AXON BEADINGS IN AUTONOMIC CHOLINERGIC NERVES

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INTRODUCTION

Many authors have described beadings of autonomic nerves in their terminal distribution in a wide variety of methylene blue–stained preparations (1, 2).

With the advent of techniques which enable catechol amine-containing nerves to be visualized by fluorescence microscopy (3, 4), the beaded nature of adrenergic nerves has been convincingly demonstrated in a number of situations in different species. Electron microscopy has confirmed that autonomic terminal nerves show successive narrowings and expansions wherein lie accumulations of mitochondria and microvesicles (5). Axons beaded in this manner and of confirmed adrenergic identity have now been described upon the adventitial surface of arteriolar smooth muscle (6). Until recently it has not been possible to differentiate with certainty between adrenergic and cholinergic terminals purely on the basis of their fine structural appearances. However, Esterhuizen et al. (7, 8) have developed a combined radioautographic and histochemical technique for the simultaneous identification of

adrenergic and cholinergic fibres in electron radioautographs. The form of cholinergic axons in their terminal distribution within the cat pancreas is the subject of the present study.

METHODS

Specimens of cat pancreas were fixed in glutaraldehyde and "stained" for acetylcholinesterase by incubation with acetylthiocholine in the presence of the specific pseudocholinesterase-inhibitor ethopropazine according to the method of Lewis and Shute (9). The specificity of this staining procedure is discussed in an earlier paper (8). Specimens were postfixed in osmium tetroxide and embedded in Araldite, and fine sections were cut with a Porter-Blum microtome. The sections were stained with lead (10) and examined in a Siemens Elmiskop I electron microscope. In addition to single sections or short series of serial sections (up to eight sections), a long series of 128 consecutive sections mounted on 16 grids was prepared.

OBSERVATIONS

Cholinergic axons were readily identified by the presence of an electron-opaque acetylcholinesterase reaction product associated with the axolemmae (Figs. 1–12). In some of the sections illustrated, the cholinergic axon is seen to contain granular vesicles of approximately 1000 A in diameter. Granular vesicles are commonly associated with adrenergic nerves, and, since it has been proposed that acetylcholine may be involved in adrenergic transmission (11, 12), the question arises whether these larger granular vesicles contain catechol amines. Indeed, Graf (13) suggests that axons in mouse heart which stain for acetylcholinesterase may be adrenergic in function simply because they contain large granular vesicles. However, the granular vesicles characteristic of presumed adrenergic nerves are nominally of about 500 A in diameter (14–16), approximately half the size of the granular vesicles observed in cholinergic axons (this paper, 13, 15, 17). Morphologically similar, larger granulated vesicles (about 1000 A in diameter) are found in adrenergic nerves (6, 8, 14, 16, 17), but the frequency with which they are observed is not significantly diminished by pretreatment of the tissues with α-methyl-m-tyrosine or reserpine (drugs which deplete noradrenaline from adrenergic nerves), whereas the number of smaller granulated vesicles (about 500 A in diameter) observed is significantly reduced (18, 19). It appears unlikely, therefore, even in adrenergic axons, that the larger granulated vesicles contain noradrenaline.

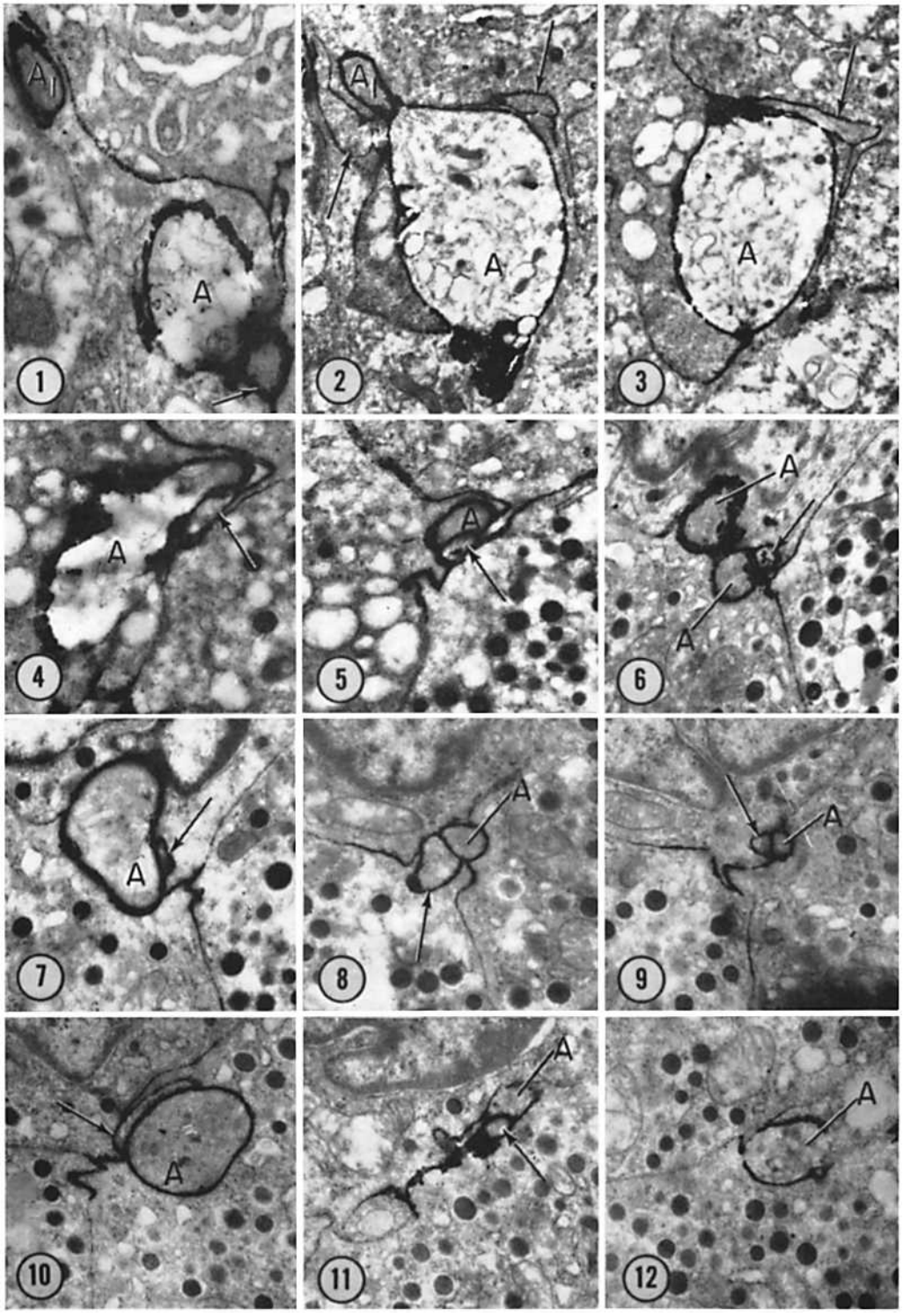
It is generally accepted that adrenergic nerves selectively accumulate noradrenaline (see reference 20). From radioautographs of noradrenaline-^{3}H-perfused autonomically-innervated tissues stained for acetylcholinesterase, it has been shown conclusively that adrenergic axons (labeled by overlying silver grains) do not stain for acetylcholinesterase and that cholinergic axons (acetylcholinesterase-stained) do not exhibit overlying silver grains (7, 8, 16, 17, 21). Moreover, many of the cholinergic axons in these studies have been observed to contain the larger variety of granulated vesicle (see reference 17). It is improbable, therefore, that the larger granulated vesicle found in some cholinergic nerves represents a store of noradrenaline. This situation emphasizes the extreme caution necessary in extrapolating solely from morphological evidence.

