
ULTRASTRUCTURE OF THE A-CELLS OF CAT ISLETS
OF LANGERHANS FOLLOWING SYMPATHETIC STIMULATION
OF GLUCAGON SECRETION

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INTRODUCTION

A previous ultrastructural study of the mechanism of glucagon secretion from the alpha cells of rabbit islets of Langerhans has suggested that the α -granules disintegrate, forming small secretory particles which migrate through the cytoplasm and pass through the plasma membrane into the extracellular space (Munger, 1962). More recently Gomez-Acebo et al. (1968) have shown that an emiocytic process (exocytosis), similar to that observed in pancreatic beta cells (Lacy, 1961), might be the mechanism of glucagon secretion from rabbit islets. In their experiments the migration of intact secretory granules to the cell surface with fusion of the granule and plasma membranes and release of the glucagon content of the granules was observed after incubation of pancreas slices *in vitro* in the presence of low concentrations of glucose. Rates of glucagon secretion might be expected to be stimulated under those conditions (Unger et al., 1962).

This report presents the results of a study of neural influences on the regulation of glucagon secretion from the alpha cells of cat islets. Ultrastructural examination was restricted to innervated islets from pancreas shown by immunoassay to be secreting glucagon at greater than basal rates, and it was thus possible to correlate the morphological findings with the findings of increased secretory activity.

MATERIALS AND METHODS

Seven young cats of either sex, weighing 2.1–2.9 kg, were used in this study. Each animal was fasted

overnight and then anesthetized by intraperitoneal injection of Nembutal (40 mg/kg). Anesthesia was further maintained (when necessary) with additional intravenous injections of small doses of Nembutal.

A laparotomy was performed and a heparin-filled polythene cannula was inserted into the splenic vein as far distally as possible so as not to obstruct any of the pancreatic veins joining onto the splenic vein. The cannula was passed up this vein so that its tip came to lie just short of the portal vein. A blood sample and a pancreas-specimen were obtained immediately after cannulation. Thereafter the cannula was kept patent by filling it with heparin-solution.

The right vagal trunk was then dissected loose, tied, and cut as high up as possible. Following this, the coeliac ganglia were dissected and all the efferents, except those running along the mesenteric and coeliac arteries, were tied and severed. The splanchnic nerves were then tied and cut.

The animal was left for at least 1 hr, during which time the viscera were kept moist with Ringer's solution at body temperature.

Stimulation

The sequence of stimulations performed on each cat is indicated in Table I. In any case the specific nerve trunk (vagus or splanchnic) was stimulated for 4 min with a square wave discharge (14 v, 100 cycles/sec, 5 msec) applied as described by Kaneto et al. (1967).

Blood Sampling and Plasma-Glucagon-Assay

Apart from the normal blood samples, taken before dissection of the nerves, further samples were collected during each stimulation as well as 5 min after the end of stimulation. As indicated in Table I,

TABLE I
Effect of Vagal and Sympathetic Stimulation on Plasma Glucagon Levels*

Cat No.	Normal	1st Vagal stimulation		1st Sympathetic stimulation				2nd Vagal stimulation			2nd Sympathetic stimulation	
		During stimulation	5 min after stimulation	During stimulation	5 min after stimulation	10 min after stimulation	20 min after stimulation	During stimulation	5 min after stimulation	10 min after stimulation	During stimulation	5 min after stimulation
3	1.4 A	1.8	1.0 B	2.0	3.6 C	2.6	—————→	—————→	—————→	—————→	3.5	3.5 D
6	1.8 A	2.0	1.2 B	1.6	1.7 C	—————→	—————→	1.2	1.3 D	1.2		
7	2.0 A	1.9	1.6	2.0	1.9	2.2	2.0	1.9	2.1			
8	2.3 A	2.4	2.1	2.0	2.8	2.5	2.3					
9	1.1 A	—————→	—————→	0.8 B	3.7	2.6 C	—————→	1.6	—————→	—————→	2.0	2.2 D
10	1.5 A	—————→	—————→	1.8	2.5 B	2.3	—————→		1.2 C			
11	2.0 A	—————→	—————→		2.4 B	—————→	—————→	2.0	2.1 C	1.6	2.1	2.7 D
Mean	1.7	2.0	1.5	1.7	2.7	2.4	2.2	1.7	1.7	1.4	2.5	2.8

* Results are given as μg equivalents of beef-pork glucagon per ml. A, B, C and D indicate times at which pancreas specimens were obtained from each animal.

blood samples were in some cases also obtained at 10 min. and 20 min after stimulation.

