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J Clin Invest. 1976;57(3):684-691. https://doi.org/10.1172/JCI108325.

Research Article

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Phosphoinositide Metabolism and Insulin Secretion from Isolated Rat Pancreatic Islets

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ABSTRACT Since many cell types have been shown to respond to extracellular stimulation with a rapid increase in phosphatidylinositol turnover, the present studies were undertaken to determine whether carbohydrate-stimulated insulin secretion from the isolated rat pancreatic islet is accompanied by detectable alterations in the phosphatidylinositol metabolism of this tissue. We have demonstrated that rat pancreatic islets incubated with tritiated myo-inositol rapidly incorporate radioactivity into islet phosphatidylinositol. Incubation of prelabeled islets with elevated concentrations of carbohydrates which stimulate insulin scretion (D-glucose and D-mannose) results in a decrease in the recovery of lipid-bound radioactivity, whereas incubation with carbohydrates which do not stimulate insulin secretion (D-galactose and myo-inositol) has no effect upon the recovery of lipid-bound radioactivity. Within 2 min of exposure of prelabeled islets to elevated concentrations of D-glucose, a decrease in the recovery of [2-^{3}H]myo-inositol-derived radioactivity in islet phosphatidylinositol can be demonstrated. When islets prelabeled with [2-^{3}H]myo-inositol are perifused with elevated concentrations of D-glucose or D-mannose (but not D-galactose or myo-inositol) a rapid and transient increase in the rate of extracellular release of water-soluble radioactivity is observed. Since a significant fraction of the radioactivity released under these conditions is in the form of myo-inositol-phosphate, cyclic myo-inositol-1,2-phosphate, and glycerophosphorylmyo-inositol, it is presumably derived from the cleavage of labeled islet phosphatidylinositol. It is speculated that alterations in the metabolism of myo-inositol-containing phospholipids may have a functional role in the process of insulin secretion from the pancreatic beta cell.

This work was presented in part at the Joint Annual Meetings of the Southern Section of The American Federation for Clinical Research, The Southern Society for Clinical Investigation, and The Southern Society for Pediatric Research, New Orleans, La., 1 February 1975 (*Clin. Res.* 23: 10A, 1975).

Received for publication 5 August 1975 and in revised form 17 November 1975.

INTRODUCTION

Phosphatidylinositol (PI) [1] is a member of a class of membrane phospholipids, the phosphoinositides, whose metabolism has been recently speculated to be related to such diverse cellular processes as ion transport (1), nerve impulse conduction (2), phagocytosis (3), lymphocyte transformation (4), and the secretion of various intracellular materials (5). In response to appropriate stimuli, a rapid and specific increase in the turnover of PI has been found to be associated with stimulated hormone secretion from a number of endocrine tissues (6–10). In murine pancreas (9), porcine thyroid (10), and rat parotid gland (11), the primary event in this phenomenon is the hydrolysis of PI to form 1,2-diacylglycerol and a mixture of myo-inositol-1-phosphate (MI-P) and myo-inositol-1,2-cyclic phosphate (cMI-P). The subsequent resynthesis of the decreased intracellular PI pool appears to occur as a secondary and somewhat delayed response (9–12). In rat brain (13), in murine cells in tissue culture (14), and in *Saccharomyces cerevisiae* (15), the degradation of PI has also been found to involve its deacylation with the consequent release of glycerophosphorylmyo-inositol (GPI). Although the functional significance of increased PI breakdown in

[1] *Abbreviations used in this paper:* cMI-P, myo-inositol-1,2-cyclic phosphate; DPI, diphosphatidylinositol; GPI, glycerophosphorylmyo-inositol; MI, myo-inositol; MI-P, myo-inositol-phosphate; PI, phosphatidylinositol; TPI, triphosphatidylinositol.

stimulated tissues has not been defined, and no role has been discovered for cMI-P (16), several workers have speculated that the hydrolysis of PI is related either to the cell-surface recognition of secretagogues (12) or to the reorganization of cellular membranes associated with stimulated hormone secretion (5, 14, 17).

Since insulin secretion from the beta cell of the pancreas is thought to occur by the process of emiocytosis (18), followed by a recycling of membrane constituents (19), the present studies were undertaken to determine whether carbohydrate-stimulated insulin secretion is accompanied by detectable alterations in the phosphoinositide metabolism of isolated rat pancreatic islets. The studies which form the basis of this report lead us to suggest that, as has been demonstrated in other secretory tissues, carbohydrate-stimulated secretion of insulin from rat pancreatic islets is accompanied by a rapid alteration in the PI metabolism of this tissue.

