Formation of intracellular canaliculi in reaggregated striated duct cells of rat submandibular gland during short-term stationary culture

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Summary. Reaggregated striated duct cells of rat submandibular gland formed deep tubular invaginations of the cell membrane, i.e. intracellular canaliculi, which are not present in the original tissue, after 24 h of the stationary culture.

There are few morphological observations on the cell membrane of dissociated cells from normal adult mammalian tissues during the short-term stationary culture. Formation of junctional complexes or similar structures were observed during short-term culture of reaggregated pancreas cells[2], acinar cells of the submandibular gland[3] and fibrocytes[4]. Amakawa and Barka reported that the cell membrane of dissociated acinar cells of rat submandibular gland, cultured for 24 h, showed a marked increase in lectin binding, compared to freshly dissociated cells[5].

In the present study, the formation of deep tubular invaginations of the cell membrane, i.e. intracellular canaliculi, which are not present in the original tissue, in reaggregated striated duct cells of rat submandibular gland during the stationary culture for 24 h are described.

Isolated cells were obtained aseptically from submandibular glands of female Sprague-Dawley rats of 4 weeks of age by a procedure described previously[3]. The dissociated cells were suspended at concentration of 5.5 to 8.8×10^5 cells per ml in a culture medium consisting of 90% Eagle's minimal essential medium with Earle's salt, 10% fetal calf serum, penicillin (100 units per ml), streptomycin (100 µg per ml) and Fungizone (0.25 µg per ml). The pH of the medium was adjusted to 7.4. 5 ml aliquots of the cell suspensions were cultured in plastic culture flasks (Falcon, type 3012) in 95% O_2 + 5% CO_2 for 24 h. After culture, the cells were resuspended by shaking and were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 1 h at 4 °C and postfixed in 1% buffered OsO_4. After dehydration in alcohol, the cells were embedded in Epon. Thin sections,