Samples were immediately cooled in an ice bath, centrifuged below 4°C , and the plasma was deep frozen.

Glucagon Assay

Plasma glucagon levels were estimated by means of an immunoassay method in which free and antibody-bound glucagon were separated by precipitation of the glucagon-antibody complex with 66% ethanol. Glucagon antisera were prepared in rabbits by the polyvinyl pyrrolidone method of Assan et al. (1965). Beef-pork glucagon for injection, for immunoassay standards, and for iodination by the method of Greenwood, Hunter and Glover (1963), was kindly provided by Dr. Walter Shaw, Eli Lilly & Co., Indianapolis, Ind.

A control plasma sample was included in each assay to provide an index of the reproducibility of the method from day to day. In eight assays performed in connection with these experiments, the mean value obtained for this sample of plasma was $2.1 \mu\text{g}/\text{ml}$, with a standard deviation of $0.15 \mu\text{g}/\text{ml}$.

Electron Microscopy

Pancreas specimens were obtained at the times indicated in Table I. All specimens were fixed in cold glutaraldehyde and "stained" for acetylcholinesterase according to the method of Lewis and Shute (1966). As a specific inhibitor of pseudocholinesterase, ethopropazine¹ in a concentration of $2 \times 10^{-4} \text{ M}$

¹ Kindly supplied by Warner-Lambert Research Institute, Morris Plains, N. J.

was added to the incubating medium. Specimens were postfixed in osmium tetroxide and embedded in Dow epoxy resin (Lockwood, 1964). Fine sections were stained with lead citrate (Reynolds, 1963) and examined in a Siemens Elmiskop 1A electron microscope.

Only islets showing acetylcholinesterase-positive (vagal) as well as acetylcholinesterase-negative (sympathetic) innervation were studied. This procedure was adopted in view of the opinion that only a certain percentage of islets are normally innervated (Henderson, personal communication).

RESULTS

The results concerning the effect of vagal and sympathetic stimulation on pancreatic venous plasma glucagon levels are presented in Table I. From this, it is clear that vagal stimulation does not have any appreciable effect on the release of glucagon. However, in five of the seven animals sympathetic stimulation resulted in a marked elevation of glucagon levels, during the 20 min period following stimulation. Thus, a mean level in the seven animals of $2.7 \mu\text{g}/\text{ml}$ was reached 5 min after stimulation, compared with a control level of $1.5 \mu\text{g}/\text{ml}$ (Table I). The electron microscopic study was therefore directed towards innervated A-cells in pancreatic specimens obtained from these five cats.

Morphological Findings

In many of the A-cells, after sympathetic stimulation, a distinct polarization of the granules to the capillary end of the cell was observed. Thus

