A morphometric study of the secretory granules of the granular duct in the submaxillary gland of the rat following stimulation with noradrenalin and isoproterenol

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Summary. In the present work, we carry out a morphometric analysis, at ultrastructural level, of the secretory granules of the granular undulated duct of the submaxillary gland of the rat, under basal conditions (Control Group or I), following stimulation for 10 minutes with 2 mg/100 g weight of Isoproterenol (Group II), and following stimulation with 2 gammas/100 g weight of Noradrenalin for the same time as in the former case. It is seen that in general, Noradrenalin produces the appearance of a greater number of small granules than does Isoproterenol or the control group; and that Isoproterenol induces the presence of larger-sized granules than does Noradrenalin.

Key words: Granular undulated duct, Submaxillary gland, Secretory granules, Noradrenalin, Isoproterenol

Introduction

The granular undulated duct, with the capacity to synthesise organic products (Vreugdenhill et al., 1980; Davis, 1988), is composed of two distinct types of cells. One of these is characterised by its basal membrane having invaginations (Gutiérrez et al., 1990), and without secretory apical cisternae in its apical pole (Tamarin and Sreebny, 1959). The other is characterised by having the basal invaginations parallel one to another, forming bundles and with secretory apical cisternae (Gutiérrez et al., 1990). Both epithelia also differ in their secretory mechanism. In the case of the cells without secretory apical cisternae, the mechanism is fusion-fission of the membrane (Martínez-Hernández et al., 1972), and in the case of the cells with secretory apical cisternae, these create a mechanism of apocrine secretion (Scott and Pease, 1959; Messelt, 1982).

Based on the fact that the secretory material recently liberated to the cytoplasm appears in very small granules (Lima and Haddad, 1981), we have studied the repercussion of Noradrenalin and Isoproterenol on the secretory granules, using a morphometric analysis.

Materials and methods

The submaxillary glands obtained from 30 male Wistar rats of approximately 250 grams each, were used to carry out the present work. Three work groups were established: Group I or Control, in which the rats were not subjected to any type of stimulus and whose glands were considered as reference; Group II, in which the animals were subjected to a beta-adrenergic stimulus by means of an intraperitoneal injection of 2 mg/100 g body weight of Isoproterenol, maintained for 10 minutes; and Group III, in which an alpha-adrenergic effect was obtained by means of an intraperitoneal injection of 2 gammas/100 g body weight of Noradrenalin, maintained for 10 minutes. Each group consisted of 10 rats.

In all cases, the animals were perfused and fixed by the method of Palade (1952) and Palay and Chan-Palay (1974). The ultrathin sections were cut at 600-900 Å, and contrasted in 2% solution of uranyl acetate according to the technique proposed by Venables and Coggeshal in 1965, followed by lead citrate according to the procedure of Reynolds (1963), in CO_2-free atmosphere.

The ultrathin sections were observed and photographed with a Siemens transmission microscope, model ELMISCOP 102. Kodak electron microscopy photographic plates of 6.5 × 2 cm were used.

Each group was divided in turn into two subgroups, depending on whether working on cells with, or without, secretory apical cisternae.

