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Phosphatidylethanolamine *N*-Methylation and Insulin Release in Isolated Pancreatic Islets of the Rat

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SUMMARY

Rat pancreatic islets methylate phosphatidylethanolamine (PE) lipids to form phosphatidylcholine (PC) with *S*-adenosyl-L-[*methy*-^{3}H]methionine as the methyl donor. Islet PE-*N*-methyltransferase had activity optima at pH 6–7 and 8–9. *S*-Adenosyl-L-homocysteine, sodium deoxycholate, and Triton X-100 inhibited methylation in islet homogenates. Addition of phosphatidyl-*N*-monomethylethanolamine and phosphatidyl-*N*,*N*-dimethylethanolamine (PDME) enhanced [^{3}H]methyl incorporation into PDME and PC, respectively. Isoproterenol, but not glucose, stimulated phospholipid methylation in islet homogenates. Propranolol inhibited the isoproterenol effect. In intact islets, glucose or isoproterenol stimulated insulin release and incorporation of [^{3}H]methyl groups from [*methyl*-^{3}H]methionine into phospholipids. Isoproterenol enhanced to a similar extent glucose-stimulated methylation and hormone release. Neither 2-deoxyglucose, tolbutamide, nor 8-bromo-cyclic AMP stimulated islet phospholipid methylation. The methyltransferase inhibitor 3-deazaadenosine inhibited both glucose and isoproterenol-stimulated methyltransferase activity and insulin release. Propranolol inhibited the β-adrenergic potentiation of glucose-induced phospholipid methylation and insulin release. These data suggest that PE-*N*-methyltransferase plays a role in amplification of the islet cell stimulus-secretion coupling response to certain secretagogues.

INTRODUCTION

Phosphatidylcholine is the major phospholipid in many endocrine tissues including the pancreatic islet (1, 2). Sequential enzymatic methylations occurring on the amino group of PE[1] phospholipids form PC in several secretory tissues, including the pituitary gland (3), adrenal medulla (4), and mast cell (5). Methylation is catalyzed by one or more PE-*N*-methyltransferases utilizing AdoMet as the methyl donor (6, 7), and methyltransferase activity is responsive to membrane active agents in the thyroid gland (8) leukocytes (9), mast cells (5, 10), hepatocytes (11), reticulocytes, HeLa cells, and astrocytoma cells (12). Phospholipid methylation and formation of PC has been proposed to decrease the microviscosity of membranes and facilitate the coupling of receptors with adenylate cyclase (13) and to affect Ca^{2+}-ATPase activity, Ca^{2+} transport, and phospholipase A_2 activity (14, 15) of membranes.

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[1] The abbreviations used are: PE, phosphatidylethanolamine; [^{3}H] AdoMet, *S*-adenosyl-L-[*methyl*-^{3}H]methionine; AdoHyc, *S*-adenosyl-L-homocysteine; PMME, phosphatidyl-*N*-monomethylethanolamine; PDME, phosphatidyl-*N*,*N*-dimethylethanolamine; PC, phosphatidylcholine; 8-Br-cyclic AMP, 8-bromo-cyclic adenosine-3′5′-monophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DOC, sodium deoxycholate.

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Recently, certain secretagogues have been shown to stimulate the turnover of PC in isolated islets of Langerhans from rats (2). Turnover of PC was attributed in part to activation of phospholipase A_2; however, it was not established whether PE-*N*-methyltransferase played any role in islet PC biosynthesis and/or turnover. The present studies were conducted to characterize the PE-*N*-methyltransferase(s) in isolated islets and to determine their sensitivity to membrane active stimuli. The results of these studies demonstrate that PC biosynthesis in islet membranes is in part determined by PE methyltransferase activity which is responsive to hormone secretagogues.

MATERIALS AND METHODS

Islets were isolated using collagenase as previously described (2). For static incubations, islets (30 islets per sample for determination of insulin release only, or 240–400 islets divided equally into paired batches per radiolabel incorporation experiment) were incubated in Krebs-Ringer bicarbonate buffer (0.5 ml) containing 16 mM HEPES, 2.8 mM glucose, 0.01% bovine serum albumin, 1 mM reduced GSH, and 10 μCi/ml (10 nmol) of [^{3}H]methionine under O_2/CO_2 (95:5%), at 37°. Islets were preincubated with radiolabel for 30 min, after which test agents were added as indicated in the text, and the incubation was continued for the time periods indicated. The incubation buffer was then carefully aspirated and the insulin content was determined by radioimmunoassay at a later time (2); the islets were immediately resuspended in cold Krebs buffer (4°) and centrifuged in a microcen-

trifuge (Eppendorf) for 10 sec with subsequent decanting of the supernatants. The lipids of the resulting islet pellet were extracted in 3 ml of acidified chloroform:methanol in preparation for TLC (see below). Islet PMME, PDME, and PC incorporated 27 ± 4% of the total radioactivity recovered in the lipid extract; the remainder of the label was recovered in the solvent front on TLC. In order to verify that label from [^{3}H]methionine was being incorporated into the ethanolamine base group, islets were allowed to incorporate label for 30 min and then the lipids were extracted as described above. The extracted lipids were then saponified in 0.5 ml of 0.1 N methanolic KOH for 2 hr at 60°, and the lipids were extracted in 3 ml of acidified chloroform:methanol (1:2, v/v). The resulting aqueous fraction was re-extracted with hexane, and the organic phases of both extractions were pooled and dried under nitrogen. Control samples were treated with methanol alone. The number of counts per min in the aqueous phase was determined by liquid scintillation spectrometry. The extracted lipids were analyzed by TLC using silica gel G plates developed in the solvent system petroleum ether:diethyl ether:acetic acid (50:50:1, v/v/v), which separates phospholipids, fatty acids, diglycerides, and triglycerides. The results showed that 93% of the total radiolabel (782 cpm) from saponified islet phospholipids was recovered in the aqueous phase with water-soluble base groups; in the absence of KOH, only 2% of the radiolabel was recovered in the aqueous fraction. In the presence or absence of KOH, 3 to 4% of the radiolabel was recovered as free fatty acids.

