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EFFECTS INDUCED BY ISOPROTERENOL DURING SYNTHESIS AND SECRETION OF THE PAROTID GLAND: EXPERIMENTAL STRUCTURAL STUDIES

Alicia Inés Malberti and Marta Elena de Crosa

Department of Histology "A", Faculty of Dentistry National University of Córdoba, Córdoba, Argentina.

ABSTRACT

The effect of isoproterenol, a β-adrenergic agonist, on the parotid gland of guinea pig was studied *in vivo*. Male guinea pigs were fasted 12 h, and then injected intraperitoneally with isoproterenol (30 mg/kg). The parotid glands were processed for light and transmission electron microscopy using conventional techniques. Morphological analysis showed massive granular secretion 2 h after isoproterenol injection and an enlarged apical surface of the plasma membrane, as indicated by the presence of microvilli. Twenty-four hours after injection of isoproterenol the gland had still not returned to its pre-stimulation state. The effect of isoproterenol decreased with time and the apical surface of the plasma membrane eventually resumed its normal (basal) appearance. These data showed that the maximun secretagogue effect of isoproterenol on parotid gland of guinea pig was evident 2 h after injection and decreased according to the time.

Key words: Guinea pig, isoproterenol, parotid gland, secretory granules, secretagogue, synthesis

INTRODUCTION

Salivary gland function is controlled by the nervous system and there is little parotid secretion in the absence of stimulation [4].

The secretory process is modulated by drugs that stimulate or inhibit secretion. Stimulation *in vivo* with high doses of the β-adrenergic agonist isoproterenol (Iso) or epinephrine results in the discharge of almost all of the secretory proteins from the parotid glands of starved rats 2 h after injection [1]. A significant increase in protein synthesis has been reported 6 h after stimulation with β-adrenergic agonists [13]. High doses of Iso decrease the amylase content of the gland by 97% after 2 h but the levels of enzyme recover by approximately 50% 21 h later when compared to unstimulated animals [3,12]. This recovery involves an increase in the rate of protein synthesis [3].

Correspondence to: Dr. Alicia Inés Malberti.
Cátedra de Histología "A", Departamento de Biología Oral, Facultad de Odontología, Universidad Nacional de Córdoba, Pabellón Argentina, Ciudad Universitaria, Córdoba, Argentina.Tel: (54) (351) 4333032, Fax: (54) (351) 4334172, E-mail: gustavonunez@sinectis.com

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Simson [16] reported an increase in the lysosomal content of acinar cells of rat parotid glands following stimulation with Iso. This drug may also induce a moderate inflammatory response, with an increase in the number of macrophages and lymph cells near the acini and intercalated ducts [5,15]. The chronic administration of Iso stimulates the proliferation of acinar cells in rat cultured salivary gland cells [19].

A thorough description of the intracellular mechanisms involved in the synthesis and secretory processes of parotid gland acinar cells is still lacking. Crosa *et al.* [8] reported that guinea pig salivary glands are structurally and functionally similar to those of humans. In this work we investigated the effect of Iso on the biosynthetic and secretory processes in the parotid gland of guinea pig fasted for 12 h.

MATERIAL AND METHODS

Male guinea pigs (200 ± 20 g) were used throughout. The animals were housed (n=3/cage) and provided with water and food (standard chow and vegetables) *ad libitum* under standard housing conditions for 15 days before the experiments. The animals were then grouped according to their body weight and starved for 12 h.

Treated animals (n=18) were injected intraperitoneally (i.p.) with isoproterenol sulfate (30 mg/kg body weight) dissolved in 0.5 ml of saline solution while the controls (n=18) received an equal volume of saline solution alone. At 0.5, 2, 4, 8, 12 and 24 h

post-injection, the animals were killed by diethyl ether inhalation and the parotid glands removed. One gland was used for structural studies by light microscopy while the contralateral gland was used for ultrastructural studies by transmission electron microscopy.