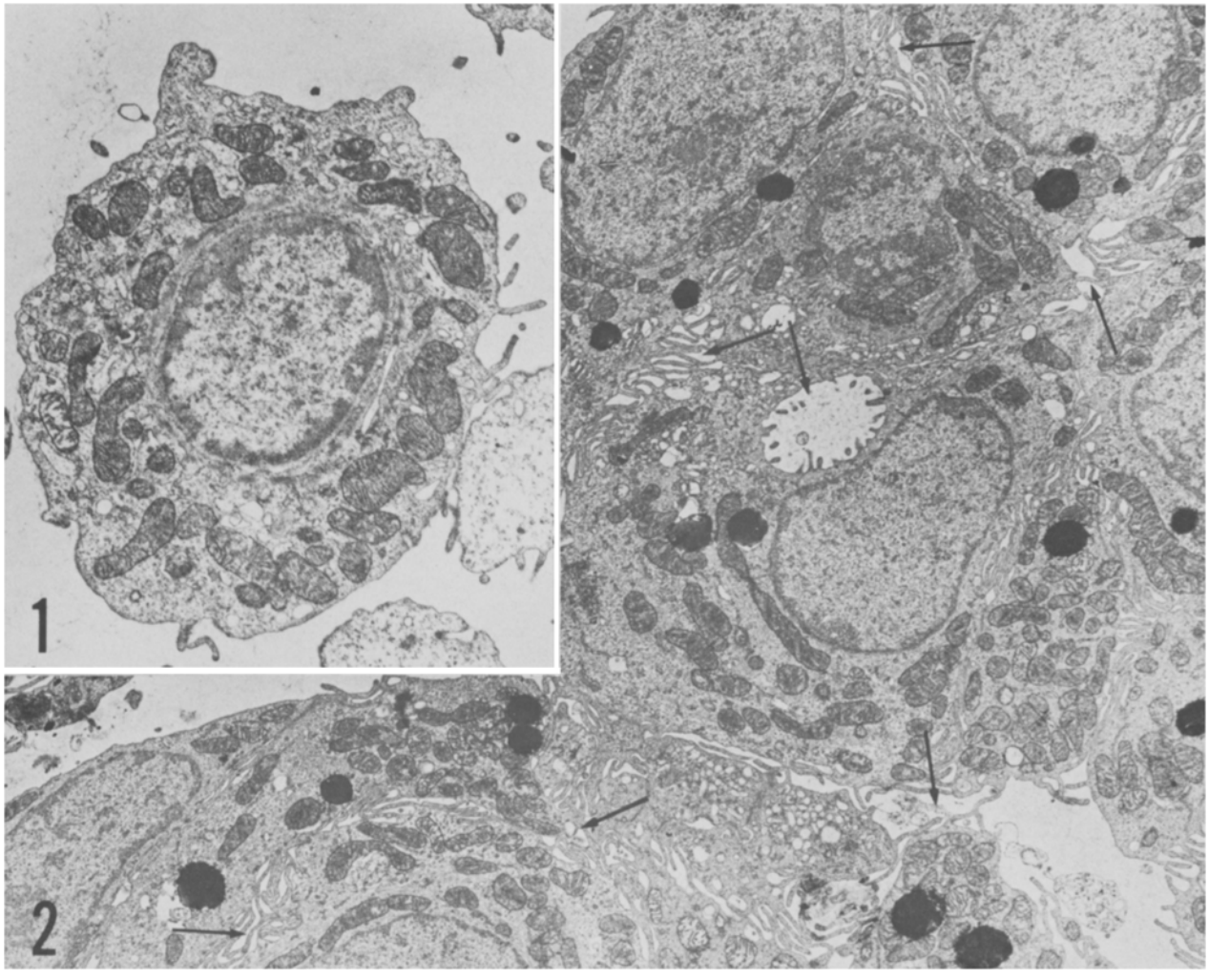

Fig. 1. Electron micrograph of an isolated striated duct cell. Few microvilli are seen. × 8800.
Fig. 2. Electron micrograph of a group of aggregated striated duct cells from a 24-h-culture of dissociated cells. Note intracellular canaliculi with numerous microvilli (arrows). × 5200.

stained with uranyl acetate and lead citrate, were examined in a Hitachi HS-8 electron microscope. - Almost all of the dissociated cells formed smaller and larger aggregates after 24 h of culture.

Immediately after dissociation, the striated duct cells showed only a few short microvilli on their surfaces (figure 1), while after 24 h of culture they formed deep tubular invaginations of the cell membrane (figure 2). Such invaginations were not seen in the original tissue. The tubular invaginations consisted of canals with numerous microvilli and were similar to the intracellular canaliculi in gastric parietal cells. - Similar structures were not seen in acinar cells and intercalated duct cells.

The characteristic structural feature of the surface of the striated duct cells is the presence of basal infoldings, short microvilli on the apical surface and folds and interdigitations of the lateral surface[6]. It is likely that the exposure to enzymes, such as collagenase and hyaluronidase in the present study, produced profound alterations of the cell surface. In fact, freshly dissociated striated duct cells do not show basal infoldings but only a few short microvilli. During 24 h of culture, a restoration of surface components lost by the enzymic digestion occurs and produces the intracellular canaliculi.

The short-term stationary culture system of dissociated striated duct cells of the submandibular gland may be useful as a model for analyzing mechanisms of the specialization and modification of the cell membrane.

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2 L. Orci, A.A. Like, M. Amherdt, B. Blondel, Y. Kanazawa, E.B. Marliss, A.E. Lambert, C.B. Wollheim and A.E. Renold, J. Ultrastruct. Res. *43*, 270 (1973).
3 S. Kanamura and T. Barka, Lab. Invest. *32*, 366 (1975).
4 C.W. Lloyd, D.A. Rees, C.G. Smith and F.J. Judge, J. Cell Sci. *22*, 671 (1976).
5 T. Amakawa and T. Barka, J. Histochem. Cytochem. *23*, 607 (1975).
6 J.A.G. Rhodin, Histology, p. 524. New York Oxford University Press, London, Toronto 1974.

