MORPHOLOGICAL CHANGES IN RAT PANCREATIC SLICES ASSOCIATED WITH INHIBITION OF ENZYME SECRETION BY HIGH CONCENTRATIONS OF SECRETAGOGUES

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ABSTRACT

Stimulation of enzyme secretion in rat pancreatic slices by cholinergic agonists or by cholecystokinin-pancreozymin (CCK-PZ) and its peptide analogs showed a biphasic dose response curve. The optimal concentrations eliciting an efficient rate of enzyme secretion were 1 μM for carbamylcholine or acetylcholine, and 5 nM and 20 nM for CCK-PZ octapeptide and CCK-PZ, respectively. At higher concentrations of secretagogues, however, the rate of secretion progressively declined, and almost complete inhibition was achieved at 1 mM of carbamylcholine or acetylcholine and at 0.1 μM of CCK-PZ or its octapeptide analog. Atropine displaced the dose-response curve for carbamylcholine to the right so that in the presence of 7 μM atropine a concentration of 1 mM carbamylcholine now gave an optimal rate of enzyme secretion. The ionophore A-23187 which bypasses the receptor and elicits enzyme secretion did not relieve the inhibition caused by supraoptimal concentrations of secretagogues, indicating that the inhibition occurs at the cellular rather than at the receptor level. Secretin had no effect on the inhibition of enzyme secretion by a high concentration of carbamylcholine, indicating that the inhibition was not caused by lack of water and electrolyte secretion. The energy-producing metabolism was not affected since the ATP level in the pancreatic slices was the same in the presence of either inhibitory or optimal concentrations of secretagogues. The inhibition of enzyme secretion was reversible since restoration of efficient enzyme secretion occurred after removal of carbamylcholine (1 mM) by washing, followed by addition of an optimal concentration of CCK-PZ octapeptide.

Morphological studies revealed that the presence of inhibitory concentrations of secretagogues caused severe distortion of the lumen structure: disruption of the filamentous system surrounding the lumen, disappearance of microvilli, and production of distended evaginations of the luminal membrane containing cellular material. These changes eventually caused a reduction in the size of the lumen which becomes plugged with secretory material. It is suggested that these changes in the microtubular microfilamentous system could account for the inhibition of enzyme secretion.
The exocrine pancreas secretes digestive enzymes together with water and electrolytes. Two receptors mediate enzyme secretion, one a muscarinic cholinergic (11), the second a receptor for the peptide hormone cholecystokinin-pancreozymin (CCK-PZ) (10). On the other hand, secretion of water and electrolytes is controlled by secretin (3, 5). It has been shown that during stimulation of enzyme secretion the secretory granules fuse with the cell membrane facing the lumen. The content of the secretory granule is then emptied to the cell exterior while the granule membrane becomes part of the cell membrane, resulting in an enlargement of the lumen perimeter (28, 29).

Secretagogues have a complex effect on enzyme secretion in the pancreas. Both cholinergic agonists as well as CCK-PZ and its peptide analogs have an optimal concentration which produces a maximal rate of enzyme secretion. At higher concentrations of secretagogues, however, the rate of enzyme secretion declines which yields a biphasic dose-response curve. This pattern of pancreatic enzyme secretion has been demonstrated in vivo both for CCK-PZ (7, 9, 18) as well as for acetylcholine (7). By use of pancreatic fragments, a biphasic dose-response curve has been found for acetylcholine (12), carbamylcholine (16, 31, 33), pilocarpine (24), CCK-PZ (33), and its analog caerulein (33, 36).

The aim of the present study was to define the morphological and biochemical characteristics of the inhibition of enzyme secretion at high concentrations of secretagogues. It is shown that the inhibition occurs at the cellular rather than at the receptor level and that it is associated with morphological changes at the luminal membrane. A preliminary report of some of this work has appeared in abstract form (32).

