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IMPAIRMENT OF INSULIN RELEASE BY METHYLATION INHIBITORS

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Abstract—The possible participation of enzymatic methylation reactions in the process of insulin release was investigated in rat pancreatic islets. The combination of 3-deazaadenosine and DL-homocysteine impaired the incorporation of ^{3}H-methyl from L-[methyl-^{3}H]methionine into endogenous islet proteins and phospholipids, but failed to affect turnover in the phosphatidylinositol cycle. The inhibitors of methylation decreased insulin release evoked by D-glucose or the combinations of D-glucose and gliclazide, L-leucine and L-glutamine, or Ba^{2+} and theophylline. The inhibitors of methylation did not impair either the oxidation of D-glucose or affect its capacity to decrease K^+ conductance, stimulate Ca^{2+} inflow and provoke ^{45}Ca accumulation in pancreatic islets. It is proposed that, in the process of insulin secretion, a methyl acceptor protein and/or phospholipid play(s) a limited modulatory role in the coupling of cytosolic Ca^{2+} accumulation to exocytosis.

The release of insulin evoked by glucose and other secretagogues represents the outcome of a series of cytophysiological events including, as the major steps in stimulus-secretion coupling, the identification of the secretagogue, an extensive remodelling of ionic fluxes and the activation of the effector system responsible for the translocation and exocytosis of secretory granules [1]. In addition to these three major steps, a number of coinciding events have been identified in stimulated islet cells. They include, for instance, changes in biosynthethic activity [2], intercellular coupling [3], membrane viscosity [4] and phospholipid turnover [5]. In the present study, we have investigated the possible significance of enzymatic methylation reactions in the secretory behaviour of the pancreatic B-cell.

MATERIALS AND METHODS

All experiments were performed with pancreatic islets removed from fed albino rats [6]. The methods used to measure insulin release [7], glucose oxidation [8], ^{45}Ca net uptake [9], cyclic AMP production [10] and ^{32}P labelling of phospholipids [11] in incubated islets, or ^{45}Ca [12] and ^{86}Rb [13] outflow and insulin release [14] from perifused islets were all described in previous publications. In the ^{32}P-labelling experiments, each incubation was terminated by addition of 1 ml ice-cold 20% (w/v) trichloroacetic acid. After 10 min centrifugation at 1000 *g* and 4°, the supernatant was discarded and the pellet extracted with 3 ml $CHCl_3$:CH_3OH:13 N HCl (200:100:1, v/v). The extracts were washed successively with 1 ml H_2O:CH_3OH (2:1, v/v) and the lipids separated by the one-dimensional thin layer chromatography method described by Fine and Sprecher [15]. Each lipid fraction was scraped into a counting vial, mixed with 2.5 ml CH_3OH and 10 ml Aquasol-2 (New England Nuclear, Boston, MA), and examined for its radioactive content by liquid scintillation.

To measure the incorporation of ^{3}H-methyl from L-[methyl-^{3}H]methionine (79 Ci/mmol) in islet proteins and lipids, groups of 150 islets were incubated for 30 min in 1.0 ml of our usual bicarbonate-buffered medium [7] containing 115 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.5% (w/v) bovine albumin and equilibrated (pH 7.4) with CO_2:O_2 (95:5), either in the presence or absence of methylation inhibitors. Alternatively the islets were preincubated for 15 min with or without the drugs prior to the addition of L-[methyl-^{3}H]methionine. Incubations were stopped by the addition of 2 ml of trichloroacetic acid (10%, v/v). After centrifugation (10 min, 1000 *g*, 4°), the precipitate was washed with 2 ml trichloroacetic acid, centrifuged again and mixed with 3 ml $CHCl_3$:CH_3OH:13 N HCl (200:100:1, v/v) to extract lipids. Following the addition of 1 ml of H_2O and vigorous mixing, the upper aqueous phase and the interface containing precipitated proteins were removed, mixed with 2 ml soluene 350 (Packard, Downers Grove, IL) to solubilize protein and 10 ml Aquasol-2, and examined for their radioactive content by liquid scintillation. The lower chloroform phase was washed with 2 ml CH_3OH:H_2O (1:2, v/v), dried in a vacuum oven, and resuspended in 0.2 ml $CHCl_3$:CH_3OH (2:1, v/v). The lipids were separated by a two-dimensional thin layer chromatography method which enables satisfactory separation of all major phospholipid classes [16]. Individual lipid spots were identified by iodine vapour, scraped into counting vials, mixed with 2.5 ml CH_3OH and 10 ml Aquasol-2, and examined for their radioactive content. In order to measure pro-