The photographs were taken at 3000, 4000 or 5000 magnification, and the corresponding factors of correction were established for each magnification. The granules were analysed morphometrically by means of an Apple II computer with graph plotter, using a program of
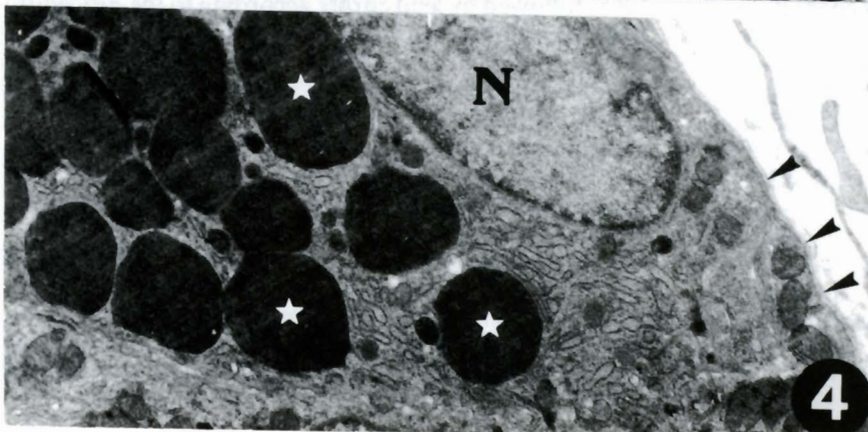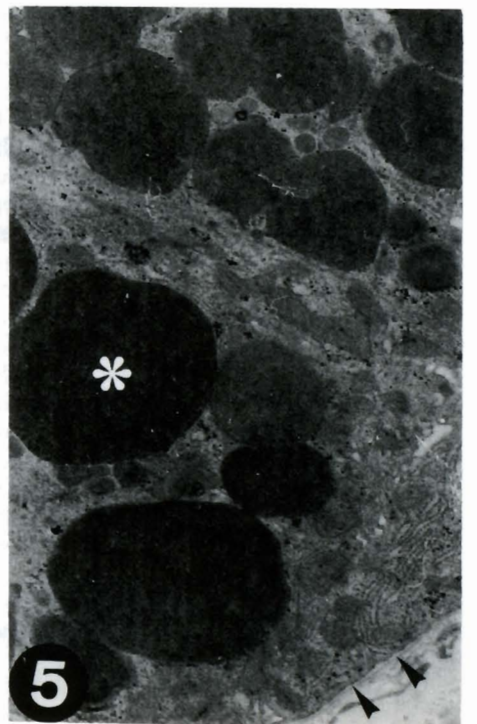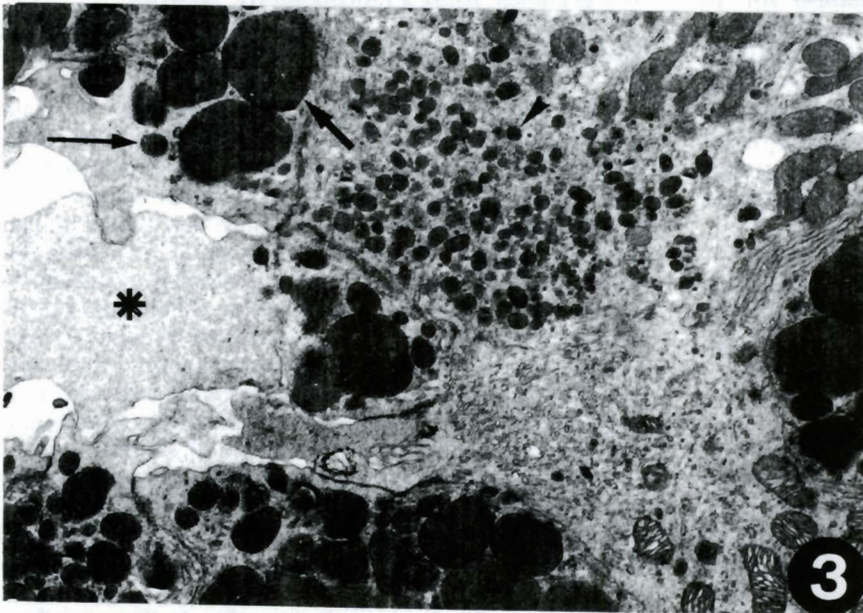
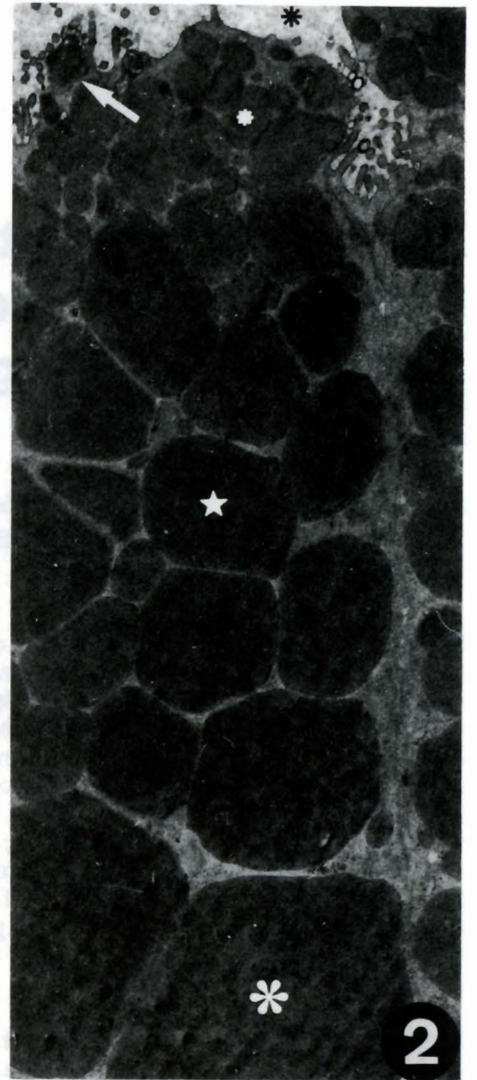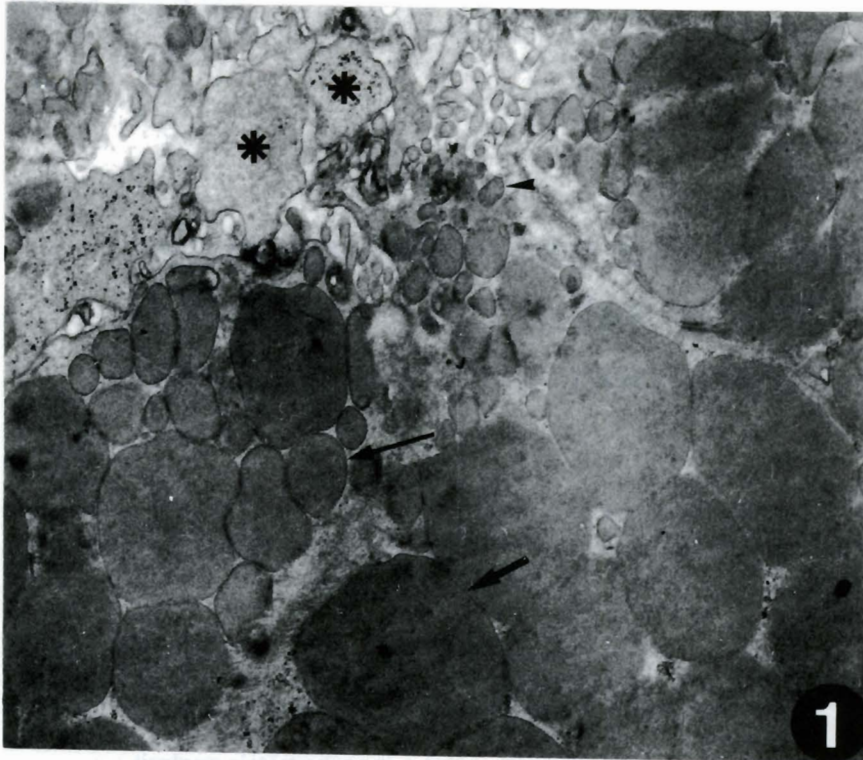*A morphometric study in the submaxillary gland*