MATERIALS AND METHODS

Pancreatic Slice System

Male albino rats, 180-220 g, fed ad libitum and kept at 24°C ± 1 were used. To minimize differences between individual animals, pancreases from at least three rats were pooled for each experiment. The rats were killed under ether anesthesia by cutting through the heart. The pancreas was removed and immediately placed in Krebs-Ringer bicarbonate medium composed of: NaCl, 118 mM; KCl, 5 mM; MgSO₄, 1 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1 mM; NaHCO₃, 25 mM; and β-hydroxybutyrate 5 mM (KRB medium). The KRB incubation medium at 37°C was continuously gassed with a mixture of 95% O₂ and 5% CO₂. Pancreases were cut into small pieces 1-2 mm² and placed in 50 ml of KRB medium gassed with the O₂/CO₂ mixture. The pooled slices were preincubated for 15 min in a rotary shaking bath at 37°C with shaking at a rate of 80 rpm. At the end of the preincubation period, which served to remove material from ruptured cells, the slices were divided into equal portions each equivalent to about one-third of a pancreas. Each portion of slices was placed in a 25-ml Erlenmeyer flask containing 3 ml of the KRB medium. The vessel was gassed for 30 s with the O₂/CO₂ mixture, tightly closed with a rubber stopper, and incubated at 37°C with shaking at 80 rpm. To initiate secretion, a concentrated solution of secretagogue was added to the medium to give the desired final concentration, and the vessel gassed and stopped. At different time intervals, 50-μl aliquots of the medium were removed for amylase determination. Whenever additions were made or aliquots removed, the vessels were gassed with the O₂/CO₂ mixture and then tightly closed. At the end of the experiments, 7 ml of H₂O were added to each vessel, and the tissue was ground with a polytron (Kinematics GMBH) homogenizer. Amylase activity was determined in aliquots of the homogenate.

Definition of Percent Secretion

Amylase activity was used as a measure of enzyme secretion. The amount of amylase released into the medium plus the amount remaining in the slices at the end of the experiment gives the total amylase initially present in the slices and is defined as 100%. The amylase secreted into the medium during any time interval is expressed as percent of the total.

Electron Microscopy

Pancreatic slices were fixed for electron microscopy in formaldehyde-glutaraldehyde fixative (14) for 3 h. After rinsing in cold 0.1 M cacodylate buffer, pH 7.2, the pancreatic fragments were postfixed in 1% osmium tetroxide, dehydrated in graded ethanol solutions and embedded in Epon. Thin sections were cut with an LKB ultramicrotome (LKB Instruments, Inc., Rockville, Md.) and stained with uranyl acetate lead citrate (30). Electron micrographs were obtained with a Philips 300 electron microscope.

Analytical Procedure

Amylase was assayed according to Bernfeld (4), and protein was determined by the method of Lowry (19) using serum albumin as standard. The amount of ATP in the slices was measured by the firefly luciferin-luciferase system (35). The concentrations of stock solutions of CCK-PZ and its peptide analogs were determined using the fluorescence of tryptophan against a standard of N-acetyl tryptophan amide (27).
Chemicals

Carbamylcholine-chloride and atropine were purchased from Mann Research Laboratories, acetylcholine-chloride from Merck Chemical Div., Merck & Co., Inc. (Rahway, N.J.) and the firefly lantern extract from Sigma Chemical Co. (St. Louis, Mo.). A sample of purified cholecystokinin-pancreozymin (CCK-PZ) was donated by Dr. V. Mutt from the Karolinska Institute, Stockholm. The carboxyl terminal octapeptide of cholecystokinin-pancreozymin (CCK-PZ octapeptide, SQ 19, 884) was a gift of Dr. M. A. Ondetti from Squibb Laboratories, and secretin was obtained from the GIH research unit, Karolinska Institute, Stockholm. The ionophore A-23187 was a gift of Dr. M. Gorman from Eli Lilly. The ionophore was dissolved in ethanol at a concentration of 0.5 mg/ml. Addition of ethanol to the slice system up to a concentration of 1% had no effect on enzyme secretion.

RESULTS

Stimulation of Pancreatic Enzyme Secretion by Various Concentrations of Secretagogues

Stimulation of enzyme secretion was optimal at a concentration of 1 μM acetylcholine or carbamylcholine. The peptide hormone CCK-PZ produced a maximal rate of enzyme secretion at 20 nM and its analog CCK-PZ octapeptide, at 5 nM. At concentrations of secretagogues above the optimum the rate of enzyme secretion was progressively decreased, giving rise to a biphasic dose-response curve. A significant inhibition was obtained at a concentration of 0.1 mM of acetylcholine or carbamylcholine and 0.1 μM of CCK-PZ or its octapeptide analog (Fig. 1).

