

NERVE-ACINAR CELL RELATIONSHIPS IN THE RAT PAROTID GLAND

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Ultrastructural studies have shown that many exocrine glands receive their autonomic innervation through close (180–250 Å) apposition of the nerve and acinar cell membranes (1, 5, 14, 16, 17, 20, 23–25). The nerve terminations or varicosities often lie in a groove on the surface of the acinar cell, or are closely apposed to two or more acinar cells by lying in the intercellular space between the cells. No specializations of the nerve or acinar cell membranes have been observed, but it is assumed that functional contacts do exist in these regions of close apposition. This report describes a type of subsurface cistern in the acinar cells of the rat parotid gland at regions of close apposition of nerves and acinar cells.

MATERIALS AND METHODS

Parotid glands were obtained from adult Sprague-Dawley rats of both sexes. The animals were perfused with a mixture of 2.5% glutaraldehyde, 2% formaldehyde in 0.1 M Na cacodylate buffer, pH 7.4(2). For routine electron microscopy, tissues were fixed for 2–6 hr in the aldehyde mixture, washed overnight in 0.1 M Na cacodylate with 7.5% sucrose, and post-fixed in 1% OsO₄ in cacodylate buffer. The tissues were stained in block with 0.5% uranyl acetate (9), dehydrated in ethanol, and embedded in Araldite (10). For cytochemical demonstration of cholinesterase activity, thin slices of tissues fixed for 2 hr and washed overnight were incubated in the medium of Karnovsky (8). Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates. Eserine (10⁻⁴ M) and BW62C47 (10⁻⁴ M) were used as inhibitors. After incubation, the tissue slices were rinsed in 0.44 M sucrose, pH 6.0, postfixed in 1% OsO₄, dehydrated, and embedded in Araldite. Thin sections were cut on a Porter-Blum microtome,

stained with lead citrate (22) and examined in a Siemens Elmiskop I at 80 kv.

RESULTS

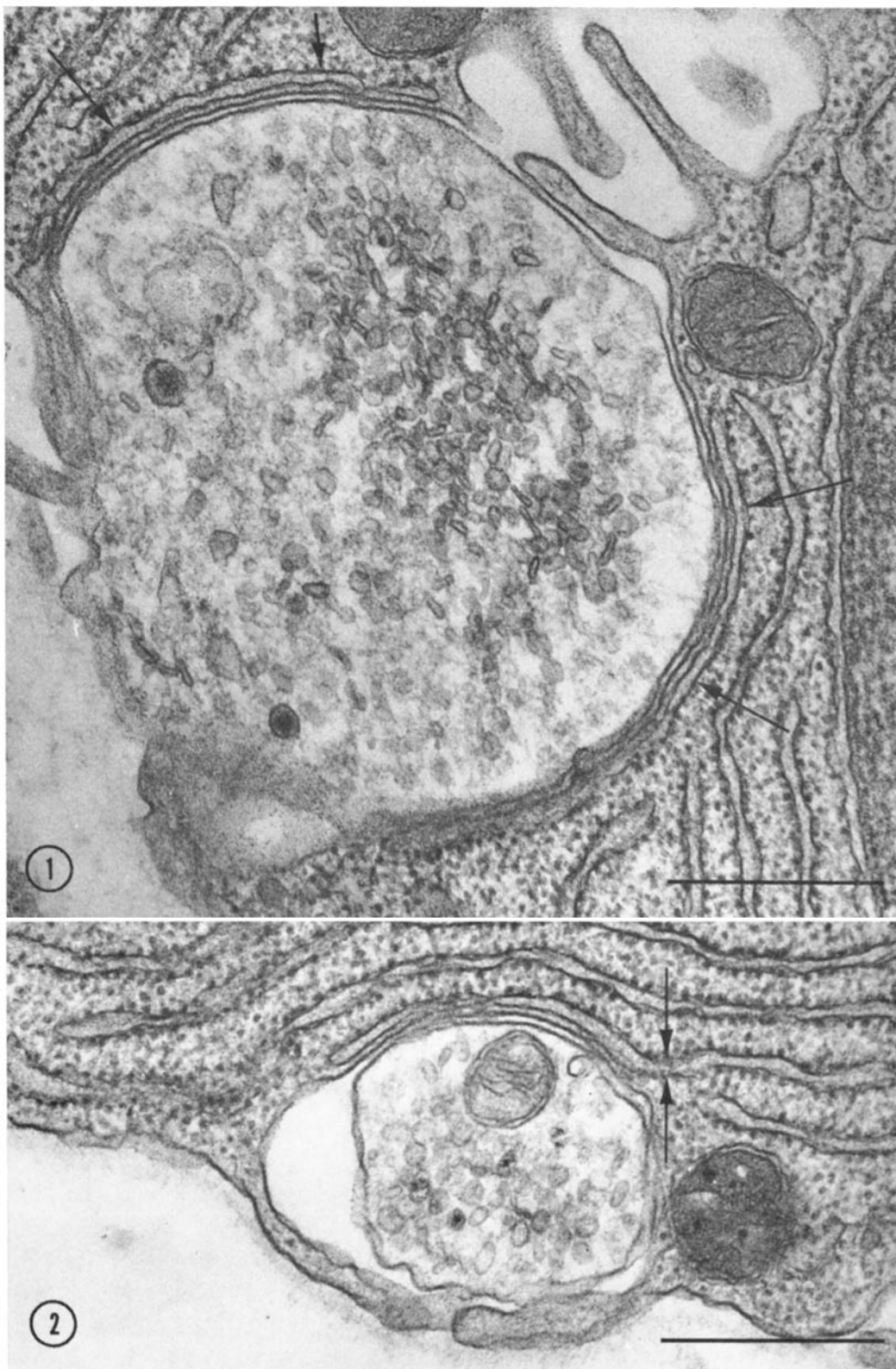
The fine structure of the rat parotid has been reported previously (11, 15, 17). Myelinated and unmyelinated nerves accompanied by Schwann cells were abundant in the connective tissue stroma. Unmyelinated axons, with or without their Schwann cell sheath, penetrated the basement membrane surrounding the acini and came into close apposition to the acinar cells (Figs. 1 and 2). The intercellular space between the nerve and acinar cell was approximately 95–120 Å (center-to-center separation of the membranes was 175–200 Å, which corresponds well with measurements reported in other studies). Axon terminals (12) were found at the basal surface of the acinar cells, where close apposition was made with only one cell, or in the intercellular space between adjacent acinar cells, where two or more cells were apposed. The terminals contained mitochondria, large dense-cored vesicles, and smaller vesicles resembling synaptic vesicles, both spherical and elliptical. The terminals were often embraced by cytoplasmic folds of the acinar cell.