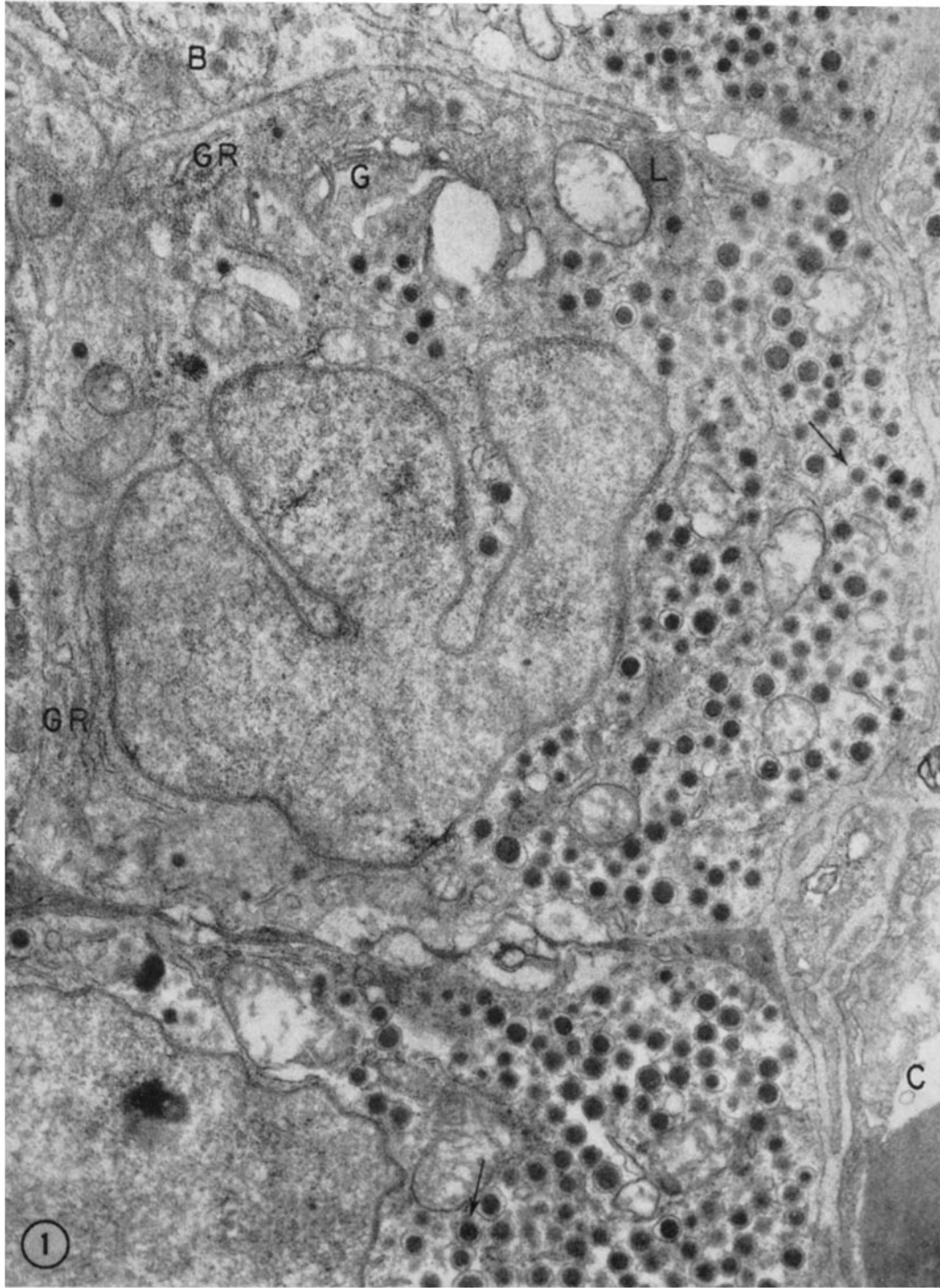


FIGURE 1 A-cells immediately after 4 min sympathetic stimulation (Specimen 9,B.) *B*, B-cell. *C*, Capillary. *G*, Goli region. *GR*, granular endoplasmic reticulum. *L*, lysosome. Linear arrangement of granules (arrows). $\times 15,000$.