Fig. 1. Granular undulated duct cells with secretory apical cisterna. Control group. Secretory apical cisternae, asterisks. Small granules, arrow head. Medium granules, thin arrow. Large granules, broad arrow. × 5,000

Fig. 2. Granular undulated duct cells without secretory apical cisternae. Control group. Apical lumen, black asterisk. Secretion of a granule into the apical lumen, arrow. Small granules, small white asterisk. Medium granules, white star. Large granules, large white asterisk. × 3,000

Fig. 3. Granular undulated duct cells with secretory apical cisterna. Group treated with Noradrenalin. Secretory apical cisterna, asterisk. Very small granules, arrow head. Small granules, thin arrow. Medium granules, broad arrow. × 3,000

Fig. 4. Basal pole of a granular undulated duct cell without secretory apical cisterna. Group treated with Noradrenalin. Nucleus, N. Basal membrane, arrow heads. Secretory granules, white star. × 3,000

Fig. 5. Basal pole of a granular undulated cell without secretory apical cisterna. Group treated with Isoproterenol. Large secretory granules, asterisk. Basal membrane, arrow heads. × 3,000

integrated stereometric analysis from Polaron, version 3.1. The data obtained from the morphometric analysis were treated statistically in an IBM System-2 computer, using the statistical package Microstat, and transferring the data to the program Harvard Graphic, version 2.1, in order to obtain the graphical representation.

