MICROFILAMENTOUS SYSTEM AND SECRETION OF ENZYME IN THE EXOCRINE PANCREAS

Effect of Cytochalasin B

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ABSTRACT

The microfilaments in the acinar cell of the exocrine pancreas are essentially located in the apical part of the cell: thin microfilaments (50 Å), cytochalasin B (CB)-sensitive, form the axis of the microvilli and a network lying beneath the apical membrane; thicker filaments (100 Å), at least partly CB-insensitive, form bundles parallel to the plasma cell membrane and the desmosomal links. CB interaction with the acinar cell of the exocrine pancreas involves at least two sites: a membrane site involved in the inhibitory effect of CB on the monosaccharide transport and a less sensitive site at the filamentous level at least partly responsible for the inhibitory effect of CB in the secretion of the exportable enzyme from the pancreatic cell. CB did not alter the energy balance of the acinar cell nor the exchanges of 45Ca between the extracellular medium and the pancreatic tissue. CB (2 x 10^{-7} and 2 x 10^{-6} M) has secretagogue properties whereas CB (2 x 10^{-5} M) has inhibitory effect on stimulated secretion and secretagogue properties. The mechanism of these secretory effects is not yet explained. The analysis presented in this investigation affords strong evidence for the involvement of the microfilamentous network in the last steps of the secretory cycle in the acinar cell of the exocrine pancreas.

The role of microfilaments in secretion has been frequently assessed, e.g. in the endocrine pancreas (Lacy et al., 1968; Orci et al., 1972), in the thyroid (Neve et al., 1970; Williams and Wolff, 1971), and in the parotid (Butcher and Goldman, 1972). It has been suggested that they could play a role in the last step in the sequence of the cellular events leading to the release of secretion granules (Orci et al., 1972).

The presence of microfilaments in the acinar cell of the exocrine pancreas has been recently recognized (Geuze and Poort, 1973); however, their role in exocrine pancreatic secretion has not yet been investigated.

Cytochalasin B (CB), a metabolite of the mold Helminthosporium dematoidenum, was originally noted by Carter (1967) to inhibit cytoplasmic division and cell motility, and to cause dramatic changes in cell shape. A wide range of other cellular activities, including secretion, has been subsequently shown to be influenced by CB (Spooner and Wessels, 1970; Allison et al., 1971;
Wagner et al., 1971; Malawista, 1971 a and b; Orr et al., 1972). Wessells et al. (1971) have emphasized the disruptive effect of CB on microfilaments, though recent evidence strongly suggests that it interacts with the surface membrane of the cell (see Everhart and Rubin, 1974). It was therefore of interest to investigate the influence of CB on the exocrine function of the pancreas.

MATERIALS AND METHODS

Pieces of rat pancreas were obtained from 2.5-month old albino rats after a 12-h fast. They were incubated for 2 h as previously described (Bauduin et al., 1969).

The incubation medium, enriched by the L-amino acid mixture of Campagne (Campagne and Gruber, 1962) and d-glucose 10 mM, was buffered with bicarbonate at pH 7.4. The gas phase was O2 95%, CO2 5%.

Secretion was quantified by the activity of chymotrypsinogen and lipase which were measured by the methods of Reboud et al. (1962) and Marchis-Mouren et al. (1965), respectively.

Enzyme secretion was induced by carbachol (K and K, Plainview, New York) or cerulein (a generous gift of Farmitalia, Milan, Italy). Drugs were added to the incubation medium at time 0.

Ethanol (0.5%) was routinely added to the controls, although it did not alter the investigated parameters.

CB was obtained from Egachemieke, Brenz, Germany. Stock solutions were prepared in ethanol at a concentration of 1 mg/ml. Dimethylsulfoxide, the usual solvent for CB, induced discharge of the exportable enzymes and altered the morphology of the cell when its concentration exceeded 0.01%.

Glucose uptake and metabolism in the pancreatic gland were investigated as follows:

(a) Conversion of glucose into proteins and CO2 was chosen as representative of glucose disposal by the pancreatic tissue incubated in vitro. Incorporation of d-[1,14C]glucose 0.06 mCi/mmol (10 μCi/ml) into proteins was measured by the method of Mans and Novelli (1961). Accordingly, only proteins precipitable by 5% TCA were taken into account. Incorporation of d-[1-14C]glucose 0.06 mCi/mmol (10 μCi/ml) into CO2 was measured as described by Hoskin (1959).