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tein methylation, the TCA-precipitated pellet was washed once with 2 ml 10% (w/v) trichloroacetic acid, once with 2 ml H_2O and finally dissolved in 0.5 ml 0.1 M borate buffer (pH 11.0) containing 7% (v/v) CH_3OH. This solution was placed in a small plastic cup inside a sealed counting vial containing 1 ml CH_3OH and incubated overnight as described by Campillo and Ashcroft [17]. The radioactive material thus liberated during protein hydrolysis was trapped as ^{3}H-methanol, which was counted to give an estimate of specific protein methylation.

All results are expressed as the mean (± S.E.M.) together with the number of individual determinations (N). The statistical significance of differences between mean values was assessed by Student's *t*-test.

RESULTS

Protein and lipid methylation. When islets were incubated for 30 min in the presence of D-glucose (1.7 mM) and L-[methyl-^{3}H]methionine (0.13 μM), the incorporation of radioactivity in islet proteins averaged 8.70 ± 1.10 fmoles/islet (N = 5), when calculated by reference to the specific radioactivity of L-methionine added to the incubation medium. The incorporation was grossly proportional to the concentration of L-methionine in the 0.13–0.67 μM range (data not shown). When 3-deazaadenosine (0.1 mM) and DL-homocysteine (0.2 mM) were present in the incubation medium, the incorporation of radioactivity in TCA-precipitate material obtained after extraction of islet lipids was decreased to 63.8 ± 3.6% of the mean corresponding control value (100.0 ± 3.3, N = 21 in both cases). At the low concentration of D-glucose used in these experiments, the incorporation of radioactivity from L-[methyl-^{3}H]methionine into TCA-precipitable material was not attributable, to any large extent, to *de novo* biosynthesis of islet proteins. Thus, over 30 min incubation in the presence of D-glucose (1.7 mM), the combination of cycloheximide (70 μM) and puromycin (200 μM), which inhibited the incorporation of L-[U-^{14}C]leucine (40 μM) into TCA-precipitable material by more than 75%, failed to affect significantly the labelling of TCA-precipitable material in islets exposed to L-[methyl-^{3}H]-methionine (0.13 μM), the experimental values averaging 98.4 ± 1.1% (N = 5) of the mean control value (10.89 ± 0.29 fmoles/islet; N = 5). The methylation inhibitors (3-deazaadenosine, 0.1 mM, and DL-homocysteine, 0.2 mM) markedly decreased the amount of radioactivity derived from L-[methyl-^{3}H]-methionine, incorporated into TCA-precipitable material and further liberated under mildly alkaline conditions, the experimental values averaging 45.7 ± 3.4% of the corresponding control value (100.0 ± 4.1%; N = 12 in each case). When the same experiments were repeated at a higher glucose concentration (16.7 mM), no significant difference was found in either the incorporation of radioactivity in the material liberated by alkaline treatment or its inhibition by the methylation inhibitors. The total incorporation of radioactivity in the TCA-precipitable material was slightly increased, however, the results obtained in the presence of 16.7 mM D-glucose being 11.2 ± 1.9% higher ($P < 0.005$) than the paired value found in the presence of 1.7 mM D-glucose (N = 6).

At the same concentrations of D-glucose (1.7 mM) and L-[methyl-^{3}H]methionine (0.13 μM) as above, the incorporation of radioactivity in islet lipids averaged 56.6 ± 4.9 amoles/islet (N = 6), of which 51% was associated with phosphatidylcholine and lysophosphatidylcholine and 35% migrated in the vicinity of phosphatidylethanolamine and lysophosphatidylethanolamine (Table 1). These data do not include the radioactivity present in the lipid extract which remained at the origin during the chromatographic separation procedure: this represented 88.9 ± 1.3% of the total radioactivity present in the extract. The incorporation of ^{3}H-methyl into islet lipids was proportional to the concentration of L-methionine in the 0.13–0.67 μM range (data not shown). When the incubation was carried out in the presence of 3-deazaadenosine (0.1 mM) and DL-homocysteine (0.2 mM), the incorporation of radioactivity into each phospholipid was severely decreased, except for the low readings recorded for phosphatidate and phosphatidylinositol. The decrease in ^{3}H-methyl incorporation was even more marked when the islets were preincubated in the presence of the methylation inhibitors prior to being exposed to radioactive L-methionine (Table 1). Over 30 min incubation, the incorporation of radioactivity in the islet lipids was not significantly different in the

