Fine Structure of a Neurosecretory Axon in a Crustacean Skeletal Muscle

We have noticed in *Carcinus* claw closer muscle a number of scattered straplike nerve endings which are completely different in appearance from the main motor nerve ending population, present in abundance on every muscle fibre. Whereas the main motor nerve endings contain uniform electron-lucent synaptic vesicles of about 500 Å diameter, the strap-like endings contain a variety of vesicles up to almost 4000 Å diameter with varying degrees of electron opacity, strongly suggestive of granular neurosecretory vesicles[1]. The strap-like nerve endings appear to belong to one or more neurosecretory axons running through the muscle.

Neurosecretory endings are known in some insect skeletal muscles[2-4] and ATWOOD et al.[5] have recently described scattered neurosecretory vesicles in the main motor nerve terminal population of some crustacean skeletal muscles. The endings described here appear to be the first evidence of discrete neurosecretory axons in crustacean skeletal muscle and their vesicle content has been examined in the hope that this would cast some light on the function of the axon.

Methods. Isolated fibres of the claw closer muscle of *Carcinus maenas* were fixed in 5% glutaraldehyde in 0.1 *M* sodium cacodylate and 0.25 *M* sucrose for 1 h. After post-fixation in 2% osmic acid for 30 min the fibres were dehydrated through an ethanol series and embedded in epon. Thin sections (500 Å) were cut on a Reichert OmU2 ultramicrotome, mounted on formvar and carbon coated grids and stained with uranyl acetate and lead citrate. Sections were examined on an AEI EM 801 at 80kV excitation voltage. The details of these methods have been published elsewhere[6].

Results. The neurosecretory axon terminals were characteristically long and strap-like (up to 1.5 mm long) making up to about 15 loose contacts with the extracellular connective tissue around the muscle fibre along its length. The photomicrograph in Figure 1 is a low-power view of a section of a neurosecretory ending at a point where it abuts onto sarcolemmal material. At this point the muscle fibre has a complex sarcolemma with a tough outer mucopolysaccharide sheath(S). However, the fibre periphery has no elaborated sub-synaptic region which could be compared with that of a normal end-plate.

The axon is packed along its length with membrane-bounded vesicles which vary from small electron lucent varieties to large electron-opaque types (Figures 2 and 3). A 1 mm length of axon terminal was scanned at high power and the vesicles were counted and measured. The segment contained 248 vesicles which were distinct enough to permit measurement and visual classification, only 6 obviously damaged vesicles were ignored.

Classified on size, the vesicles fall into 4 peaks at 1200, 1900, 3100 and 3700 Å, but the middle vesicle range from 1600 to 2100 Å was not morphologically homogeneous.

Classification of vesicles by size and appearance and their relative abundance

Type of vesicle	Mean size (Å)	Relative abundance[a]
Large, very dense	3100	14
Large, granular	3700	5
Medium, very dense	1800	23
Medium, granular	1700	16
Medium, light granular	2000	3
Small, clear	1300	39

[a] Expressed as a percentage of the total vesicle count.

The Table shows a more rational classification based on both size and appearance, and it can be seen that 6 distinct types are present. It is clear that these axon terminals have a very complex vesicle content, and since the different types probably contain different neurosecretory products, the nature of the products, their synthesis and the target areas affected by their release will prove very difficult to analyse.

Discussion. Whereas ATWOOD et al.[5] only noticed a few neurosecretory granules mixed in with the normal synaptic vesicles in crustacean nerves, this study clearly shows that discrete neurosecretory axons do occur in crab skeletal muscle. Although no very close association was seen between the axon and the muscle fibres, the presence of such large numbers of vesicles makes it unlikely that the axon was routed through the muscle to some other target structure, and we must conclude that the endings are probably associated with the functioning of the muscle.

OSBORNE et al.[4] noticed that most of the neurosecretory material released from the neurosecretory endings in insect and frog muscle was liberated into the haemolymph or into the connective tissues which separate the muscle fibres. This is the most likely explanation of the fate of the vesicles in the endings described here, since no synaptic-like contacts with the muscle are present, but the fibres are surrounded by an elaborate connective tissue sheath (Figure 1).

That neurosecretory material may be ejected into the extracellular space near the muscle fibres is suggested by the presence of the moderate-sized clear vesicles which may constitute 40% of the total vesicle count. These vesicles, at 1300 Å are far larger than the 500 Å clear synaptic vesicles of crustaceae[5] and there is now much evidence that the moderate-sized clear vesicles are involved in the neurosecretory release process[7]. If neurosecretory vesicles release their products by exocytosis (see SMITH[7-9]) then a membrane retrieval process will be essential, and the clear vesicles may be the end products of this process[7]. Either the clear vesicles may bud inwards from the general nerve membrane[4] or they may form from the neurosecretory vesicle as its contents are released. These vesicles may well be involved in the resynthesis of neurosecretory material in conjunction with the neurofilament system[10, 11].

The presence of neurosecretory vesicles in nerve terminals associated with skeletal muscles is so widespread that it can be no longer ignored, and its is clear that we are far from understanding the nature and function of synaptic and neurosecretory vesicles in Arthropods, since these vesicles are far more diverse than formerly supposed.