Light microscopy (LM)

The glands were fixed for 12 h in 10% formol buffered with cetyl pyridine chloride pH 7. The samples were embedded in paraffin and stained with hematoxylin-eosin (HE) and toluidine blue (pH 3.8). Some sections were also stained with the periodic acid-Schiff (PAS) reaction. The sections were examined and photographed with a Leitz Orthoplan microscope.

Transmission electron microscopy (TEM)

The samples were fixed in Karnovsky solution, buffered with sodium cacodylate pH 7.2, for 4 h at room temperature and then postfixed in 1% osmium tetroxide (2-12 h) followed by dehydration in acetone. The glands were finally embedded in araldite. Ultrathin sections were stained with lead citrate and uranyl acetate and were examined in a Siemens Elmiskop transmission electron microscope.

RESULTS

Structural and ultrastructural analyses Controls: 12 h fast + saline solution (no food)

Figure 1A shows the parotid gland of the control animals starved for 12 h. Basophilic acini were present but there was no secretory material in the lumen of the excretory and secretory-excretory ducts. The apical region of the cytoplasm was filled with granular material (Fig.1B). PAS or toluidine blue staining confirmed the lack of secretion in the lumen of the ducts (Fig.1B).

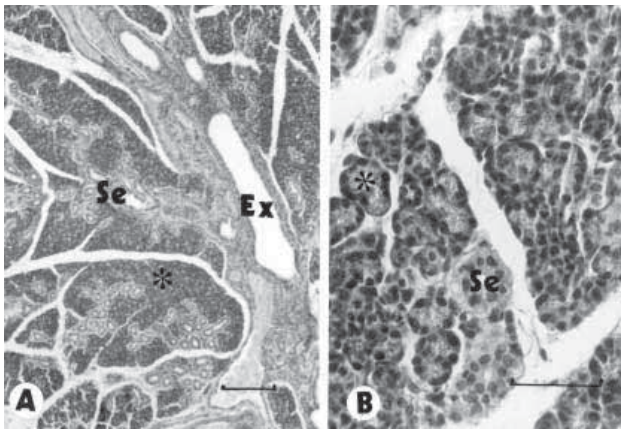

Figure 1. Controls. **(A)** Excretory (Ex) and secretory-excretory (Se) ducts without secretory material in the lumen. Basophilic acini (*). HE. Bar = 500 µm. **(B)** Granular material (*) in the apical region of the cytoplasm. HE. Bar = 50 µm.

