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INOSITOL TRISPHOSPHATE PRODUCTION AND AMYLASE SECRETION IN MOUSE PANCREATIC ACINI

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SUMMARY: Dispersed mouse pancreatic acini prelabelled with (^{3}H)-myoinositol generated (^{3}H)-inositol trisphosphate (^{3}H-IP$_3$), (^{3}H)-IP$_2$ and (^{3}H)-IP$_1$ in response to both cholinergic and cholecystokinin analogues. The generation of (^{3}H)-IP$_3$ was very rapid, reaching a maximal value within 5 seconds following hormone stimulation. Stimulation with 10^{-3}M carbachol increased (^{3}H)-IP$_3$ to a value which was 13 times that found in unstimulated acini. These results indicate that the mechanism of stimulus-secretion coupling in mouse pancreatic acini may proceed by a mechanism similar to many other systems, including rat pancreatic acini. This sequence includes hormone-stimulated phosphatidylinositol turnover and Ca^{2+} mobilization, i.e. secretagogue-stimulated generation of IP$_3$ which induces the subsequent release of intracellular Ca^{2+}. These observations differ from those recently reported by Hokin-Neaverson and Sadeghian (J. Biol. Chem. 259: 1346, 1984), in which no hormone stimulated IP$_3$ generation was detected in mouse pancreatic acini.

The agonist-stimulated turnover of PI was first demonstrated in the cells of the exocrine pancreas (1). Subsequently, the process of PI hydrolysis and the concommitant generation of inositol phosphates and diacylglycerols has been demonstrated in a wide variety of cell types (2,3). Rubin et al (4,5) recently reported that rat pancreatic acini produced IP$_3$, IP$_2$, and IP$_1$, in response to carbachol or caerulein. Additionally, Streb et al (6) observed that 1,4,5-IP$_3$ was capable of releasing Ca^{2+} from intracellular stores in permeabilized rat acinar cells. Similar findings have been reported in other cell types, including hepatocytes (7,8) and a microsomal fraction of insulinoma cells (9). Although poorly understood, it is generally believed that the hormone-induced increase in cytosolic Ca^{2+} level is closely coupled to the secretory response. In many systems, including pancreatic acinar cells, this conclusion has been supported by the observation that secretion can be caused by raising cytosolic Ca^{2+} levels with ionophores or by

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Abbreviations: PI, phosphatidylinositol; IP$_3$, inositol trisphosphate; IP$_2$, inositol bisphosphate; IP$_1$, inositol monophosphate; CCK, cholecystokinin, HEPES, N-2-Hydroxyethylpiperozine-N^1-2-ethanesulfonic acid.

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microinjection of Ca^{2+} (10,11). Thus, for most systems, the sequence of events appears to be (a) agonist-receptor binding, (b) PI hydrolysis with release of IP_3 (as well as other inositol phosphates and diacylglycerol), (c) Ca^{2+} release and an increase in the cytosolic Ca^{2+} level, and (d) secretion.

Recently, Hokin-Neaverson and Sadeghian (12) reported that they were unable to detect IP_3 or IP_2 in mouse pancreatic acinar cells following CCK stimulation. They suggested that mouse acinar cells might generate only IP_1 in response to agonist stimulation, and that the mechanism of stimulus-secretion coupling in mouse pancreatic acini may differ from that observed in the rat, as well as other systems which utilize the phosphatidylinositol hydrolysis pathway.

In the present communication we report studies which have re-investigated the mechanism of stimulus-secretion coupling in mouse pancreatic acini by examining inositol phosphate generation in response to secretagogues. These studies suggest that secretagogue-induced increases in cytosolic Ca^{2+} and secretion from mouse pancreatic acini, as in the rat pancreas and many other systems, involves IP_3 generation.

METHODS AND MATERIALS

Dispersed pancreatic acini were prepared from female CD-1 mice (12-20 gms) by collagenase digestion and gentle shearing (13). Pancreata from 7 to 12 mice were pooled for each experiment. Incubations were performed in HEPES-Ringer buffer (pH 7.4) composed of: 113 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM Na_2HPO_4, 10 mM Hepes, 15 mM glucose, 1.26 mM $CaCl_2$, 0.1 mg/ml soybean trypsin inhibitor, and 1 mg/ml bovine serum albumen. The buffer was saturated with O_2 and gassed during all incubations with 95% O_2 and 5% CO_2.