(b) Incorporation of glucose into pancreatic pieces was determined on a sample of the homogenates digested overnight with soluene in the ratio 1:1 (vol/vol). Counting was performed in a Nuclear Chicago liquid scintillation spectrometer Mark II using the following scintillation mixture: Omnifluor (New England Nuclear, Frankfurt, Germany) in toluene 4 g per liter, 15 ml per flask.

(c) Sugar transport was estimated with 2-deoxy-D-glucose. Tissues were incubated in a glucose-free medium supplemented with 2-deoxy-D-[1,14H]glucose 0.6 μCi/mmol (0.6 μCi/ml), 6.0 mCi/mmol (6 μCi/ml) in tracer experiments. At the end of the incubation, the tissue was washed free of extracellular deoxy-D-glucose in the usual incubation medium for 10 min at 20°C (110 strokes per min). The uptake of deoxyglucose was measured on a sample of the homogenized tissue.

(d) The conversion of 2-deoxy-D-glucose to 2-deoxy-D-glucose 6 phosphate was determined by incubating the pancreatic pieces for 15 or 45 min with 2-deoxy-D-[1,14C]glucose 5 mCi/mmol (1 μCi/ml). At the end of the incubation, after washing the tissue for 10 min in the usual incubation medium, the pancreatic pieces were homogenized in water (2 ml); perchloric acid (12 M) was added to make the final concentration 0.4 M. Further processing was achieved according to the method of Kletzien and Perdue (1973): deoxy-D-glucose and deoxy-D-glucose phosphate were separated by thin layer chromatography. The activity was measured in a radiochromatogram scanner Packard model 7200 (Packard Instrument Co., Inc., Downers Grove, Ill.). Quantification was made by planimetry.

The ATP content of the incubated gland was estimated by the luciferin-luciferase method (Stanley and Williams, 1969). The incubation was stopped by heating the tissue in 1 ml of boiling water for 3 min; then, the tissue was homogenized and centrifuged; the ATP content was measured in the 17,000 g x 45 min supernate.

The calcium exchanges in the pancreas in vitro were investigated by an adaptation of the method described by Case and Clausen (1973).

(e) Uptake of calcium. Each pancreas was cleaved into 10 pieces (about 100 mg each). A preincubation of 10–15 min was achieved in a calcium-free medium supplemented with 0.5 mM EGTA. The pieces were transferred to individual flasks containing 44Ca 2.5 μCi/mmol (0.5 μCi/ml) in the appropriate medium (control or CB-supplemented) and their weights determined. At the end of the incubation period, they were washed, then homogenized in water (1 ml); the incorporated radioactivity was measured in triplicate on samples of the homogenates as described above. Five pieces were used for the control loading curve, and the other five gave the loading curve in the treated gland. This procedure avoids the variation of calcium uptake due to interanimal variation which would otherwise completely obscure the effect of the treatment.

(f) Efflux of calcium. Since the slope of the loading curve considerably decreases after 45 min of incubation, this time interval was selected as an appropriate loading period.

Each pancreas was cleaved into eight pieces which were incubated together in the usual incubation medium containing 44Ca 25 mCi/mmol (5 μCi/ml) for 45 min after a preincubation of 10 min in a calcium-free, 0.5 mM EGTA-supplemented medium. A wash of extracellular 44Ca was achieved by two successive incubations of 7.5 min in calcium-free, 0.5 mM EGTA-supplemented medium. The efficacy of the washing was assessed by the comparison of the activity removed in the combined wash.
and the activity trapped in the extracellular space as calculated from the activity of the loading incubation medium and the volume of the extracellular space. The pieces were then transferred to the incubation medium containing the appropriate calcium concentration and weighed. Every 10 min a sample of the medium was removed to allow estimation of the released radioactivity and exportable enzymes (amylase activity was determined using the method of Danielson, 1974) and replaced by an equivalent amount of the appropriate incubation medium. Eight flasks were incubated in each series. Drugs were added after the fifth sample (50 min) had been taken. Sampling was further continued for 80 min. At the end of the incubation period the tissue was removed, briefly blotted on filter paper and homogenized. Samples of homogenates were counted in triplicate after dissolution in soluene. The initial activity in the tissue was calculated from the residual activity of the tissue at the end of the incubation period and the activity released into the medium. At each incubation time, the residual activity in the tissue was calculated from the activity released into the incubation medium and expressed in percent of the activity in the pancreatic fragment at time 0 arbitrarily fixed at 100%. Corrections were applied for the dilution of the medium due to the replacement of the sample by an equal volume of cold, fresh medium (40 μl vs. 3 ml of incubation medium).