METHODS

Isolation of pancreatic islets. Islets of Langerhans were prepared from the pancreases of fed male Sprague-Dawley rats by the technique of Lacy et al. (20). Islets were transferred to polypropylene center wells (Kontes Glass Co., Vineland, N. J.) containing 0.1 ml of Krebs-Ringer bicarbonate solution, pH 7.4, (modified to contain 1.0 meq/liter calcium) which contained bovine plasma albumin (0.3 g/100 ml, wt/vol, Armour Pharmaceutical Co., Chicago, Ill.), unlabeled myo-inositol (MI) (0.01 mg/ml, Sigma Chemical Co., St. Louis, Mo.), and (unless otherwise indicated) 1.0 mg/ml D-glucose (Sigma Chemical Co.) with a gas phase of oxygen (95%) and carbon dioxide (5%). This solution is subsequently referred to as "standard buffer solution." Batches of 20–30 islets were collected for incubation studies, and 50–135 islets were used for perifusion experiments. The elapsed time between excision of the pancreas and the beginning of each experiment was monitored and did not exceed 60 min.

In vitro incubation studies. To study the rate of incorporation of [2-^{3}H]MI into total islet lipid and into the individual phosphoinositide fractions, an additional 0.1 ml of standard buffer solution which contained [2-^{3}H]MI (2.84 Ci/mmol, New England Nuclear, Boston, Mass.) was added to the center wells, which were incubated at 37°C in a metabolic shaker for the times indicated in Fig. 1. The center wells were then transferred to tubes containing 3.0 ml HCl (0.1 N) in methanol and 2.0 ml of H_2O (3). Chloroform (6.0 ml) was added and the tubes shaken intermittently for 1 h. A 2.0-ml sample of $MgCl_2$ (1 M) was added, the tubes shaken and centrifuged and the upper phase removed by aspiration (this step was repeated four times). With each set of experiments, a control center well containing no islets was processed in an identical fashion to assure that the washing procedure eliminated all counts derived from water-soluble [2-^{3}H]MI from the lower phase. In the absence of islets, the radioactivity recovered from the lower phase did not differ from that of background.

To determine [2-^{3}H]MI incorporation into total islet lipid, duplicate 1.0-ml aliquots of the lower phase were pipetted into scintillation vials, dried under nitrogen, dissolved in 10 ml of scintillation fluid (toluene-Triton X-100, 2:1, vol/vol, PPO 11 g/liter, and POPOP 0.1 g/liter), and counted in a liquid-scintillation spectrometer (Searle Analytic, Inc., Atlanta, Ga.) (Fig. 1).

The phosphoinositide classes were separated by thin-layer chromatography (21). A 3.0-ml portion of the lower phase from the lipid extraction procedure was dried under nitrogen, dissolved in a small volume of chloroform: methanol: H_2O (75:25:2, vol/vol/vol), and streaked in a 5-cm band on 20 × 20-cm glass plates precoated with 500-μm layers of silica gel H (Applied Science Labs, Inc., State College, Pa.). The plates were equilibrated with a mixture of chloroform, methanol, and 4.3 N ammonium hydroxide (90:65:20, vol/vol/vol) and ascending chromatography carried out with the same solution. PI, diphosphatidylinositol (DPI), and triphosphatidylinositol (TPI) isolated from porcine brain by the method of Hendrickson and Ballou (22) as well as from bovine brain (kindly provided by Professor J. N. Hawthorne) were employed for standardization. The location of the phosphoinositides was determined by exposure of the developed plates to iodine vapor. With this system, the R_f of PI was 0.72, DPI gave rise to two bands with R_fs of 0.61 and 0.50, and TPI migrated as a single band with an R_f of 0.31. After chromatography, the plates were dried at room temperature for 2 h and 1-cm bands of silica gel were removed with a spatula and counted as described above. The incorporation of [2-^{3}H]MI into the individual phosphoinositides was estimated by the radioactivity recovered in the areas corresponding to the migration of the phosphoinositide standards (Fig. 1). The mean recovery of phosphoinositide-bound radioactivity in this system was found to be 59.5±13.9%, and the relative recoveries of PI, DPI, and TPI were assumed to be similar to the recovery of total lipid-bound radioactivity.