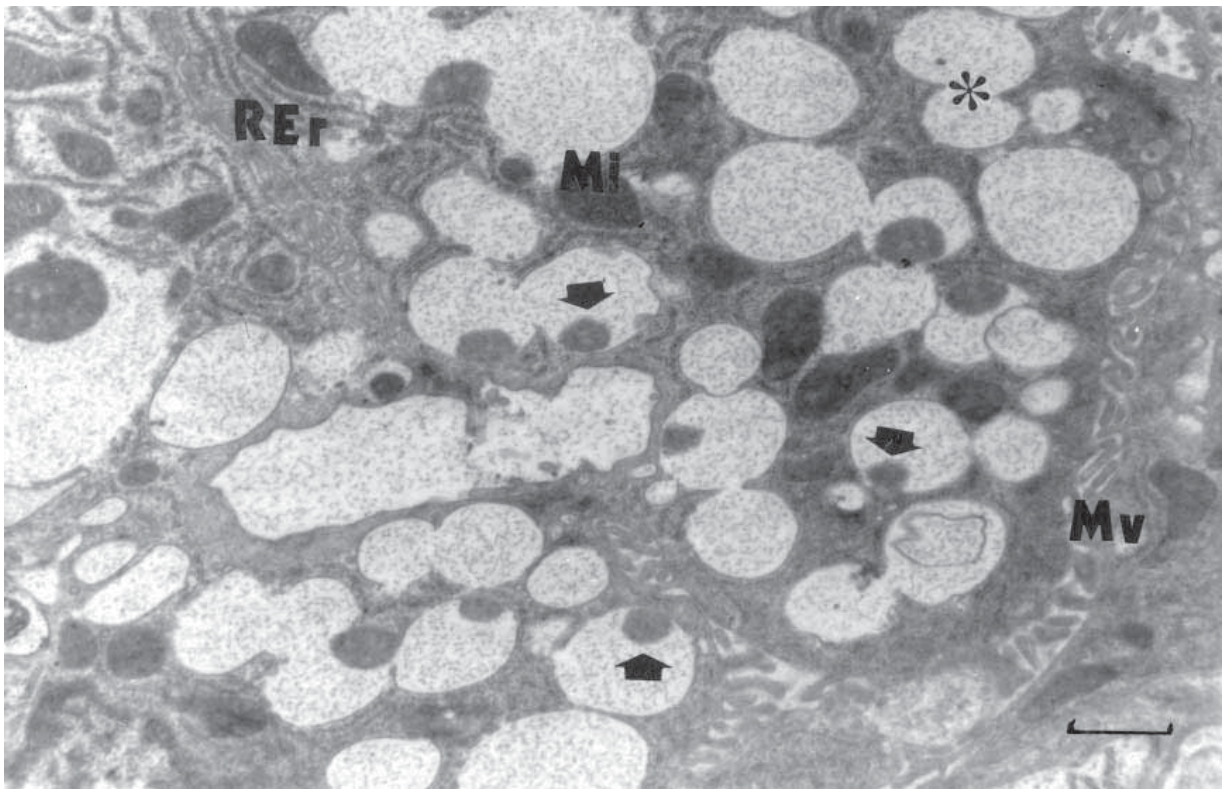

Figure 2. TEM of controls. Dense bodies (¢) within the secretory vesicles. Rough endoplasmic reticulum (REr) related to the secretory granules. Microvilli (Mv) in the lumen of the intercellular canaliculi. Mi = mitochondria, * = fused granules. Bar = 2 µm.

The appearance of control samples examined by transmission electron miscroscopy (TEM) is shown in Figure 2. The cytoplasm was filled with granules of different shapes and many of the secretory vesicles contained an electron dense body displaced to one side. The rough endoplasmic reticulum (RER) and large mitochondria were surrounded by secretory granules. Numerous microvilli were present in the lumen of the intercellular canaliculi. Figure 3 shows an empty striated duct with short microvilli in the apical membrane of the cells. In unstimulated 24 h controls, the gland produced secretion spontaneously.

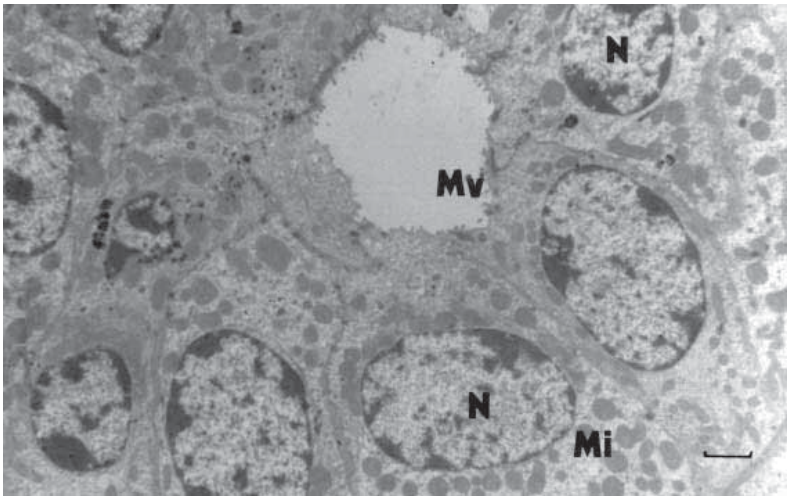

Figure 3. TEM of controls. Striated duct without secretion in the lumen and with short microvilli (Mv) in the apical membrane of the cells. Mi = mitochondria, N = nucleus. Bar = 2 μm.

Stimulation with isoproterenol

Thirty minutes after stimulation with Iso, the gland parenchyma was homogeneous in appearance. The acinar cells were intensely basophilic and there was no secretory material in the lumen of the excretory and secretory-excretory ducts.