**Electron Microscope Examination**

Small pancreatic fragments (7th to 1/3 of the incubating pieces) were fixed in 2% glutaraldehyde buffered with 0.2 M sodium cacodylate pH 7.3 and postfixed in 1% osmium tetroxide in the same buffer. They were dehydrated in a graded sequence of alcohols and then embedded in Araldite. Thin sections were cut on a Reichert OM U ultramicrotome, doubly stained with uranyl acetate and lead citrate, and examined with a Philips EM 300 electron microscope.

**Expression of Results**

Because of the nongaussian distribution of the parameters, means and standard deviations of the mean have been calculated on the logarithms of the data. Results are expressed as antilogarithms of the mean ± the standard deviation of the mean (Dumont, 1964).

**RESULTS**

**Influence of CB on Exocrine Pancreatic Secretion**

**NON-STIMULATED SECRETION (FIG. 1):** CB enhanced the release of exportable enzymes-lipase and chymotrypsinogen from incubated pancreas. This secretagogue-like effect was maximal in the 0.5–2.0 μg/ml range. It gradually disappeared with higher CB concentrations and was no longer discernible at 10 μg/ml.

**MODERATELY STIMULATED SECRETION (FIG. 2 A):** Cerulein (3 ng/ml) tripled the basal secretion of chymotrypsinogen or lipase. The dose-response curve of this stimulated secretion was bell shaped. No statistically significant difference from control (cerulein-stimulated secretion in the absence of CB) was recorded at any concentration of CB from 0.1 to 10 μg/ml. However, low CB concentrations tended to increase the release of exportable enzyme above control level while high concentrations (10 μg/ml) were somewhat inhibitory. The bimodal effect was objectified by the statistically significant difference between the enzyme release produced in the presence of 0.1 (or 1 μg/ml) and 10 μg/ml CB concentration (P < 0.01 in paired test).
Figure 2 Effect of CB on stimulated chymotrypsinogen secretion in the rat exocrine pancreas. (A) Moderately stimulated secretion. Secretion has been stimulated by cerulein 3 ng/ml. Results are means ± SEM of 7 experiments. They are expressed in percent of the release of chymotrypsinogen induced by cerulein in the absence of CB. Control: 32.5 ± 2.5 units per 100 mg tissue per h. (B) Maximally stimulated secretion. Secretion has been stimulated by carbachol 0.1 mM. Results are means ± SEM of 23 experiments. They are expressed in percent of the release induced by carbachol in absence of CB. Control: 69.8 ± 8.9 units per 100 mg tissue per h.

Maximally Stimulated Secretion (Fig. 2 b): Maximal enzyme release (about five times the control level in the present experiments) was achieved with 0.1 mM carbachol. Strong inhibition of the maximal enzyme release was observed with CB concentration ≥ 5 μg/ml. The enzyme release no longer differed from the secretion inducible by CB alone at 10 μg/ml.

Reversibility of CB Effect on Exocrine Pancreatic Secretion

0.01 mM carbachol increased the spontaneous release of chymotrypsinogen by 167% (n = 4). The increase was 157% when the tissue had been preincubated with 10 μg/ml CB for 1 h before the stimulation of secretion was primed with 0.01 M carbachol in a CB-free medium. One wash of 3-4 min was done before transferring the tissue to CB-free medium. This reversibility was confirmed by electron microscope examination of the tissue (see below).

Ultrastructure of the Exocrine Pancreas: Microfilamentous System

The ultrastructure of the acinar cell of the incubated pancreas was indistinguishable from the nonincubated control (Figs. 3 and 4). When incubated in the absence of CB, the apical region of the acinar cell was filled with numerous zymogen granules; emiocytosis was an infrequent occurrence. An ectoplasmic band, generally devoid of cytoplasmic organelles, extended just beneath the luminal membrane. Its major component was a heterogeneous population of filaments; microfilaments about 100 Å in diameter were usually packed in bundles parallel to the acinar lumen. They ran close to the membrane from one side of the cell to the other. The other component of the microfilamentous network consisted of microfilaments 50-70 Å in diameter; they were spread throughout the ectoplasmic band without any preferential orientation. A similar material filled the core of the numerous microvilli which protruded into the acinar lumen. The similarity of the microfilaments of the microvilli and the thin filaments of the ectoplasmic band was further supported by the following observation: occasionally in normal pancreatic tissue, but very frequently in cells recovering from CB exposure, some of the thin filaments gathered in the ectoplasmic band at the root of the nascent microvillus. In more advanced stages of the formation of the process, it was clear that they formed the axis of the microvilli.