Results

The results obtained in our study were the following:

A-control group

In this group, 618 granules of different sizes were analysed in the subgroup of cells with secretory apical cisternae, and a total of 723 granules of different sizes from the subgroup of cells without secretory apical cisternae. In the subgroup of cells with secretory apical cisternae, some of the secretory granules were very large, with the smaller ones situated close to the secretory apical cisternae; there was a great diversity of granular size (Fig. 1). In the case of the subgroup without secretory apical cisternae, we usually saw large granules at the level of the mid-basal portion of the cell and an increase in the number of medium- and small-sized ones in the apical portions (Fig. 2).

The percentage distribution of the different diameters of granules from this group is shown in Table 1, and their graphical representation in Figures 6 and 7. At the same time, the study of the mean areas and standard deviation; mean perimeters and standard deviation; and mean diameters and standard deviation of the granules of this work group and subgroups are shown in Table 2 and represented graphically in Figures 12, 13 and 14.

B-group treated with noradrenalin

In this group, a total of 647 secretory granules from cells with secretory apical cisternae were analysed, and a total of 655 secretory granules from cells without secre-

tory apical cisternae. In the subgroup with secretory apical cisternae, we saw a very marked increase in the number of very small diameter granules in the apical pole (Fig. 3). At the same time there was a lesser presence of large diameter granules (Fig. 3). In the subgroup without secretory apical cisternae, the presence of small- and medium-sized granules could be observed, the latter even in the portions close to the basal membrane (Fig. 4).

The percentage distribution of the different diameters of granules from this work group, in its two different subgroups, is shown in Table 1, and represented graphically in Figure 8 and 9. At the same time, the study of the mean areas and standard deviation; mean perimeters and standard deviation; and mean diameters and standard deviation of the granules of this work group and subgroups are shown in Table 2 and represented graphically in Figures 12, 13 and 14.

C-group treated with isoproterenol

In this work group, a total of 699 secretory granules in the ducts with cells having secretory apical cisternae were analysed, and a total of 678 granules in the ducts with cells without secretory apical cisternae. In both subgroups, as the basal portion was neared, we saw an increase in the diameter of the secretory granules, as shown in Figure 5, which corresponds to a cell without secretory apical cisterna.

The percentage distribution of the different diameters of granules from this work group and subgroups, is shown in Table 1, and their graphical representation in Figure 10 and 11. The study of the mean areas, mean perimeters, and mean diameters, with their respective standard deviations, of the secretory granules are shown in Table 2 and represented graphically in Figures 12-14.

Discussion

The groups subjected to the action of Noradrenalin, both with secretory apical cisternae and without, present a greater number of small granules in their cytoplasm than those stimulated with Isoproterenol (Figs. 3-5, 8-11) and (Table 1). The fact that the smallest granules contain the most recently synthesized secretory material (Lima and Haddad, 1965), leads us to think that this increase is due to the stimulus produced by Noradrenalin, coinciding with Vreugdenhill and collaborators (Vreugdenhill et al., 1981), in that this drug is a potent inducer of synthesis in the duct.

In the groups treated with Noradrenalin, a great number of small granules should be expected than in the controls. This result is not seen in the cells with secretory apical cisternae (Figs. 6, 8) and (Table 1). Given that each cell has its own secretory rhythm (Tamarin and Sreebny, 1965), this justifies, in our opinion, the fact that a non-stimulated group (the control) has a greater capacity of synthesis than another more basally depressed group which is stimulated with Noradrenalin.