Two hours after the injection of Iso the acini continued basophilic with well defined contours but the secretory ducts now contained abundant material in their lumen. PAS staining showed that this material contained glycoproteins, and staining with toluidine (Fig. 4) revealed that the secretion was basophilic. The acinar cells had the characteristic structures involved in protein synthesis and secretion, i.e., an intensely basophilic cytoplasm and granules with metachromatic contents in the apical region.

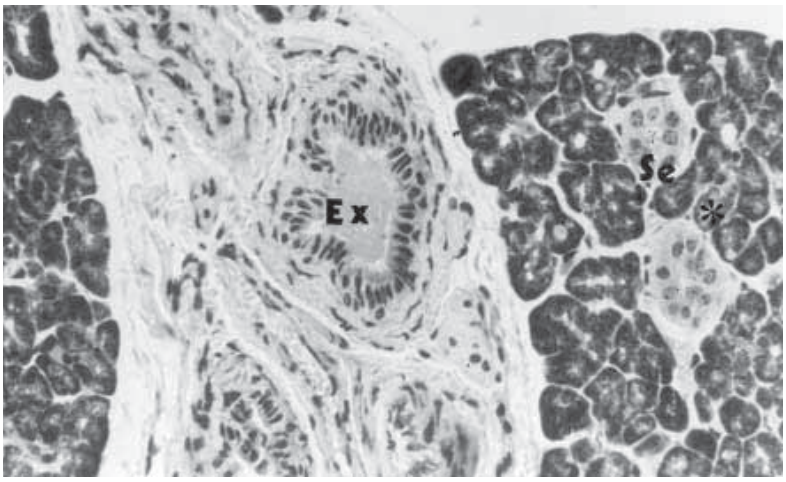

Figure 4. Toluidine blue staining 2 h after stimulation with Iso. Excretory (Ex) and secretory-excretory (Se) ducts with basophilic secretion in the lumen. Acinar cells (∗) with basophilic cytoplasm and, in the apical region, granules with metachromatic content. Toluidine blue. Bar = 50 μm.

TEM indicated that the apical region next to the acinar lumen was filled with granules of different electron densities, as in the controls, although only a few of them have an electron dense body (Fig. 5). The lumen of the acini contained abundant microvilli, secretory material and membranous structures.

Four hours after the injection of Iso acidophilic secretory material was observed and the acinar cells contained cytoplasmic vacuoles. The PAS reaction revealed glycoprotein material in the lumen of the ducts whereas toluidine blue staining detected basophilic secretory material in the ductal lumen and the vacuole acinar cytoplasm. Figure 6 shows the ultrastructural modifications at this time point. Electrolucid secretory granules and mitochondrial modifications were seen and the RER arrangement was irregular. There were abundant microvilli towards the acinar lumen.

After 8 h, some acini were filled with large vacuoles and there was secretory material in the duct lumen. After 12 h, there was little secretion in the ducts and the acinar cytoplasm was basophilic. PAS and toluidine blue staining failed to detect secretory material in the lumen of the ducts.

