POSTSYNAPTIC SPECIALIZATION OF SMOOTH MUSCLE AT CLOSE NEUROMUSCULAR JUNCTIONS IN THE GUINEA PIG SPHINCTER PUPILLAE

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While postsynaptic specialization of various kinds has been described at the skeletal neuromuscular junction (1, 2) and at nerve synapses (5), no well-defined postsynaptic structures have been established for smooth muscle at autonomic neuromuscular junctions (3, 4) Postsynaptic structures of three kinds have been described, although these have been reported to be inconsistent features not present at all junctions: (*a*) Aggregations of micropinocytotic vesicles opposed to the nerve varicosities in smooth muscle cells have been described in arterioles of the pancreas (15) and in the muscle coat of the intestine (4, 14). (*b*) Areas of increased density of postsynaptic membrane (26, 17) or desmosome-like structures (11) at the close-contact area between axons and muscle cells have been reported. However, these appear to be a rare feature and may represent mechanical attachment points between axon and muscle cell rather than structures related to the neurotransmission process (11) (*c*) Subsurface cisternae have been observed in the region of smooth muscle closely opposed (less than 200 A) to some nerve terminals in the vas deferens (20, 16, 4)

In the present study, postsynaptic regions at neuromuscular junctions in the guinea pig sphincter pupillae have been examined. This tissue was chosen because it is relatively densely innervated with close (about 200 A) neuromuscular junctions (21), and because the need for rapid neuromuscular transmission seems likely to be greater in this situation than in visceral or vascular systems.

Irises from adult albino guinea pig were fixed in 0 05 M phosphate-buffered (pH 7.4) 4% glutaraldehyde for 20 min, washed in phosphate buffer for 2 hr, and postfixed in osmium tetroxide for 1 hr. They were block-stained in 2% uranyl acetate solution (27) before dehydration and embedding

