High Uptake of myo-Inositol by Rat Pancreatic Tissue in Vitro Stimulates Secretion

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A recent study by Hokin-Neaverson, M., Sadeghian, K., Majumder, A. L., and Eisenberg, F. (1975) Biochem. Biophys. Res. Commun. 67, 1537-1544, demonstrates that free myo-inositol in the pancreas is significantly increased during intense cholinergic stimulation of secretion. Incubation of rat pancreatic tissue in medium with 100 mM myo-inositol increases 10-fold the endogenous content of free myo-inositol and elicits a prompt and sustained 50% increase in the rate of release of amylase activity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis reveals that the electrophoretic pattern of the protein mixture released in the presence of 100 mM myo-inositol is the same as that of the secretory output released in the presence of 10 μM carbachol. Microscopic examination of tissue pieces indicates that there is no significant decrease in the zymogen granule content of the pancreatic acinar cells during incubation in medium with 100 mM myo-inositol. Jamieson, J. D., and Palade, G. E. (1967) J. Cell Biol. 34, 597-615, have shown that pulse-labeled secretory proteins in guinea pig pancreas first appear in zymogen granules 1 hour postpulse, becoming maximally accumulated in these storage sites by 2 hours postpulse. myo-Inositol (100 mM) stimulates release of pulse-labeled secretory proteins only if incubation in medium with 100 mM myo-inositol is initiated any time during the first 80 min postpulse. The findings thus indicate that a high uptake of myo-inositol by rat pancreatic tissue in vitro selectively stimulates the release of just those secretory proteins being packaged in newly forming zymogen granules.

In 1953, Hokin and Hokin first reported that there is a marked increase in phosphatidylinositol synthesis associated with cholinergic stimulation of amylase secretion by the pancreas (1). Subsequent studies have provided a detailed description of phosphatidylinositol metabolism in relation to pancreatic secretion. Secretagogues elicit not just a marked increase in phosphatidylinositol synthesis, but also a net decrease in the phosphatidylinositol content of the pancreas (2). Increased phosphatidylinositol synthesis occurs promptly (as soon as 2 min after stimulation) and almost exclusively in the rough ER and Golgi complex (9). Calcium ion deprivation, which markedly reduces amylase secretion, only partially inhibits increased phosphatidylinositol synthesis (4). The concentrations of secretagogues required to stimulate phosphatidylinositol synthesis and degradation are higher than those sufficient to stimulate amylase secretion (9).

The generally accepted interpretation of these findings is that increased phosphatidylinositol turnover and stimulated enzyme secretion do not have a causal relationship and therefore are two phenomena which merely occur in parallel in response to different types of secretagogues. Lapetina and Michell recently suggested, however, that a physiologically significant consequence of enhanced phosphatidylinositol turnover may be the production and accumulation of intracellular cyclic inositol 1,2-monophosphate (6). In response to this proposal, Hokin-Neaverson et al. have found that free inositol is the sole water-soluble product of acetylcholine-stimulated phosphatidylinositol degradation; free inositol increases in amounts which are equal to the net loss of phosphatidylinositol content of the tissue (7). There is thus direct evidence that intense physiological stimulation of secretion by the pancreas is accompanied by a significant increase in the intracellular level of free inositol.

Our experimental approach to the hypothesis that the significance increase in free inositol which occurs during intense physiological stimulation plays a role in intense stimulated secretion has been to determine the rate of uptake of [3H]myo-inositol by rat pancreatic tissue in vitro as a function of the concentration of myo-inositol in the medium and the rate of release of pulse-labeled secretory proteins as a function of the amount of myo-inositol taken up. The results of our studies demonstrate that high extracellular concentrations of myo-inositol, in the absence of physiological secretagogues, (a) increase free inositol in rat pancreatic tissue to levels which are attained only during exhaustive physiological stimulation and (b) stimulate secretion.

EXPERIMENTAL PROCEDURES

Tissue Preparation

Pancreatic tissue for each experiment was obtained from a single male albino rat, weighing 200 to 300 g, killed by a blow to the head after
an overnight fast (16 to 24 hours). The pancreas was quickly excised and immersed in oxygenated KHBS (8) at 0-2°. After mesenteric and fat were trimmed away, the pancreas was cut into pieces (~1 x 2 mm) with fine scissors and the tissue pieces washed four times with fresh KHBS.