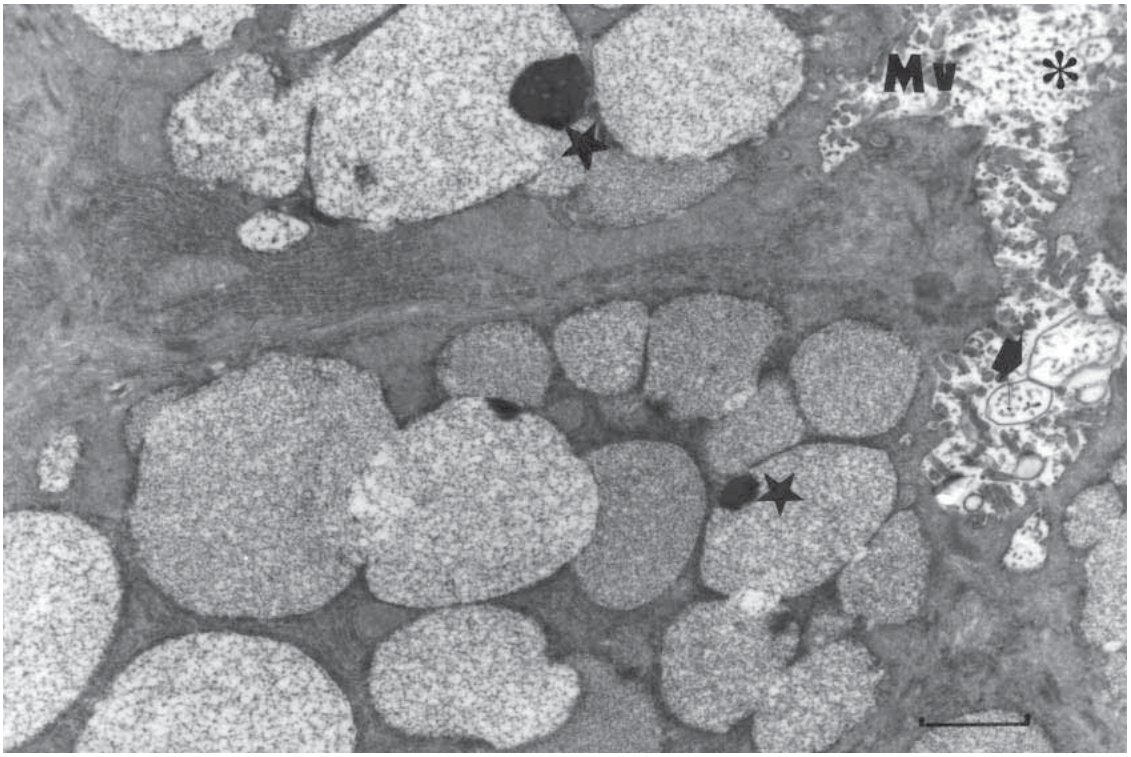

Figure 5. TEM 2 h after stimulation with Iso. Electron dense body (★) within secretory granules. Abundant microvilli (Mv) in the lumen of the acini, together with secretory material (*) and membrane figures (◗). Bar = 2 μm.

HE staining showed that 24 h after stimulation the gland parenchyma was structurally similar to the controls, i.e., empty secretory ducts and slightly basophilic acini. The toluidine blue staining (Fig. 7) revealed a basophilic secretion towards the lumen in striated ducts and the acini were metachromatic in the apical region of the cytoplasm. TEM (Fig. 8) showed granule fusion and dense bodies within the granules. The apical surface of the acinar cells had microvilli and there was secretion towards the lumen.

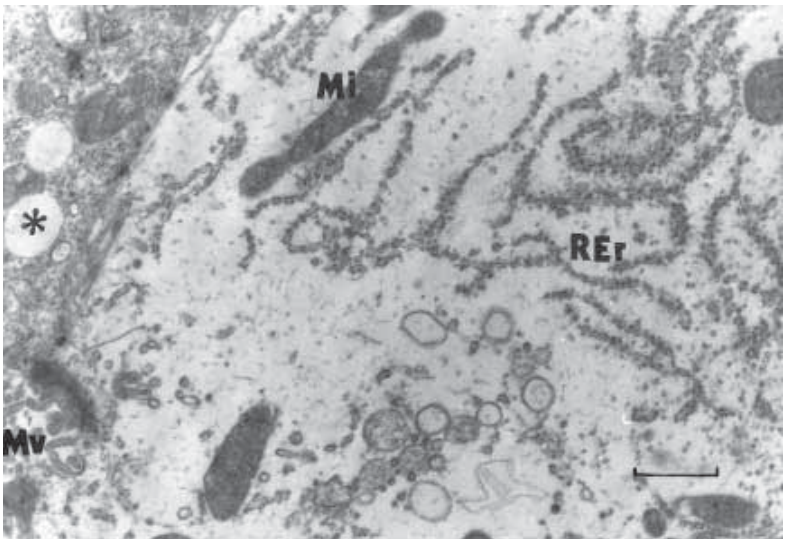

Figure 6. TEM 4 h after stimulation with Iso. Electron dense lumen secretion granules (*) and structural modifications in the mitochondria (Mi). Rough endoplasmic reticulum (REr) with cisternal arranged irregularly. Note the abundant microvilli (Mv) towards the acinar lumen. Bar = 1.5 μm.