Measurement of initial rates of enzyme secretion for the first 15 min of stimulation revealed that during this period there was little, if any, inhibition of enzyme secretion with increasing concentrations of secretagogues. Apparently, the development of the inhibition of enzyme secretion was a time-dependent process. Thus, a typical biphasic dose response curve of enzyme secretion was evident only when incubation times longer than 15 min were used. It should be noted, however, that the maximal rate of enzyme secretion in the first 15 min was somewhat smaller than that achieved with longer incubation times (Fig. 2).

Relationship between the Effects of the Cholinergic and Peptide Hormone Agonists on Pancreatic Enzyme Secretion

An attempt was made to evaluate whether a high concentration of agonist selectively inhibits
stimulation of enzyme secretion through the homologous, or possibly also through the heterologous, receptor. Addition of an optimal concentration (5 nM) of CCK-PZ octapeptide to slices incubated in the presence of an inhibitory concentration of cholinergic agonist (carbamylcholine 1 mM) did not increase the rate of enzyme secretion (Fig. 3). Addition to the slice system of CCK-PZ octapeptide and carbamylcholine, both at optimal concentrations, gave essentially the same rate of enzyme secretion as that caused by 1 μM carbamylcholine (cf. Fig. 4). In a complementary experiment the slow rate of enzyme secretion caused by an inhibitory concentration of CCK-PZ octapeptide (0.1 μM) was not increased by addition of an optimal concentration of carbamylcholine (not shown). The addition of inhibitory concentrations of both CCK-PZ octapeptide and carbamylcholine caused an additive inhibition of enzyme secretion (Fig. 4).

**Effect of the Ionophore A-23187 and Atropine on Inhibition of Pancreatic Enzyme Secretion by High Concentrations of Secretagogues**

The ionophore A-23187 has been shown to bypass the receptor and elicits efficient enzyme secretion from rat pancreatic slices (8). Stimulation of enzyme secretion by calcium acting through the ionophore was found to be dependent on efficient energy production and was specific for digestive enzymes. It was therefore inferred that the ionophore bypasses the receptor-me-
Additive inhibitory effect of high concentrations of secretagogues on pancreatic enzyme secretion. Concentrations of secretagogues were: carbamylcholine 1 μM (O---O), CCK-PZ octapeptide 0.1 μM (□—□), carbamylcholine 1 mM (●—●), carbamylcholine 1 mM + CCK-PZ octapeptide 0.1 μM (■—■), and a control system with no addition (O—O).

Addition of the ionophore to slices incubated in the presence of an inhibitory concentration of carbamylcholine (1 mM) did not increase the rate of secretion, whereas in the absence of carbamylcholine the ionophore caused efficient enzyme secretion (Fig. 5). These experiments suggest that the inhibition of secretion caused by high concentrations of secretagogues occurs at a step later than activation of the receptor. In the present work, the ionophore was used to test whether the inhibition of enzyme secretion occurs at the receptor level. Addition of the ionophore to slices incubated in the presence of an inhibitory concentration of carbamylcholine (1 mM) did not increase the rate of secretion, whereas in the absence of carbamylcholine the ionophore caused efficient enzyme secretion (Fig. 5). These experiments suggest that the inhibition of secretion caused by high concentrations of secretagogues occurs at a step later than activation of the receptor. The question remained, however, whether the inhibition was mediated by specific interaction of the secretagogue with its receptor or whether it was due to a nonspecific action of secretagogues when present at high concentration. To investigate this possibility, the effect of atropine, a specific competitive inhibitor of the muscarinic cholinergic receptor, was tested on stimulation of secretion by increasing concentrations of carbamylcholine. As shown in Fig. 6, atropine displaced the dose response curve for carbamylcholine to the right, but did not change the biphasic characteristics of the response. Comparison of the curves in the presence and absence of atropine shows that in the presence of atropine higher concentrations of carbamylcholine were required to produce an optimal rate of enzyme secretion. In the absence of atropine, however, these high concentrations of carbamylcholine produce an inhibition of enzyme secretion. Taken together, the experiments with the ionophore and atropine indicate that although the inhibition of enzyme secretion is mediated by the receptor, the putative inhibited step is a late event in the secretory pathway and most likely occurs at a cellular rather than at the receptor level. The intracellular messenger which...
Figure 6  Effect of atropine on stimulation of amylase secretion by various concentrations of carbamylcholine. Pancreatic slices were preincubated for 10 min in the absence (○—○) or presence of atropine 0.1 μM (●—●) and 1 μM (■—■). At zero time, carbamylcholine was added to give the indicated concentrations. Rates of amylase secretion were measured as in Fig. 1.

