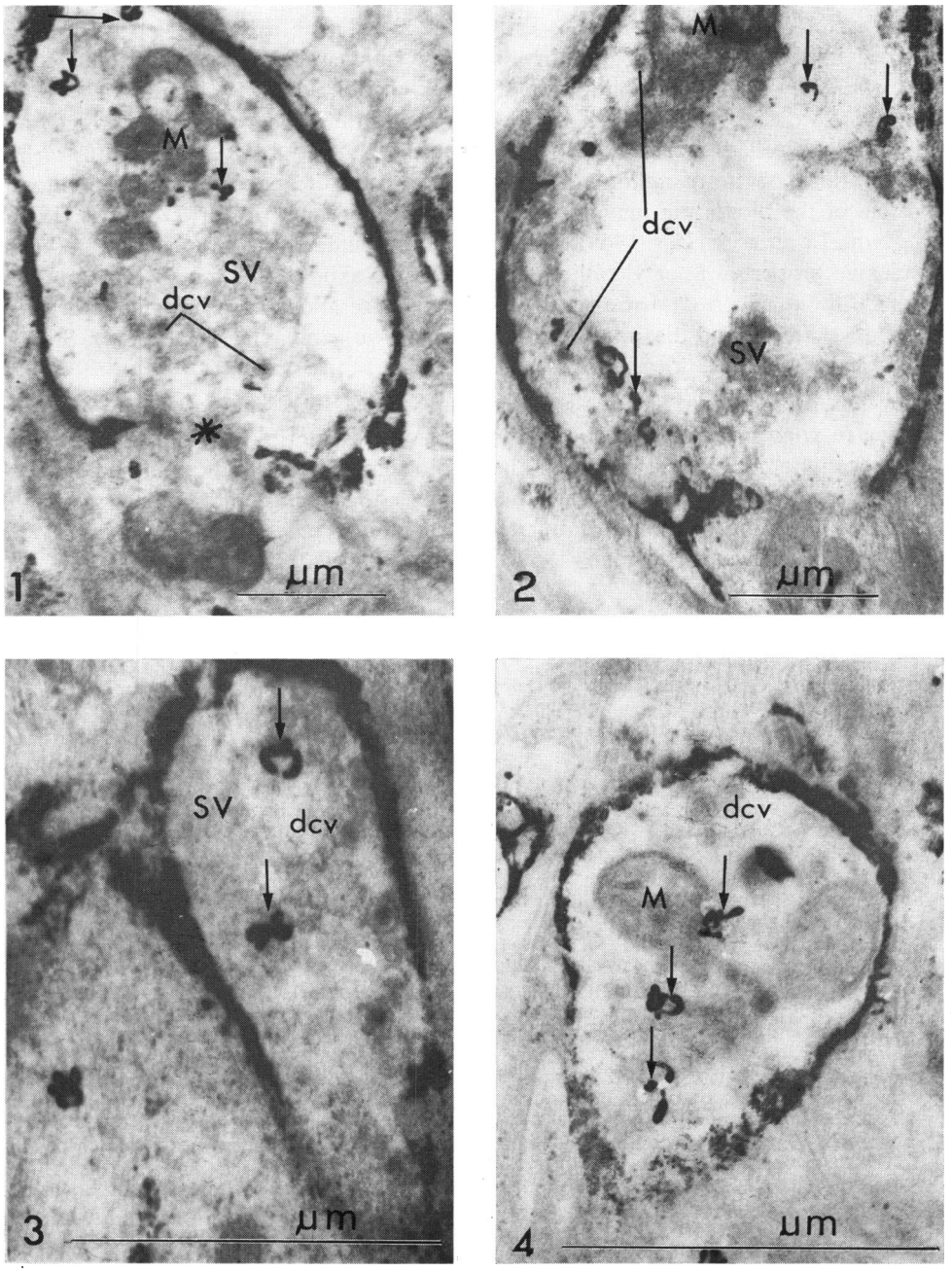
Results

Although the relations of Schwann cell processes to axons and to dendrites are often similar, thus presenting difficulties in the interpretation of electron micrographs, it was possible to identify dendrites by their content of free ribosomes and granular reticulum. Axons were identified by their terminal features and by their covering of cholinesterase reaction product. Besides concentrations of mitochondria, these preganglionic terminals contained vesicles, most of which were clear and 300–500 Å in size while a few, measuring 1,000 Å approximately, were dense-cored. Concentrations of silver grains indicating the presence of [^{3}H]-noradrenaline, were found over profiles of acetylcholinesterase-positive nerve terminals (Figs. 1–4).

Statistical analysis of grain counts per unit area over axon profiles and over adjacent (cellular and extracellular) background showed that [^{3}H]-noradrenaline was accumulated in preganglionic terminals to a significantly larger extent ($P<0·001$) than by other adjacent structures (Table 1). No silver grain concentrations and by inference no [^{3}H]-noradrenaline accumulations were detected over principal ganglion cells or their dendritic processes.

TABLE 1. *Silver grain distribution over acetylcholinesterase containing (AChE) terminals in cat inferior mesenteric ganglia*

	AChE+ve terminals	Adjacent cellular background (including principal ganglion cells)	Principal ganglion cell cytoplasm and dendritic processes	Cellular and extracellular background
No. of grain counts	23	23	12	12
Mean no. of silver grains per unit area	0·293±0·051 (S.E.M.)	0·122±0·017 (S.E.M.)	0·089±0·002 (S.E.M.)	0·105±0·002 (S.E.M.)
P-value (Student's *t* test)	<0·001		0·3–0·4	



FIGS. 1–4. Electron autoradiographs of preganglionic nerve terminals in cat inferior mesenteric ganglia. Material was incubated in the presence of [^{3}H]-noradrenaline and processed for the demonstration of acetylcholinesterase activity. The nerve terminals contain mitochondria (M), clear synaptic vesicles (SV) and large dense-cored vesicles (dcv); the accumulation of silver grains (arrows) indicates [^{3}H]-noradrenaline. The asterisk indicates a tangential section of an axodendritic synapse.

Discussion

Our observations, which suggest that preganglionic nerve terminals can concentrate exogenous noradrenaline, may be complementary to the work of Marrazzi (1939), Bulbring (1944), Eccles & Libet (1961) and Knoll & Vizi (1970), who described inhibitory effects of noradrenaline upon preganglionic transmission in sympathetic ganglia. In addition to this, Christ & Nishi (1969 & 1971) and Nishi (1970) have claimed that the preganglionic cholinergic terminals in sympathetic ganglia are endowed with α-adrenoceptors. Our present findings may be contrasted with earlier observations on postganglionic autonomic nerves in the pancreas and the smooth muscle of the nictitating membrane (Esterhuizen, Graham, Lever & Spriggs, 1968; Graham, Lever & Spriggs, 1968) where [^{3}H]-noradrenaline uptake was found to be an exclusive property of acetylcholinesterase-negative axons containing a large number of small (300–500 Å d) dense-cored vesicles. These axons are sympathetic because they degenerated and disappeared after sympathetic ganglionectomy (Lever, Spriggs & Graham, 1968).

In the present experiments noradrenaline, which was present in the medium during *in vitro* incubation of pieces of posterior mesenteric ganglion, was taken up by preganglionic axon terminals. The significance of this finding should be considered in the light of the possible sources of endogenous noradrenaline within the ganglion including the small granulated chromaffin type cells described by several authors (Matthews & Raisman, 1969; Jacobowitz, 1970).

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