Isolated microfilaments could also be seen along the lumen or extending between the zymogen granules (Fig. 4). Though their length could not be defined precisely, it could reach 0.7 μm or more. Again, no specific orientation was apparent.

Influence of CB on Exocrine Pancreatic Ultrastructure

Non-stimulated Secretion: No ultrastructural changes were observed when the gland had been exposed to concentrations of CB lower than 5 μg/ml for 1 or 2 h (Fig. 5), though enzyme release was increased. Exposure to higher concentrations of CB (Figs. 6 and 7) resulted in a dramatic alteration of the juxtaluminar area of the cell: the microvilli had nearly completely disappeared. The microfilamentous network was apparently disrupted and replaced by cores of granular material. These electron-lucent areas were not infrequently surrounded by a tightly packed bundle of filaments, apparently similar to the 100 Å filaments described in the untreated tissue. Though very short filamentous material could still occasionally be observed in the electron-lucent core, no thin microfilaments of measurable size were ever
seen in any area of the acinar cell treated with high concentrations of CB.

**Stimulated Secretion:** The gland stimulated by 0.1 mM carbachol or 10 ng/ml cerulein showed the typical aspect of enlarged acinar lumina filled with secretion material (Fig. 8). Numerous pictures of emiocytotic processes developed along the apical membrane of the cell. The ectoplasmic band, as well as the microfilamentous system did not differ from the control.

CB altered the ultrastructure of the stimulated gland whenever its concentration was greater than 5 pg/ml as in the unstimulated gland. The acinar lumen contour was no longer convoluted; the margins were smooth and unusually regular as in the nonstimulated, CB-treated gland. Very few microvilli were still present; they were always reduced in size (Figs. 9 and 10). Emiocytosis had nearly completely disappeared; the lumen was empty or contained very sparse material contrasting with the untreated, stimulated gland. Thin microfilaments had vanished; microfilaments, 100 Å in diameter and generally packed in bundles, frequently surrounded electron-lucent cores of granular material as in the unstimulated gland exposed to CB (10 pg/ml).

**Reversibility of CB Effect on Exocrine Pancreatic Ultrastructure**

When the gland had been exposed for 1 h to 10 pg/ml CB, then transferred to CB-free incubation medium for 1 h after a wash of 3–4 min, the ultrastructural aspect had returned to normal: the electron-lucent cores could no longer be seen, the thin filaments had reappeared, and the apical membrane had lost its apparent rigidity and was normally convoluted. This reversal was gradual: 30 min after transfer into the CB incubation medium, thin filaments were clearly visible in the ectoplasmic band as well as in the root of newly forming microvilli.

**Influence of CB on the Energy Supply of the Exocrine Pancreas**

**Influence of CB on the Energy Balance of the Exocrine Pancreas:** As shown in Table I, the energy balance, estimated by the ATP content of the gland, was not altered by CB in the investigated concentration range (0.1–10 pg/ml). Stimulation of the secretion of the pancreas by secretagogues such as 0.1 mM carbachol resulted in a 25% decrease of the ATP level in the tissue. The same decrease was observed when the stimulation was achieved in the presence of CB, even when the concentration of CB reached 10 pg/ml, a concentration that strongly inhibits the secretory response.

**Influence of CB on Glucose Utilization in the Exocrine Pancreas:** CB ≥ 1 pg/ml decreased the incorporation of radioactivity from D-[U-14C]glucose into whole homogenates of unstimulated gland (Table II). In the stimulated gland the incorporation was already decreased in the presence of 0.1 pg/ml of CB. CB (≥ 1 pg/ml) provoked a slight but statistically significant decrease of D-[U-14C]glucose incorporation into the TCA-precipitable proteins of the unstimulated gland (Table III). The conversion of glucose into proteins was more sensitive to CB when the gland was under carbachol 0.1 mM stimulation: the inhibition was already statistically significant with the 1 pg/ml concentration of CB.

The influence of CB on the conversion of D-[1-14C]glucose into CO2 is illustrated in Table IV: the conversion of D-[1-14C]glucose was decreased by CB (≥ 1 pg/ml), and this inhibitory effect was also more obvious in glands treated with carbachol 0.1 mM.

