Quantitative Analyses of the Constituent Membranes of Parotid Acinar Cells and of the Changes Evident after Induced Exocytosis

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Summary. A morphometric study has been made at the EM level of Isoproterenol (IPR) induced secretion of rabbit parotid glands in vivo. Emphasis has been placed here on the membrane content of acinar cells and the changes which occur following induced degranulation. In particular it was hoped to establish whether the preservation of zymogen granule membrane as intact electron microscopically visible subunits and the subsequent reutilisation of this membrane is a plausible hypothesis from a quantitative morphological standpoint.

After two hours IPR had caused > 95% depletion of granules. About 1343 μm²/cell of granule limiting membrane temporarily fused with the apical plasmalemma during this time and by two hours 1158 μm²/cell of this had been eliminated. Only a small increase in intracellular smooth membrane area was recorded after degranulation and we find no evidence that the zymogen granule membrane is stored indefinitely as smooth membrane fragments either in the region of the Golgi apparatus or elsewhere in the cytoplasm.

IPR caused changes in RER membrane area (+ 37.7%, 1406 μm²/cell), which is a possible, but we consider implausible relocation site of granule membrane.

The possible mechanism of the removal of 'excess apical membrane' and the ultimate fate of the zymogen granule membrane is discussed.

Key words. Parotid gland — Membranes, Exocytosis — Isoproterenol — Amylase — Stereology, Electron microscopy.

Introduction

The exportable enzymes of the parotid gland are accumulated and stored in the cytoplasm of acinar cells as membrane-bound zymogen granules. A single injection of the β activator Isoproterenol (IPR) causes, within 2 hours, complete depletion of α amylase activity, one of the principle exportable enzymes of the gland (Byrt, 1966). It is now widely accepted that the mechanism of enzyme secretion involves the fusion of the zymogen granule limiting membrane with the apical plasmalemma and the discharge of the granule contents by exocytosis (Amsterdam, Ohad and Schramm, 1969; Simson, 1969). Thus there is a temporary increase in the size of the acinar lumen, which is particularly marked in induced degranulation as numerous granules discharge almost simultaneously. Eventually the lumen returns to its normal size as a result of the elimination of excess granule-derived membrane from its perimeter (Amsterdam, Ohad and Schramm, 1969).

It has been suggested that this eliminated membrane may be reutilised by the cell, but as yet the precise mechanism has not been established. Either the membranes may be degraded and the constituents reutilised (Fawcett, 1962; Hokin, 1968) or, as originally proposed by Palade (1959), intact fragments of membrane may become interiorised and returned to the Golgi zone to be stored and subsequently incorporated into newly-formed condensing vacuoles (Amsterdam,
Ohad and Schramm, 1969; Jamieson and Palade, 1971). However, biochemical evidence does not entirely substantiate either of these hypotheses. Although some membrane phospholipids seem to be reutilised (Hokin, 1968), Amsterdam et al. (1971) reported concomitant synthesis of membrane protein and exportable protein in the rat parotid, suggesting that the granule membranes may be synthesised de novo.

To our knowledge no measurements have been made at the electron microscope level of the membrane content of exocrine tissue. As part of a program on the metabolism and fate of zymogen granule membranes we have made a morphometric study at the electron microscopic level of rabbit parotid acinar cells before and after induced degranulation. In the first instance, we have studied the changes in membrane composition which are apparent two hours after the onset of degranulation. This time was not arbitrarily chosen, but was arrived at following pilot experiments. It represents the time at which the α-amylase content of the gland is at its lowest (see also Byrt, 1966). At this time too, the acinar cells’ surface to volume ratio has returned approximately to normal following the temporary enlargement of the apical plasmalemmal area caused by the zymogen granules membranes fusing with it. Once the acinar cells have recovered thus far from the effects of degranulation, the cells enter a prolonged resynthesis phase which finally ends with the complete re-establishment of the granule stores, but which starts with a period when morphological changes occur relatively slowly. Thus at two hours the timing of sampling is less critical and the results more reproducible as morphologically detectable events are occurring at a significantly slower rate than during the degranulatory phase. The changes apparent at two hours should serve to summarise the changes induced by IPR during the degranulatory phase of the secretory cycle, and in particular the absolute amount of membrane involved in the degranulatory process can be determined. It was hoped also to establish whether the reutilisation of intact membrane is a plausible hypothesis from a quantitative morphological standpoint and to determine the possible membrane relocation sites within the acinar cell.

Because the degree of granule loading in the glands of rabbits varies widely even after starvation, it was considered best to carry out stereological analyses on the paired glands of individuals, one gland from each animal being removed surgically as a control before the secretagogue was administered. Only after complete analyses on individual animals had been completed were the results pooled to give overall mean results. The difficulty of carrying out the surgical procedure necessarily limited the number of separate experiments that were possible.

In this study special attention has been paid to the tissue preparation procedure, since it has been found that the glands react adversely to non-isotonic media, even to the extent of tissue disintegration and membrane vesiculation.

Materials and Methods

Abbreviations. The following abbreviations have been adopted in the ensuing text:

- \( N \) number per unit volume, \( S \) surface area per unit volume, \( V \) volume per unit volume, \( \bar{d} \) mean organelle profile diameter, \( \bar{D} \) mean organelle diameter, \( L \) intercept length per unit length of test line, \( P \) interceptions per unit length of test line, \( s/v \) surface to volume ratio, \( IPR \) DL (1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride, RER rough endoplasmic reticulum.