**Labeling Procedures**

**Uptake and Incorporation of [3H]myo-Inositol into Phospholipid**—Tissue pieces (total wet weight 15 to 25 mg) were kept for 15 min in 2.5 ml of ice-cold oxygenated KHBS containing radio-labeled myo-inositol at a given concentration before initiating incubation at 37°. After a selected interval of incubation at 37°, the ice-cold KHBS containing the tissue pieces was quickly washed with 2.5 ml of fresh KHBS before being homogenized with a Teflon homogenizer in 5.0 ml of ice-cold 10% (w/v) trichloroacetic acid. The homogenate was centrifuged at 2500 x g for 10 min and the radioactivity of an aliquot of the supernatant determined as a measure of the free myo-inositol in the tissue pieces. The ice-cold trichloroacetic acid precipitable material was washed once with ice-cold 5% trichloroacetic acid and then suspended in 5.0 ml of ice-cold chloroform/methanol (2/1, v/v). After 2 to 3 hours at 4°, the suspension was blended on a Vortex mixer with 1.06 ml of 0.05 M KCl and centrifuged at 30,000 x g for 10 min. The upper phase was removed by aspiration and the lower phase washed twice with 2.5 ml of theoretical upper phase (chloroform/methanol/0.05 M KCl, 3/48/47 v/v/v), before being dried and counted for radioactivity as a measure of the amount of radiolabeled myo-inositol incorporated into phospholipid (9). The material at the boundary between the two phases was dissolved in 0.1 ml 0.5 N NaOH for determination of protein content according to the procedure of Lowry et al. (10).

**Pulse-labeling Secretory Proteins**—Our protocol was a modification of that employed by Jamieson and Palade (11) in their studies of the intracellular transport of secretory proteins in the pancreatic exocrine cell. All the tissue pieces for each experiment were kept in ice-cold KHBS before being transferred to 2.0 ml of fresh KHBS for incubation at 37° for 5 min. Thirty microcuries of L-[3H]leucine (0.5 to 0.8 μCi) were added and incubation continued at 37° for another 2 min, after which the tissue pieces were washed three times with 5.0 ml of fresh KHBS containing 1.0 mM L-leucine (postpulse medium) and finally divided among a number of 25-ml Erlenmeyer flasks containing 1.05 ml of 0.05 M KCl and centrifuged at 30,000 x g for 10 min. The cleared supernatant was discarded and the pellets dissolved in 0.2 ml of 0.5 N NaOH and 1.0 ml of NCS tissue solubilizer for determination of the amount of pulse-labeled secretory proteins still retained by the tissue pieces.

**Amylase Assay**

Amylase was assayed on samples of homogenates and incubation media in 0.2% Triton X-100 containing 0.02 M NaCl and 0.02 M sodium phosphate (pH 6.9) according to the procedure of Bernfeld (12). All measurements were made under conditions giving a linear relationship between measured activity and protein (enzyme) concentration.

**Polyacrylamide Gel Electrophoresis Procedure**

To collect the mixture of secretory proteins discharged under different incubation conditions, tissue pieces were incubated in 2.0 ml of KHBS containing unlabeled myo-inositol or carbamylcholine or both, for 3 hours at 37°. In those experiments in which the electrophoretic pattern of the discharged secretory proteins was to be determined, incubation was conducted in 25-ml Erlenmeyer flasks treated with Siliclad, in order to minimize adsorption of secretory proteins to glass. Every hour during the 3-hour incubation the medium was entirely replaced with fresh medium; the medium collected was chilled and supplemented with benzamidine to a final concentration of 1 mM (to prevent zymogen activation (13)). The media collected from the three 1-hour intervals were mixed and cleared of cellular debris by centrifugation at 100,000 x g for 1 hour at 4°. The cleared supernatant was dialyzed against 50 mM ammonium bicarbonate and 1 mM benzamidine (pH 7.6) for 4 to 5 hours at 4° and lyophilized. The resulting white powder was dissolved in 1% SDS, 1% β-mercaptoethanol, 10% glycerol, 0.01% bromphenol blue (tracking dye), and 60 mM Tris-HCl (pH 6.7), boiled for 2 min and subjected to gel electrophoresis in 5 mm inner diameter glass tubes, using Maizel's (14) discontinuous "sodium dodecyl sulfate-disc" system and 13% acrylamide in the running gel. Gels were run at room temperature at a constant 80 V and finally stained at room temperature in 0.2% Coomassie brilliant blue R, dissolved in 25% isopropyl alcohol and 10% acetic acid. Background destaining was accomplished in 10% isopropl alcohol and 10% acetic acid.