In the acinar cells, at the area of close apposition of some of the nerve terminals, a cistern of endoplasmic reticulum closely paralleled the acinar cell membrane (Figs. 1 and 2). This cistern was separated from the cell membrane by 105–155 Å. The cisternal membrane facing the cytoplasm had attached ribosomes, while the one facing the cell membrane was devoid of ribosomes.

Scale bar (Figs. 1–3) is 0.5 μ.

FIGURE 1 A nerve terminal lying in the intercellular space between two parotid acinar cells is closely apposed to both of them. A cistern of endoplasmic reticulum closely parallels the acinar cell membrane at each area of apposition (arrows). Ribosomes are present on the cytoplasmic surface of the cisterns but are absent on the side facing the cell membrane. ×65,700.

FIGURE 2 A nerve terminal is embraced by cytoplasmic folds of the basal surface of an acinar cell. The specialized portion of the endoplasmic reticulum paralleling the area of apposition is continuous with the endoplasmic reticulum in the rest of the cell (arrows). ×68,600.



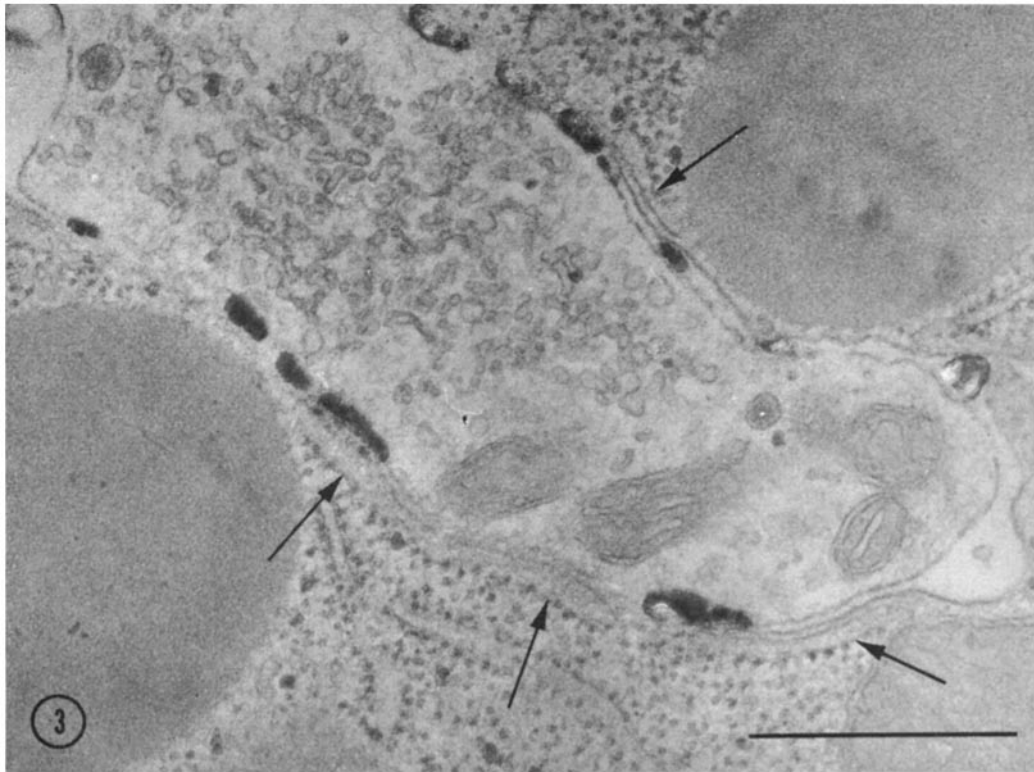


FIGURE 3 Acetylcholinesterase reaction product lies in the intercellular space at the areas of apposition of the nerve with the acinar cells. Cisterns of endoplasmic reticulum parallel the nerve for much of the area of apposition (arrows). $\times 68,600$.

In some cases the cistern only partially covered the area of apposition. Similarly, identification of the same terminal in serial sections showed that portions of the area of apposition were covered by a cistern, while other portions were not. Occasionally a cistern was seen paralleling the cell membrane where a Schwann cell process or a cytoplasmic fold of the acinar cell intervened between the acinar cell and the nerve. Fortuitous sections demonstrated continuity between these specialized cisterns and the granular endoplasmic reticulum, studded on both surfaces with ribosomes (Fig. 2).

In tissues incubated for demonstration of cholinesterase activity, using acetylthiocholine iodide as substrate, reaction product was found in the intercellular space between the axon or terminal and its surrounding Schwann cell or acinar cell (Fig. 3). In some cases, the location of the reaction product was correlated with the presence of an ER cistern (Fig. 3); in others, no cistern was

apparent adjacent to the site of deposition of reaction product. Not all areas of nerve-acinar cell apposition had an accumulation of reaction product.

Nonspecific deposits were sometimes found in the connective tissue spaces, especially associated with basement membranes or collagen fibers near nerve bundles. Deposition on collagen under certain conditions of fixation was previously noted by Karnovsky (8). Sections pretreated and incubated with butyrylthiocholine iodide and BW62C47 (a specific acetylcholinesterase inhibitor) had no reaction product associated with the nerves or areas of nerve-acinar cell apposition.

