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Effects of Administration of Pilocarpine on the Acinar Cells of Parotid and Mandibular Glands of the Mice

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Introduction

It is well known that the salivary glands are received nerve fibers from both the sympathetic and parasympathetic nerves and salivary secretion is under the control of them, and salivary secretion is promoted weakly by the former and strongly by the latter.

In the parotid and mandibular glands of mice[6], rats[1,3–7] and cats[2], there are several reports on the administration of pilocarpine which is a cholinergic drug having an enhancing effect on salivary secretion. However, in these reports there have been no detailed descriptions on the relationships between the secretory granules and cytoplasmic organelles.

In this report, after the administration of pilocarpine, we observed the changes of fine structure of the parotid and mandibular acinar cells and then discussed about the formation and extrusion of secretory granules.

Materials and Methods

Adult 40 male and 40 female ICR–JCL mice (90 days old) were used in these experiments. Thirty male and 30 female mice were administered pilocarpine hydrochloride (0.2 mg/kg.B.W.) subcutaneously. Ten male and 10 female intact mice were used as control at age of 90 days old. The animals were sacrificed at 10, 30 and 60 minutes after injection. Then, the parotid and mandibular glands were removed immediately and the tissues were fixed with the mixture of 1.25% glutaraldehyde and 1% osmium tetroxide in phosphate buffer, at pH 7.4. After the fixation, the tissues were dehydrated in ethanol and then were embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and were examined with JEM–100C transmission electron microscope.

For light microscopy, after fixation in Zenker-formol, the tissues were embedded in paraffin and sectioned at six microns. The sections were stained with Hematoxylin and Eosin (H·E), periodic acid Schiff (PAS) and pH 2.5 Alcian blue (AB), respectively.

Results

I. Parotid gland

The control parotid acinar lumina were narrow and inconspicuous. The acinar cells were pyramidal in shape and contained acidophile granules, being PAS-positive and AB-negative. The acinar cells were composed of light, dark and specific light cell, of which the former two containing

bipartite granules with electron dense corpuscle and moderately dense matrix (Fig. 1). The granular endoplasmic reticulum (gER) and free ribosomes were distributed widely throughout the cytoplasm, and the former was dilated relatively in the supranuclear and apical portions. The mitochondria were scattered sparsely. The Golgi apparatus which was composed of vesicles and vacuoles occupied an extensive area of cytoplasm in supranuclear portion. Specific light cell contained the granules which were similar to those of light and dark cells, and the cytoplasmic organelles were under developed.

Ten minutes after the administration (Fig. 2), the acidophile granules of acinar cell were decreased and basophile areas were increased in the cytoplasm. In some acinar cells, large vacuoles exhibiting PAS-negative were observed in the supranuclear and apical portions. At ultrastructural level, the number of secretory granules were decreased and the intercellular canaliculi were dilated slightly. The most of secretory granules showed no significant differences in nature and size as compared with controls, while the less and moderately dense granules were seen rarely. The dilated gER and Golgi apparatus with vesicles and vacuoles were relatively well developed. The appearances of free ribosomes and mitochondria were similar to those of control.

Thirty and 60 minutes after the administration, the acidophile granules were decreased more and more, and the cytoplasm showed a basophile as compared with those observed 10 minutes after the administration. The secretory granules exhibited the granular similar to control, homogeneous ones with high density (Fig. 3) and bipartite ones with the peripheral rim of moderate density which was different from the control's (Fig. 4). The dilated gER were observed in the supranuclear portion. Golgi apparatus was composed of lamellae, vacuoles and vesicles in the supranuclear portion. The appearances of free ribosomes and mitochondria were similar to those of control.

II. Mandibular gland

The control mandibular acinar cells were slightly basophile and moderately positive to PAS and AB. The round or ovoidal nuclei were situated in the basal region. The acinar cells had the cytological characteristics of a typical seromucous cell (Fig. 5). They contained less dense secretory granules, well-developed lamellar arrays of gER and a few mitochondria with flattened cristae. The intercellular canaliculi, one of the cytological characteristics in the seromucous acini, were detected in them. The myoepithelial cells were observed around the acini but no nerve terminals were detected in the acini.