**Materials**

All chemicals were of reagent grade and were purchased from the following sources: [3H]myo-inositol (1 to 5 Ci/mmol) and L-[3H]leucine (30 to 50 Ci/mmol) from New England Nuclear; carbamylcholine chloride, myo-inositol, atropine sulfate, bovine serum albumin, ammonium persulfate, sodium dodecyl sulfate, bromphenol blue, and Coomassie brilliant blue R from Sigma; benzamidine hydrochloride hydrolyte from Matheson, Coleman and Bell, Siliclad from Clay Adams, protein molecular weight markers from Schwarz/Mann; acrylamide, N,N'-methylenebisacrylamide and N,N,N',N'-tetramethylene-thiénylidenamine from Eastman Kodak; and NCS tissue solubilizer from Amersham/Searle.

**RESULTS**

**Uptake of [3H]myo-Inositol**—To examine the effect of extracellular myo-inositol concentration on [3H]myo-inositol uptake, tissue pieces were kept for 15 min in ice-cold KHBS with varying concentrations of myo-inositol before initiating incubation at 37°. This procedure was followed in order to permit equilibration of the myo-inositol concentration between the extracellular spaces of the tissue pieces and medium. Fig. 1 shows that the amount of free [3H]myo-inositol in the tissue pieces remains constant after the first 30 to 60 min incubation at 37°, and is directly proportional to the myo-inositol concentration in the KHBS for concentrations ranging from 1 μM to 100 mM.

The trichloroacetic acid-soluble [3H]myo-inositol recovered from the tissue pieces represents both radiolabeled myo-inositol taken up by the pancreatic acinar cells and that residing in the extracellular spaces of the tissue pieces. To obtain an estimate of the amount of free [3H]myo-inositol in the extracellular spaces, tissue pieces were labeled for 1 hour with [3H]myo-inositol in KHBS containing either 1 μM or 100 mM myo-inositol, washed, and then incubated for 3 hours in KHBS containing unlabeled myo-inositol at a concentration of both 1 μM and 100 mM. Fig. 2 shows that the free [3H]myo-inositol found in tissue pieces after incubation in KHBS with 1 μM or 100 mM myo-inositol decreases by 50 to 75% during the first 30 min of the chase incubation; this percentage represents the fraction of free radiolabeled myo-inositol trapped in the extracellular spaces of the tissue pieces. The free [3H]myo-inositol remaining after chase incubation appears to have been taken up by the cells and not bound to cell surfaces or elements in the extracellular spaces since the kinetics of loss of free [3H]myo-inositol from tissue pieces labeled in KHBS with 1 μM Tris-HCl containing 10 mM of carbamylcholine or both, are not affected when the concentration of unlabeled myo-inositol during chase incubation is increased 100,000-fold to 100 mM. Taking these results into account, the data shown in Fig. 1 indicate that, on the average, after 1 hour incubation 0.1 to 0.2 μmol of myo-inositol is taken up per g of tissue pieces.
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Since there is 0.66 pmol of myo-inositol per g of tissue, wet tails of the procedures are given in the text. The results are the average obtained from a single pancreas were kept for 15 min in 2.5 ml of ice-cold tissue for every pmol of myo-inositol per ml in the medium.