DISCUSSION

The specialized cisterns of endoplasmic reticulum located directly adjacent to areas of nerve-gland cell apposition are very similar to the subsurface cisterns (SSC) described by Rosenbluth and others in the central nervous system (CNS) (6, 13, 18).

Similar structures have been reported in certain sensory cells (19), ameloblasts (7), hepatocytes (21), renal proximal tubular cells (3), and Sertoli cells (4). In the CNS, the SSC's are situated slightly closer to the cell membrane, usually at a distance of 40–100 Å (13), rather than the 105–155 Å observed in the present study. Also, the association of ribosomes with the cytoplasmic surface of the SSC was variable in the CNS, but constant in the rat parotid gland. There appears to be no association of mitochondria with the cytoplasmic surface of the cisterns in the parotid gland as there often is in the Purkinje cell (6), and no opposing cistern in the nerve process as seen in adjacent cells in other tissues (3, 4, 7, 21).

Rosenbluth (13) and Herndon (6) have suggested that the SSC's may be involved in the transfer of metabolites into and out of the cell. The continuity of these cisterns with the granular endoplasmic reticulum could channel materials into and out of the ER system. In the parotid gland, it seems likely that they are involved in some manner with nerve-acinar cell interactions since they have been observed only in relationship to areas of close apposition between nerves and acinar cells. The localization of acetylcholinesterase reaction product in areas of close apposition between nerves and acinar cells is indicative of a relationship more important than topography alone. Stimulation of the acinar cell could occur at all points along the areas of close apposition, or may be restricted to certain portions of that area. In the absence of membrane specializations typical of synapses of the CNS, it is tempting to speculate that the presence of these subsurface cisterns denotes the site of functional contact between the nerve and its effector cell. However, they may have other functions, such as synthesis of a neurotrophic substance which directs the establishment, and maintenance, of acinar cell innervation.

At present it is not known whether these subsurface cisterns are peculiar to salivary glands or are universally found in all exocrine glands. They are also found in von Ebner's gland (unpublished observation), although they are not so frequent. A survey of other exocrine glands, further cytochemical studies, and correlations of the cisterns with cholinergic or adrenergic nerves

would provide further information as to their possible function.

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