Ten minutes after the administration, the acinar cells got slightly smaller in size and the secretory granules were decreased, and the cytoplasmic basophile region was increased. In some cells, vacuoles and large granules fused with one another were observed in the cytoplasm. At ultrastructural level, the secretory granules were accumulated in close relation to the lumen and intercellular canaliculi and were extruded from the cell by a merocrine typed secretion. A few large vacuoles suggesting their being formed by the mutual fusion out of the secretory granules were observed. The gER were dilated slightly and Golgi apparatus composed of vesicles was relatively well developed in comparison with those of control acinar cells.

Thirty minutes after the administration, concerning the acinar cells, the most remarkable ultrastructural features were noted to be the greatly dilated gER and numerous large vacuoles (Fig. 6). The secretory granules were decreased more and more, and the vacuoles were remarkable in number. The vacuoles were full of variety in size ranging from small ones to large ones, and in some cells, the cytoplasm was filled with vacuoles containing the fine flocculent materials of moderate density and the secretory granules were extruded into these vacuoles by a merocrine typed

secretion (Fig. 7). The gER were remarkably dilated and the dilated gER-cisternae contained the materials which are similar to those of the secretory granules, on the other hand, the granules related to the dilated gER-cisternae were observed rarely (Fig. 7).

Sixty minutes after the administration, the secretory granules were strikingly decreased in number and the number of vacuoles was full of variety, but the large vacuoles as seen in 30 minutes after the administration were small in number (Fig. 8). The secretory granules were similar to those of 30 minutes after the administration both in nature and in size. The gER were dilated but the degree of dilation were generally small as compared with those after 30 minutes.

No significant differences in the effect of pilocarpine-administration were noted between male and female animals in both parotid and mandibular glands.

Discussion

Several physiological and histochemical reports have been made public on the pilocarpine-administration of the parotid and mandibular glands in the mice[6] and rats[1,3-7]. On the other hand, as to the report on the ultrastructure of the parotid glands in mice treated with pilocarpine we have only the one described by Parks[6].

In the present pilocarpine-treated parotid acinar cells, the decrease of secretory granules, the dilation of gER-cisternae and the heterogeneous granules invisible in control acinar cells were observed at 10 and 30 minutes after administration. The appearances of the former were similar to those described in Parks's report[6]. About the heterogeneous granules, it is assumed that the granules may be some immature forms as described in rabbit[8] and chinese hamster[9]. On the other hand, there has been no report on the dilation of gER-cisternae in pilocarpine-treated parotid acinar cells. Such appearances as mentioned above were assumed to have been due to the structural changes brought forth by the functional acceleration of salivary secretion.

As far as we know, no attempt has even been done on the fine structures of the pilocarpine-treated mouse mandibular gland. After the administration of pilocarpine, the decrease of secretory granules, increases not only of vacuoles but of the well-developed Golgi apparatus, dilation of gER-cisternae and granules related to gER-cisternae were observed in mandibular acinar cells.

Concerning the granules related to gER-cisternae, it is generally mentioned that the secretory materials synthesized in gER are transferred to Golgi apparatus and then are condensed into large, dense secretory granules. These granules above mentioned are assumed to be either immediately extruded from the cell as secretory granule or are related to Golgi membrane and thereafter extruded from the cell. However, owing to the fact that no mandibular acinar cells in control showed such appearances, these assumed that there exist some granules which are synthesized in gER-cisternae and extruded immediately from the acinar lumina and intercellular canaliculi in the phases of strikingly high secretory function.