![Graph showing uptake of [3H]myo-inositol](image)

Fig. 1. Uptake of [3H]myo-inositol by tissue pieces incubated in KHBS with varying myo-inositol concentrations. Tissue pieces obtained from a single pancreas were kept for 15 min in 2.5 ml of ice-cold KHBS containing varying concentrations of [3H]myo-inositol (1 µM, ○; 10 µM, □; 100 µM, ■; 1 mM, △; 10 mM, ▲; and 100 mM, Δ) before initiating incubation at 37°C. Details of the procedures are given in the text. The results are the average of three experiments.

The kinetics of release of pulse-labeled secretory proteins during 4 hours postpulse stimulation with 10 µM carbachololine are shown in Fig. 3. Data collected from six separate experiments show that 10 µM carbachololine elicits through out the postpulse period a 2.6 ± 0.5-fold increase in the rate of release of incorporated radiolabeled leucine. Fig. 4 shows the percent release of pulse labeled secretory proteins after 4 hours postpulse stimulation with varying concentrations of carbachololine. This dose-response curve compares very favorably with that determined by Scheele and Palade (16) using their pancreatic lobule system, and shows that the concentration of carbachololine which elicits maximal stimulation of release of pulse-labeled secretory proteins is 10 µM.

Fig. 5 shows the percent release of label after 4 hours incubation in postpulse medium containing varying concentrations of myo-inositol. The percent of label released in postpulse medium with 1 µM to 40 mM myo-inositol is the same as that released under control conditions. At a concentration of 60 mM myo-inositol there is a 1.9-fold increase in the label released and at concentrations of 80 and 100 mM there is a 2.25-fold increase. The percent of label released in the presence of both 100 mM myo-inositol and 10 µM carbachololine is approximately a third greater than that released by either agent alone. We thus find that the extracellular myo-inositol concentrations which result in a minimum 10-fold increase in the endogenous intracellular level of free inositol are those which stimulate secretion.

The stimulation of pancreatic secretion elicited by high extracellular concentrations of myo-inositol appears similar in several respects to the stimulation elicited by carbachololine. First, there is no difference in the composition of the secretory output released in the presence of 100 mM myo-inositol from that released under control or secretagogue-stimulated conditions (17). Fig. 6 shows that the electrophoretic pattern of the protein mixture released in the presence of 100 mM myo-inositol is the same as those for the mixtures released in the presence of 10 µM carbachololine or in the absence of any stimulatory agent. Furthermore, the relative proportions at which the major protein components are released in the presence of 100 mM myo-inositol appear similar to those released in the presence of 10 µM carbachololine.

Secondly, 100 mM myo-inositol and 10 µM carbachololine each evoke similar kinetics of stimulated discharge of amylase activity, the only difference being in the magnitude of the stimulation (Fig. 7). The rate of amylase release in the presence of both 100 mM myo-inositol and 10 µM carbachololine is the same as that which occurs in the presence of 10 µM carbachololine alone.

Thirdly, the stimulatory effect of 100 mM myo-inositol exhibits the same Ca²⁺ dependency as that exhibited by 10 µM carbachololine. The results in Table I show that incubation of tissue pieces, pulse-labeled with L-[3H]leucine, in Ca²⁺-free KHBS with EDTA during the first 30 min of the postpulse period, completely inhibits stimulated secretion by both agents.

There are, however, certain characteristics of myo-inositol stimulation which distinguish it from carbachololine stimulation. First, we find that 100 mM myo-inositol-stimulated release of pulse-labeled secretory proteins does not begin until 2 hours postpulse, in both the presence and absence of carbachololine (Fig. 8). In both unstimulated and maximally physiologically stimulated tissue, the initial traces of a packet of pulse-labeled secretory proteins do not appear in zymogen granules until 30 min postpulse; an hour is required for the majority of the packet to accumulate in these storage sites (18).

The 2-hour delay in the onset of 100 mM myo-inositol-stimulated release suggests that such a high extracellular...
myo-inositol concentration alters the kinetics of the intracellular transport of secretory proteins through the rough ER and Golgi complex, extending their normal transit time through these compartments.

Secondly, we find that 100 mM myo-inositol cannot stimulate the release of pulse-labeled secretory proteins after they have accumulated in mature zymogen granules. Jamieson and Palade have shown that secretory proteins in pancreatic exocrine cells are transported from the site of their synthesis in the rough ER to condensing vacuoles of the Golgi complex, extending their normal transit time through these compartments.