In experiments utilizing tissue homogenates, islets were sonicated (Kontes Ultrasonic cell disrupter) for approximately 10 sec in 0.3 M sucrose containing 10 mM HEPES, pH 7.4, at 4°. The methyltransferase assay contained either sodium acetate (0.1 M) (pH 4 and 5), Tris-maleate (0.1 M) (pH 6,7, 7.4. 8, 8.5), or Tris-HCl (0.1 M) (pH 7.4, 8, 8.5), Tris base (0.1 M) (pH 9 and 10), $MgCl_2$, PE, PMME, PDME as indicated in the text, 10–40 μg of tissue protein, and 1 μCi (0.1 nmol) of [^{3}H]AdoMet per 0.1-ml final reaction volume. [^{3}H]Methyl recovery in phospholipids increased in proportion to addition of 6 μg to 72 μg of homogenate protein. Methyl incorporation was allowed to proceed for up to 60 min and was stopped by addition of acidified extraction solvent. Blank values, obtained in the absence of protein, were subtracted from tissue sample values in every experiment. Radioactivity in PMME, PDME, and PC accounted for 15 ± 3% of the total tritium recovered in the lipid extracts.

Phospholipids were extracted from islets or islet homogenates as previously described (3) using chloroform:methanol:hydrochloric acid (2:1:0.02, v/v/v) containing 0.05 mg/ml butylated hydroxytoluene, followed by 2 ml of 0.1 M KCl in 50% methanol. The organic extracts were dried under nitrogen and resuspended in TLC solvents (for immediate phospholipid characterization using silica gel G plates (Analtech, Inc., Newark, DE) developed in solvent system I containing chloroform:propionic acid:1-propanol:H_2O (2:2:3:1, v/v/v/v) (11). Selected experimental samples were also developed in the second dimension using the solvent system chloroform:methanol:acetone:acetic acid:water (5;1:2:1:0.05, v/v/v/v/v) and recovery of [^{3}H]methyl in PMME, PDME, and PC was 86% of that recovered from samples run in one dimension in solvent system I only. PE, PMME, PDME, and PC standards (2.5 μg each) were added to extractions as carrier lipids and for identification of phospholipid zones on TLC using iodine staining. Radioactivity in the separate phospholipid zones was determined by liquid scintillation spectrometry. Background counts (16 dpm) were subtracted from samples, and quench was negligible. Counting efficiency was 55–63% (Beckman LS1800; EP scintillation cocktail), and each sample was counted for 20 min at less than 1.5% error.

Statistical analyses were performed using values of the geometric mean and Student's *t* test for paired or unpaired samples (two-tailed) or analysis of variance (one-way). All paired comparisons were made between real data values of disintegrations per min, femtomoles of methyl incorporation/mg of protein, or microunits of insulin/ml; differences between sets of data are expressed as per cent changes, and reflect statistical analysis of real data values.

S-Adenosyl-L-[*methyl*-^{3}H]methionine (10–15 Ci/mmol) and L-[*methyl*-^{3}H]methionine (1 Ci/mmol) were obtained from ICN Chemical and Radioisotope Division, Irvine, CA. Phospholipid standards (egg PC, or transphosphatidylated egg lecithins) were from GIBCO, Inc., Grand Island, NY or Sigma Chemical Co., St. Louis, MO.

RESULTS

Phospholipid methylation in islet homogenates. Incorporation of [^{3}H]methyl groups into PMME, PDME, and PC of islet homogenates was time-dependent, with methylation occurring most rapidly between 15 and 30 min (Fig. 1). The pH profile of methyltransferase showed optima of activity between pH 6 and 7 and 8 and 9 (Fig. 2). The pH profile for methylation of endogenous phospholipids (Fig. 2, A, B, and C) was similar to that determined after the addition of exogenous phospholipid methyl acceptors (Fig. 2, D, E, and F). Substitution of Tris base for Tris-maleate buffer from pH 7.4–8.5 resulted in an activity profile similar to that shown in Fig. 2 (data not shown). Methyltransferase activity was not dependent upon the presence of cations with EDTA (1 mM) present, and addition of up to 1 mM Mg^{2+} did not significantly affect [^{3}H]methyl incorporation in phospholipids when compared to methylation in the absence of added cation (data not shown). A higher concentration of Mg^{2+} (10 mM) depressed [^{3}H]methyl incorporation in PMME, PDME, and PC by 23 ± 11, 31 ± 12 ($p < 0.05$), and 52 ± 17% ($p < 0.05$) of maximum methylation in the absence of added cation.