In the groups treated with Isoproterenol, larger-sized
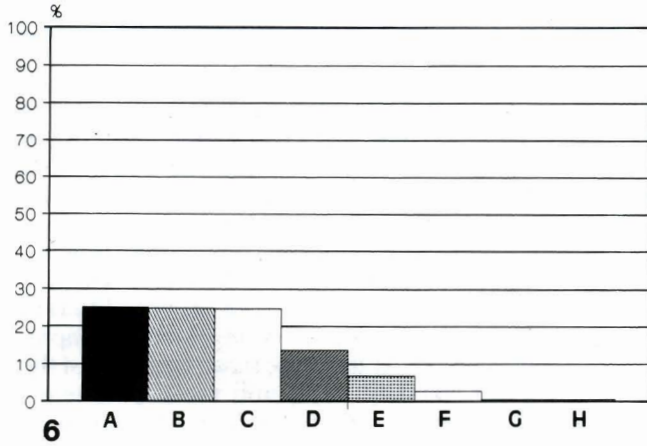
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Fig. 6. Control group with secretory apical cisterna. Percentage distribution of the granules by diameter.
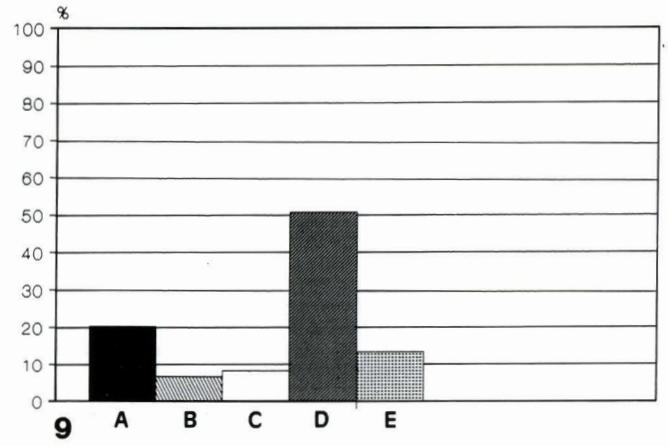

Fig. 9. Group treated without Noradrenalin without secretory apical cisterna. Percentage distribution of the granules by diameter.
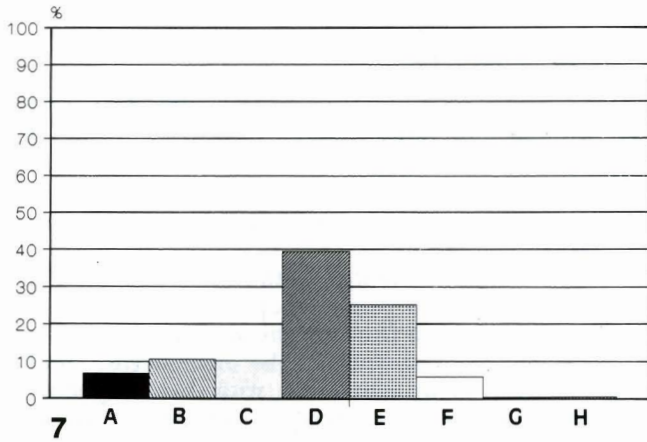

Fig. 7. Control group without secretory apical cisterna. Percentage distribution of the granules by diameter.
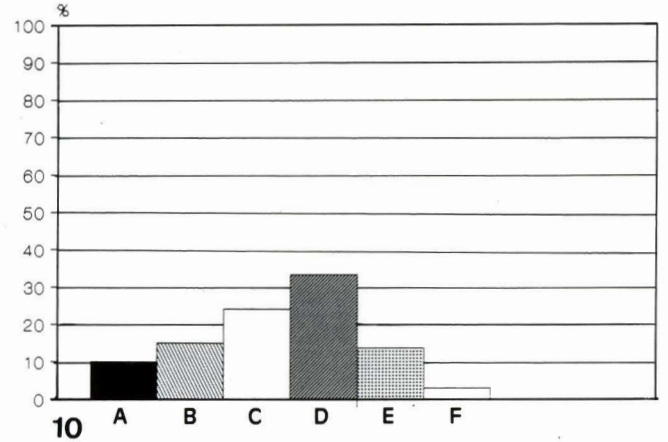

Fig. 10. Group treated with Isoproterenol with secretory apical cisterna. Percentage distribution of the granules by diameter.
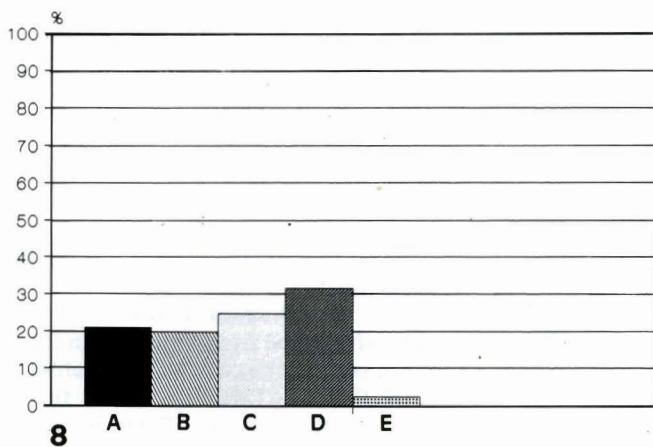