About the existences of vacuoles, in mouse Parks[6] described about the parotid acinar cells not reporting about the mandibular ones. What Parks[6] assumed was the fact that the vacuoles are to be produced by imbibing water as well as flat membranous vesicles and coalescence of secretory granules. It has not been examined in this study that the vacuoles were produced either by liquefaction and coalescence of secretory granules or by imbibition of water. However, a few vacuoles showing that apparently they were formed by the coalescence and liquefaction of secretory granules were observed.

From these investigations, it was ascertained that after the administration of pilocarpine, the

secretory granules of parotid and mandibular acinar cells are decreased in number, the gER-cisternae are dilated and the Golgi apparatus is developed, and moreover in the phases of strikingly high secretory function it was guessed to become directly the secretory granules out of the gER.

Summary

Following the pilocarpine-administration, the fine structures of the mouse parotid and mandibular acinar cells were studied by light and electron microscopies.

Ten minutes after the administration of pilocarpine the decrease of secretory granules, dilated gER-cisternae and a few heterogeneous granules were observed in parotid acinar cells. These changes were remarkable 30 minutes after administration.

In mandibular acinar cells, the slight decrease of secretory granules and a few vacuoles were observed 10 minutes after administration, and then, 30 minutes after administration a striking decrease of secretory granules, greatly dilated gER and numerous large vacuoles were observed. The secretory granules were extruded into these vacuoles by a merocrine typed secretion, and the granules related to the dilated gER-sacs were observed rarely.

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Explanation of figures

Fig. 1. Acinar cell of the parotid gland of the untreated control. These cells contain bipartite granules. The gER and Golgi apparatus are well developed.

Fig. 2. Acinar cells of the parotid gland 10 minutes after pilocarpine-treatment. The intercellular canaliculi and gER are dilated.

Fig. 3. Acinar cells of the parotid gland 30 minutes after pilocarpine-treatment, showing a few electron dense granules and dilated gER.

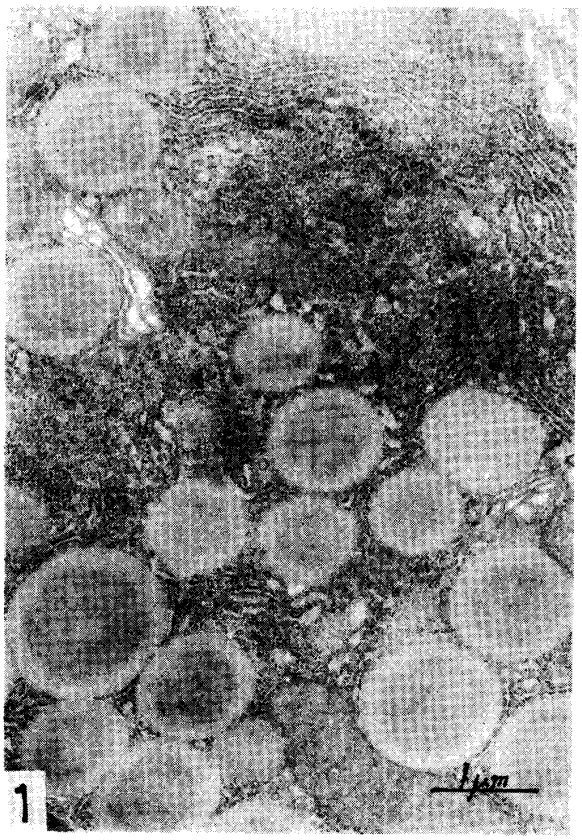
Fig. 4. Acinar cells of the parotid gland 30 minutes after pilocarpine-treatment. Secretory granules exhibit the bipartite granules with the peripheral rim of moderate density.

Fig. 5. Acinar cells of the mandibular gland of the untreated control. The low dense secretory granules are present in apical portion.

Fig. 6. Acinar cell of the mandibular gland 30 minutes after pilocarpine-treatment. Many large vacuoles and dilated gER are observed.

Fig. 7. Acinar cell of the mandibular gland 30 minutes after pilocarpine-treatment. The secretory granules containing the flocculent material are extruded into the large vacuole. Many vacuoles, greatly dilated gER-sacs and granule which are closely related to gER-sac are observed.