Methyltransferase activity was affected by the presence of exogenous phospholipid. Optimum [^{3}H]methyl

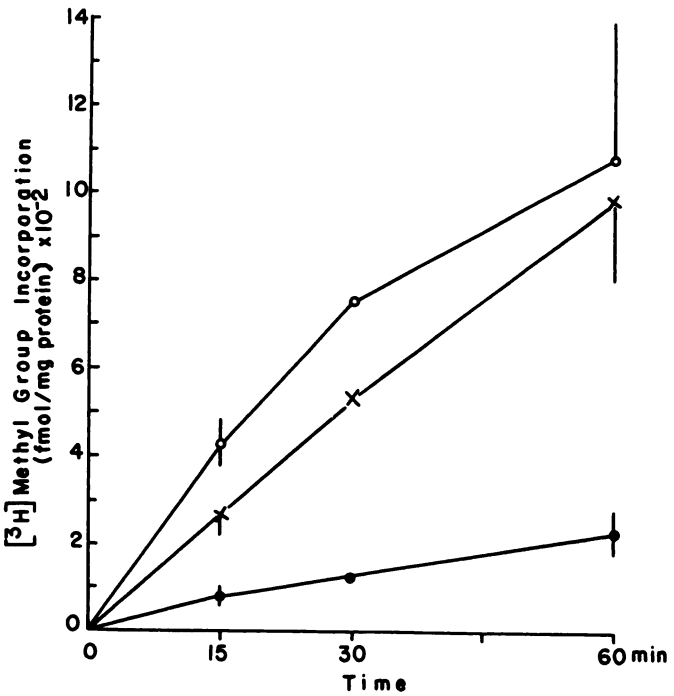

FIG. 1. *Time course of [^{3}H]methyl incorporation into islet phospholipids*

Aliquots of islet homogenate (23 ± 2 μg of protein) were incubated with [^{3}H]AdoMet (1 μCi), $MgCl_2$ (1 mM), and 50 μg each of PE, PMME, and PDME, at pH 9.0 for up to 60 min. Values for [^{3}H]methyl incorporation into PMME (●), PDME (○), and PC (×) are means ± SE in femtomoles/mg of protein for 4–16 different experimental determinations.

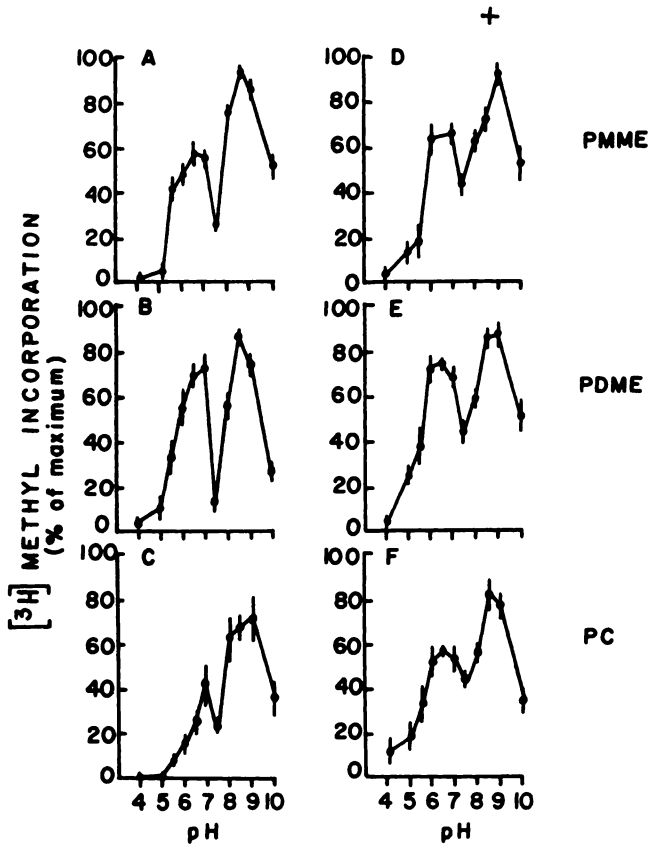

FIG. 2. *Effect of pH on [³H]methyl incorporation into phospholipids*
Aliquots of islet homogenate (16 ± 2 μg of protein) were incubated with [³H]AdoMet (1 μCi), and the presence (D, E, F) or absence (A, B, C) of 50 μg each of PE, PMME, and PDME, for 1 hr. Values are means ± SE for [³H]methyl incorporation in PMME (A, D), PDME (B, E), and PC (C, F) for 7–8 independent experimental determinations. Maximum [³H]methyl incorporation values for PMME, PDME, and PC in the absence of exogenous lipid were 800 ± 100, 1816 ± 462, and 1042 ± 100 fmol/mg of protein, respectively; maximum values in the presence of exogenous lipids were 969 ± 281, 2622 ± 397, and 1672 ± 481 fmol/mg of protein, respectively.

incorporation during 60 min was attained when 50 μg each of PE, PMME, and/or PDME was included in the assay at pH 9. Addition of 50 μg each PE, or PMME, or PDME enhanced [³H]methyl incorporation into their corresponding methylated phospholipids by 283 ± 87, 371 ± 96 ($p < 0.05$), and 844 ± 90% ($p < 0.001$) of incorporation, respectively, observed in the absence of exogenous lipid. Since each exogenous phospholipid primarily affected the recovery of [³H]methyl groups in the next higher methylated phospholipid, it is likely that PE, PMME, and PDME enhanced specific steps in the methylation sequence. When 50 μg each PE, PMME, and PDME were added together to the assay, [³H]methyl incorporation in the next higher methylated phospholipids did not significantly differ from the values obtained after adding the lipids separately.

Agents which stimulate or inhibit methyl incorporation. Further characterization of PE methyltransferase activity in islets was possible using various inhibitors. AdoHcy, which competitively inhibits PE methylation (6), depressed basal islet methyltransferase activity by as much as 80% (Table 1). The detergents DOC and Triton X-100 also markedly inhibited methyl group incorporation into phospholipids (Table 1).