Moreover, we found that 100 mM myo-inositol cannot stimulate the release of pulse-labeled secretory proteins after they have accumulated in mature zymogen granules. Jamieson and Palade have shown that secretory proteins in pancreatic exocrine cells are transported from the site of their synthesis in the rough ER to condensing vacuoles of the Golgi complex, where they are intensively concentrated, the condensing vacuoles being converted to zymogen granules (11, 18, 19). They have shown by electron microscopy autoradiography that the time course of the passage of pulse-labeled secretory proteins through these intracellular compartments is such that during the first 10 min postpulse, most of the pulse-labeled secretory proteins are confined to regions containing rough ER; by 20 min to the periphery of the Golgi complex; by 40 min to the condensing vacuole, and finally from 60 to 120 min postpulse, they accumulate in the zymogen granules. Accordingly, we measured the stimulatory effects of 100 mM myo-inositol and 10 μM carbamylcholine when applied at increasing times after initiation of the postpulse incubation. Data collected from four separate experiments showed that if transfer to media with 100 mM myo-inositol occurs at any time from 40 to 80 minutes after the pulse-label period, the amount of label released above controls is 90 to 110% that released if the transfer is made immediately (5 min) after the pulse-label period (Table II). There is, however, a dramatic decrease in the capacity of 100 mM myo-inositol to stimulate the release of a packet of pulse-labeled secretory proteins during the period 80 to 100 min after their synthesis. In the case of 10 mM carbamylcholine, the decrease in its stimulatory effect is less severe, as the amount of label released above controls is 70-80% that released by tissue pieces transferred to medium with 10 μM carbamylcholine immediately after the pulse-label period.

Light microscopic examination supports this evidence that 100 mM myo-inositolstimulates the release of secretory proteins from a pool other than that of mature zymogen granules. Figs. 9 and 10 show that in tissue pieces which have been incubated for 4 hours in medium with 100 mM myo-inositol, both tissue architecture and cellular ultrastructure remain well preserved; there is, however, no significant decrease (certainly not more than 25%) in the zymogen granule content of the cells. We find, as expected, that there is a marked depletion of zymogen granules in most cells in tissue pieces which have been incubated for 4 hours in medium with 10 μM carbamylcholine, either in the presence or absence of 100 mM myo-inositol (Fig. 11 a and b).

Thirdly, stimulation of secretion by 100 mM myo-inositol is associated with an inhibition of [3H]myo-inositol incorporation into phosphatidylinositol. The effect of extracellular myo-inositol concentration on the rate of incorporation of [3H]myo-inositol into phospholipid was examined during the same experiments conducted to study [3H]myo-inositol uptake. Fig. 12 shows that incorporation proceeds linearly during 2 hours incubation at 37° in KHBS with myo-inositol concentrations ranging from 1 μM to 10 mM; the rate of incorporation decreases as the myo-inositol concentration is increased. There is no significant incorporation in medium with 100 mM myo-inositol.

It is difficult to assess from these results the absolute rates of incorporation in the tissue pieces since we do not know the specific activities (μCi/μmol) of the intracellular myo-inositol...
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FIG. 5. Percent of pulse-labeled proteins released in KHBS with varying myo-inositol concentrations. The amounts of pulse-labeled proteins released in the media were measured 4 hours postpulse. The symbol (■) indicates the percent of label released in the presence of 100 mM myo-inositol and 10 μM carbamylcholine. The short and long arrows indicate the percent of label released under control conditions and in the presence of 10 μM carbamylcholine, respectively.

