CONFRONTING SUBSURFACE CISTERNAE IN CHICK EMBRYO SPINAL GANGLIA

PEDDRICK WEIS. From the Department of Anatomy, New Jersey College of Medicine and Dentistry, Jersey City, New Jersey 07304

Subsurface cisternae (SSC's) have been described in several types of conducting tissue (1–6), and in a cultured neoplastic cell line (7). In some of these cases (2, 3, 7), paired SSC's appeared opposite each other in apposing cells, a configuration referred to as subsurface confronting cisternae (7). This note demonstrates the existence of SSC's in a transient confronting configuration in a developing normal vertebrate tissue, the sensory neuroblast of the chick.

MATERIALS AND METHODS

Lumbosacral spinal ganglia of chick embryo, from eggs incubated 4–12 days at 38°C, were fixed in 1% OsO_4 in Hanks balanced salt solution or in Dalton's chrome-osmium (8) with 0.01 M $CaCl_2$ added for 1 hr, dehydrated in a graded series of ethanol, embedded in Maraglass (9), and sectioned on a Porter-Blum microtome with glass knives. Thin sections were mounted on Formvar- and carbon-coated grids and examined in a Philips EM-100B electron microscope (Philips Electronics and Pharmaceutical Industries Corp., New York). The mounted sections were stained with uranyl acetate and lead citrate (10). Since neuroblasts differentiate sooner in the ventrolateral area of the ganglia (11), observations were restricted to this region to obtain a temporal sequence of developmental events.

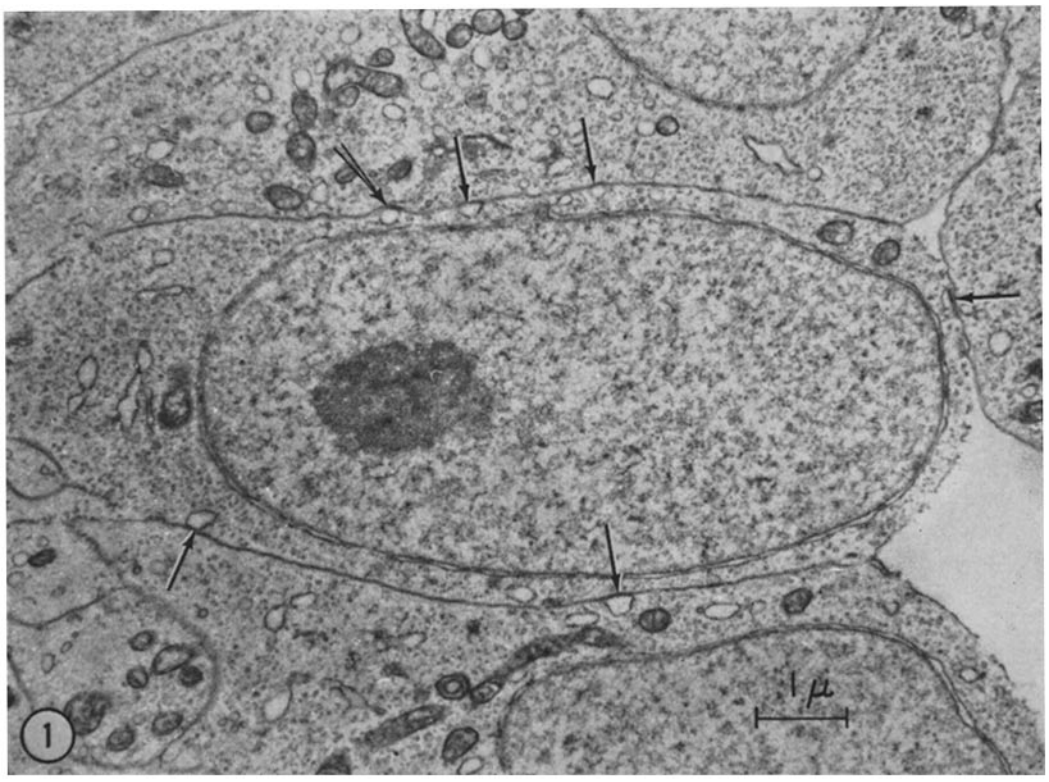

FIGURE 1 Lumbosacral spinal ganglion from chick embryo incubated for 4 days. OsO_4 fixation. Subsurface cisternae indicated by arrows; paired cisternae at double-stemmed arrow. x 11,800.