A glucose concentration (17 mM) capable of eliciting maximum insulin release in intact islets (16) failed to alter significantly [³H]methyl incorporation into any of the phospholipids with homogenate preparations (Fig. 3). The nonmetabolizable, nonsecretory sugar galactose also failed to affect the methylation of phospholipids and served as an osmotic control in these experiments (Fig. 3). The β-adrenergic agonist and insulin secretagogue isoproterenol increased methyl group incorporation into PMME, PDME, and PC by up to 6-fold (Fig. 3). Accumulation of [³H]PMME and [³H]PC was most notable in preparations treated with the β-adrenergic agonist. The effects of isoproterenol were blocked by the β-adrenergic antagonist propranolol (Fig. 3).

Phospholipid methylation in intact islets. Intact islet phospholipids were labeled with methyl groups donated by [³H]methionine. Incorporation of [³H]methyl from methionine into PMME, PDME, and PC of intact islets occurred most rapidly within 5 min; after 60 min, [³H]methyl incorporation was further increased by only 40 to 60% (Fig. 4).

[³H]Methyl incorporation in phospholipids was investigated in islets exposed to [³H]methionine and various agents with known effects on hormone release. The physiological insulin secretagogue glucose (17 mM) enhanced [³H]methyl incorporation into PMME, PDME, and PC after 60 min (Table 2). However, agents that do not stimulate insulin release, such as a substimulatory glucose concentration (2.8 mM) or the nonmetabolized sugar mannitol, elicited similar levels of phospholipid [³H]methyl incorporation which were significantly lower ($p < 0.05$) than those elicited by 17 mM glucose (Table 2). 3-Deazaadenosine, an inhibitor of PE methyltransferase activity in intact cells, completely inhibited glucose-stimulated methylation and reduced [³H]methyl incorporation in phospholipids to levels attained in the presence of 3-deazaadenosine and control glucose (2.8 mM) levels (Table 2). The sulfonylurea tolbutamide, and 8-Br-cyclic AMP, although proven stimuli for secretion in islets (17, 18), did not enhance methylation. [³H]Methyl incorporation in PMME, PDME, and PC combined fractions of islets treated with 1 mM tolbutamide (337 ± 63 dpm/200 islets) or 5 mM 8-Br-cyclic AMP (266 ± 5 dpm/200 islets) for 60 min was 73 ± 5 ($p < 0.01$, $n = 9$) and 75 ± 6% ($p < 0.01$, $n = 9$), respectively, of paired control (2.8 mM glucose) islet tritium levels. When islets were exposed to tolbutamide (1 mM) for 5 min, [³H]methyl incorporation was 99 ± 3% of control ($p > 0.05$, $n = 3$).

The time-dependency of secretagogue-induced phospholipid methylation also was investigated. Glucose stimulated methylation in a biphasic manner over 60 min, with the first peak of methylation detectable within 2.5 min after the onset of stimulation and a second peak of methylation detectable after 30–60 min (Fig. 5A). [³H]Methyl incorporation in PMME, PDME, and PC was increased by 118 ± 7 ($p < 0.05$), 148 ± 13 ($p < 0.02$), and 120 ± 51% ($p < 0.05$) of control, respectively, after 2.5 min of glucose (17 mM) stimulation. Insulin release in

TABLE 1

Inhibitors of [^{3}H]methyl incorporation from AdoMet

[^{3}H]Methyl incorporation into phospholipids after 60 min was determined in the presence of islet homogenate (20 ± 2 μg of protein), [^{3}H] AdoMet (1 μCi), and 50 μg each PE, PMME, and PDME, Tris buffer (0.1 mM), pH 9, and the absence (control) or presence of AdoHcy, DOC, or Triton X-100 as indicated. Values are means ± SE for the number of independent determinations (n). Significance levels ($^a p < 0.01$, $^b p < 0.02$, $^c p < 0.05$) were determined by Student's t test for paired values.

Treatment pairs	[^{3}H]Methyl incorporation					
	PMME	(n)	PDME	(n)	PC	(n)
	fmol/mg protein					
Control	686 ± 150	(8)	3490 ± 1590	(5)	1060 ± 635	(6)
AdoHcy (0.1 mM)	166 ± 73^a	(8)	802 ± 190^c	(5)	99 ± 38^b	(6)
	(29 ± 11%)		(36 ± 13%)		(20 ± 8%)	
Control	828 ± 111	(6)	2140 ± 587	(6)	1490 ± 540	(7)
Triton X-100 (0.1%)	362 ± 100^b	(6)	83 ± 55^b	(6)	62 ± 42^a	(7)
	(47 ± 10%)		(5 ± 3%)		(14 ± 11%)	
Control	425 ± 130	(5)	1450 ± 746	(5)	1160 ± 444	(5)
DOC (1 mM)	167 ± 92^b	(5)	760 ± 475^c	(5)	563 ± 352^c	(5)
	(26 ± 11%)		(26 ± 17%)		(31 ± 12%)	