Table I  Effect of Various Concentrations of Carbamylcholine on ATP Levels in Pancreatic Slices

| Incubation time min | No addition | 1 μM Carbamylcholine | 1 mM Carbamylcholine | No. of exp |
|---------------------|-------------|----------------------|----------------------|-----------|
| 10                  | 9.1         | 9.3                  | 8.3                  | 2         |
| 30                  | 10.8        | 9.5                  | 8.6                  | 2         |
| 60                  | 10.0        | 9.1                  | 8.0                  | 4         |
| Mean ± S.D.         | 9.9 ± 1.7   | 9.2 ± 1.1            | 8.2 ± 0.8            | 8         |

At the incubation times indicated in the table, slices were transferred to 4% perchloric acid and homogenized. ATP and protein were determined as described in Materials and Methods.

causes inhibition of enzyme secretion has not yet been identified.

Cellular Effects of Inhibitory Concentrations of Secretagogues

Since pancreatic enzyme secretion is highly dependent on efficient ATP production (13), it was important to evaluate the effect of inhibition of enzyme secretion on the energy-producing machinery. As shown in Table I, the level of ATP remained constant during 60 min of incubation. Moreover, there were no significant differences between slices incubated in the presence of inhibitory or optimal concentrations of secretagogue. Thus, it is unlikely that the inhibition of enzyme secretion was due to a deleterious effect on the energy-producing systems.

Homogenization of the slice systems followed by sedimentation of the particulate fraction showed an appreciable amount of soluble amylase. This was found whether the slices were incubated in the presence of an optimal or an inhibitory concentration of secretagogue. In part, this soluble amylase is due to destruction of granules during homogenization. In both systems, however, the relative amount of amylase in the particular fraction of slices incubated in the presence of secretagogue was significantly lower than when the slices were incubated in its absence (Table II). Apparently, the soluble amylase also reflects discharged extracellular amylase which is present in the duct system.

At high concentration of carbamylcholine, addition of secretin had no effect on the rate of enzyme secretion (Fig. 7). This indicated that it is unlikely that the inhibition of enzyme secretion was due to lack of water and electrolyte secretion. In the parotid gland, activation of the muscarinic cholinergic receptor by a high concentration of
TABLE II

Effect of Various Concentrations of Carbamylcholine on the Stability of Pancreatic Secretory Granules

| Incubation time (min) | No addition | 1 μM Carbamylcholine | 1 mM Carbamylcholine |
|-----------------------|-------------|----------------------|----------------------|
| 20                    | 37.2        | 41.9                 | 42.6                 |
| 40                    | 35.1        | 43.7                 | 43.3                 |
| 60                    | 36.9        | 43.6                 | 46.8                 |
| 90                    | 32.5        | 43.6                 | 39.4                 |
| 120                   | 36.4        | 44.3                 | 47.1                 |

Mean ± S.D. 35.6 ± 2.9 43.4 ± 3.9 43.8 ± 4.0

At the time indicated in the table, the slices were washed and transferred into cold 0.3 M sucrose (5 ml/system) containing diphenyl-p-phenylene diamine 0.2 μg/ml. Homogenization was achieved by 10 strokes in a tight Teflon glass homogenizer. The homogenate was centrifuged for 15 min at 1,400 g, and the amount of amylase was determined in the supernate and the pellet. Centrifugation at higher speed revealed that the 1,400 g × 15 min pellet contained essentially all the particulate amylase. Each number represents the mean of two experiments. The mean ± SD at the bottom of each column represents 10 determinations taken at the times indicated in the table.

Carbamylcholine (0.1 mM) causes a release of potassium from the acinar cell (34). In the pancreas, however, carbamylcholine (1 mM) did not cause potassium release (not shown).

It was of interest to find out whether the inhibition of pancreatic enzyme secretion is a reversible process. To that intent, slices were incubated for 30 min in the presence of 1 mM carbamylcholine, after which they were thoroughly washed and transferred to fresh medium containing atropine (1 μM) and allowed to recover for a period of 10 min. With this treatment, the slices resumed their ability to respond to an optimal concentration of CCK-PZ octapeptide, and the rate of enzyme secretion in the inhibited system became indistinguishable from that of a control system incubated in the absence of carbamylcholine and then treated in the same way as the test system (Fig. 8).