Table 1. Effect of 3-deazaadenosine and DL-homocysteine upon the incorporation of ^{3}H-methyl from L-[methyl-^{3}H]methionine (0.13 μM) into islet phospholipids

	Control 30 min (N = 6)	3-Deazaadenosine (0.1 mM) and DL-homocysteine (0.2 mM) 30 min (N = 3)*	15 + 30 min (N = 3)*
Phosphatidylcholine	20.71 ± 0.83†	7.13 ± 0.63[d]	4.82 ± 0.16[d]
Lysophosphatidylcholine	8.32 ± 0.75	4.96 ± 0.21[a]	3.03 ± 0.39[b]
Phosphatidylethanolamine	14.40 ± 0.97	7.08 ± 0.35[c]	3.31 ± 0.56[d]
Lysophosphatidylethanolamine	5.49 ± 0.45	2.90 ± 0.16[d]	1.24 ± 0.23[b]
Phosphatidate	4.68 ± 0.42	5.21 ± 0.71	3.11 ± 0.18
Phosphatidylinositol	2.98 ± 0.32	3.50 ± 0.53	1.85 ± 0.03

* The islets were either incubated for 30 min in the presence of the two inhibitors and L-[methyl-^{3}H]methionine (second column), or preincubated for 15 min in the presence of the inhibitors and then incubated for 30 min in the presence of both the inhibitors and L-[^{3}H-methyl]methionine (third column).

† Mean values (± S.E.M.) are expressed as amoles/islet and are shown together with the statistical significance (a: $P < 0.05$; b: $P < 0.025$; c: $P < 0.005$; d: $P < 0.001$) of differences between experimental and control values.

Table 2. Effect of 3-deazaadenosine and DL-homocysteine upon insulin release evoked by D-glucose (16.7 mM)

Inhibitors (mM)	Insulin output (μU/90 min per islet)
Nil	310.9 ± 10.5 (65)*
3-Deazaadenosine (0.1)	279.8 ± 7.0 (48)[a]
DL-Homocysteine (0.2)	262.5 ± 13.7 (46)[b]
3-Deazaadenosine (0.1) + DL-homocysteine (0.2)	229.9 ± 7.8 (68)[c]

* Mean values (± S.E.M.) are shown together with the number of individual determinations (in parentheses) and the statistical significance (a: $P < 0.03$; b: $P < 0.01$; c: $P < 0.001$) of differences between experimental and control values.

presence of D-glucose 1.7 and 16.7 mM, respectively. Thus, the ratio between paired values (high/low glucose incubation) averaged 0.98 ± 0.02 (N = 4).

Insulin release. Insulin release evoked by D-glucose (16.7 mM) was inhibited by either 3-deazaadenosine or DL-homocysteine (Table 2). In the presence of both these inhibitors, the rate of insulin release was significantly lower ($P < 0.05$) than in the presence of each drug alone. The combination of the two inhibitors failed to affect significantly the basal release of insulin found at a low concentration of D-glucose (2.8 mM), but inhibited insulin secretion evoked by either nutrient or non-nutrient secretagogues (Table 3). The relative extent of inhibition was comparable in the presence of 7.0 mM D-glucose (23.9%), 16.7 mM D-glucose (26.1%), 7.0 mM D-glucose and 0.06 mM gliclazide (27.4%), and 10.0 mM L-leucine and 10.0 mM L-glutamine (29.4%). However, when insulin release was stimulated by the combination of Ba^{2+} (2 mM) and theophylline (1.4 mM) in the absence of Ca^{2+}, the inhibition of secretion attributable to 3-deazaadenosine and DL-homocysteine was more marked, averaging 54.0%.

Oxidative and ionic data. The methylation inhibitors failed to affect significantly D-glucose oxidation, whether at low or high glucose concentration (Table 3). They also failed to inhibit ^{45}Ca net uptake or cyclic AMP production by the islets. On the contrary, at high glucose concentration (16.7 mM), the combination of 3-deazaadenosine and DL-homocysteine slightly increased ^{45}Ca net uptake (Table 3).