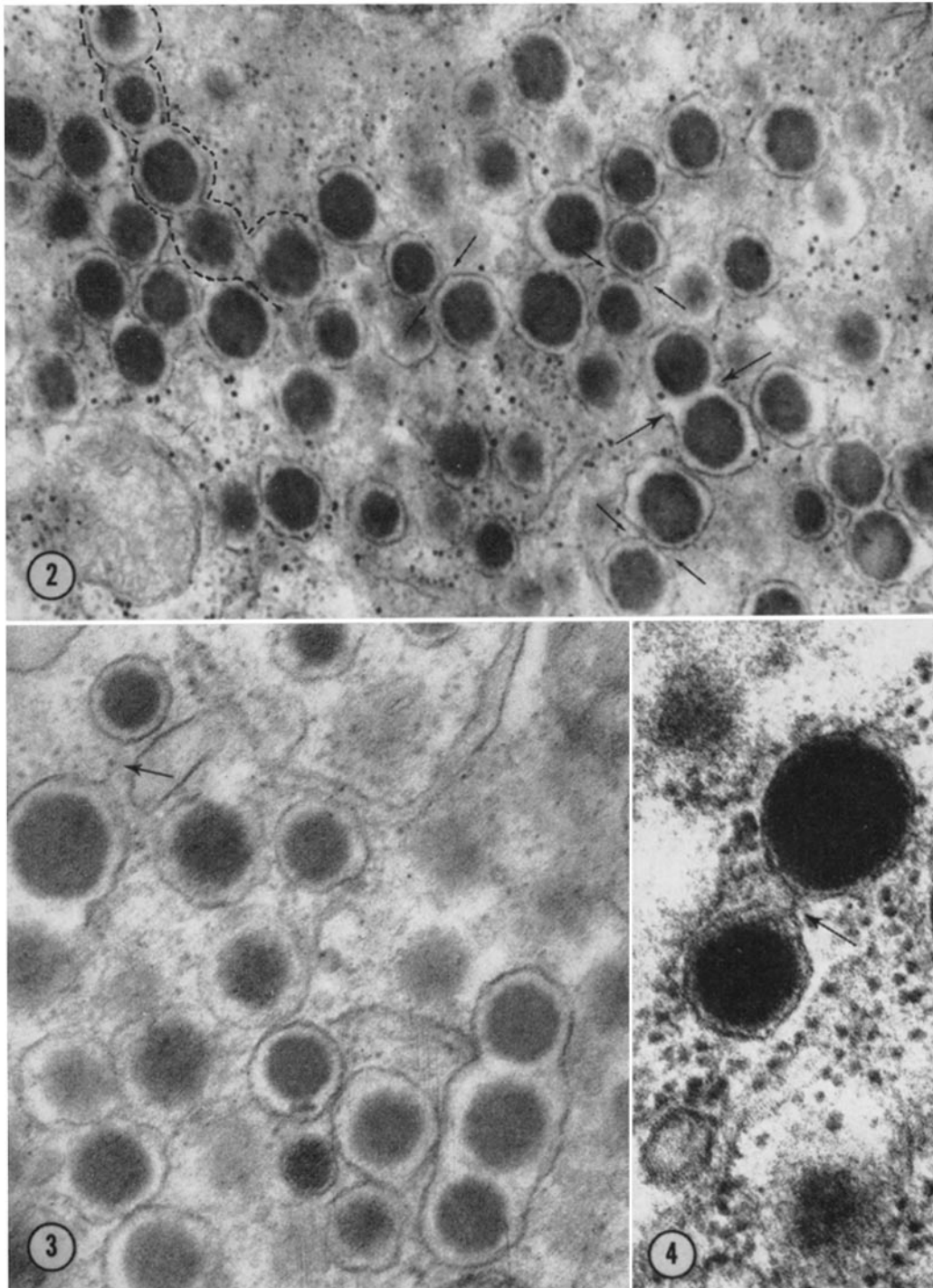


FIGURE 2 A-cell from specimen 9,B. Linear arrangement of granules (stippled lines). Close contact of granule membranes (small arrows). Fusion of granule membranes (large arrows). $\times 42,000$.

FIGURE 3 A-cell from specimen 9,B. Three granules with their membranes fused. Arrow points to micro-tubule (or fibril) between two granules. $\times 60,000$.

FIGURE 4 Another example of a fibrous connection (arrow) between two granules (Specimen 3,C) $\times 80,000$.

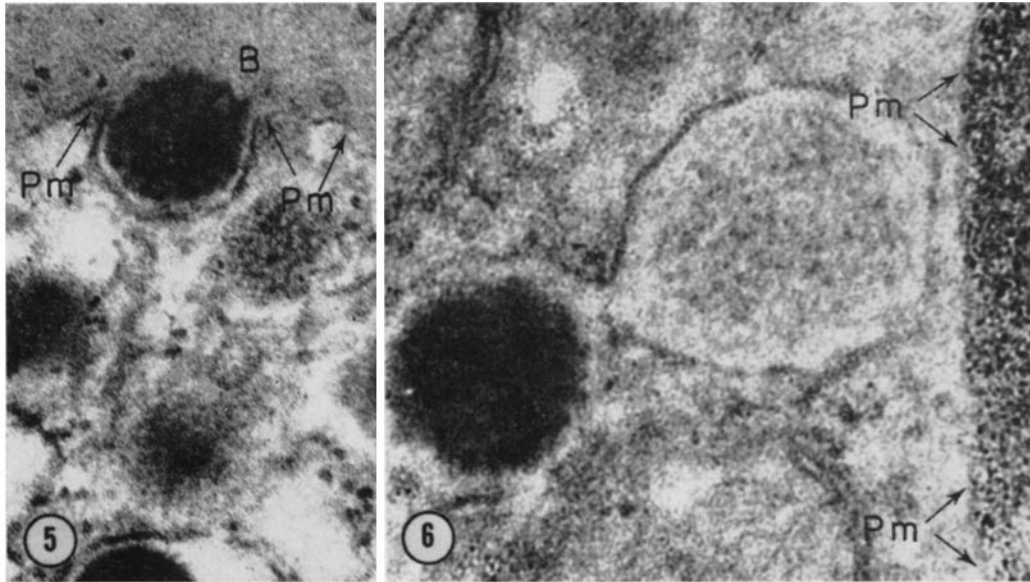


FIGURE 5 Exocytosis of A-granule (Specimen 3,D). *Pm*, plasma membrane. *B*, pericapillary space. $\times 80,000$.