Fig. 8. Acinar cells of the mandibular gland 1 hour after pilocarpine-treatment. The dilated gER are present. No large vacuoles are present.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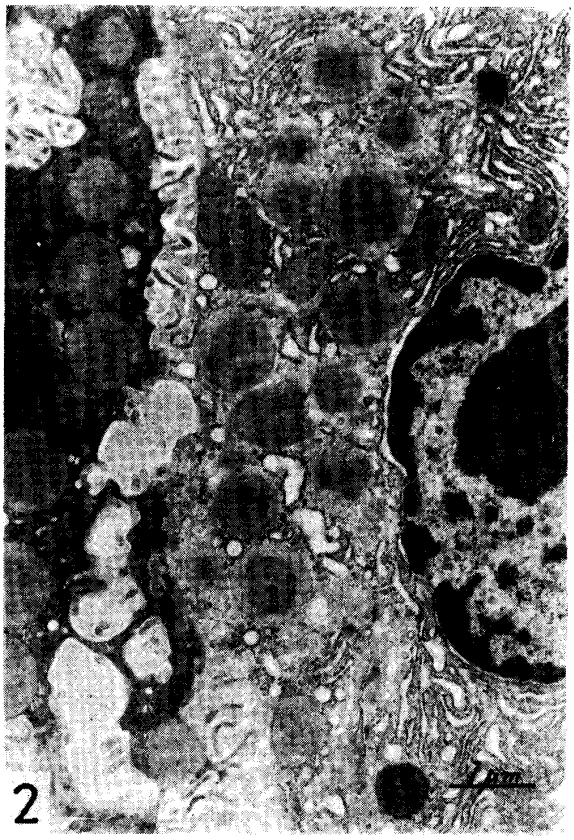
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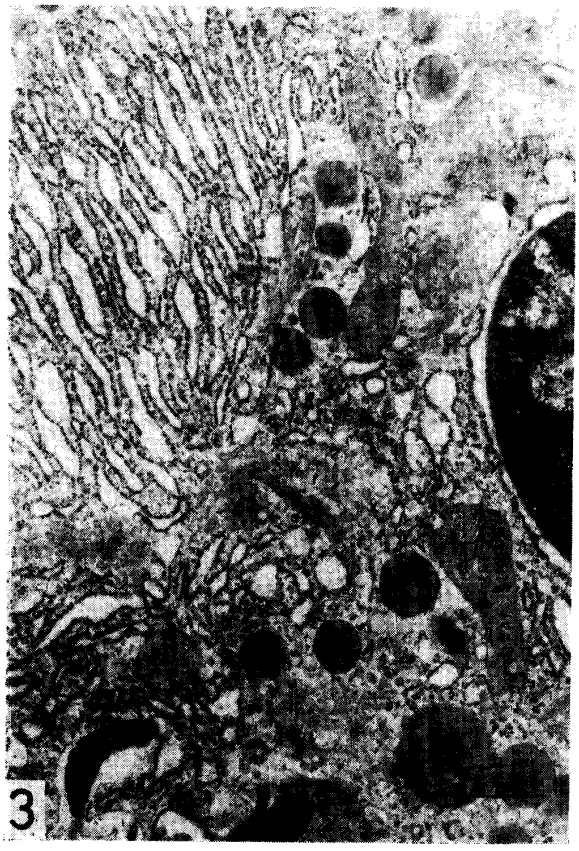