In perifused islets, the methylation inhibitors, when present throughout the perifusion period, failed to affect either the basal value for ^{86}Rb fractional outflow rate or the decrease in effluent radioactivity evoked by a rise in glucose concentration from zero to 4.2 mM (Fig. 1) or 16.7 mM (Fig. 2). Likewise, when administered from the 45th to 69th min of perifusion, the methylation inhibitor failed to cause any obvious change in the pattern of ^{86}Rb outflow from glucose-deprived or glucose-stimulated islets (Fig. 3). A rise in glucose concentration from zero to 8.3 or 16.7 mM is known to provoke an initial fall followed by a secondary rise in ^{45}Ca efflux from the perifused islets. The methylation inhibitors did not alter this dual response whether to 8.3 mM (Fig. 4) or 16.7 mM D-glucose (Fig. 5). In

Table 3. Effects of 3-deazaadenosine and DL-homocysteine upon insulin release, glucose oxidation, ^{45}Ca net uptake and cyclic AMP production by pancreatic islets

Secretagogues (mM)	Control	3-Deazaadenosine (0.1 mM) + DL-homocysteine (0.2 mM)
Insulin release (μU/90 min per islet)		
D-Glucose (2.8)	13.4 ± 6.3 (19)*	16.5 ± 12.6 (19)
D-Glucose (7.0)	87.1 ± 6.0 (20)	66.3 ± 3.9 (19)[b]
D-Glucose (16.7)	310.9 ± 10.5 (65)	229.9 ± 7.8 (68)[d]
D-Glucose (7.0) + gliclazide (0.06)	128.5 ± 8.7 (19)	93.3 ± 7.3 (20)[c]
L-Leucine (10.0) + L-glutamine (10.0)	264.3 ± 7.7 (18)	186.5 ± 6.2 (18)[d]
Ba^{2+} (2.0) + theophylline (1.4); no Ca^{2+}	149.0 ± 6.8 (18)	68.5 ± 4.1 (18)[d]
D-[U-^{14}C]Glucose oxidation (pmoles/120 min per islet)		
D-Glucose (2.8)	10.6 ± 0.8 (8)	10.3 ± 0.4 (8)
D-Glucose (16.7)	38.0 ± 1.3 (8)	34.9 ± 1.7 (8)
^{45}Ca net uptake (pmoles/90 min per islet)		
D-Glucose (2.8)	0.98 ± 0.07 (10)	1.05 ± 0.08 (10)
D-Glucose (16.7)	3.86 ± 0.19 (19)	4.62 ± 0.25 (19)[a]
L-Leucine (10.0) + L-Glutamine (10.0)	2.72 ± 0.17 (10)	2.63 ± 0.13 (10)
Cyclic AMP content and output (fmoles/90 min per islet)		
D-Glucose (16.7)	9.7 ± 1.9 (6)	11.2 ± 1.6 (6)
D-Glucose (16.7) + 3-isobutyl-1-methylxanthine (1.0)	215.9 ± 21.5 (9)	202.3 ± 10.0 (10)

* Mean values (± S.E.M.) are shown together with the number of individual observations (in parentheses) and the statistical significance (a: $P < 0.03$; b; $P < 0.01$; c: $P < 0.005$ and d: $P < 0.001$) of differences between experimental and control values.

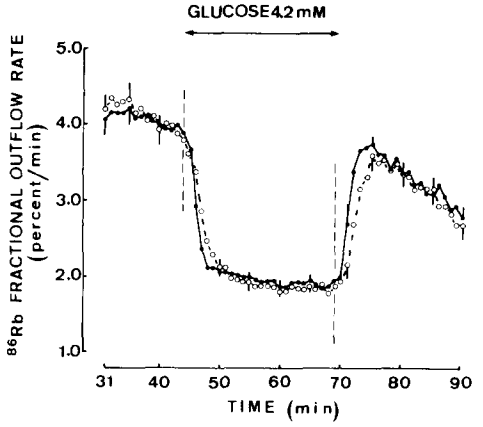

Fig. 1. Effect of a rise in glucose concentration from zero to 4.2 mM upon ^{86}Rb FOR from islets perifused for 90 min in the absence (○, - - -) or presence (●, ——) of DL-homocysteine (0.2 mM) and 3-deazaadenosine (0.1 mM). Mean values (± S.E.M.) refer to 4 individual experiments.