To study the effect of an elevated concentration of D-glucose upon the recovery of radioactivity in the individual phosphoinositides, paired batches of islets were incubated with [2-^{3}H]MI for 1 h as described above. The incubation medium was removed by aspiration with a finely drawn-out pipette under a dissecting microscope.[2] To each paired batch, 0.2 ml of standard buffer solution which contained either a low (1.0 mg/ml) or a high concentration of D-glucose (5.0 mg/ml) was added. The islets were subsequently incubated for the times indicated in Table I, and islet phosphoinositides extracted and separated as described above.

The effect of elevated concentrations of various carbohydrates upon the recovery of [2-^{3}H]MI-derived radioactivity in total islet lipid was evaluated in a third series of experiments. Batches of islets were incubated for 60 min with [2-^{3}H]MI (0.64 Ci/mmol) as described above. The incubation medium was replaced with 0.4 ml of standard buffer solution containing either various concentrations of D-glucose or with 3.0 mg/ml solutions of D-galactose, D-mannose, or unlabeled MI and incubation continued for an additional 30 min (Table II). An aliquot of the medium (0.2 ml) was removed for the determination of insulin content by radioimmunoassay with Sephadex-bound antibody (Pharmacia Fine Chemicals, Inc., Upsala, Sweden), using crystalline rat insulin (Novo Research Institutes, Copenhagen, Denmark) to establish nine-point standard curves, and total islet lipid was extracted and counted as described above.

Perifusion experiments. To determine whether the decreased recovery of lipid-bound radioactivity observed after

[2] In ten consecutive experiments this procedure was found to remove all but 0.0036±0.0013% of total radioactivity. Of the residual islet-associated radioactivity, 85±4% was water-soluble and the remainder was lipid bound.

exposure of prelabeled islets to stimulatory concentrations of D-glucose or D-mannose was accompanied by an extracellular release of radioactivity derived from the breakdown of labeled PI, two series of perifusion experiments were performed. In the initial series, islets were incubated with standard buffer solution containing [2-^{3}H]MI and 0.3 mg/ml D-glucose for 2 h. The islets were then transferred to 5-μm cellulosic filters (Gelman Instrument Co., Ann Arbor, Mich.) contained within plastic perifusion chambers (Millipore Corp., Bedford, Mass.). The chambers were maintained at 37°C and perifused at the rate of 0.9 ml/min. The effluent was collected in graduated centrifuge tubes maintained in an ice bath.

The islets were washed by perifusion with standard buffer solution containing 0.3 mg/ml D-glucose for 30 min. The effluent was collected over 5-min intervals for the initial 20 min and at 1-min intervals for the final 10 min of the wash period. The perifusate was then changed to either a fresh 0.3 mg/ml D-glucose solution or to a solution containing 5.0 mg/ml D-glucose and perifusion continued for 30 min. The effluent was collected over 1-min intervals for the initial 10 min following the change, and over 5-min intervals thereafter. In six consecutive experiments, at the end of the wash period 46.7±4.1% of the remaining islet-associated radioactivity was lipid-bound and the remainder was water-soluble.

The release of water-soluble radioactivity from the islets was determined in 0.5-ml aliquots of the effluent diluted with 10 ml of Aquasol (New England Nuclear) (Fig. 2). The insulin content of the effluent was determined as described above, and the D-glucose concentration of the effluent was determined by the glucose oxidase technique to determine the dead time of the perifusion system (2 min).

The release of water-soluble radioactive MI, MI-P, cMI-P, and GPI was determined by high-voltage paper electrophoresis (24). Perifusion experiments were performed as described above, and the effluent collected over 3-min intervals. The release of total water-soluble radioactivity was determined in 0.5-ml aliquots and the remaining effluent from each collection period was lyophilized, dissolved in 0.5 ml of H_2O, deproteinized, and desalted by passage through a 1 × 25-cm column of Biogel P-2 (Bio-Rad Laboratories, New York, N. Y.). The fractions containing radioactivity were pooled, lyophilized, dissolved in a small volume of H_2O, and spotted on 15 × 50-cm sheets of S and S no. 2316 paper (Schleicher & Schuell, Inc., Keene, N. H.). The papers were saturated with a mixture of pyridine: acetic acid: H_2O (4: 100: 895, vol/vol/vol) and electrophoresis carried out at 18 V per cm for 8 h with the same solvent system (24). The migration of MI, MI-P, (Sigma Chemical Co.), cMI-P (prepared by the method of Pizer and Ballou [25]), and GPI (Supelco, Inc., Bellefonte, Pa.) standards were determined for each run by means of a silver nitrate staining technique (26). With this system, the average migration of MI was 2.6 cm; GPI, 23.6 cm; MI-P, 26.9 cm; and cMI-P, 29.4 cm. Strips corresponding to the migration of the standards were counted in 10 ml of Aquasol. Since radioactive standards of MI-P, cMI-P, and GPI were not available, the recovery of these compounds through the isolation procedures was assumed to be the same as that of total water-soluble [2-^{3}H]MI-derived radioactivity (43.4±3.5%), and the calculations based on this assumption.