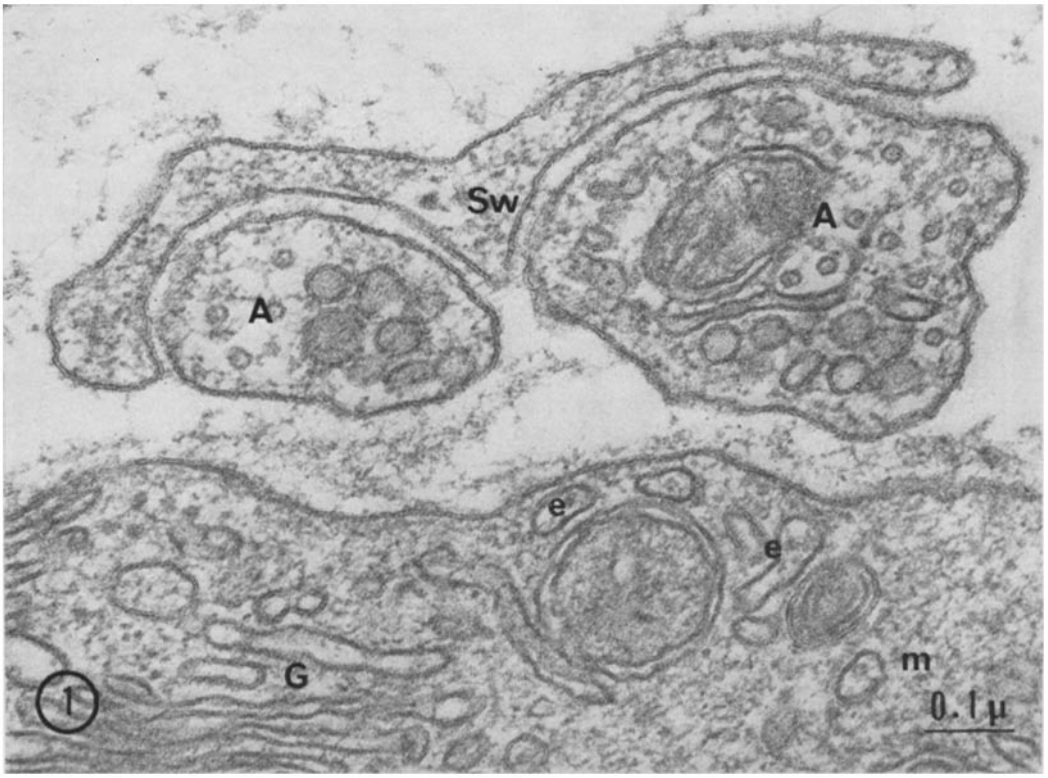


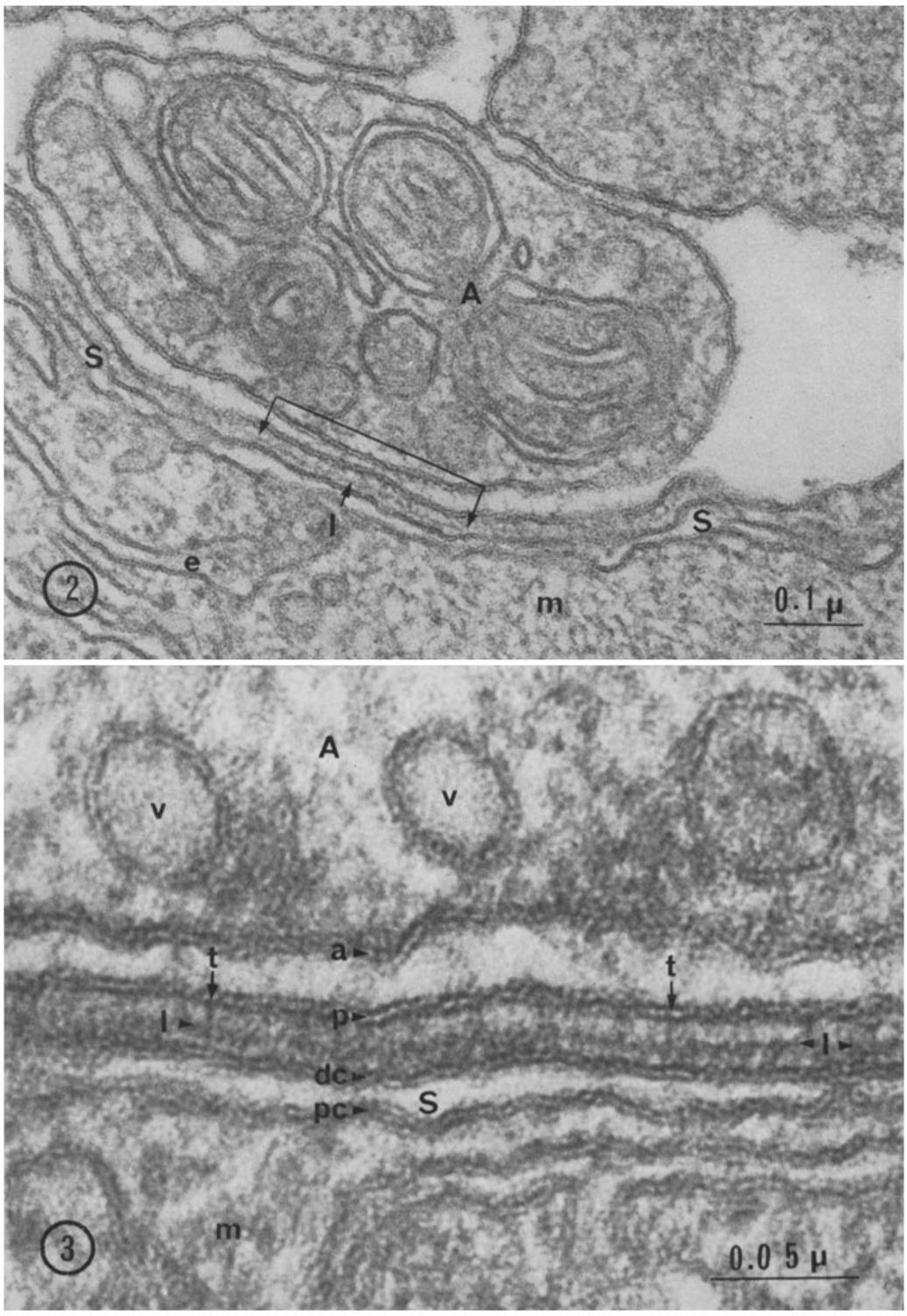
FIGURE 1 Two axons (*A*), partly enclosed by a Schwann cell sheath (*Sw*), are separated by a wide gap (500 A) from the surface of the smooth muscle cell (*m*) in the guinea pig sphincter pupillae. Basement membrane material is interposed in the gap. Note that there is no structural specialization in the peripheral cytoplasm of the muscle cell opposing the axons, although some of the smooth endoplasmic reticulum (*e*) appears to be closely applied to the muscle membrane. *G*, Golgi complex. × 120,000.

in Araldite. Ultrathin sections were doubly stained in uranyl acetate in 90% methanol for 30 min and lead citrate for 5 min (28) and examined with an Hitachi HU 11B electron microscope.