DISCUSSION

The basal secretion by the unstimulated parotid gland was low, and probably involved exocytosis and other pathways that did not require the prior accumulation of secretory proteins in the cytoplasm [4]. The parotid gland is stimulated physiologically by processes such as food intake, but can also be stimulated pharmacologically by substances acting through membrane receptors. Stimulation with a β-adrenergic secretagogue results in a massive discharge of secretory granules [3,11-13]. As shown here, the stimulating effect of Iso on secretion started 2 h after injection since abundant secretory material was observed in the duct system. The observation that the secretory material was positive in the PAS reaction, but acidophilic following staining with HE and

basophilic with toluidine blue suggested that Iso induced the secretion of a complex pattern of substances by the gland.

Itoh [10] reported a bimodal distribution in the size of rat parotid gland granules and Bedi *et al.* [2] indicated a preferential discharge of the oldest secretory granules which were smaller and denser than newer granules. Simson *et al.* [17] reported similar findings for the rat parotid gland after stimulation with Iso. In contrast, 2 h after the injection of Iso in guinea pigs, the granules were heterogeneous in size and electron density and were located close to the cell surface and ready to be released. These findings suggested that Iso was a non-selective inducer of granules secretion.

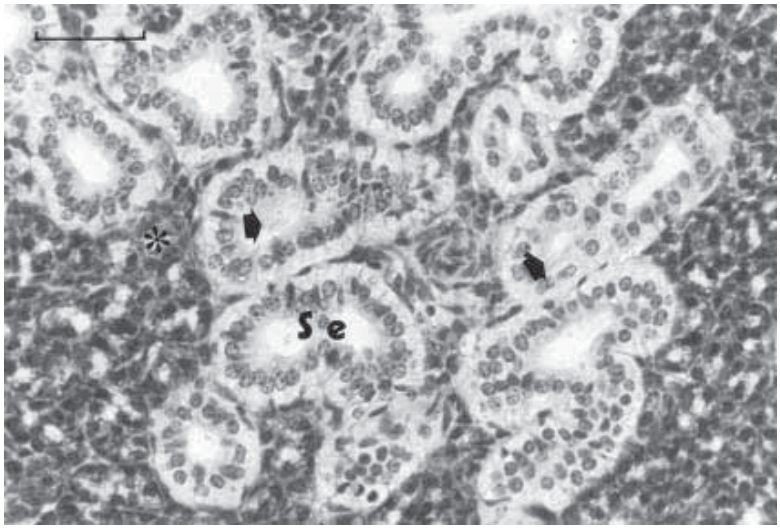

Figure 7. Toluidine blue staining 24 h after stimulation with Iso. Note the striated ducts (Se) with basophilic secretion towards the lumen (◗) and the metachromatic acini in the apical region of the cytoplasm (∗). Bar = 50 μm.