Ultrastructural Changes in Pancreatic Slices Incubated in the Presence of High Concentrations of Secretagogues

Morphological studies of the exocrine pancreas were of great importance in elucidating the various cellular steps in the secretory pathway (29).

Since the biochemical studies indicated that inhibition of enzyme secretion occurs at the cellular rather than at the receptor level, it was hoped that in this case, too, the inhibition would be characterized by distinct morphological changes.

The pancreatic secretory unit is composed of several acinar cells lining, with their apical part, a narrow tubule known as the acinar lumen. Upon stimulation of enzyme secretion the acinar lumen is enlarged (Fig. 9). This has been shown to be due to fusion of secretory granules with the cell membrane lining the lumen (28). In contrast, slices incubated for 1 h in the presence of an inhibitory concentration of carbamylcholine (1 mM) show severe reduction in the size of the lumen (Fig. 10). Keeping in mind that the development of inhibition of enzyme secretion takes about 15 min, it was decided to study the early stages of the inhibition morphologically. The resting slice system (Fig. 11) shows a lumen with a diameter equal to the size of a secretory granule.

![Figure 7](image-url)
The lumen is lined with microvilli and can be recognized by junctional complexes all around it. It should be noted that the immediate area around the luminal membrane does not contain ribosomes (Fig. 11). This area, known as the terminal web, is rich in filamentous structures some of which penetrate the microvilli and thus act to maintain their structure. Slices incubated for 5 min in the presence of an inhibitory concentration of carbamylcholine show evaginations of the apical cell membrane towards the lumen. An evagination can be recognized by its irregular shape, and the presence of cellular material including ribosomes. An initial stage with only one evagination is shown in Fig. 12. A more progressive stage of evagination is shown in Fig. 13, also taken from slices incubated for 5 min in the presence of 1 mM carbamylcholine. In this micrograph, three of the four acinar cells have produced evaginations while the cell on the left side still keeps its normal structure with typical microvilli containing their filamentous structure. Complete disappearance of microvilli and disruption of the filamentous structure occurs after 15 min of incubation in the presence of 1 mM carbamylcholine (Fig. 14). The irregular evaginations are filled with cellular material, and some of them transverse the lumen diameter and touch the membrane of an opposite cell. At this stage, the lumen area can be identified only by the junctional complexes around it (Fig. 14).

An extreme modification of the lumen is produced after 30 and 60 min of incubation in the presence of an inhibitory concentration of carbamylcholine. There is little, if any, further morphological changes between 30–60 min of incubation under inhibitory conditions. At this stage the lumina are severely reduced in size, with ribosomes adjacent to the inner luminal membrane. A concentrated amorphous substance can be seen trapped within the collapsed lumen and may represent plugging of the lumen with secretory material (Figs. 15 and 16). It should be pointed out that production of the evaginations was confined to the cell membrane facing the lumen, while other parts of the cell membrane, mitochondria and nuclei seem to be well preserved (Fig. 10). Essentially the same morphological changes were observed when CCK-PZ octapeptide was used instead of carbamylcholine (not shown). After 60 min of incubation in the presence of 1 mM carbamylcholine, there seems also to be an increase in the number and size of autophagic vacuoles. However, this aspect was not investigated in the present study.

A critical question was whether atropine, at a concentration which prevents the biochemical parameter of inhibition of enzyme secretion caused by 1 mM carbamylcholine, also prevents the morphological changes which were found to occur under this condition. As shown in Fig. 17, pancreatic slices incubated for 60 min in the presence of 7 μM atropine and 1 mM carbamylcholine revealed a morphologically normal lumen area with well-preserved microvilli. Furthermore, good correlation was also found between reversal of the carbamylcholine inhibition of secretion and the morphological reappearance of the lumen with numerous microvilli (Fig. 18). The reversal of the inhibition both biochemically and morphologically, thus, shows that upon removal of the inhibitory effect the cell has the capacity to reform its normal lumen morphology.

**DISCUSSION**

Inhibition of pancreatic enzyme secretion at high concentrations of secretagogues has been described for several in vivo and in vitro systems...
Figure 9  Pancreatic acinus of secreting slice system incubated for 1 h in the presence of carbamylcholine (1 μM). The larger lumen (L) containing secretory material is lined with numerous microvilli. Only few intensely stained secretory granules are seen. × 4,800.