The second series of perifusion experiments was performed to compare the effects of 10-min pulses of various carbohydrates upon the release of water-soluble radioactivity from islets prelabeled with [2-^{3}H]MI. Batches of islets were incubated for 60 min with [2-^{3}H]MI and 1.0 mg/ml D-glucose as described above. After washing for 30 min with standard buffer solution containing unlabeled MI and 1.0 mg/ml D-glucose, the islets were perifused for a 10-min period with either a fresh solution of 1.0 mg/ml D-glucose (controls) or with solutions containing elevated concentrations of either D-glucose, D-galactose, D-mannose, or MI (Figs. 3 and 4).

RESULTS

[2-^{3}H]IM incorporation into islet lipids. When islets were incubated with [2-^{3}H]MI, the incorporation of radioactivity into total lipid increased with time in a nearly linear fashion (Fig. 1). At all time intervals studied, the majority of radioactivity incorporated into total lipid was recovered in the PI fraction, which accounted for more than 80% of the lipid-bound radioactivity with incubations of 45 min or longer. In contrast, the radioactivity incorporated into DPI and TPI did not increase with time and accounted for a small proportion of the incorporation of [2-^{3}H]MI into total islet lipid (Fig. 1).

Effect of an elevated D-glucose concentration upon recovery of [2-^{3}H]MI in islet phosphoinositides. When islets preincubated with [2-^{3}H]MI were exposed to 5.0 mg/ml D-glucose, a prompt decrease in the recovery of radioactivity in islet PI was observed (Table I). After 2 min of exposure to the elevated D-glucose concentration, the mean recovery of radioactivity in the PI fraction fell to 82% of that observed in the matched controls and continued to fall with more prolonged stimu-

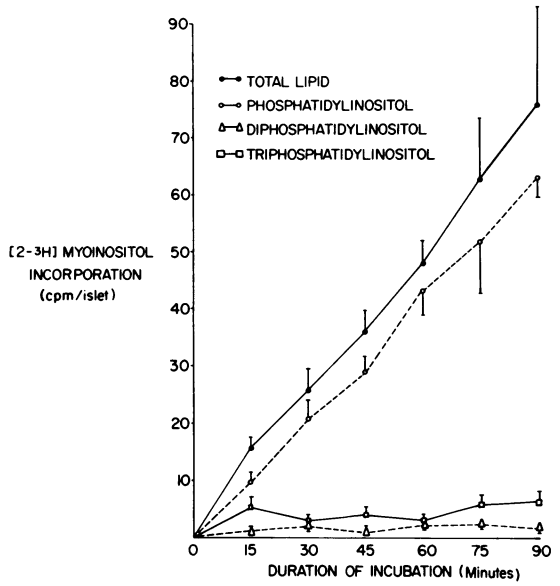

FIGURE 1 Incorporation of [2-^{3}H]MI into total lipid, PI, DPI, and TPI of isolated rat pancreatic islets. Each point represents the mean values obtained in eight individual experiments and the vertical bars represent the standard errors of the means (23).