We can, however, estimate relative rates of incorporation. The pancreas maintains in vivo a rather considerable permeability barrier to intracellular free inositol (15); it is therefore likely that the tissue pieces maintain their endogenous content of free inositol and any increases resulting from uptake of myo-inositol from the extracellular medium. We adjusted the specific activities of [3H]myo-inositol in the media so that they were inversely proportional to the myo-inositol concentration (i.e., the specific activity in medium with 1 mM myo-inositol was adjusted to be 1000 times greater than that in medium with 1 mM myo-inositol). We followed this procedure so that the amount of microcuries of radiolabeled myo-inositol taken up per g of tissue would be independent of the myo-inositol concentration in the medium, since myo-inositol uptake is directly proportional to myo-inositol concentration. We would therefore expect that if the amount of radiolabeled myo-inositol taken up is small relative to the endogenous content, the rate of incorporation of [3H]myo-inositol into phospholipid should not vary significantly with the concentration of myo-inositol in the medium. The data shown in Fig. 12 indicate, however, that there is a 70% decrease in the rate of incorporation in tissue pieces incubated in medium with 1 mM myo-inositol compared to that by tissue pieces incubated in 1 μM myo-inositol; in 1 mM myo-inositol the intracellular free inositol increases by only approximately 10%. We thus are led to conclude that the absence of any significant incorporation of [3H]myo-inositol into phospholipid by tissue pieces incubated in medium with 100 mM myo-inositol is not a consequence of the relatively low specific activity of the intracellular myo-inositol pools in these tissue pieces, but rather a result of a marked inhibition of phosphatidylinositol synthesis.

Finally, we find that if atropine, a specific pharmacological antagonist of acetylcholine (2), is added at a concentration of 10 μM to postpulse medium, it has no effect on 100 mM myo-inositol-stimulated secretion. As expected, atropine completely inhibits the stimulatory effect of carbamylcholine.

DISCUSSION

We believe that the experimental findings cited here and those which have been reported by Hokin and Hokin and their colleagues (1-5) not only tentatively account for myo-inositol-stimulated secretion, but, more importantly, implicate a central role of enhanced phosphatidylinositol turnover in secretagogue-stimulated secretion. If we compare the dose-response curves determined by ourselves and Scheele and Palade (16) for the stimulated discharge of pulse-labeled secretory proteins by carbamylcholine, with that determined by Hokin (5) for the stimulated discharge of amylase, we find the data is consistent in demonstrating that the carbamylcholine concentrations which maximally stimulate the discharge of pulse-labeled secretory proteins are the same as those which stimulate phosphatidylinositol turnover; they are, however, 1 to 2 orders of magnitude greater than those eliciting maximal stimulation of amylase discharge. We suggest that these
represent 100% stimulation. The release of amylase during 3 hours incubation in KHBS (O—O) and KHBS with 10 μM carbamylcholine (●—●), 100 mM myo-inositol (□—□), and 10 μM carbamylcholine and 100 mM myo-inositol (■—■) was determined as described in the text. The results are given as per cent of the sum of enzymatic activity in tissue pieces and medium and represent the average of three experiments.

Fig. 8 (right). Kinetics of release of pulse-labeled proteins in KHBS with 100 mM myo-inositol. The per cent of label released during 4 hours postpulse incubation in KHBS (O—O), KHBS with 10 μM carbamylcholine (●—●), 100 mM myo-inositol (□—□), and 10 μM carbamylcholine and 100 mM myo-inositol (■—■) was determined as described in the text. The results are the average of three experiments.

TABLE I
Effect of Ca**+-deprivation on the stimulation of secretion by 10 μM carbamylcholine and 100 mM myo-inositol

| Incubation condition | Per cent of pulse-labeled proteins released by 4 hours postpulse |
|----------------------|---------------------------------------------------------------|
|                      | In Krebs-Henseleit bicarbonate saline | In Ca**+-free KHBS | In Ca**+-free KHBS when the tissue pieces are incubated in Ca**+-free KHBS with EDTA for the first 30 min postpulse |
| Control              | 12.4 | 10.0 | 6.7 |
| 10 μM carbamylcholine| 38.7 | 22.5 | 13.6 |
| 100 mM myo-inositol  | 36.3 | 9.0  | 8.0  |

