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EFFECTS OF INSULIN SECRETAGOGUES AND INHIBITORS ON PHOSPHOLIPID METABOLISM IN LANGERHANS' ISLETS OF RAT PANCREAS

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SUMMARY: Glucose stimulation of [^{32}P]-prelabelled pancreatic islets induced an increased incorporation of radioactivity into phosphatidylinositol. Glucagon and agents that increases cAMP in the islet did not affect phosphatidylinositol turnover, in spite of increased release of insulin. Furthermore, the potent inhibitor of insulin release, somatostatin did not alter phospholipid metabolism. Colchicine inhibited glucose-stimulated turnover of phosphatidylinositol as well as insulin release. These results may suggest that: (1) cAMP and phosphatidylinositol turnover are involved in different transmembrane control system for regulating insulin release; and (2) the function of microtubules modulate phospholipid metabolism.

The mechanism by which glucose induces insulin secretion has not been elucidated yet. Glucose is reported to augment cyclic AMP levels in pancreatic islets, leading to an increase in insulin secretion (1,2). However, since the amounts of insulin secreted and of cyclic AMP accumulated in the islets are not always parallel (3,4), it seems unlikely that cyclic AMP is a sole mediator for glucose-induced insulin secretion. In a recent paper, Freinkel *et al* (5) demonstrated that the pancreatic islets increased insulin secretion and [^{32}P]labelling of the membrane phospholipid, especially of phosphatidylinositol, in response to elevated glucose concentration in the medium. This process has been

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The abbreviations used are: PI: phosphatidylinositol, IBMX: 3-isobutyl-1-methylxanthine, DBcAMP: dibutyryl cyclic AMP.

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considered to be an increased breakdown of phosphatidylinositol to form unsaturated diacylglycerol and postulated to play an important role in the transmembrane control of insulin secretion by glucose (6). Recently, a new species of protein kinase (C-kinase), which is activated in the presence of Ca^{2+} and membrane phospholipid, has been identified in various tissues including rat pancreatic islets (7). Preceding reports (8-9) have suggested that this protein kinase which may be tightly coupled with phosphatidylinositol turnover regulate in the process of stimulus-secretion coupling in human platelets.

Based on these findings we studied effects of substances which stimulate or inhibit insulin secretion on the [^{32}P]labelling of phosphatidylinositol in order to clarify relationships between the phosphatidylinositol turnover and cyclic AMP accumulation in the islets.

MATERIALS AND METHODS

Pancreatic islets were isolated from male Wistar rats (250-300 g) fed ad libitum by the collagenase (Worthington Biochemical Corp., type IV) digestion method (10). [^{32}P] labelling of phospholipid in the islets was studied according to the method as described, by Freinkel et al. (5). Twenty islets were preincubated for 90 min in a Krebs Ringer bicarbonate buffer containing 3.75 mM glucose, 0.25% BSA and 50-70 μCi [^{32}P] at 37°C under the gas phase of 95% O_2-5% CO_2. Following the preincubation, test substances were added to the medium and the islets were incubated further for 60 min. An aliquot of the medium was then removed for insulin radioimmunoassay. Phospholipids were extracted from the islets with chloroform/methanol (2:1), and then applied for a thin layer chromatography in Skipski's solution (11). Following autoradiography, each fraction was scraped, dissolved in Brays' solution and counted in a liquid scintillation counter. Cyclic AMP content in the islets was assayed by the method of Steiner et al. (12). IBMX and DBcAMP were purchased from Sigma Chemical Co., glucagon from Novo Research Institute and ^{32}Pi from the Radiochemical Center (Japan). Insulin concentrations in the medium were measured by the polyethylene glycol radioimmunoassay (13) using rat insulin (Novo Research Institute) as a standard.

RESULTS AND DISCUSSION

As shown in Table 1, addition of glucagon (10 μM) or IBMX (1 mM) to the medium containing 5.5 mM glucose resulted in increase in both cyclic AMP contents in the islets and insulin secretion. An enhanced insulin release was also observed by adding DBcAMP (2 mM) to the medium. However, these substances failed to affect the [^{32}P] incorporation into phosphatidylinositol. Elevation of glucose concentrations from 5.5 to 16.7 mM caused increases in the [^{32}P]

Table 1.

Effect of cyclic AMP on [^{32}P] incorporation into PI and insulin release

Glucose (mM)	Test Compound	PI fraction (%)	IRI (μU/islet/60 min)	cAMP (pmol/20 islets/60 min)
5.5		35.6 ± 1.7	43.5 ± 8.9	0.02 ± 0.01
5.5 + 10 μM glucagon		37.8 ± 3.5	141.1 ± 27.7	0.11 ± 0.05
5.5 + 1 mM IBMX		35.7 ± 3.0	85.3 ± 32.1	0.59 ± 0.28
5.5 + 2 mM dbcAMP		36.1 ± 3.6	111.5 ± 20.0	n.d.
16.7		57.6 ± 3.0	206.3 ± 26.1	0.43 ± 0.10
16.7 + 10 μM glucagon		56.9 ± 0.2	272.5 ± 30.0	n.d.
16.7 + 2 mM dbcAMP		53.9 ± 4.1	378.8 + 6.3	n.d.

The number of [^{32}P] incorporation into PI are expressed as percent of total radioactivity that was incorporated into the phospholipid fraction as 100. The results are means ± SEM for the observation of 6 experiments.
n.d. = (not determined).

incorporation into phosphatidylinositol as well as insulin secretion and cyclic AMP levels in the islets. Addition of glucagon (10 μM) or DBcAMP (2 mM) to the medium containing 16.7 mM glucose further augmented insulin secretion but did not alter [^{32}P] labelling of phosphatidylinositol. These results indicate that increase of cyclic AMP may not modulate the phosphatidylinositol turnover in the islets. Recently, another line of evidence has shown that cyclic AMP does not influence PI turnover in hepatocytes (14). Although the tissue response to cyclic AMP may vary in different tissues, these results including our data suggest that cyclic AMP and PI turnover play an independent role in controlling cellular activities.