The utilization of glucose by the pancreatic gland in vitro was thus more sensitive to CB treatment when the secretory function of the acinar cell was stimulated. Not only was the threshold concentration of CB that was able to reduce the conversion of glucose into TCA-precipitable proteins and CO2 decreased, but also the magnitude of the inhibition was greater: this was more evident with the 10 pg/ml concentration. Alteration of both glucose transport and its metabolism could be involved in the action of CB. To further characterize the mode of action of CB on glucose utilization by the pancreatic gland, we took advantage of the properties of the glucose analogue, 2-deoxyglucose. This metabolite is transported by the same carrier system as glucose, is phosphorylated by hexokinase but is not further metabolized (Kletzien and Perdue, 1973; Smith and Gorski, 1968).

Table V shows that the incorporation of radioactivity from tracer deoxyglucose into the pancreatic tissue was strongly inhibited by CB; the threshold effective concentration was lower than 1 pg/ml. The presence of 10 mM glucose in the incubation medium strongly decreased the uptake
of deoxyglucose by the pancreatic tissue. The inhibitory effect of CB, though less evident, was still clearly observed. The effect of CB in the secreting gland was the same as in the nonstimulated tissue. The effect of CB on deoxyglucose transport was rapid, as shown in Fig. 11. In the presence of 1 mM deoxy-D-glucose, the magnitude of the inhibition varied between 35 and 50% in the nonstimulated gland after 45 min of incubation and even exceeded these values in 0.1 mM carbachol-stimulated tissue (up to 65%). The statistical significance of this observation was not further investigated. To securely establish that the action of CB on sugar transport took place at the level of the cellular membrane, it was necessary to exclude an interaction of CB with the hexokinase which phosphorylates the sugar. This possibility was ruled out by the observation that the ratio of free deoxyglucose to deoxyglucose 6 phosphate radioactivity was not different in the CB-treated gland (0.38) and the control (0.31) after 45 min of incubation.

Influence of CB on Calcium Exchange in Exocrine Pancreas

**Calcium Uptake:** Fig. 12 illustrates the 45-min initial phase of the 45Ca loading. This loading period achieved an incorporation of 45Ca in the pancreatic tissue equivalent to 80-85% of the level realized after a 2-hr loading period.

**Calcium Efflux:** Calcium efflux is the result of very complex exchange systems. Since the efflux was expressed by the residual activity of the tissue in percent of the initial 45Ca content of the gland, the experiments were designed to define this initial content as well as possible. To avoid contamination by the extracellular medium, two washes were performed in calcium-free medium supplemented with a calcium chelator. The 45Ca load was accomplished in a low calcium concentration medium to minimize alteration of the easily exchanging intracellular pool(s) and to equilibrate the gland with an extracellular medium which allows a full response of the tissue to the secretagogues (Matthews and Petersen, 1972; Kanno, 1972). The uptake of released 45Ca by the tissue did not play a significant role in the recorded curves since the decrease of 45Ca content in the pancreatic fragments was not accelerated by the chelator (EGTA 0.5 mM) in calcium-free medium.

The fraction of calcium activity lost by the tissue gradually decreased so that the activity retained in the tissue tended to reach a plateau after 50 min of incubation: no more than a 10-15% loss of initial radioactivity occurred in the last 80 min of incubation.
TABLE I

Influence of CB on the ATP Level of the Pancreatic Gland In Vitro.

| Cytchalasin B | 0 μg/ml | 1 μg/ml | 10 μg/ml |
|--------------|---------|---------|----------|
| Control      | 100     | 104.0 (12) | 118.9 (8) |
|              |         | 95.4 - 113.2 | 108.3 - 130.4 |
| Carbachol    | 74.0 (6) | 71.5* (8) | 72.9* (8) |
| 0.1 mM       | 62.3 - 88.0 | 62.5 - 81.8 | 64.3 - 82.5 |

Results are expressed in percent of control. Mean minus SEM and mean plus SEM have been calculated on the logarithms of the data (see Materials and Methods). Control = ATP concentration in the pancreatic tissue (0.21 x 10^-e M). Statistical significance + P < 0.05.

DISCUSSION

The presence of microfilaments in the acinar cell of the exocrine pancreas was reported on briefly by Geuze and Poort (1973). The present investigation clearly shows that an important and heterogeneous network of filaments exists in the apical region of this pancreatic cell. It is composed of several populations of microfilaments which can be recognized according to their location, size, organization and sensitivity to CB.