TABLE II
Effect of initiating incubation in media with 10 μM carbamylcholine or 100 mM myo-inositol at increasing times postpulse

| Stimulant     | Per cent of stimulatory effect exhibited by stimulants when applied at indicated times postpulse (min) |
|---------------|--------------------------------------------------------------------------------------------------|
|               | 5      | 40     | 60     | 80     | 100    | 120    |
| 10 μM carbamylcholine | 100 | 107    | 110    | 104    | 75     | 74     |
| 100 mM myo-inositol   | 100  | 108    | 98     | 97     | 4      | 0      |

relationships are most simply understood by postulating that there occur within pancreatic acinar cells two pools of secretory proteins available for immediate discharge, and the regulatory system governing the stimulus-secretion coupling of one pool is activated by enhanced phosphatidylinositol turnover, while the regulatory system of the other pool is not.
Fig. 9 (top). Low power light micrograph of a section of a tissue piece incubated for 4 hours in KHBS with 100 mM myo-inositol. All of the cells have a zymogen granule content typical of that observed in tissue removed from a rat after an overnight fast. x 630.

Fig. 10 (bottom). Low magnification electron micrograph of the apical region of a pancreatic acinar cell from a tissue piece incubated for 4 hours in KHBS with 100 mM myo-inositol. Several principal ultrastructural features of the exocrine cell are indicated: rough ER (rer), piled Golgi cisternae (gc), condensing vacuole (cv), and zymogen granules (z). m, mitochondrion. x 20,000.

According to this proposal, physiological secretagogues, during maximal stimulation, increase secretion by tapping both pools of secretory proteins. myo-Inositol-stimulated secretion, however, draws upon the pool of newly forming zymogen granules only. This distinction in the mechanics of carbamylcholine- and myo-inositol-stimulated secretion accounts for both the similarities and the differences between the two stimulatory effects. Stimulation of amylase secretion by myo-inositol should be as prompt but not as great as that elicited by carbamylcholine since in this instance the secretory protein occurs within both pools from the onset of stimulation onward. When we measure the release of secretory proteins pulse-
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FIG. 11. a and b, low power light micrographs of two sections of a tissue piece incubated for 4 hours in KHBS with 100 mM myo-inositol and 10 μM carbamylcholine. Within the tissue pieces regions can be found in which (a) zymogen granule extrusion from the cells has been almost complete and (b) zymogen granule extrusion has been less extensive. Comparison of b and Fig. 9 (the tissue pieces shown in both this figure and Fig. 9 were obtained from the same pancreas), shows that even in these cells which still retain some zymogen granules after 4 hours incubation in medium with 100 mM myo-inositol and 10 μM carbamylcholine, there has nevertheless been a significant reduction in the zymogen granule content elicited by the additional presence of 10 μM carbamylcholine. × 630.

labeled in vitro, however, we are monitoring a packet of secretory proteins which must be transported from the rough ER and processed through the Golgi complex before entering the pool of newly forming zymogen granules. Not until this temporally defined packet has begun to enter this first pool, a journey which on the average requires 1 hour, can both carbamylcholine and myo-inositol stimulate its release. If, now, the packet is allowed to progressively accumulate in the second pool of mature zymogen granules, a process which spans the second postpulse hour, then only carbamylcholine can stimulate its release. These predictive features of the proposal are consistent with the results shown in Fig. 7 and Table II.

The proposal also accounts for the difference in the sensitivity of the two stimulatory effects to inhibition by atropine. Since 10 μM atropine has no effect on the 10-fold increase in the intracellular free inositol which occurs during incubation in medium with 100 mM myo-inositol, we would expect atropine
to have no effect on myo-inositol-stimulated secretion. Carbamylcholine, on the other hand, elicits an increase in intracellular free inositol only indirectly through its capacity to stimulate phosphatidylinositol degradation, and this response, as Hokin-Neaverson (2) has recently shown, is blocked by atropine.

We believe that there is sufficient evidence to suggest at this time, but not yet prove, that enhanced phosphatidylinositol turnover diverts the inclusion of newly forming zymogen granules from the pool of mature zymogen granules and instead stimulates their immediate discharge, this action being mediated by free inositol. We are planning to confirm by electron microscope autoradiography that incubation of pancreatic tissue in medium with 100 mM myoinositol (a) alters the kinetics of the intracellular transport of secretory proteins and (b) preferentially stimulates the discharge of newly forming zymogen granules. It is our belief that such evidence will more directly validate the view that phosphatidylinositol metabolism plays important roles in the secretory process, especially with respect to the role we have discussed here.

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