Somatostatin inhibited insulin release by glucose (16.7 mM) in a dose dependent manner (Table 2). This decrease in insulin secretion was not associated with changes in [^{32}P] incorporation into phosphatidylinositol fraction. On the other hand, addition of 100 ng/ml somatostatin was seemed to lower cAMP contents

Table 2.

Effect of somatostatin on [^{32}P]incorporation into PI and insulin release by 16.7 mM glucose.

Glucose (mM)	Somatostain	PI fraction (%)	IRI (μU/islet/60 min)
16.7	- (6)	51.0 ± 7.1	193.8 ± 13.3
16.7	1 ng/ml (6)	55.4 ± 6.7	135.3 ± 49.8
16.7	10 ng/ml (6)	56.2 ± 8.8	109.2 ± 15.8
16.7	100 ng/ml (6)	52.9 ± 4.7	65.9 ± 15.6
16.7	1000 ng/ml (6)	50.7 ± 3.3	60.4 ± 14.4

The number of [^{32}P]incorporation into PI are expressed as percent of total radioactivity that was incorporated into the phospholipid fraction as 100. The results are means ± SEM for the observation of 6 experiments.

in the islets from 0.27 ± 0.08 (mean ± S.E.M. pmol/20 islets) to 0.12 ± 0.07 (n = 6 not significant). The mechanism by which somatostatin inhibits insulin release has not been fully elucidated. Several studies have suggested that inhibition of insulin secretion by somatostatin is mediated via decreases in cyclic AMP levels in the islets (15,16). Oliver et al (17) demonstrated that somatostatin modulated glucagon-dependent activation of cyclic AMP-dependent protein kinase in the islets. Furthermore, we found that somatostatin failed to affect C-kinase activity in the islets (unpublished observation). Thus, it seems likely that the inhibition by somatostatin of insulin release occur by a mechanism which is independent from the phosphatidylinositol turnover and phospholipid-dependent protein kinase.

It has been well-known that the microtubular-microfilamentous system is involved in the process of insulin secretion (18). Schellenberg et al (19,20) have revealed that concanavalin A-induced phosphatidylinositol turnover in lymphocytes is inhibited by colchicine and proposed that the microtubular-microfilamentous system may regulate the phosphatidylinositol turnover. We

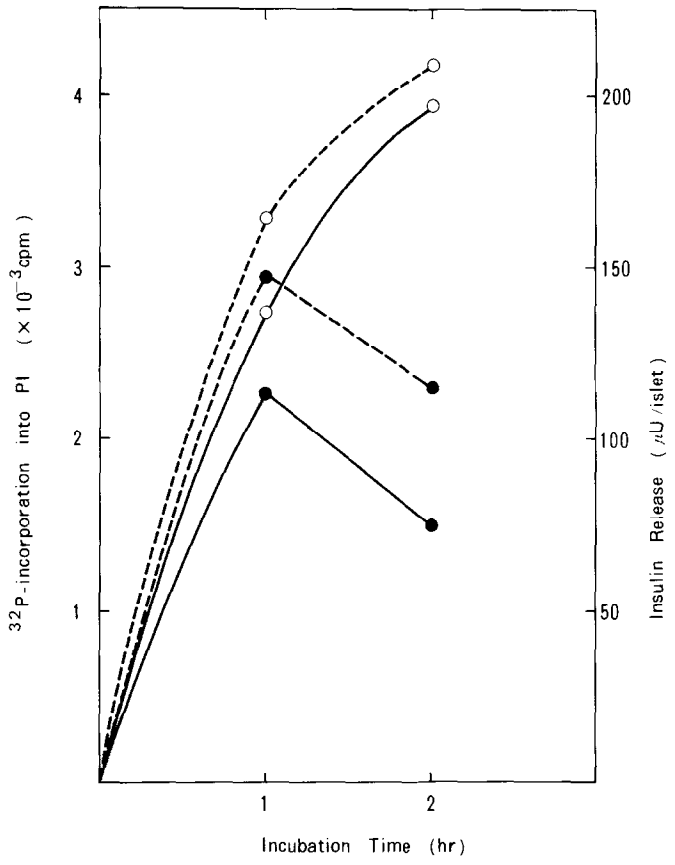

Figure 1. Time course of [^{32}P] incorporation into PI (_____) and insulin release (-----) by 16.7 mM glucose in the presence of (closed circles) or absence of (open circles) colchicine. Pancreatic islets were incubated with colchicine (100 μM) in the medium containing 16.7 mM glucose for one or 2 hrs. Colchicine did not exert any significant effects on both glucose-induced insulin release and [^{32}P] incorporation into phosphatidylinositol at the 1 hr incubation. In the separate experiment, however, after the 2 hrs incubation, both insulin secretion and [^{32}P] incorporation were inhibited by colchicine. The results are means of quadriplicate experiments.

confirmed their findings in islets of rat pancreas as shown in Figure 1. Colchicine reduced insulin secretion as well as [^{32}P] labelling of phosphatidylinositol in response to glucose, indicating that the microtubular-microfilamentous system may be involved in regulating the phosphatidylinositol turnover and insulin secretion from the islets.

The present study suggests that cyclic AMP and phosphatidylinositol turnover are independent systems that control insulin secretion from pancreatic islets, and glucose regulates insulin secretion through at least two different pathways.

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