TABLE I

Recovery of [2-^{3}H]MI in PI, DPI, and TPI from Eight Sets of Paired Batches of Islets Preincubated with [2-^{3}H]MI for 60 min and Then Exposed to Low (1.0 mg/ml) or High (5.0 mg/ml) D-Glucose for Various Time Intervals

Phosphoinositide fraction	Concentration of D-glucose	Recovery of [2-^{3}H]MI				
		Duration of incubation (min)				
		0	2	5	15	30
	mg/ml			*cpm/islet*		
PI	1.0	43.3±4.2	45.8±7.6	46.7±6.5	53.9±11.5	63.0±3.2
	5.0	—	37.8±7.1	30.6±6.3*	35.5±6.5‡	32.8±3.8*
DPI	1.0	2.3±0.3	2.3±0.6	2.6±0.9	2.4±0.4	1.7±0.4
	5.0	—	1.8±0.3	2.9±1.1	2.2±0.4	1.7±0.5
TPI	1.0	3.1±0.7	4.8±1.3	3.7±1.3	6.1±1.3	6.5±1.7
	5.0	—	6.3±1.9	2.8±0.8	5.9±2.7	4.8±1.1

* P of paired differences <0.001.
‡ P of paired differences <0.01.

lation (66% of control after 5 and 15 min and 52% of control after 30 min). In contrast, [2-^{3}H]MI-derived radioactivity recovered in DPI and TPI was not influenced by exposure to 5.0 mg/ml D-glucose (Table I). At all time intervals studied, more than 80% of the decrease in recovery of [2-^{3}H]MI in total islet lipid could be accounted for by the decrease in radioactivity recovered in the PI fraction.

Effect of various carbohydrates upon insulin secretion and recovery of [2-^{3}H]MI in total islet lipid. When islets preincubated with [2-^{3}H]MI were exposed to D-glucose in concentrations of 2.0 mg/ml or higher, a significant increase in insulin output was observed (Table II). Those concentrations of D-glucose (2.0, 3.0, or 5.0 mg/ml) which stimulated insulin secretion also produced a significant decrease in the recovery of [2-^{3}H]MI-derived radioactivity in total islet lipid (Table II). Similarly, D-mannose increased the mean output of

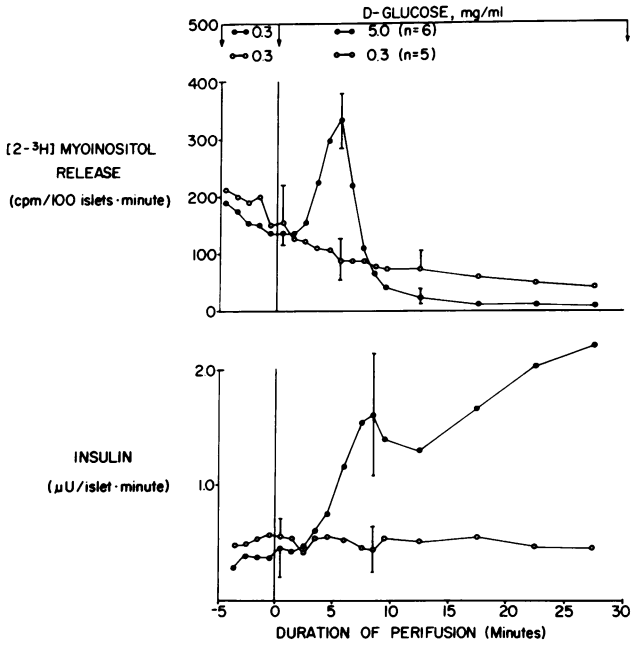

FIGURE 2 Efflux of water-soluble radioactivity derived from [2-^{3}H]MI during perifusion of prelabeled islets with an elevated concentration of D-glucose. The upper panel represents the release of [2-^{3}H]MI-derived radioactivity from prelabeled islets exposed at time 0 to solutions containing either 0.3 or 5.0 mg/ml D-glucose. The lower panel represents the mean insulin output observed in the same experiments.

TABLE II

Insulin Output and Recovery of Lipid-Bound [2-^{3}H]MI from Prelabeled Rat Pancreatic Islets Incubated for 30 min with Various Carbohydrates

Carbohydrate	Concentration	Insulin output	Total lipid-bound [2-^{3}H]MI
	mg/ml	*μU/islet·30 min*	*cpm/islet*
D-Glucose	1.0 (12)*	20.1±2.6	13.2±2.1
	2.0 (8)	68.2±6.9‡	6.2±0.9§
	3.0 (8)	76.7±5.0‡	6.0±1.2§
	5.0 (8)	70.3±10.5‡	6.3±0.7§
D-Galactose	3.0 (9)	8.9±2.4§	12.0±1.3
D-Mannose	3.0 (8)	51.7±15.0‖	5.7±0.9§
MI	3.0 (8)	16.4±2.9	17.8±5.1

* The number of observations is given in parentheses.
‡ $P < 0.001$.
§ $P < 0.01$.
‖ $P < 0.05$.