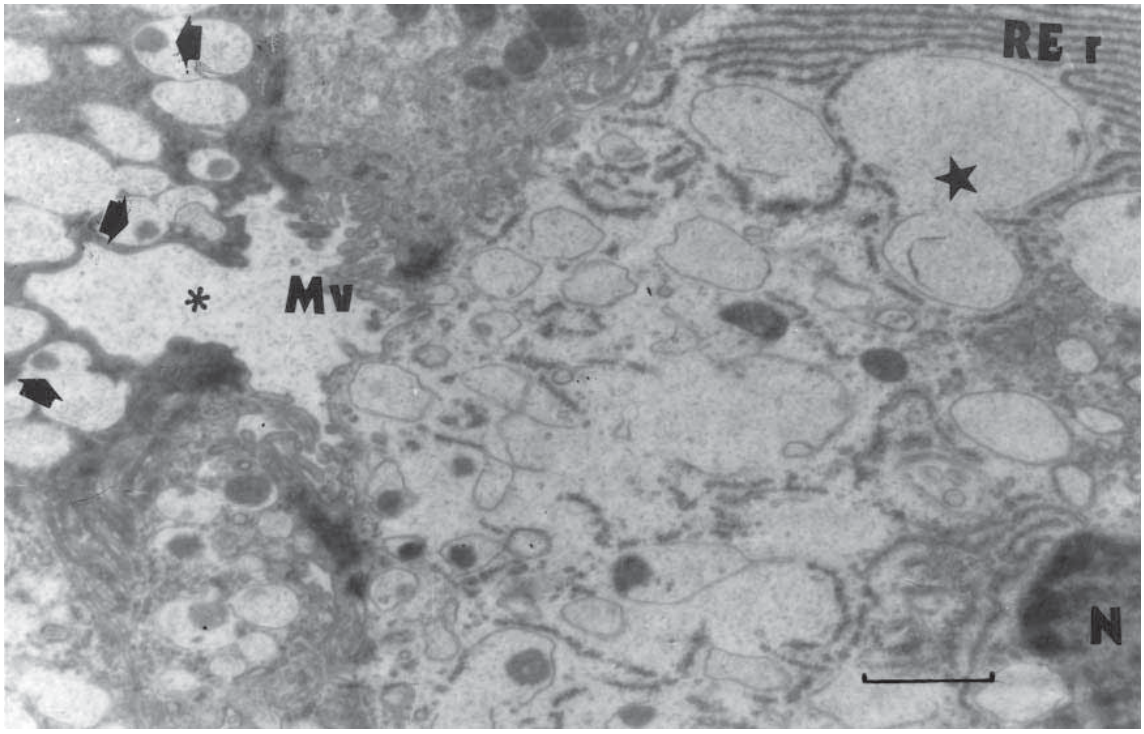

Figure 8. TEM 24 h after stimulation with Iso. Note the fused granular (★) and dense bodies (◗) within the granules. Microvilli (Mv) on the apical surface of the acinar cells direct the secretion towards the lumen (∗). N = Nucleous, REr = rough endoplasmic reticulum. Bar = 2 μm.

In controls starved for 12 h, a large number of secretory granules contained a dense body in their cytoplasm. These bodies, probably metalloenzymes [17] were uncommon in the granules of glands 2 h after stimulation with Iso and were not seen in granules formed ≥ 8 h after stimulation. Simson *et al.* [17] reported that these dense bodies contained amylase binding lipids. TEM also indicated that some granules formed after 2 h contained a dense body. The massive secretion induced by Iso at 2 h post-injection resulted in an enlarged apical surface of the plasma membrane, as indicated by the presence of microvilli. This effect has also been reported by Amsterdam *et al.* [1] for the rat parotid gland 0.5 h after treatment with Iso, and

by Williams and Cope [18] who observed an increased apical membrane in rabbits 2 h after secretion induced by Iso compared to non stimulated glands. Segawa *et al.* [14] reported that in human cultured salivary glands Iso increased the transformation of microvilli of the lumen membrane and that the apical surface area of the membrane eventually decreased progressively with time.

Since little membranous material was seen in the acinar lumen, it is possible that fragments of the granular membrane may be expelled to the lumen during exocytosis. Cope and Williams [6,7] and Williams and Cope [18] reported a complete recovery of the storage granules in rabbit parotid gland 12-16 h after the injection of Iso. In contrast, in guinea pigs there was still complete restoration of the parenchyma by 24 h post-injection. These findings agree with our previous conclusions [9] that secretion and the recovery of granule contents are not synchronous throughout the gland parenchyma.

Although no detailed morphometric analysis was done here, the acinar lumen showed shorter and fewer microvilli after 24 h than after 2 h post-injection. Thus the changes in acinar lumen content induced by Iso depended on the time elapsed after injection.The longer the time after stimulation, the lower effect of the drug and the secretion present produced no marked alterations in the apical surface of the plasma membrane.

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