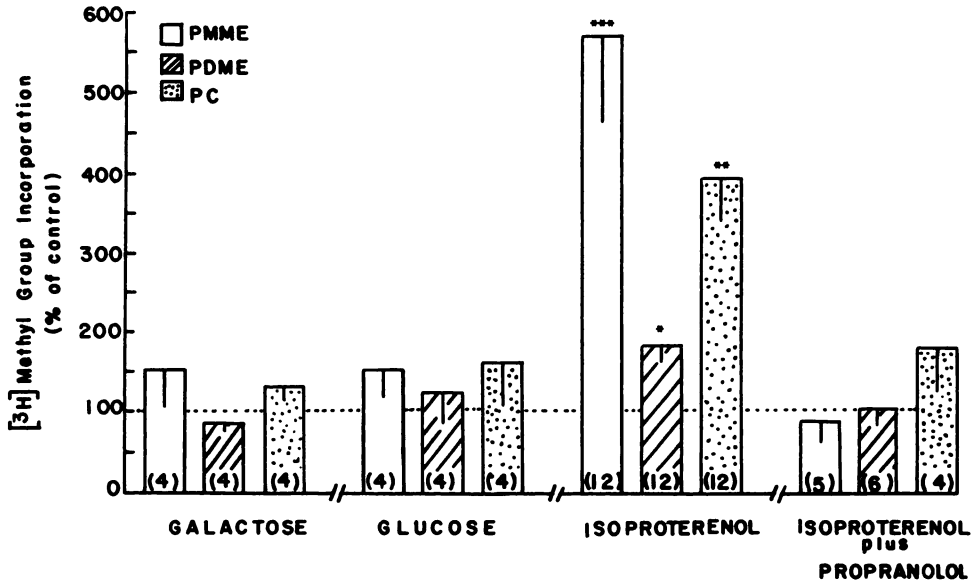

FIG. 3. *Effects of various agents on [^{3}H]methyl incorporation into phospholipids*

Aliquots of islet homogenate (17 ± 2 μg of protein) were incubated with [^{3}H]AdoMet (1 μCi), 50 μg each of PE, PMME, and PDME, 1 mM $MgCl_2$, and the absence (control) or presence of 17 mM galactose, 17 mM glucose, 0.1 mM isoproterenol, or 0.1 mM isoproterenol and 0.2 mM propranolol, for 15 min at pH 9.0. Values are means ± SE percentage of control for [^{3}H]methyl group incorporation into PMME, PDME, and PC. [^{3}H]Methyl control values for PMME, PDME, and PC were 116 ± 35, 513 ± 93, and 226 ± 63 fmol/mg of protein, respectively. Significance levels ($^* p < 0.05$, $^{**} p < 0.02$, $^{***} p < 0.01$) determined by Student's t test for paired values. Number of independent experimental determinations is shown in parentheses.

response to glucose also was elevated significantly within 2.5 min of stimulation, and hormone release continued to be stimulated throughout 60 min of islet exposure to glucose (Fig. 5A).

In contrast to the glucose response, isoproterenol-induced phospholipid methylation was characterized by a short lag followed by enhanced phospholipid methylation within 5 min of the onset of stimulation; thereafter, tritium incorporation declined until a second period of stimulated [^{3}H]methyl incorporation was observed after 60 min (Fig. 5B). [^{3}H]Methyl recovery in PMME, PDME, and PC after exposure to isoproterenol (0.1 mM) for 5 min increased by 147 ± 12 ($p < 0.02$), 134 ± 7 ($p <$ 0.02), and 119 ± 11% of control, respectively. Pretreatment of islets with 3-deazaadenosine (20 μM) inhibited the methylation response to isoproterenol, such that [^{3}H] methyl incorporation with isoproterenol (0.1 mM) and 3-deazaadenosine after 5 min was 89 ± 12% of unstimulated control islet [^{3}H]methyl levels. Insulin release in response to isoproterenol was significantly elevated within 2.5 min after the onset of stimulation, and insulin levels remained elevated throughout 60 min (Fig. 5B). When islets were exposed to a lower concentration of isoproterenol (10 μM) for 5 min, phospholipid [^{3}H]methyl incorporation increased to 123 ± 11% of control ($p <$

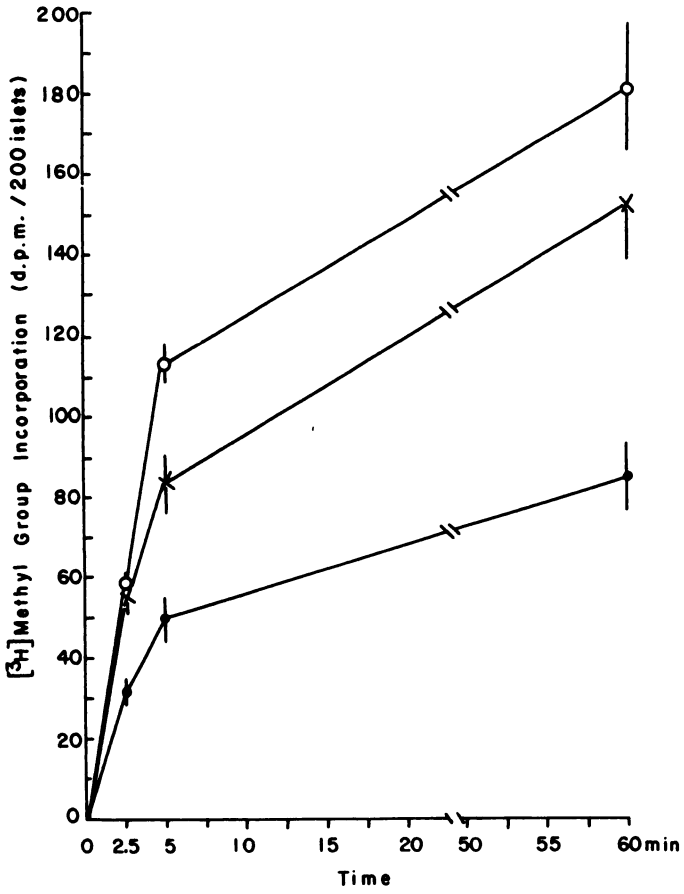

FIG. 4. *Time course of intact islet [³H]methyl incorporation into phospholipids*

Islets were incubated in Krebs-Ringer bicarbonate HEPES buffer, pH 7.4, containing glucose (2.8 mM), GSH (1 mM), and [³H]methionine (10 μCi) for up to 60 min. Values for [³H]methyl incorporation into PMME (●), PDME (○), and PC (×) are mean ± SE disintegrations per min per 200 islets in 6–20 independent experimental determinations.