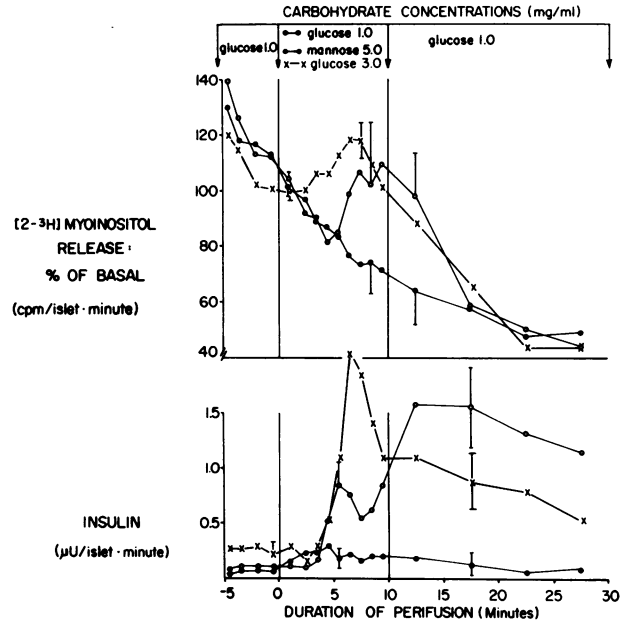

FIGURE 3 Effect of a 10-min pulse of 3.0 mg/ml D-glucose, 5.0 mg/ml D-mannose, or of 1.0 mg/ml D-glucose on the release of water-soluble radioactivity derived from [2-^{3}H]-MI (upper panel) and insulin output (lower panel) from islets prelabeled with [2-^{3}H]MI. Each point represents the mean values observed in five experiments. Because of the variation observed with different batches of islets, the release of radioactivity is expressed as a percentage of the mean rate of release observed during the first 3 min of each experiment.

insulin and significantly decreased the recovery of lipid-bound radioactivity. In contrast, neither D-galactose nor unlabeled MI elicited either insulin release or a decrease in lipid-bound radioactivity (Table II). Thus, in this experimental model, those carbohydrates which stimulated insulin secretion resulted in a significant decrease in [2-^{3}H]MI-derived lipid-bound radioactivity, whereas exposure of islets to carbohydrates which did not elicit insulin secretion had no effect upon lipid-bound radioactivity.

Release of water-soluble [2-^{3}H]MI from perifused islets. Despite the time required for preincubation with [2-^{3}H]MI, a brisk release of insulin from perifused islets was observed within 1 min of exposure to rising D-glucose concentrations (Fig. 2). The release of insulin was biphasic, with the peak of the first phase occurring between the 8th and 9th min and the second phase beginning after the 15th min of the experiment Fig. 2). The amount of insulin released by the islets compared favorably with that previously observed by Lacy et al. (20) under similar experimental conditions.

The release of water-soluble radioactivity from islets prelabeled with [2-^{3}H]MI and perifused with solutions containing 0.3 mg/ml D-glucose progressively declined throughout the experiment (Fig. 2). However, within 1 min of exposure of the islets to rising D-glucose concentrations, a marked increase in the rate of release of water-soluble radioactivity was observed, which rose to a peak within 4 min and returned to the control rate over the succeeding 3 min. The total net release of radioactivity derived from [2-^{3}H]MI between the 2nd and the 8th min of these experiments averaged 730 cpm per 100 islets.

Characterization of the water-soluble radioactivity released from prelabeled islets. Analysis of [2-^{3}H]MI and its phosphorylated derivatives in the effluent from prelabeled islets revealed that the majority (99.3%) of the water-soluble radioactivity released during the initial wash period was in the form of nonphosphorylated MI. However, after exposure to elevated D-glucose, an increase in the rate of release of the phosphorylated derivatives of MI was observed between the 3rd and 9th min of these experiments. During this time interval, the net release of total water-soluble radioactivity derived from [2-^{3}H]MI was found to average 564 cpm/100 islets, of which 262 cpm/100 islets was recovered as MI, 156 cpm/100 islets as GPI, 68 cpm/100 islets as MI-P and 78 cpm/100 islets as cMI-P.