Filaments of the microvilli and part of the filamentous component in the ectoplasmic band lining the apical membrane are 50 Å in diameter. They appear similar to the thin, or actin, filaments reported on by Ishikawa et al. (1969); they are sensitive to CB as in many other cell types (Scheuder, 1972; Spooner and Wessells, 1970; Wessells et al., 1971; Aversperg, 1972; Manasek et al., 1972; Orci et al., 1972). The filaments 100 Å in diameter form a more heterogeneous group; in the ectoplasmic band they are organized into bundles whereas the thin filaments have no preferential orientation. They may well be similar to the intermediate filaments described by Ishikawa et al. (1968). They differ from the filamentous material of the apical junctions of the cell by their sensitivity to CB, though some of them maintain their usual appearance when surrounding the electron-lucent core induced by CB.

FIGURE 8 Rat exocrine pancreas incubated for 2 h in the presence of carbachol (0.1 mM). The acinar lumen (Lu) is filled with dense secretory material. Microvilli (mv) are numerous; the ectoplasmic band is not different from control (Fig. 3): the microfilamentous network displays its thin (50–60 Å) and thicker (100 Å) components. The former (arrowheads) are scattered without any preferential orientation; the latter (arrows) are most frequently packed into bundles of parallel components grossly parallel to the apical membrane. Z = zymogen granule. × 25,100.

FIGURES 9 and 10 Rat exocrine pancreas incubated for 2 h in the presence of carbachol (0.1 mM) and CB (10 μg/ml).

FIGURE 9 Enlarged acinar lumina (Lu) are empty or contain a sparse material; microvilli (mv) are very rare and considerably reduced in size (see also Fig. 10). The thin filaments have completely disappeared; instead, an electron-lucent material (fm) is located just beneath the apical cell membrane. Many zymogen granules (Z) are very close to the membrane. × 14,000.

FIGURE 10 Nearby the electron-lucent cores made up of granular or of very small-sized filaments (fm), thick filamentous material and microtubules (arrows) can be seen having the same characteristics as in the carbachol-stimulated tissue: their association in bundles of parallel filaments is maintained. Lu = acinar lumen, Z = zymogen granules. × 40,000.
The microfilamentous equipment of the exocrine acinar cell is thus as complex as in any other cell type in which it has been thoroughly investigated, such as the macrophage (Reaven and Axline, 1973), embryonic muscle cell (Ishikawa et al., 1968), or frog bladder (Carasso et al., 1973).

The role of microfilaments in the secretion process has been studied in several tissues (Lacy et al., 1968; Neve et al., 1970; Williams and Wolff; 1971; Schofield, 1971; Orci et al., 1972; Douglas and Sorimachi, 1972; Butcher and Goldman, 1972; Leclercq-Meyer et al., 1974). CB has been a very useful tool in those investigations. However, recently several investigators (Bluemink, 1971; Holtzer et al., 1972; Krishan, 1972) have directed attention to the cell membrane as being a possible
TABLE V

Effect of CB on 2-Deoxy-D-[1-3H]Glucose Uptake in the Pancreatic Gland In Vitro. Influence of Carbachol

| Cytochalasin B | 0 µg/ml | 1 µg/ml | 5 µg/ml | 10 µg/ml |
|----------------|--------|--------|--------|--------|
| D-Glucose (0 mM) | 100 | 48.3 | 44.8 | 28.9 |
| D-Glucose (10 mM) | 37.7 | 29.1 | 30.3 | 25.9 |
| (100) (77.2) (80.3) (68.7) |
| D-Glucose (0 mM) + | 88.5 | 46.5 | 36.4 | 30.2 |
| Carbachol (0.1 mM) | (100) | (52.6) | (41.1) | (34.1) |
| D-Glucose (10 mM) + | 42.2 | 28.1 | 26.0 | 30.6 |
| Carbachol (0.1 mM) | (100) | (68.7) | (60.8) | (72.4) |

Pancreatic pieces were incubated for 2 h at 37°C with 0.12 mM 2-deoxy-D-[1-3H]glucose (0.6 µCi/ml). Results are the means of two experiments. They are expressed in percent of control: control = 383,000 dpm/100 mg. In parentheses are the results expressed in percent of the incorporation under the treatment mentioned on the corresponding line (no CB).

primary site of action of CB. The most often cited evidence is the alteration of transport of various metabolites, especially glucose and derivatives (Estensen and Plageman, 1972; Kletzien et al., 1972; Taverna and Langdon, 1974). The elegant investigation of Kletzien and Perdue (1973) has shown that in the chick embryo fibroblast the CB effect on monosaccharide transport is indeed a membrane-specific effect.