0.05), and insulin release was increased to 163 ± 9% of control ($p < 0.02$).

In order further to define the relationship of PE methylation to insulin release, islets were exposed to the methyltransferase inhibitor 3-deazaadenosine. 3-Deazaadenosine significantly inhibited the early phase (5 min) of insulin release elicited by glucose and isoproterenol (Table 3). Although glucose stimulated phospholipid methylation and insulin secretion after 60 min (Fig. 5, Table 3), the inhibition of methylation by 3-deazaadenosine was not accompanied by a significant reduction in insulin release after 60 min (Table 3). Thus, the early and late phases of glucose-induced insulin release were affected differently by 3-deazaadenosine.

In experiments designed to determine the effects of isoproterenol on glucose-induced hormone secretion and phospholipid methylation, isoproterenol enhanced both the rapid glucose-induced phospholipid methylation response and insulin release during 2.5 min of incubation (Table 4). Isoproterenol did not significantly affect glucose-induced [³H]methyl incorporation and insulin release when propranolol was present (Table 4).

In order to determine the specificity of the effects of glucose on methylation and insulin release, islets were exposed to 2-deoxyglucose, which is transported into cells but is not phosphorylated and metabolized. [³H]Methyl incorporation in phospholipids of islets exposed to 2-deoxyglucose (17 mM) for 2.5 min (114 ± 10 dpm/200 islets) was 87 ± 15% ($p > 0.05$) of basal levels (141 ± 10 dpm/200 islets) in three independent determinations. Insulin release was unaffected by 2-deoxyglucose (103 ± 7% of release from paired control islets with 2.8 mM glucose). Thus, in intact islets, 2-deoxyglucose did not mimic the effects of glucose on phospholipid methylation or insulin release.

TABLE 2

Effect of various agents on intact islet phospholipid methylation

Islets were incubated in Krebs-Ringer bicarbonate HEPES buffer containing 2.8 mM glucose (control), [³H]methionine (10 μCi), and the agents indicated for 60 min. 3-Deazaadenosine (3-DZA) was added to islets 10 min prior to time zero for stimulus addition. Values are means ± SE for the number of paired independent determinations indicated (n). Significance levels determined by Student's t test (paired) using disintegrations per min values ([a]$p < 0.05$ compared to paired control; [b]$p < 0.05$ compared to paired mannitol; [c]$p < 0.05$ compared to paired 17 mM glucose).

Treatment pairs	[³H]Methyl incorporation			
	PMME	PDME	PC	(n)
	dpm/200 islets			
Control	111 ± 20	248 ± 42	183 ± 33	(5)
Glucose (17 mM)	132 ± 13[a]	343 ± 51[a]	269 ± 34[a]	(5)
	(128 ± 13%)	(144 ± 13%)	(153 ± 14%)	
Mannitol (17 mM)	75 ± 25	220 ± 84	167 ± 64	(5)
Glucose (17 mM)	157 ± 30[b]	348 ± 85[b]	285 ± 66[b]	(5)
	(260 ± 65%)	(211 ± 39%)	(251 ± 65%)	
Glucose (17 mM)	156 ± 46	394 ± 103	421 ± 117	(4)
Glucose (17 mM) *plus* 3-DZA (20 μM)	80 ± 27[c]	166 ± 44[c]	169 ± 32[c]	(4)
Control	105 ± 37	239 ± 84	229 ± 48	(4)
3-DZA (20 μM)	69 ± 19[a]	147 ± 52[a]	158 ± 34[a]	(4)
	(74 ± 1%)	(63 ± 5%)	(70 ± 4%)	

DISCUSSION

In the present study, the identification and characterization of islet PE transmethylation reactions using AdoMet as a methyl donor and determination of the rapid response of phospholipid methylation to insulin secretagogues suggest that PE methylation is an early contributory event in islet hormone release. Islet phospholipid metabolism has been characterized previously as participating in stimulus-secretion coupling since secretagogues stimulate phospholipid fatty acid (2, 19) and phosphate turnover (20, 21), in addition to the biosynthesis of phospholipids from glycerol (22). The biochemical characterization of islet transmethylation reactions that included inhibition by the AdoMet competitive inhibitor AdoHcy, 3-deazaadenosine, and the detergent action of DOC and Triton X-100 is consistent with the effects of these agents on PE-*N*-methyltransferase activity in other tissues (5, 6, 11, 23–27). In addition, pH optima of 6 and 9, and cation independence for methyl-