Effect of pulses of various carbohydrates on the release of radioactivity derived from [2-^{3}H]MI. Ex-

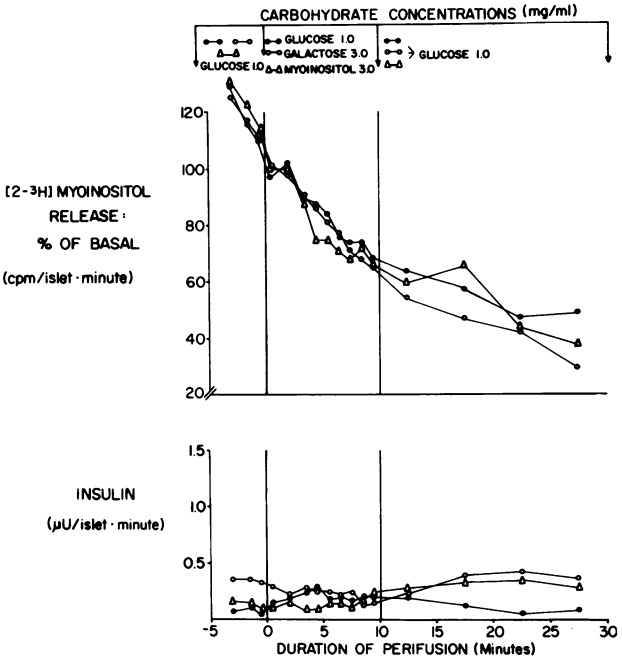

FIGURE 4 Comparison of the effect of a 10-min pulse of 3.0 mg/ml D-galactose ($n = 5$), 3.0 mg/ml MI ($n = 3$), or 1.0 mg/ml D-glucose ($n = 5$) on insulin secretion and the release of radioactivity derived from [2-^{3}H]MI from prelabeled perifused islets. The experimental conditions are otherwise similar to those described in Fig. 3.

posure of prelabeled islets to an elevated concentration of either D-glucose or D-mannose for 10 min resulted in a significant increase in the rate of release of water-soluble radioactivity and an increase in the rate of insulin secretion (Fig. 3). Pulses of a lower concentration of either D-glucose (2.0 mg/ml) or of D-mannose (3.0 mg/ml) gave rise to lesser increments in the rate of insulin secretion and the release of water-soluble radioactivity (data not shown). In contrast, when islets were exposed to pulses of D-galactose or MI there was no change in the rate of insulin secretion or in the rate of release of water-soluble radioactivity (Fig. 4). Thus, those carbohydrates which stimulated insulin secretion also evoked the extracellular release of water-soluble radioactivity derived from [2-^{3}H]MI from prelabeled islets, while those carbohydrates which did not stimulate insulin secretion did not provoke an increase in the rate of release of [2-^{3}H]MI-derived radioactivity.

DISCUSSION

The present studies have demonstrated that exogenous tritiated MI is rapidly incorporated into the lipids of isolated rat pancreatic islets and that the majority is recovered as PI. When islets preincubated with radioactive MI are exposed to a stimulatory concentration of D-glucose, a rapid and progressive decrease in the recovery of labeled MI in the PI fraction is observed. A relationship between this decrease in recovery of lipid-bound [2-^{3}H]MI and insulin secretion is suggested by the observations that both phenomena occur after exposure of islets to stimulatory concentrations of D-glucose or D-mannose, but do not occur when islets are exposed to high concentrations of D-galactose or MI.

Two lines of evidence support the hypothesis that the observed decrease in islet lipid-bound radioactivity associated with carbohydrate-stimulated insulin secretion is due to the hydrolysis of PI. First, within minutes of exposure of perifused islets to elevated D-glucose or D-mannose concentrations, a marked increase in the rate of release of water-soluble radioactivity is observed which (at least in the case of D-glucose) bears a close temporal relationship to the observed decrease in labeled islet PI. Second, a significant fraction of the [2-^{3}H]MI released within the first few minutes of exposure of islets to elevated D-glucose concentrations is in the form of MI-P and cMI-P, which are presumably derived from the hydrolysis of PI through a phosphodiesterase or phosphotransferase mechanism (9, 10, 12, 24, 27) and could not result from a leakage of intracellular free MI secondary to a carbohydrate-induced increase in islet membrane permeability (28). That the release of radioactivity in response to carbohydrate stimulation is not due simply to the passive extracellular diffusion or exchange of labeled intracellular MI is further supported by the observation that high extracellular unlabeled MI concentrations have no demonstrable effect upon either islet lipid-bound [2-^{3}H]MI or upon radioactive MI release. The release of GPI observed in these experiments may reflect either an activity of the PI degrading system (29) or could result from the action of a deacylating phospholipase within the islets (13, 15, 29, 30).