It is thus a prerequisite that the mode of action...
FIGURE 13. \(^{45}\)Ca content of rat pancreas during washout. In the first series (13 A), the fragments after loading with \(^{45}\)Ca (5 µCi/ml) and washings (see Materials and Methods) were transferred to vials containing standard buffer with the concentration of calcium ranging from 0 to 2.5 mM (0; 0.1; 0.5; 2.5 mM). Addition of CB (10 µg/ml) is indicated by the arrows. In the second series (13 B), the fragments were treated as in series A but carbachol (0.1 mM) was added to controls after 50 min and carbachol 0.1 mM + CB (10 µg/ml) (arrows) in the "treated" glands. Vials contained standard buffer with the concentration of calcium ranging from 0 to 2.5 mM as in series A. The \(^{45}\)Ca activity remaining in the tissue at a given time was calculated and expressed as a percentage of the total content at the onset of washout. Each curve is representative of one experiment; (●) control, (×) CB-treated gland.

In the exocrine pancreas, the comparative analysis of the ultrastructure, exportable enzyme release and metabolic events suggests two different sites of action of CB on the acinar cell.

of CB be analyzed carefully before any conclusion is drawn concerning the involvement of the microfilamentous system in the cellular process under investigation.
The effect of CB on pancreatic exocrine secretion is bimodal: stimulation of basal secretion by low concentrations, and inhibition of stimulated secretion by high concentrations.

A low concentration of CB (2 x 10^-7 M to 4 x 10^-6 M) increases the extrusion of the exportable enzymes to a limited extent but does not alter the ultrastructural aspect of the microfilamentous system of the acinar cell. It also inhibits the uptake of the monosaccharide by the exocrine pancreas as in many other cell types—fibroblasts (Kletzien et al., 1972); hepatoma cells (Estensen and Plageman, 1972); erythrocytes (Bloch, 1973; Taverna and Langdon, 1974). The difference between the CB sensitivity of the transport system and that of the microfilaments was the same as that already reported by Kletzien and Perdue (1973) in erythrocytes and by Axline and Reaven (1974) in macrophages.

The inhibition of monosaccharide transport strongly suggests the plasma membrane as a site of action of CB. This assertion is emphasized by the absence of alteration of hexokinase activity by CB, in agreement with the reports of Mizel and Wilson (1972) on Hela cells, Zigmond and Hirsch (1972a) on macrophages, and Gorski and Raker (1973) on the uterus.

A functional alteration of the ectoplasmic microfilaments by low concentrations of CB cannot, however, be ruled out. If any, the alteration should be a modification of their tensile state, since no alteration of their orientation was ever displayed in the ultrastructural examination. Besides its membrane action, CB also interacts with the microfilamentous system of the exocrine acinar cell: at high concentration of CB, the thin microfilaments disappeared and electron-lucent cores were formed. Concomitantly, the pattern of the secretory response was completely altered. The correlation between the inhibition of the stimulated secretion and the alteration of the filaments by CB suggests the implication of the thin filamentous network in the secretory function of the exocrine pancreas.

Deprivation of available energy or change in the cellular calcium distribution could also alter the aspect of the contractile filaments and inhibit the secretory cycle. Should such changes result from exposure to CB, they could be responsible for the secretory and ultrastructural effect of CB. The inhibition of glucose utilization by the pancreatic gland, an effect mostly due to alteration of the monosaccharide transport by CB as in adipocytes (Loten and Jeanrenaud, 1974) or in phagocytizing macrophages (Zigmond and Hirsch, 1972a), could suggest a shortage of energy fuel in the gland exposed to CB.

An inhibition of ATP synthesis by CB in mitochondria has been reported by Lin et al. (1973). However, no alteration of the tissue ATP level resulted from exposure of fibroblasts to CB (Warner and Perdue, 1972). As in the fibroblast, CB has no effect on the energy balance in the pancreatic gland at rest or stimulated by secretagogue. Since the exportable enzyme release was concomitantly altered by CB, deprivation of energy cannot be an eventual common factor in the filamentous and secretory response to CB.