Fig. 8. Group treated with Noradrenalin with secretory apical cisterna. Percentage distribution of the granules by diameter.
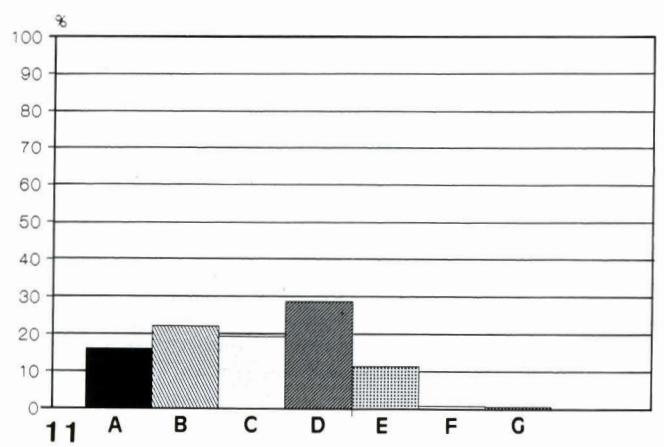

Fig. 11. Group treated with Isoproterenol without secretory apical cisterna. Percentage distribution of the granules by diameter.

Table 1. Percentage classification by diameter of the secretory granules studied in the different groups.

DIAMETERS	CONTROL		NORADRENALIN		ISOPROTERENOL	
	ASC + %	ASC–%	ASC + %	ASC–%	ASC + %	ASC–%
A.– 0.00 – 0.25 µm	25.23	6.94	21.13	20.33	10.09	16.08
B.– 0.26 – 0.50 µm	24.95	10.72	19.87	6.77	15.14	22.39
C.– 0.51 – 1.00 µm	24.91	10.41	24.92	8.47	24.29	19.55
D.– 1.01 – 2.00 µm	13.87	39.70	31.83	50.84	33.45	29.00
E.– 2.01 – 3.00 µm	6.96	25.50	2.52	13.59	13.88	11.55
F.– 3.01 – 4.00 µm	2.83	6.10	0.0	0.0	3.15	0.80
G.– 4.01 – 5.00 µm	0.63	0.63	0.0	0.0	0.0	0.63
H.– 5.01 – 6.00 µm	0.63	0.0	0.0	0.0	0.0	0.0

ASC = Apical Secretory Cisterna.

Table 2. Relationship by groups and subgroups of the arithmetic means and standard deviations of the areas, perimeters and diameters of the granules studied.

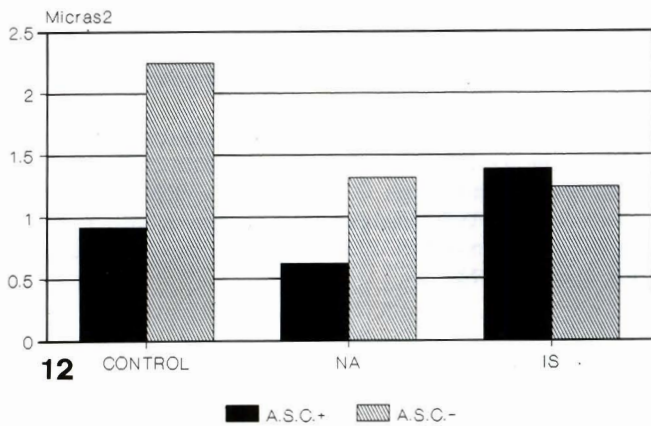|	CONTROL		NORADRENALIN		ISOPROTERENOL	
	ASC +	ASC –	ASC +	ASC –	ASC +	ASC –
N.º GRANULES	618	723	647	655	669	678
AREAS						
MEAN ± SD	0.92 ± 1.91	2.25 ± 2.11	0.63 ± 0.68	1.32 ± 1.25	1.39 ± 1.66	1.24 ± 1.81
PERIMETERS						
MEAN ± SD	2.46 ± 2.35	4.75 ± 2.65	2.40 ± 1.57	3.53 ± 2.15	3.47 ± 2.29	2.13 ± 2.40
DIAMETERS						
MEAN ± SD	0.83 ± 0.88	1.59 ± 0.91	0.80 ± 0.56	1.20 ± 0.75	1.16 ± 0.79	1.03 ± 0.81