The ratio of MI-P and cMI-P (0.87:1) released after exposure of prelabeled islets to elevated D-glucose was similar to that observed by others after the incubation of PI with particulate cerebral cortex (27) or water-soluble hepatic (24) and lymphocyte (31) PI-degrading enzyme activity and with that observed in studies of the porcine thyroid gland (10). The possibility that the islets contain MI-P phosphomonoesterase and cMI-P phosphodiesterase activities which could have contributed to the observed pattern of release of radioactive MI and its phosphorylated derivatives will require further investigation.

Freinkel et al. have recently observed that the majority of the radioactivity released from rat islets preincubated with [^{32}P]orthophosphate in response to carbohydrate stimulation is in the form of inorganic phosphate and that none can be shown to be associated with the water-soluble phosphorylated derivatives of MI (28). It should be pointed out that if the amount of released radioactivity associated with MI-P and cMI-P in their experiments was similar to the amount of radioactivity found to be associated with these compounds in the present study, it would represent less than 1/1,000 of the total release of ^{32}P which they observed and could have escaped detection by their analytical techniques.

The present studies indicate that approximately half of the total amount of MI-derived radioactivity incorporated into islet phospholipids is rapidly depleted during the 30 min after exposure to a stimulatory concentration of D-glucose and that nearly two-thirds of this breakdown occurs within the first 5 min of stimulation. While the rate and magnitude of this decrease in recovery of lipid-bound [2-^{3}H]MI is striking, it is similar to the decreases in PI observed in other secretory tissues after stimulation (9, 11). Unfortunately, the small amount of PI present in isolated islets does not permit quantification of the changes in islet PI content or in its specific activity. Therefore, it is not clear whether the PI degraded in response to D-glucose stimulation represents a small pool of PI which turns over rapidly, or whether the observed changes reflect alterations in the entire PI content of the islets.

While our observations may appear to be in conflict with the studies of Fex and Lernmark in which an acute effect of insulin secretagogues upon the rate of incorpo-

ration of ^{32}P into PI could not be demonstrated (32, 33), it should be pointed out that the present study describes the effect of D-glucose upon the breakdown of prelabeled PI, whereas the earlier studies were concerned with the resynthesis of PI after islet stimulation. Since PI resynthesis may be both physically and temporally removed from the acute phenomena which accompany the stimulated secretion of intracellular materials (12), it is not surprising that although these workers could not demonstrate an acute effect of D-glucose upon islet PI synthesis (32, 33), we and others (34) have demonstrated an acute effect upon its rate of breakdown.

In other secretory tissues, the degradation of PI has been speculated to play a functional role as part of a transport mechanism in which the cell membrane could be opened at discrete locations through the action of a MI-specific phospholipase C (5). More recently, it has been suggested that the deacylation of PI would provide an even more effective mechanism by which openings in the granule or plasma membranes could be produced (14). In addition, Michell has recently speculated that the stimulated cleavage of PI may be related to the mechanism by which information is transmitted from cell-surface receptors to the interior of the cell (12). Since PI metabolism has been thought to be related to the movement of calcium ions and the generation of nerve membrane potentials (1), it is tempting to speculate that an analogous D-glucose-induced alteration in islet PI metabolism may play a role in the generation of the calcium fluxes (35) and the bioelectrical alterations (36, 37) which accompany insulin secretion from rat pancreatic islets. While the present studies indicate that carbohydrate stimulation of rat pancreatic islets results in a rapid alteration in the metabolism of MI-containing phospholipids, the functional relationship of this phenomenon to the process of insulin secretion remains to be determined.

ACKNOWLEDGMENTS

The authors are grateful to Professor J. N. Hawthorne for his generous gift of phosphoinositide standards and to Dr. Albert Winegrad for critically reading the manuscript. The technical assistance of Mrs. Barbara L. Poole, Willard R. Starnes, and the late Miss Etherene Pearson is appreciated. We thank Mrs. Mary Kay Miles and Mrs. June P. Reid for preparing the manuscript.

This work was supported in part by a grant from the Veterans Administration (MRIS 1472), a grant from the Juvenile Diabetes Foundation, and grant RR-32 from the General Clinical Research Centers Branch, Division of Research Resources, National Institutes of Health, Bethesda, Md.

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