Acini were incubated for 1 hour with 100 uCi/ml (^3H)-myoinositol and 1 mM $MnCl_2$. They were then washed once and resuspended in buffer (without BSA but including 10 mM LiCl) at a concentration of approximately 35 ug DNA/ml. After a 10 minute preincubation, 1 ml aliquots of acini were placed into tubes containing agonists and the incubations were continued for the time indicated. Incubations were terminated by the addition of 2 ml cold 0.6N perchloric acid. The inositol phosphates were extracted, separated by ion exchange chromatography, and counted as described by Rubin (5). Lipid soluble radioactivity was extracted from the perchloric acid pellet with chloroform: methanol (2:1) and 2M KCl. Amylase secretion was measured by the method of Bernfeld (14) as described previously (13). DNA was measured by the method of LaBarca and Paigen (15).

Inositol phosphate generation is reported the percent of the initial dpm for the sample of interest. Amylase secretion is calculated as the net percent stimulation, which is the percent of the total amylase in a sample released in response to agonist in 10 min.

CD-1 mice were obtained from Charles River Breeding Labs (Boston, MA). Collagenase (CLSPA) and soybean trypsin inhibitor were purchased from Cooper Biomedical (Freehold, NJ). (^3H)-myoinositol was from American Radiolabelled Chemicals (St. Louis, MO). Dowex 1-X8 (formate form) was purchased from Biorad (Richmond, CA). Caerulein was from Farmitalia Carloerba (Milan, Italy). All other chemicals were from common sources.

RESULTS AND DISCUSSION

Figures 1 and 2 demonstrate that mouse pancreatic acini which have been preloaded with (^{3})H-myoinositol generate (^{3}H)-IP_3, as well as (^{3}H)-IP_2 and (^{3}H)-IP_1, following treatment with carbachol, a cholinergic agonist, or caerulein, a CCK-analogue. These measurements were made following 5 minutes of stimulation in the presence of 10 mM LiCl. Our studies indicate that the carbachol-induced increase in IP_3 levels are maximal within 5 seconds and remain constant through 5 minutes (Figure 3).

The dose response curve for carbachol-stimulated generation of (^{3}H)-inositol phosphates is shifted to the right of the carbachol-stimulated amylase secretion dose response curve (Figure 1). This relationship has been observed in numerous systems when comparing IP_3 generation and the physiological response (5). An additional finding is that the IP_3 dose response curve increases monotonically over the dose range examined. This is in contrast to amylase secretion which increases to a maximum and decreases at higher agonist concentrations, a phenomenon often referred to as high dose

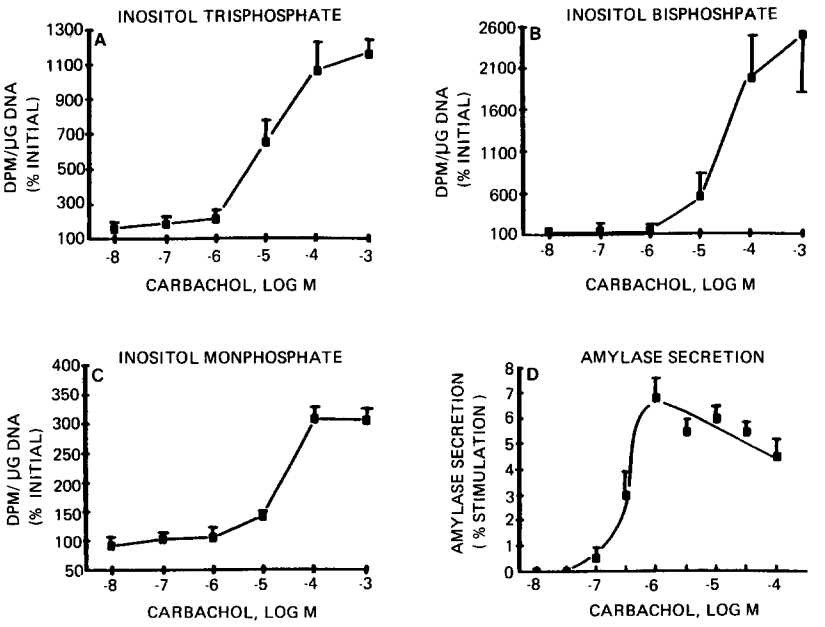

FIGURE 1. Inositol production (A,B,C) and amylase secretion (D) in mouse pancreatic acini following carbachol treatment. Levels of (^{3}H)-inositol phosphates were measured at 5 min in the presence of 10 mM LiCl. Amylase was measured as the net stimulated secretion at 10 min. Methods are described in the text. Points represent mean ± SE for 3 separate experiments in which samples at each point were analyzed in duplicate.