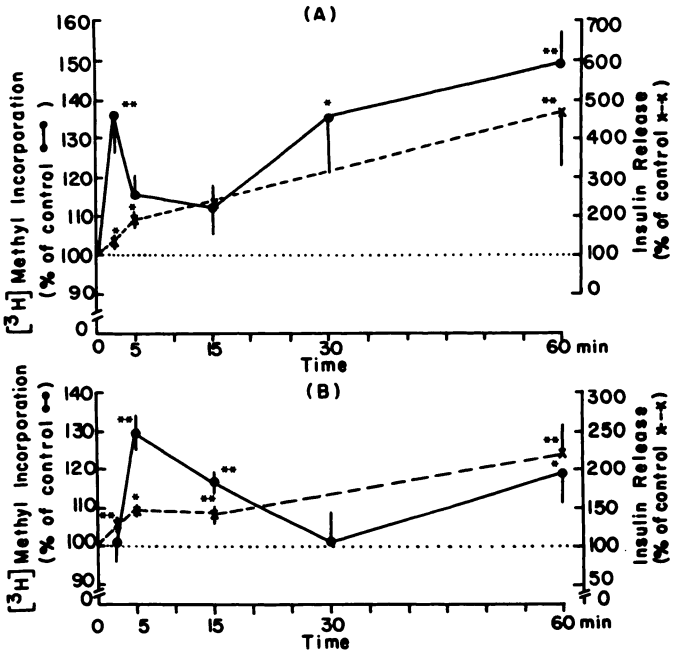

FIG. 5. *Effects of glucose and isoproterenol on insulin release and [³H]methyl incorporation into lipids in intact islets*

Islets were incubated for various lengths of time in Krebs-Ringer bicarbonate HEPES buffer, pH 7.4, containing [³H]methionine (10 μCi), GSH (1 mM), glucose (2.8 mM, control), or in (A) glucose (17 mM), or with (B) the addition of isoproterenol (0.1 mM). [³H]Methyl incorporation into PMME, PDME, and PC combined fractions is represented as mean ± SE percentage of paired control disintegrations per min for (A) glucose or (B) isoproterenol-treated islets. Insulin release (microunits/ml) is represented as mean ± SE percentage of control in (A) glucose or (B) isoproterenol-treated islets. Significance levels ($^*p < 0.05$, $^{**}p < 0.01$) determined by Student's *t* test (paired) for disintegrations per min or microunits of insulin/ml values. The number of independent experimental determinations are 4–9.

ation are consistent with the kinetics of PE-methyltransferases in certain other tissues (4, 6, 7, 23, 26–28).

Exogenous PMME and PDME either stimulated methyltransferase methylation of endogenous phospholipids or behaved as methyl acceptors, as reported for brain methyltransferase (7, 27). On the other hand, the addition of PE to islets as well as other (4, 7, 27) crude methyltransferase preparations demonstrated modest effects of this phospholipid on subsequent methylation steps, perhaps due to saturating levels of membranous PE or to the hindered accessibility of exogenous PE to the methyltransferase. As in other tissues (23), the conversion of PE to PMME in islets is likely to be rate limiting since PMME accumulated to a lesser degree than did PDME or PC. In islet homogenates [³H]methyl incorporation into PC initially lagged behind the incorporation into PDME. However, methyl incorporation into PDME approached steady state levels more rapidly than did the incorporation into PC, indicating that PC formation depended in part upon metabolism of PE and/or PMME as well as on preexisting PDME (29). [³H]PC probably did not accumulate to a greater extent than [³H]PDME in intact islet membranes due to PC degradation by phospholipases (2).

The sensitivity of islet phospholipid [³H]methyl incor-

TABLE 3

Effect of 3-deazaadenosine on insulin release

Islets (30 per treatment) were incubated in Krebs-Ringer bicarbonate HEPES buffer containing 2.8 mM glucose (basal) and the other agents as indicated. Aliquots of incubation medium were removed at the specified times and insulin content was determined by radioimmunoassay. Values are means ± SE for insulin release as a percentage of insulin levels at the time of agent addition (zero time). Mean zero time insulin value for all experiments combined was 105 ± 6 microunits/ml. Significance levels determined by analysis of variance ($^ap < 0.05$ compared to basal) or by Student's *t* test ($^bp < 0.05$ compared to glucose (17 mM); $^cp < 0.02$ compared to isoproterenol (0.1 mM)) for per cent zero time values. Numbers in parentheses (*n*) represent different experimental determinations.

	Insulin release			
	5 min	(*n*)	60 min	(*n*)
	% zero time			
Basal	107 ± 17	(4)	164 ± 14	(9)
3-Deazaadenosine (20 μM)	109 ± 20	(4)	219 ± 37	(4)
Glucose (17 mM)	193 ± 20[a]	(5)	955 ± 129[a]	(5)
Glucose (17 mM) plus 3-Deazaadenosine (20 μM)	131 ± 11[b]	(5)	867 ± 176[a]	(5)
Isoproterenol (0.1 mM)	193 ± 28[a]	(7)		
Isoproterenol (0.1 mM) plus 3-Deazaadenosine (20 μM)	111 ± 7[c]	(7)		

TABLE 4

Insulin release and methyl group incorporation into islet phospholipids

Islets were incubated for 2.5 min in Krebs-Ringer bicarbonate HEPES buffer containing [³H]methionine (10 μCi), glucose (17 mM), glucose (17 mM) plus isoproterenol (0.1 mM), glucose (17 mM) plus propranolol (0.2 mM), or glucose (17 mM) plus isoproterenol (0.1 mM) plus propranolol (0.2 mM) as indicated. In experiments where propranolol was used, the drug was added 10 min prior to glucose and isoproterenol. For determination of per cent change with drug treatment, experiments were conducted as paired incubations with glucose (17 mM)-stimulated islets. Radiolabel incorporation into PMME, PDME, and PC is represented as disintegrations per min per 200 islets, and insulin release values are normalized per 200 islets. Values are means ± SE for the number of independent determinations represented as *n*. Significance level determined by Student's *t* test for paired disintegrations per min or microunits of insulin/ml values; $^ap < 0.05$.