The importance of calcium in the secretory process is well-known (Douglas, 1968; Rubin, 1970). Stimulation of ecbolic secretion of the pancreas is accompanied by an increased efflux of calcium, which implies a redistribution of calcium in the exocrine acinar cell (Case and Clausen, 1973). It is obvious that alteration of calcium distribution could also alter the contractile state of the microfilaments. The action of CB on calcium exchange in the pancreatic gland has been examined in tissue equilibrated with a large range of extracellular calcium concentrations (0.25 mM) in order to sensitize, if possible, the distribution of calcium among rapidly exchanging pools to the action of an external agent. CB did not alter the efflux of calcium in exocrine tissue in the resting or stimulated secretory state whereas it inhibited the secretory response to the secretagogues. Notwithstanding the complexity of the regulation of calcium dynamics in cells, those data do not support the hypothesis of alteration of calcium distribution as a common factor in the genesis of the ultrastructural and secretory action of CB.

The implication of the filament network in the secretion of the exocrine pancreas is thus the most likely explanation of our results. As emphasized by Perdue (1973), plasma membrane stabilization might be the microfilaments' most important function. The close apposition of the thin filaments and the plasma membrane (McNutt et al., 1971; Buckley and Porter, 1967; Schroeder, 1972; Spooner et al., 1971; Perdue, 1973) and their

1 Alteration of the metabolic disposal of glucose, though suggested by the brisk fall in the conversion of glucose into TCA-insoluble protein by increasing the CB concentration from 2 x 10^-4 M to 2 x 10^-3 M, could only be of minor importance as compared to the monosaccharide transport inhibition.
contractile nature which is supported by compelling evidence (Hatano et al., 1967; Ishikawa et al., 1969; Pollard et al., 1970; Nachmias, 1972; Perdue, 1973), could explain the alteration in membrane functions by change in the tension of the filament network.

The direct interaction of the cytochalasins with actin, and the ATPase activity of the actin-heavy meromyosin complex (Spudich and Lin, 1972) or myosin (Puszkin et al., 1973), though not recognized by all (Forer et al., 1972), suggest an alteration of the tensile state of the filaments by CB.

In the exocrine pancreas, as in other tissues, the action of CB on secretion could be explained by its interaction with the filamentous system exclusively. Its secretagogue action would result from a facilitated approach of the secretion granule to the cell membrane by permeation of the filamentous network. Its antisercretagogue action would be caused by the functional disruption of the thin filaments which prevents them from playing their role in the orientation (passive role) or the driving (active role) of the secretory granules towards their membrane site of extrusion (Orci et al., 1972). The broadened acinar lumen, the unusual regularity of its contours, and the disappearance of the microvilli as well as the accumulation of zymogen granules close by the apical membrane suggest an alteration of membrane properties. Since this membrane effect is correlated with alteration of the filaments, it is probably a mere consequence of the action of CB on the cell web.

However, the exocrine acinar cell, like other mammalian cells (Lin et al., 1974), possesses at least a second site of action of CB. The existing evidence limits its role to the inhibition of monosaccharide and eventually nucleotide transport (Kletzien et al., 1972; Plageman and Estensen, 1972). In the exocrine pancreas, the alteration of glucose handling by the gland plays no detectable role in the secretory action of CB.

The importance of the extrofilamentous action of CB is made particularly clear in the endocrine pancreas. The mechanism of the potentiation of submaximally stimulated insulin release by CB (Orci et al., 1972; Malaisse et al., 1972; Lacy et al., 1973), when the filaments are functionally paralyzed, is still obscure. However, the role of glucose as a secretagogue in insulin release (Grodsky et al., 1963) suggests that the recently reported (McDaniel et al., 1974) alteration of its handling in endocrine pancreatic cells by CB could be an important part of the action of CB. Two recent reports also have emphasized the importance of the extrofilamentous action of CB in this tissue: an inhibitory action of CB on insulin release takes place when the concentration of CB is increased far above that needed to achieve complete functional disruption of the cell net (Schauder and Frerichs, 1974); the increase of insulin release under CB exposure occurs in saurian endocrine pancreas when no filamentous system could have been involved (Rothen, 1973). Only further investigations will decide the role of the extrofilamentous action of CB in the exocrine acinar cell.

In conclusion, strong evidence has been obtained in support of the involvement of the thin filament network in the secretory function of the exocrine acinar cell. The action of CB has been shown to be located at two sites that have different sensitivity. The more sensitive site, at the cell membrane level, is involved in the inhibitory action of the drug on monosaccharide transport. The less sensitive site, at the filamentous level, is involved in the action of the drug on enzymatic secretion.

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