this series of experiments, the methylation inhibitors also failed to affect significantly insulin output in response to the low concentration of glucose (8.3 mM; Fig. 4) but impaired the early and late phase of the secretory response to glucose in high concentration (16.7 mM; Fig. 2). Thus, after correction for the basal value (min 40–44), the integrated output of insulin (min 45–69) averaged, in the presence of the methylation inhibitors, 74.0 ± 13.6% of the mean control value ($P < 0.08$).

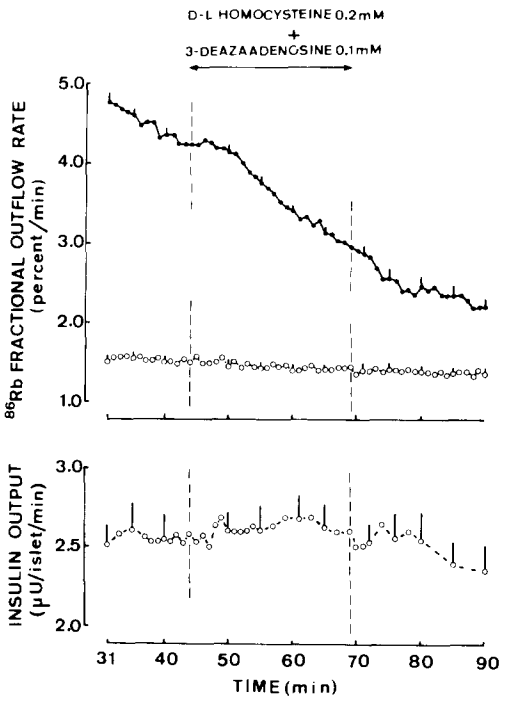

Fig. 3. Effect of DL-homocysteine (0.2 mM) and 3-deazaadenosine (0.1 mM) administered from min 45 to 69 upon ^{86}Rb FOR from islets deprived of glucose (●) or exposed to 16.7 mM D-glucose (○) and upon insulin release from islets exposed to 16.7 mM D-glucose (lower panel). Mean values (± S.E.M.) refer to 4 individual experiments.

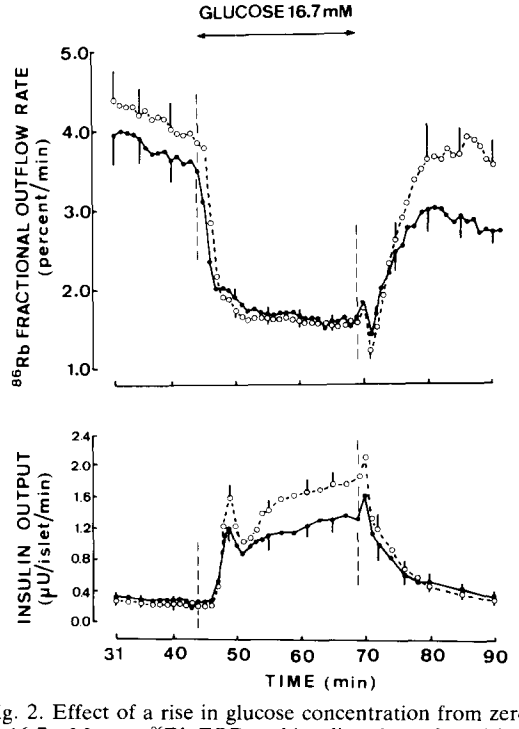

Fig. 2. Effect of a rise in glucose concentration from zero to 16.7 mM upon ^{86}Rb FOR and insulin release from islets perifused for 90 min in the absence (○, - - -) or presence (●, ——) of DL-homocysteine (0.2 mM) and a 3-deazaadenosine (0.1 mM). Mean values (± S.E.M.) refer to 4 individual experiments.