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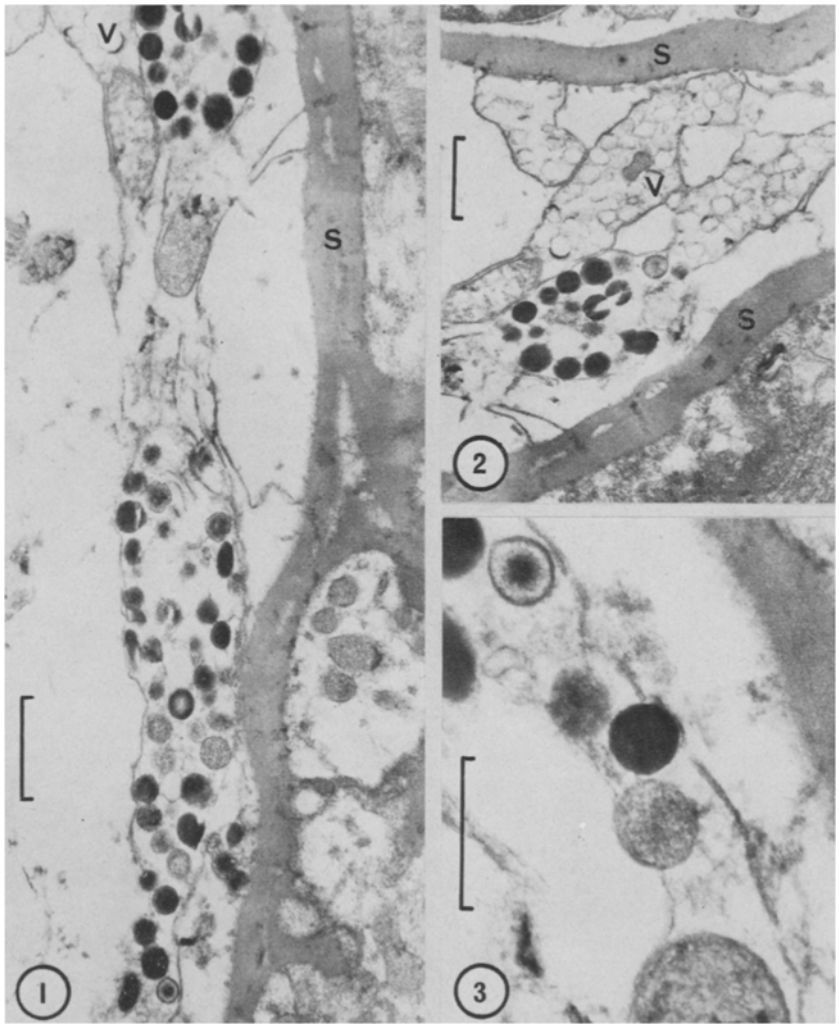

Fig. 1. Longitudinal section through part of a strap-like neurosecretory axon terminal. The axon, somewhat convoluted along its length, makes loose contact at several points with the extracellular connective tissue sheath(S) surrounding the muscle fibre (not shown). The axon, at this point, is packed with large granular or dense membrane-bounded vesicles. A few small clear vesicles(V) are evident in the upper left-hand corner. ×14,000; scale 1 μm.

Fig. 2. Portion of a neurosecretory axon running in a cleft between 2 fibres completely bounded by extracellular sheath material(S). Note the large aggregations of medium-sized electron-lucent vesicles(V). ×11,100; scale 1 μm.

Fig. 3. High-power view of some neurosecretory vesicles showing the variations in size and appearance. ×42,000; scale 0.5 μm.

Several reports[12–14] have now identified substances such as noradrenaline, dopamine and 5-hydroxytryptamine in neurosecretory vesicles and ATWOOD et al.[5] have tentatively suggested that the contents of neurosecretory vesicles may mediate the well established trophic effect of nerve on muscle[15]. If this is true, then the only difference between the endings described here and those of ATWOOD et al.[5] is that in *Carcinus* the trophic vesicular element has become separated from the synaptic vesicular element at the peripheral sites.

Résumé. Un axone neuro-sécréteur à extrémités localisées dans le muscle squelettique du crane *Carcinus* est décrit. Ces extrémités contiennent 6 sortes de vésicules ayant les diamètres de 1300 a 3700 Å. Il est suggéré que les petites vésicules correspondent à la phase de rétablissement de la membrane, pendant la décharge des granules neuro-sécréteurs. On suppose aussi que cet axone peut-être l'intermédiaire d'une influence trophique du nerf sur le muscle.

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Immunological Activity of Transplanted Spleens in *Xenopus laevis*

Adults of *Xenopus laevis*, like other amphibians[1], are known to show immunological responses when maintained at a temperature of 20°C or above. These responses include the production of circulating antibody to soluble or to particulate antigen[2,3]. Allograft rejection occurs[2] and its immunological basis is shown by the specific accelerated rejection of second set grafts[4].

In our experience with skin transplants, *Xenopus* maintained at 24°C behaves in a manner broadly comparable with mammals and birds. We have found that autografts,