Small bundles of axons in the sphincter pupillae partly invested by Schwann cell processes are usually separated from the underlying smooth muscle cells by a gap more than 500 A, with basement membrane material interposed Although these axons contain a significant number of synaptic

FIGURE 2 A subsurface cisterna (*S*) beneath the plasma membrane of the muscle cell (*m*) in the close-contact (within 200 A) area between the naked axon (*A*) and the muscle cell (*m*). The cytoplasmic zone (area indicated by arrows), intercalated between the muscle cell membrane and the distal membrane of the cisterna, is consistent in width (150–170 A) and contains a continuous electron-opaque intermediate layer (*I*). × 150,000.

FIGURE 3 High-power electron micrograph showing the organized cytoplasmic zone containing the intermediate layer (*I*) about 40 A thick between the plasma membrane (*p*) of muscle cell (*m*) and the distal membrane (*do*) of the cisterna (*S*). The intermediate line is about 80 A apart from the plasma membrane and about 40 A from the distal cisternal membrane. Electron-opaque filamentous material (*i*) appears to traverse the cytoplasmic zone. The plasma membrane (*a*) of the terminal axon (*A*) is separated from the muscle surface by a narrow gap (about 200 A). *v*, synaptic vesicle; *pc*, proximal membrane of the cisterna. × 450,000


A
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l
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vesicles, the region of smooth muscle cells that apposes the axons does not exhibit detectable postsynaptic specialization (Fig. 1). However, at the close neuromuscular junctions, where single terminal axons, usually free of Schwann cell investment, approach within 200 A of the muscle membrane without intervention of basement membrane, subsurface or subsynaptic cisternae are often encountered just beneath the muscle membrane opposed to the terminal axon These cisternae are up to 1.5–2 μ long and from 50 to 150 A wide except at their bulbous lateral edges (Fig. 2)

A characteristic feature of the subsurface cisternae in the sphincter pupillae muscle is the occurrence of a continuous electron-opaque intermediate line interposed between the muscle membrane and the distal membrane of the cisternae (Fig. 2). The cytoplasmic zone containing the intermediate line is of consistent width (150–170 A), whereas the remaining part of the cytoplasmic zone between the cell membrane and the cisternae is less organized with an uneven width ranging from 50 to 200 A. Fig. 3 shows a high resolution micrograph of this highly organized cytoplasmic zone beneath the terminal axon. The electron-opaque layer, which is about 40–60 A thick, runs between the muscle membrane and the distal membrane of the subsurface cisternae. This layer is not centrally placed; it is closer to the distal membrane of the cisternae (about 40 A) and is separated by about 80 A from the muscle membrane. Filamentous elements appear to traverse the space between the muscle membrane and the distal membrane of the cisternae In a few cases, subsurface cisternae with this kind of specialization were also seen in areas of muscle surface where no nerves were visibly related. However, it appears that they are mostly confined to neuromuscular junctions.

Subsurface cisternae have been described in a variety of other cell types: neurons in peripheral and central nervous systems (22, 9, 23), sensory cells in receptor organs (12, 24), Sertoli cells (6), cultured oral cells (13), and parotid acinar cells at the area of close apposition of nerve terminals (8). In a study of subsynaptic cisternae in the central nervous system, Rosenbluth (22) made a brief reference to a comparable structure, which he described as a faint intermediate line consisting of cementing substance, or minute cross-bridges in the space between the plasma membrane and subsurface cisternae. Siegesmund (23) has also demonstrated electron-opaque fine granular material in this space in the neurons in the central nervous system. However, no descriptions of highly organized structures of this kind have been reported in relation to subsurface cisterns of other systems Comparable structural organization appears to be represented in triads or diads in skeletal muscle (25, 19). In this system, the cisternae (terminal cisternae of the sarcoplasmic reticulum) and the transverse tubules or plasma membrane are connected by organized electron-opaque structures, and it has been suggested that they are involved in impulse transmission during excitation-contraction coupling (18).

Further studies with high resolution electron microscopy are necessary to determine whether subsurface cisternae, in general, have the kind of organized substructure described in this report. The functional significance of the subsurface cisternae in the sphincter pupillae is not known. However, by analogy with subsurface cisternae described in other systems, whether or not these cisternae have structural specialization, they may be involved in the transmission process (7), possibly by regulating the physiological properties of the postsynaptic membrane (22, 10).

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