Figure 10  Pancreatic slices incubated for 1 h in the presence of carbamylcholine (1 mM). The lumen (L) is small, is surrounded by numerous secretory granules, and contains dense secretory material. Microvilli cannot be seen. × 5,700.
FIGURE 11 Acinar lumen of resting pancreatic slices incubated for 1 h in KRB medium. The small lumen (L) is surrounded by secretory granules and contains microvilli (MV). The cell web adjacent to the luminal membrane is free of ribosomes and in some areas shows a filamentous structure (F). Junctional complexes (J) and desmosomes (D) are shown. (× 45,000).

FIGURES 12-13 Acinar lumen of pancreatic slices incubated for 5 min in the presence of an inhibitory concentration of carbamylcholine (1 mM). The lumen (L) is identified by the junctional complexes (J) all around it. In Fig. 12, the lumen contains only one evagination (E) which can be recognized by its irregular shape. The evagination is filled with cellular material including ribosomes and is devoid of
filamentous structure. Many microvilli (MV) containing filaments are still preserved. × 28,700. In Fig. 13, the microvilli and the filamentous structure (F) are preserved in only one cell (at the left side of the lumen). In the other acinar cells, the microvilli are replaced by irregular evaginations containing ribosomes. Desmosomes, D. × 47,600.

**FIGURE 14** Acinar lumen of pancreatic slices incubated for 15 min in the presence of an inhibitory concentration of carbamylcholine (1 mM). About half of the lumen area (L) is occupied by multiple, distended evaginations (E) filled with cellular material. No microvilli can be seen. Junctional complexes, J. Desmosomes, D. × 52,700.
Figure 15-16 Acinar lumen of pancreatic slices incubated for 1 h in the presence of an inhibitory concentration of carbamylcholine (1 mM). The lumen (L) surrounded by junctional complexes (arrow) and desmosomes (D) is plugged with dense secretory material. There is severe reduction in the size of the lumen, apparently due to pushing of the cellular content on the lumen membrane. Many ribosomes are located just adjacent to the luminal membrane. Fig. 15, × 37,000; Fig. 16, × 50,000.
Figure 17 Acinar lumen of pancreatic slices incubated for 1 h in the presence of atropine plus carbamylcholine. Incubation procedure was as described in Fig. 6, using concentrations of 7 μM atropine and 1 mM carbamylcholine. A typical acinar lumen (L) lined with microvilli is seen. The junctional complexes are marked by arrows. × 33,500.

Figure 18 Morphological reversal of the carbamylcholine inhibition of enzyme secretion. Pancreatic slices were incubated for 30 min in the presence of 1 mM carbamylcholine, then transferred to a fresh medium and treated as described in Fig. 8. After further incubation for 20 min in the presence of 5 nM CCK-PZ octapeptide, the slices were processed for electron microscopy. There is reappearance of the lumen (L) which is lined with numerous typical microvilli (MV). The lumen can be identified by junctional complexes (arrows), desmosomes (D). × 27,000.
using various experimental animals. It was thought to be one aspect of desensitization of the receptor which recently received considerable attention in studies of the adenylate cyclase system (23). As shown in the present work, several lines of evidence indicate that the inhibition of pancreatic enzyme secretion does not occur at the receptor level. A high concentration of cholinergic agonist inhibited stimulation of enzyme secretion by an optimal concentration of CCK-PZ analog known to act on a different receptor. The same cross inhibition was observed when a high concentration of CCK-PZ octapeptide was used in addition to an optimal concentration of carbamylcholine. Furthermore, when inhibitory concentrations of either carbamylcholine or CCK-PZ octapeptide were used, it was not possible to increase the rate of enzyme secretion by the calcium ionophore A-23187 which, in the presence of calcium, bypasses the receptor and elicits efficient enzyme secretion (8). These experiments led to the conclusion that the inhibition of enzyme secretion occurs at a stage later than activation of the receptor, or introduction of calcium into the cell. A possible explanation for the observed inhibition based on a reduction in the intracellular level of ATP was excluded since the level of ATP was the same whether an inhibitory or an optimal concentration of secretagogue was used.