Fig. 12. Graphical representation of the mean areas of the groups: control, treated with Noradrenalin and treated with Isoproterenol.
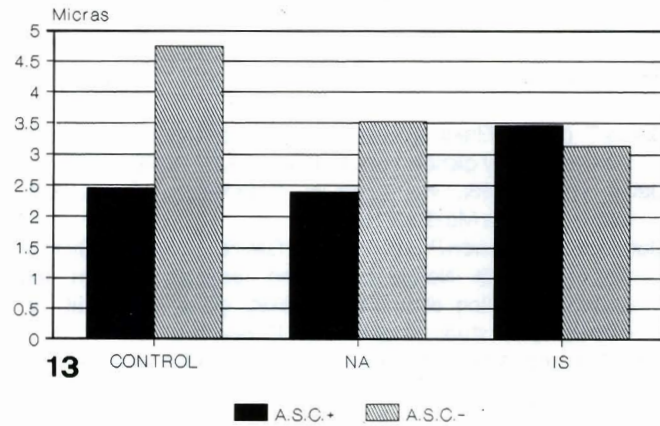

Fig. 13. Graphical representation of the mean perimeters of the groups: control, treated with Noradrenalin and treated with Isoproterenol.

granules are observed than in those treated with Noradrenalin (Figs. 3-5, 8-11, Table 1). Given that Isoproterenol reorganises the granular content and reduces the capacity of its osmotically active molecules in the acinus but not in the granular duct (Gutiérrez et al., 1989), we consider the presence of these large granules secondary to the retention of liquid in them. Authors such as Barca and Simson show this retention of liquid in cells treated with Isoproterenol (Barca, 1971; Simson, 1979).

The different granule sizes observed in the cytoplasm originate from the gradual fusion of the recently synthesized granules between themselves and those already present in the cytoplasm (Lima and Haddad, 1965).

There is a constant tendency for the largest granules to be sited in the basal cytoplasm and the smallest in the apical (Lima and Haddad, 1965).

There is a constant tendency for the largest granules to be sited in the basal cytoplasm and the smallest
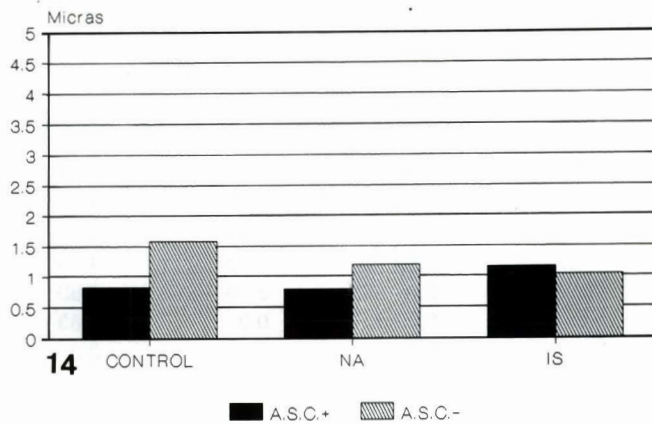
Fig. 14. Graphical representation of the mean diameters of the groups: control, treated with Noradrenalin and treated with Isoproterenol.

in the apical (Lima and Haddad, 1965).

On the other hand, given the well-known role that calcium (Ca^{2+}), cAMP and phosphatidyl inositol phosphate (PIP) play in the secretion of exocrine glands such as the pancreas (Poggioli and Putney, 1982), parotid (Petersen and Maruyama, 1983) and submaxillary (Putney, 1986), we admit the possibility that these mediators are also involved in the secretory mechanisms of these granule cells of the excretory portion of the submaxillary gland.

In this study, we have not been able to present markedly significant differences between the behaviour of the cells with secretory apical cisternae and those without secretory apical cisternae in any of the work groups.

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