In single sections the diameters of cholinergic axon profiles showed great variation. The larger profiles were frequently identified with concentrations of microvesicles and mitochondria within the axoplasm. Where cholinergic axons were transected in longitudinal section, there was evidence that they possessed expansions and narrowings. There was also an indication, from short series of consecutive sections, that the dimensions of cholinergic axons may change abruptly along a short length of nerve.

In the present attempt to produce a long series of consecutive sections, not every section was secured, for technical reasons; although the series was thus incomplete, it was still possible to follow selected cholinergic axons for a distance of approximately 10 μ.

Since it is clearly impracticable to illustrate in a short report all the sections examined, Figs. 1–12 have been chosen as representative. However, it must be emphasized that our observations were based on an examination through the whole series.

Incontrovertible evidence that cholinergic axons showed successive beadings and narrowings was obtained. It has been possible to trace continuity of one axon (A) throughout the series. It is clear from Figs. 1 and 2 that the two profiles A_1 and A represent transections of this same axon. When axon A is followed through Figs. 3–12, it exhibits successive swellings and narrowings of outline. These swellings or beadings along its course are shown in Figs. 2–4, 7, 10, 12 and are characterized by concentrations of microvesicles.



FIGURES 1–12 Representative sections in a long series through an axon (*A*) related to endocrine cells in the cat pancreas. Axon A shows successive beadings and narrowings through the series. Acetylcholinesterase reaction product is the dense material surrounding the axon and other cell profiles (arrows, probably Schwann cell process). For more detailed description, see text. The approximate depth (assuming 800-A thick sections) in the tissue block seen in Figs. 1–12 were 0.5, 1.1, 1.8, 2.2, 2.5, 3.4, 3.7, 4.2, 5.6, 7.2,8.8, and 10.1 μ, respectively. × 12,000.

While there is no doubt concerning the axonal nature of the profiles labeled *A*, it has not been possible to identify unequivocally the other profiles (arrows) also surrounded by the acetylcholinesterase reaction product. The fact that none of these other profiles could be traced through more than a few sections eliminates the possibility of their being independent axons. Such appearances are likely produced by Schwann or parenchymal cell processes in cross-section. In Fig. 6 two profiles have been labeled (*A*) as axon cross-sections; the evidence for this identity is their convergence into the single axon beading (*A*) seen in Fig. 7. In other words, the profiles *A* in Fig. 6 should be regarded as sections through short processes of the axon beading *A* in Fig. 7.

It would appear that the cell process (arrows) alongside the axons in Figs. 6–10 has undergone rotation through approximately 180° in passing from the level of Fig. 6 to that of Fig. 10.

It is accepted that adrenergic axons exhibit successive beadings and narrowings along their lengths, and that transmitter release may occur at these beadings (4, 6, 22, 23). Whether the para-axonal outlines (arrows) in Figs. 1–11 are regarded as Schwann cell processes, and this is most likely, it is clear that they provide only an incomplete covering for the terminal axon *A*. As in the case of adrenergic terminal axons, the denuded areas of axolemma over axon beadings (*A*) may provide the sites of transmitter release; this suggestion is reinforced by the presence of microvesicles in these beadings (Fig. 10). It would appear, therefore, that the mode of termination of postganglionic cholinergic autonomic nerves differs from that of the typical cholinergic somatic ending as seen in skeletal muscle (24, 25) for example.

While short complete series of fine sections are necessary to elucidate cytological structure, the present study underlines the importance of much longer series in solving problems in electron microscopical histology.

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