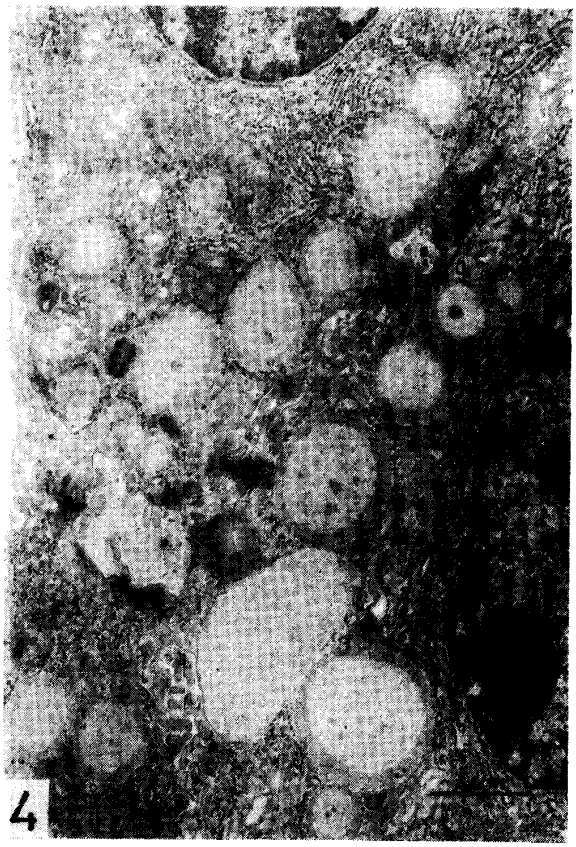

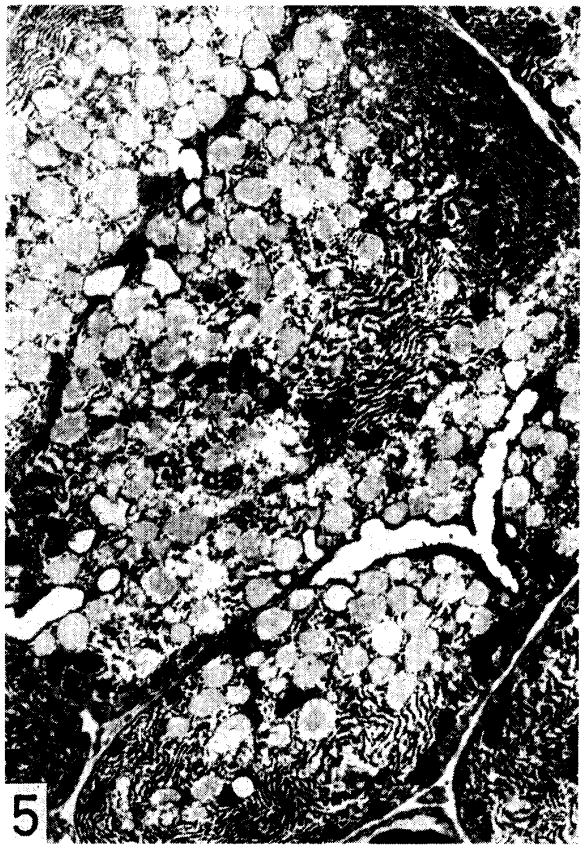
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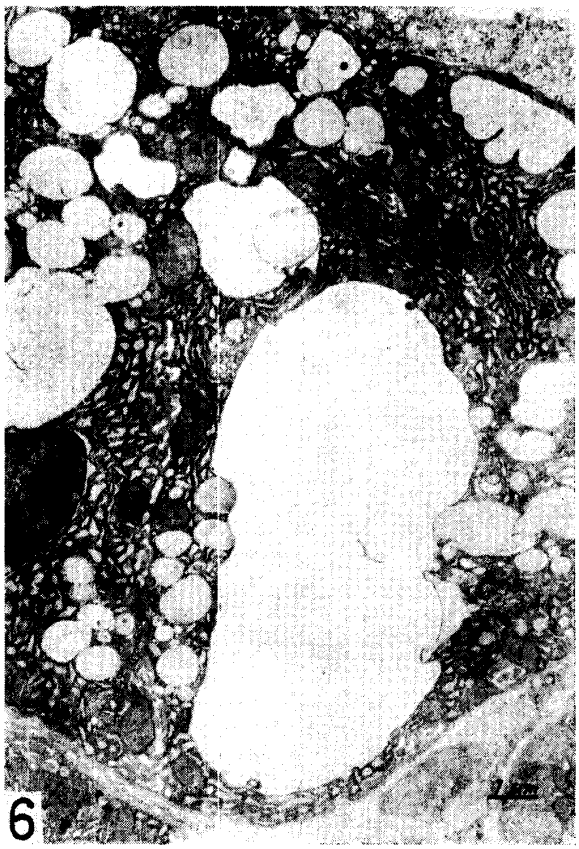
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