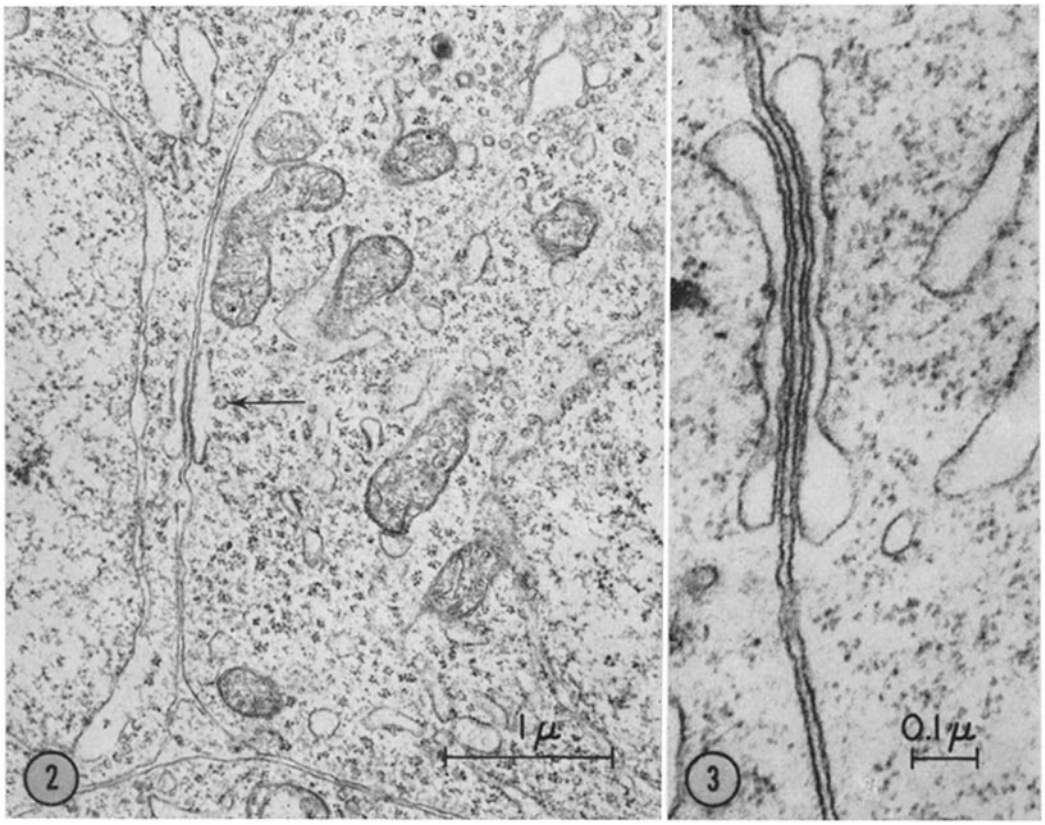

FIGURE 2 Spinal ganglion from chick embryo of 5 days' incubation. Arrow points to spiral array of ribosomes on surface of diverticulum from an SSC in confronting configuration. OsO_4 fixation. x 21,800.

FIGURE 3 Spinal ganglion from chick embryo at 6 days' incubation, showing a confronting pair of SSC's in apposed neuroblasts. Chrome-osmium fixation. x 84,000.