Inhibition of neural tube closure by ionophore A23187 in chick embryos[1]

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Summary. Ionophore A23187 inhibited closure of the chick neural tube through its effects on cytoskeletal components.

Ionophore A23187, a carboxylic antibiotic isolated from *Streptomyces chartreusensis,* promotes transport of divalent cations, especially Ca^{++}, across biologic membranes[2]. This antibiotic has been shown to activate several calcium-dependent biologic processes. Some of these include: DNA synthesis in sea urchin eggs[3], secretion in mast cells[4,5], and mitogenesis in lymphocytes[6]. More recently, Moran[7] and Moran and Rice[8] found that ionophore counteracted the inhibitory effect of papaverine, a smooth muscle relaxant, on amphibian neurulation and proposed that the availability of free Ca^{++} is crucial in controlling closure of the neural tube. The present study was undertaken to investigate effects of ionophore on closure of the neural tube in chick embryos.

Materials and methods. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8_+ of development[9]. A total of 96 embryos were explanted by New's[10] technique and grown on thin albumen (= nutrient medium) with or without 25 µg/ml ionophore A23187, a concentration found to selectively inhibit closure of the neural tube. Embryos were examined at intervals during incubation to determine the degree of neural fold fusion. Some were fixed in Bouin's fluid, stained with Delafield's hematoxylin and kept as whole mounts. Others were processed for microscopic studies as described below.

For histological studies, embryos were fixed in Bouin's fluid, embedded in paraffin, serially sectioned at 6 µm, and stained with Delafield's hematoxylin and eosin. For electron microscopy, embryos were fixed in half strength Karnovsky's[11] fixative for 2 h at room temperature. After a thorough washing in 0.08 M cacodylate buffer (pH 7.2), embryos were postfixed for 1 h in 1% osmium tetroxide. After an additional brief rinse in buffer, embryos were stained 'en bloc' with 1% uranyl acetate, dehydrated in graded ethanol series, embedded in Spurr's[12] resin, and sectioned. Sections (silver/pale gold) were contrasted with aqueous uranyl acetate and lead citrate and examined with a RCA EMU-4 electron microscope.

Results and discussion. In stage 8_+ embryos, apposing neural folds have not yet made contact except in the future hindbrain region[13]. However, a brief exposure (1-2 h) of these embryos to 25 µg/ml ionophore often resulted in prematurely fused neural folds even in the somite region. In this region, affected neuroepithelial cells were highly elongated, wedge-shaped, and exhibited numerous apical foldings (figure 1), features which are usually found only in the brain region at this stage of development (figure 2). In addition, the terminal web, a band of contractile apical microfilaments, was densely organized (figure 1). Ionophore is known to promote both Ca^{++} influx and release of bound Ca^{++}[2]. High concentrations of intracellular free Ca^{++} can augment the contraction of microfilaments[14], cytoskeletal components known to be responsible for apical constriction and the wedge-shaped appearance of neural tube cells[13,15]. Therefore, it seems likely that the observed premature changes in cell shape and uplifting of neural folds in ionophore-treated embryos are consequences of enhanced microfilament contraction.

The effect of ionophore on the developing neuroepithelium was found to be time dependent. After 16 h of incubation, affected cells usually lost their highly elongated shape and the neuroepithelium as a whole became thinner than that of corresponding controls (compare figures 3 and 4). This effect was most pronounced in the portion of the neuroepithelium adjacent to the notochord (figure 3). Revel and Brown[16] have observed that fine fibrils firmly anchor the neural tube to the notochord. These 2 structures were often widely separated in ionophore-treated embryos (figure 3). Also, the cells forming the floor of the neural tube were less elongated than usual, suggesting that anchorage to the notochord may account in part for their high columnar, 'pulled down' appearance during the uplifting of neural