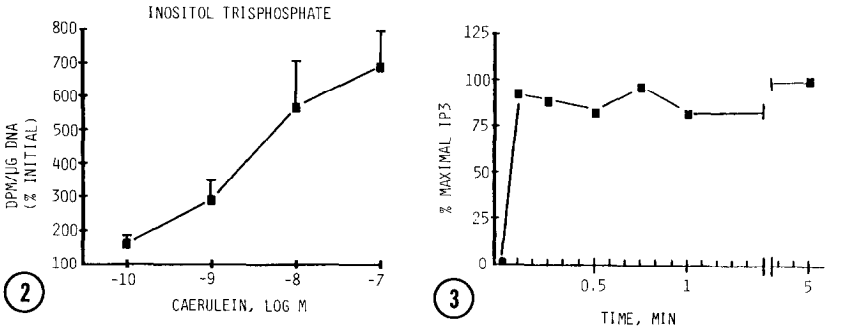

FIGURE 2. (^{3}H)-IP$_3$ generation in mouse pancreatic acini in response to caerulein at 5 min in the presence of 10 mM LiCl. Methods are described in the text. Data are mean ± SE for 4 separate experiments in which samples at each concentration of caerulein were analyzed in duplicate.

Figure 3. Time course of (^{3}H)-IP$_3$ generation in mouse pancreatic acini in response to 10^{-3}M carbachol. Points are the percent of maximal IP$_3$ levels from data compiled from three separate time course experiments in which the samples at each time were analyzed in duplicate.

inhibition. This disparity was also observed for IP$_3$ production and amylase secretion in rat pancreatic acini (5).

These data suggest that mouse pancreatic acinar cells respond to agonist stimulation in a manner analogous to that of rat acinar cells. We observed that agonist stimulation caused mouse acini labelled with (^{3}H)-myoinositol to generate increased levels of (^{3}H)-IP$_3$. This IP$_3$ has the capability of inducing an increase in the cytosolic free Ca^{2+} concentration in rat pancreatic acinar cells (6) and a cytosolic Ca^{2+} rise in response to endogenously produced IP$_3$ would explain the quin2-measurable Ca^{2+} increase reported by ourselves and others in mouse pancreatic acini following secretagogue stimulation (13,16).

Our findings are in contrast to those of Hokin-Neaverson and Sadeghian (12) who did not detect any IP$_3$ or IP$_2$ in their mouse acinar cell preparation after hormone stimulation. We are unable to explain the discrepency between their findings and our own, but consider as possibilities: (1) a decreased stimulability of their cell preparation, perhaps due to exposure to EDTA or acetylcholine during the acinar cell preparation; or (2) a low level of labelling of cellular phospholipids, such that the radioactivity in the (^{3}H)-IP$_3$ and (^{3}H)-IP$_2$ fractions might have been too low to measure. The first point is of concern in view of the low rate of amylase secretion they observed in response to acetylcholine stimulation. We consider that the latter concern may also be of

importance, since we also initially encountered problems in detecting (^{3}H)-IP_3 generation due to insufficient (^{3}H)-myoinositol incorporation. We found it necessary to load with relatively high amounts of (^{3}H)-myoinositol in order to measure (^{3}H)-IP_3 generation in the mouse exocrine pancreas. We did not find it necessary to stimulate with agonist and then block with an antagonist during preparation of the acini, as had been done by Hokin-Neaverson and Sadeghian, in order to obtain sufficient loading of radioactivity.

In summary, we have observed that mouse pancreatic acini labelled by incubation with (^{3}H)-myoinositol generate (^{3}H)-IP_3 in response to secretagogue stimulation. This observation strongly suggests that the sequence of events in stimulus-secretion coupling in the mouse pancreatic acinar cell is similar to that observed in rat acinar cells, as well as in a number of other systems.

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