The extremely high concentration of secretagogues which were required to cause the inhibitory effect raises the question whether the secretagogues enter the cell and inhibit enzyme secretion through action on receptors inside the cell. In this regard, the work by Cheng and Farquhar (6) is relevant since these authors demonstrated the presence of adenylate cyclase in intracellular membranes of rat liver, suggesting that components which are usually found on the cell membrane could also be present inside the cell. The following experiments suggest, however, that the inhibition of enzyme secretion is initiated by an action of the secretagogues on receptors at the cell membrane. Atropine, a competitive inhibitor which specifically blocks the muscarinic receptor, changes the response of the pancreas so that 1 mM carbamylcholine becomes an optimal concentration for stimulation of enzyme secretion. This experiment shows that, even at a high concentration, carbamylcholine must act on specific muscarinic receptors accessible to atropine. Furthermore, CCK-PZ which is made up of 33 amino acids was as effective in causing inhibition of enzyme secretion as its octapeptide analog. It is not likely that a peptide of this size would penetrate the membrane and act inside the cell. Taken together, the experiments suggest that although the inhibition of enzyme secretion apparently occurs inside the cell, it is initiated by interaction of the secretagogues with their receptors on the cell membrane. The experiments also indicate that, in order to produce maximal enzyme secretion, only a fraction of the pancreatic cell receptors have to be occupied. In support of this suggestion are the observations that depolarization, 44Ca efflux (22) and the enhanced incorporation of 32P into phosphatidylinositol (12) required a higher concentration of secretagogues than that required to produce a maximal stimulation of enzyme secretion. The presence of receptors in excess of the amount required to produce maximal response was first suggested by Nickerson (25) who coined the term "receptor reserve." It was also observed in the rat parotid for stimulation of enzyme secretion through the β-adrenergic receptor (1).

More conclusive evidence against inhibition at the receptor level is the morphological finding that inhibition of enzyme secretion was accompanied by dramatic structural changes at the acinar lumen. At present, it is not known what precise molecular mechanism causes the collapse of the acinar lumen, aside from the fact that the morphological changes occur only at the apical part of the cell membrane and both the inhibition and the structural changes could be reversed by removal of the inhibitory secretagogue. A clue to the mechanism comes from experiments on the early morphological changes occurring during the development of inhibition. These experiments indicate that the initial change is disruption of the filamentous structure around the lumen, disappearance of the microvilli, and concomitant production of evaginations of less-ordered structure. This sequence of events suggests that the first change is probably disaggregation of the cytoskeleton which supports the luminal membrane. This leads to weakening and protrusion of the cell membrane to form evaginations which eventually severely reduce the lumen diameter. It has been suggested that the microtubular-microfilamentous system is important in the process of glucose-stimulated insulin secretion from beta-cells of the pancreas (17). Using cytochalasin B in studies of insulin secretion, Orci et al. (26) put forward the hypothesis that the "cell web" (a microtubular-microfilamentous structure underlying the plasma
membrane) acts as a barrier preventing the fusion of secretory granules with the cell membrane. According to this hypothesis, agents that stabilize the “cell web” inhibit secretion, whereas agents that cause disruption of the filamentous structure, initially, result in an enhancement of secretion of secretory granules with the cell membrane.

The morphological picture at a concentration of cytochalasin B which inhibited enzyme secretion showed disruption of the microfilamentous structure of the “cell web” and microvilli, but these changes had not progressed to cause collapse of the acinar lumen. As pointed out (2), the effects of cytochalasin B were not confined to the process of enzyme secretion. Both the uptake of glucose and the intracellular transport of exportable protein were markedly inhibited. For these reasons, the finding that secretagogues acting on specific receptors at the cell membrane lead to profound changes in the “cell web” and microvilli at the lumen is of particular significance. It is possible that the morphological changes caused by high concentrations of secretagogues represent an exaggeration of one phase of the normal sequence of events occurring under optimal conditions of enzyme secretion. This concept suggests that disaggregation of the microfilamentous structure during optimal secretion is counteracted by an opposite reaction which maintains the acinar lumen morphology. In support of this hypothesis is the finding that once the inhibitory effect is removed, the lumen readily regains its normal structure. Thus, the finding that disruption of the microtubular-microfilamentous system by high concentrations of secretagogues coincides with inhibition of enzyme secretion suggests that this structure is involved in the discharge process. Alternatively, it is also possible that high concentrations of secretagogues affect another yet unknown component essential for discharge of secretory material.

An interesting question is whether the microtubular-microfilamentous system has an implication in pancreatic diseases. It has recently been reported that excessive doses of pancreatic secretagogue cause experimental pancreatitis in vivo (15). Thus, inhibition of enzyme secretion could be an important factor in the pathogenesis of pancreatitis. Clearly, more work should be done to find out whether the microtubular-microfilamentous system has a pathophysiological role in various diseases of the exocrine pancreas.

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