FIGURE 6 Two members from a granule chain (Specimen 3,D). The contents of the one nearest the plasma membrane shows almost complete fragmentation or dissolution. *Pm*, plasma membrane partially obscured by reaction product diffused from nearby acetylcholinesterase-positive nerve terminal area. $\times 140,000$.

the basal parts of the cells in Fig. 1 are practically devoid of granules, with a few granules being still present in the Golgi-region of the central cell.

Groups of several secretory granules showed a linear arrangement or chain formation. The membranes of adjacent granules were either in close contact (Figs. 1 and 2) or actually fused (Figs. 2 and 3). In single sections we have seen up to three granules enclosed in a common membrane. Careful study of serial sections, however, enabled us to count as many as nine granules with their membranes fused to one another.

In Figs. 3 and 4 are depicted what look like fibrous connections between the granules. They resemble the microtubules or fibres described by Lacy et al. (1968) between the granule membrane and the cell membrane of B-cells.

A few A-cells showed exocytosis of entire granules (Fig. 5). It should be noted that this phenomenon was always accompanied by fragmentation or partial dissolution of the granule content. Fragmentation or partial dissolution was also seen in granules prior to their release, especially in some of the members of granule chains (Fig. 6).

DISCUSSION

The estimation of glucagon concentrations in plasma by immunoassay is complicated by the presence, in plasma as well as in the gastrointestinal tract, of a material which cross reacts with glucagon antisera, but which is not chemically identical to glucagon (Unger and Eisentraut, 1968). Thus, only "glucagon-like immuno-reactivity" can be measured in sera. However, a large proportion of the immunoassayable material present in splenic venous plasma might be expected to be glucagon secreted by the pancreatic alpha cells, and minimal interference by cross-reacting substances from the gut has been assumed in these experiments. We therefore feel justified to correlate the morphological findings with an increase in glucagon release by the A-cells.

On the basis of our morphological findings, we present the following hypothesis for a mode of release of glucagon: (a) polarization of the secretory granules to the capillary end of the cell; (b) linear arrangement of group of granules; (c) fusion of granule membranes, thereby forming a system of

intracytoplasmic tubules, (d) partial disintegration of the granule content into smaller particles (microgranules); (e) fusion of membranes of granules, that come to lie at the periphery of the cell, with the cell membrane; and (f) release of the entire granule or of microgranules originating in the individual members of the granule chains.

The mechanism for the polarization and linear arrangement of secretory granules should probably be looked for in the microtubules or fibers seen between the membranes of adjacent granules. These structures might have a contractile ability, thereby drawing granules towards each other and towards the cell membrane as suggested by Lacy et al. (1968) in their work on the release of B-granules. Fusion of granules into chains prior to secretion has previously been reported to occur in exocrine pancreas (Ichikawa, 1965) and in the rat parotid (Amsterdam, Ohad, and Schramm, 1969).

Concerning the supposed disintegration or partial dissolution of the granule contents, it must be pointed out that Munger (1962) has described the formation of secretory particles (microgranules) and their passage through the cytoplasm and cell membrane of the A-cell, the perivascular space, and the endothelial cells of blood capillaries. Increased release of glucagon was obtained by Synthalin A administration; this agent is known to be toxic, causing hydropic degeneration in the A-cells of rabbits after a single subcutaneous injection (Bencosme et al., 1957). In the present study, we have seen secretory particles on the inside of granule membranes, but it was impossible to identify free-floating glucagon particles in the cytoplasm.

Exocytosis has convincingly been demonstrated in rabbit A-cells by Gomez-Acebo et al. (1968) during physiological stimulation of glucagon secretion by low concentrations of glucose. We suggest that exocytosis following the formation of granule chains also provides the ultimate mode of glucagon secretion in response to sympathetic stimulation of A-cells in the cat.

SUMMARY

Electrical stimulation of the sympathetic innervation of the anesthetized cat pancreas resulted, in five of seven animals, in marked elevations of the pancreatic venous glucagon concentrations. The ultrastructure of the alpha cells of innervated islets of Langerhans of these animals was therefore examined in order to establish morphological changes

which might correlate with this increased secretory activity.

The main observations included a polarization of the secretory granules to the vascular pole of the cell, formation of granules into chains, and fusion of the granule membranes both within the chains and with the plasma membrane. The contents of the granules could then diffuse along the intracellular tubules formed in this way into the extracellular space; the possible relevance of these findings to the secretory process is discussed.

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