OBSERVATIONS

Migrating neural crest cells have begun to coalesce into ganglia by the 4th day of incubation. In those neuroblasts which have not yet made contact with each other, the ribosomes are mostly in a nonattached, polysomal configuration, while those neuroblasts which have made contact with other neuroblasts have begun to accumulate rough endoplasmic reticulum. SSC's can be seen simultaneously with the appearance of the first cisternae of the ergastoplasm. At first, they appear both singly and as confronting pairs (Fig. 1), but by the 5th day, they occur only in the configuration of confronting pairs (Fig. 2). This configuration is formed not only at places of contact between somas of adjacent neuroblasts, but also at dendrosomatic and dendrodendritic contacts. These relationships persist through to the 7th day of incubation, at which time satellite cells have begun to encapsulate the neuroblasts. As the satellite cells' processes separate the neuroblasts from each other, the pairs of confronting SSC's are likewise separated. The relative positions of the SCC's are not maintained; perhaps the SCC's are moved about by cytoplasmic streaming. By day 12 the ventrolateral neuroblasts are 2–3 times the diameter of 7-day neuroblasts, and the SSC's are relatively scarce.

Throughout this developmental sequence, the SSC's maintain the appearance described by Rosenbluth (1). The superficial membrane is smooth and is separated from the neuronal plasmalemma by a space approximately one-half that of the 200 A intercellular space (Fig. 3). The deeper membrane is sparsely studded with ribo-

somes. The cisternal depth is quite shallow, although some SSC's have lumens which are dilated partially or completely. This dilation may be a result of fixation. There is no consistent association of other organelles with the SSC's, as is seen in mature mammalian neurons (1), nor is there any indication of any local difference in the intercellular material.

DISCUSSION

Several functions have been suggested for SSC's. These include alteration of the neuronal plasma membrane for either facilitation (1) or inhibition (6) of electrical transmission, or for passage of metabolites (1, 3). The only finding related to the function of SSC's is the localization of acetylcholinesterase within the lumen (12).

Confronting SSC's of rough endoplasmic reticulum have been described in cultured neoplastic cells (7), but no functions have been suggested for this configuration. Confronting SSC's of smooth endoplasmic reticulum are found as dyads with cytoplasmic tubular extensions in striated muscle of the bloodworm *Glycera*, where they may have a metabolic function (3). Berger has found confronting SSC's of smooth endoplasmic reticulum in paired cones of a teleost retina (2); he suggests that these SSC's, which cover virtually the entire contacting area of the paired cells, may insulate the cells from each other by presenting six, instead of the usual two, membranes between the cytoplasmic areas of the paired cells. In the case of chick sensory neuroblasts, however, any such effect would be minimal since the SSC's underlie only a minor portion of the cell surface.

The fact that the SSC's do come together and stay together points to either a localized difference in the extracellular material whose influence extends through the plasma membranes on either side, a mutual attraction of the SSC's, or localized differences in the plasma membranes resulting in an adhesive quality which attracts both SSC's and similar areas on opposing membranes. Such external adhesiveness could be involved in contact inhibition, as suggested by Abercrombie (13). Since the SSC's are found in the confronting configuration soon after migration of the particular neuroblasts has ceased, such relationship to contact inhibition must be considered.

Contact inhibition has also been related to intercellular communication by Loewenstein and Penn (14) who have demonstrated that electrical communication between cells of regenerating amphibian skin is established shortly after cell migration ceases. There is a greater time lag between cessation of migration by chick neuroblasts and the subsequent appearance of confronting SSC's than in the sequence of cessation of movement and the development of intercellular communication in amphibian skin. Nevertheless, these two types of tissue differ greatly, and so it does not seem unreasonable that SSC's, which could be associated with localized differences in the plasma membrane, could affect permeability and the transmission of ions. This idea is not new; similar functions for SSC's have been discussed by Rosenbluth (1) in relation to intercellular communication though not in relation to contact inhibition. Loewenstein and Penn (14) have suggested that tight junctions provide the pathways for communication. Such membrane specializations exist in the chick ganglion (15) but occupy a relatively small amount of cell surface as compared with the SSC's.

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Note Added in Proof:

In a recent paper describing the development of the endoplasmic reticulum in chick embryo spinal ganglia, Pannese has noted that, at the thoracic level, "primitive neuroblasts" develop SSC's at 4 days of incubation. He also shows a confronting pair. (Ennio Pannese, 1968. *J. Comp. Neurol.* **132:** 331.)

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