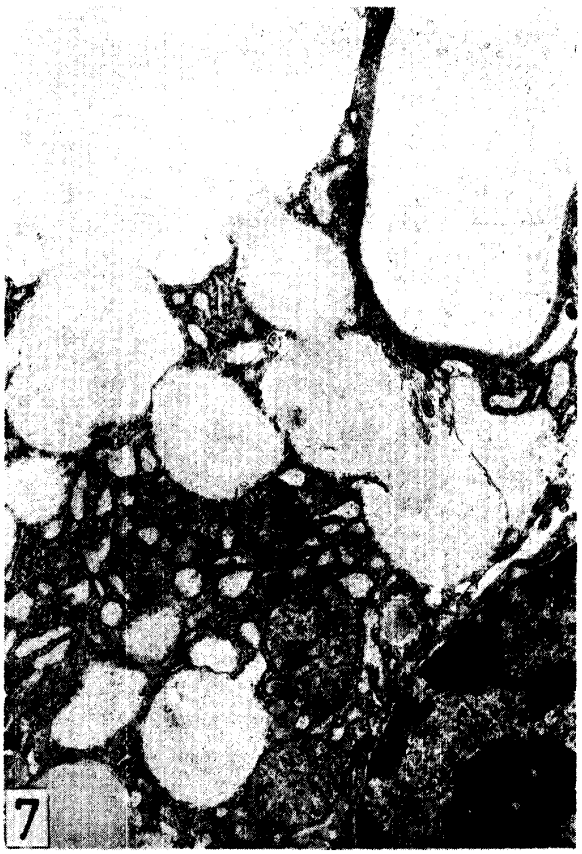
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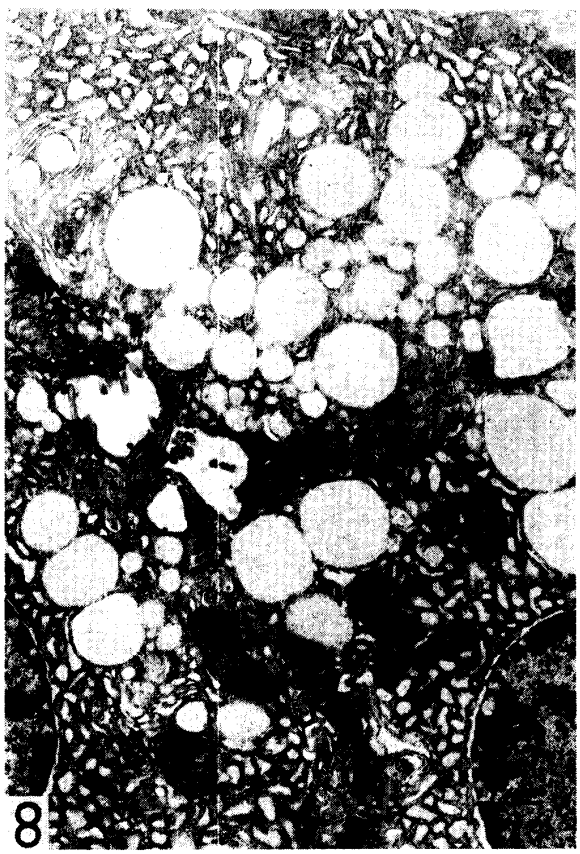
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