Treatment pairs	[³H]Methyl incorporation	Insulin release
	dpm	*microunits/ml*
Glucose	179 ± 21	270 ± 59
Glucose plus isoproterenol	224 ± 26[a]	345 ± 65[a]
	(127 ± 10%)	(133 ± 13%)
	n = 5	*n* = 5
Glucose	153 ± 47	283 ± 52
Glucose plus propranolol	205 ± 39	379 ± 104
	(115 ± 5%)	(112 ± 16%)
	n = 4	*n* = 3
Glucose	179 ± 29	230 ± 39
Glucose plus isoproterenol plus propranolol	208 ± 21	282 ± 72
	(120 ± 9%)	(126 ± 17%)
	n = 5	*n* = 5

poration to β-adrenergic agonism was demonstrated for intact islets as well as broken cell preparations. In homogenates, isoproterenol enhanced methylation by up to 5-fold, whereas in intact cells, where substrate availability and AdoMet levels are physiologically regulated, isoproterenol evoked only a 30% increase in methyl group incorporation. Furthermore, since enhanced methyl recovery in islet PMME and PDME occurred within 5 min after isoproterenol stimulation, these methylated intermediates may modulate the secretory response. Enhanced accumulation of the methylated lipid intermediates can inhibit Ca^{2+}-ATPase activity (15), thus promoting Ca^{2+} mobilization in cells and modulating the secretory response. In intact islets, isoproterenol stimulated the sequential methylation of PE, whereas in homogenates isoproterenol especially enhanced accumulation of PMME and PC. Calcium-dependent phospholipase hydrolysis of PC in the absence of added calcium in homogenate preparations may not occur to as great an extent as in intact cells, thus favoring the accumulation of PC in homogenates. β-Adrenergic receptor agonism enhances methylation in a variety of other tissues and propranolol blocks this effect (12). Unlike isoproterenol, glucose at secretory concentrations failed to affect PE methylation in broken cell preparations. On the other hand, glucose potently stimulated PE methylation in intact islet cells. The latter response appeared to depend upon glucose phosphorylation and/or oxidation, since 2-deoxyglucose failed to mimic the glucose effect.

The results also suggest that isoproterenol and glucose affect methyltransferase activity in islets in diverse ways. Isoproterenol evokes a response via receptor stimulation, whereas glucose metabolism may affect enzyme activity directly or affect methionine uptake and/or metabolism and thus the cellular AdoMet/AdoHcy ratio. Since isoproterenol enhanced methyl incorporation in response to a maximal glucose concentration, the two secretagogues may have different mechanisms of action. Since isoproterenol enhanced the early phase of glucose-induced insulin release, lipid methylation may be a common mediator of the secretory effects of the two agents and contribute to enhanced adenylate cyclase activity or changes in Ca^{2+} mobilization (14, 15, 25).

In order to determine if PE methylation and insulin secretion were correlated, a time course comparison of both events was carried out. A two-phase insulin release profile is not evident in static islet incubations; however, methyl incorporation appeared to mimic the insulin secretion profile identifiable in a dynamic perifusion system in which islets release insulin within 2 to 5 min following glucose stimulation, after which the rate of release declines for several minutes and then increases to a maximum release rate within 60 min (16). The recovery of [^{3}H]methyl phospholipids may have declined in stimulated islets due to increased hydrolysis by activated phospholipase A_2 or increased levels of AdoHcy following the initial period of stimulation. These data suggest that PE methylation may amplify the glucose recognition signal and modulate insulin release. Inhibition of the early phase of insulin release coincident with methyltransferase inhibition by 3-deazaadenosine is supportive of a role for PE methylation in insulin release; however, 3-deazaadenosine may affect other islet methylation reactions which affect secretion.

Certain of the data suggest, however, that PE methylation is not a *sine qua non* for secretion. Inhibition of methylation by 3-deazaadenosine did not affect the late phase of glucose-induced insulin release. And, although 3-deazaadenosine inhibited insulin release in response to isoproterenol during a time when methylation would have been maximally stimulated, a small early increase in insulin release induced by isoproterenol was not accompanied by detectable changes in methylation. In addition, the insulin secretagogues tolbutamide (2) and 8-Br-cyclic AMP (18) inhibited islet methyltransferase activity. These latter agents may affect secretion at a cell locus distal to the generation of methylated phospholipids. In other tissues, cyclic AMP analogues have no effect (12) or inhibit PE methylation (30), perhaps due to protein kinase-regulated changes in AdoMet/AdoHcy concentrations. Tolbutamide also enhances adenylate cyclase activity (31). However, since isoproterenol and glucose stimulate methyltransferase activity and enhance adenylate cyclase activity (32), it seems unlikely that cyclic AMP mediates the dissimilar effects of isoproterenol, glucose, and tolbutamide on methyltransferase.

In conclusion, islet PE-*N*-methyltransferase appears to be a modulator of the early cellular events regulating insulin release. Phospholipid methyl incorporation may amplify events leading to insulin secretion through alterations in membrane viscosity and fusogenicity, ion transport, or enzyme activation (13–16). These functional alterations may be brought about by either enhanced levels of methylated phospholipids and/or the high level of unsaturated fatty acids in membrane PC.

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