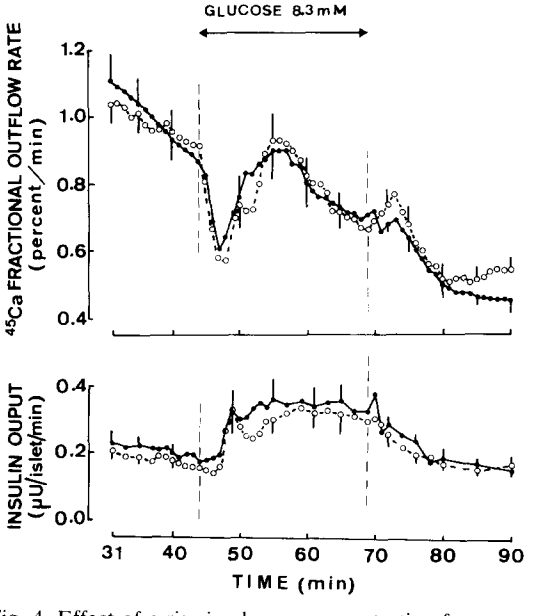

Fig. 4. Effect of a rise in glucose concentration from zero to 8.3 mM upon ^{45}Ca FOR and insulin release from islets perifused for 90 min in the absence (○, - - -) or presence (●, ——) of DL-homocysteine (0.2 mM) and 3-deazaadenosine (0.1 mM). Mean values (± S.E.M.) refer to 5–6 individual experiments.

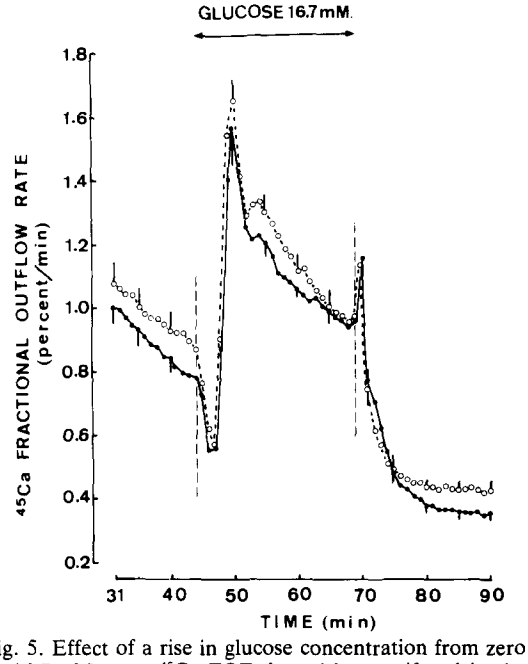

Fig. 5. Effect of a rise in glucose concentration from zero to 16.7 mM upon ^{45}Ca FOR from islets perifused in the absence (○, – – –) or presence (●, ——) of DL-homocysteine (0.2 mM) and 3-deazaadenosine (0.1 mM). Mean values (± S.E.M.) refer to 6–8 individual experiments.

However, when the islets were exposed throughout the perifusion period to a high concentration of glucose (16.7 mM), the administration of 3-deazaadenosine and DL-homocysteine did not cause any obvious immediate alteration of insulin output (Fig. 3).

Phosphatidylinositol cycle. In the last series of experiments, we have examined the effect of 3-deazaadenosine and DL-homocysteine upon the stimulation of the phosphatidylinositol cycle by D-glucose or carbamylcholine. In the absence of the methylation inhibitors, a rise in glucose concentration from 2.8 to 22.2 mM increased the ^{32}P content of the fraction containing phosphatidylinositol and phosphatidate, whilst failing to affect significantly the ^{32}P content of phosphatidylcholine, lysophosphatidylcholine and phosphatidylethanolamine (Table 4). At low glucose concentration (2.8 mM), carbamylcholine (2.0 mM) caused an even more marked increase in the ^{32}P content of phosphatidylinositol and phosphatidate. The methylation inhibitors failed to exert any significant effect upon labelling of islet phospholipids whether in response to the increase in glucose concentration or addition of carbamylcholine (Table 4).

DISCUSSION

The stepwise enzymatic methylation of phosphatidylethanolamine to phosphatidylcholine is thought to play an important role in agonist–receptor interactions in certain cell types [18]. The drugs 3-deazaadenosine and L-homocysteine cause the intracellular accumulation of 3-adenosylhomocysteine and *S*-3-deazaadenosylhomocysteine, which are potent inhibitors of methylation reactions [19]. By doing so, these agents may inhibit the functional response of target cells to certain external signals [18]. The inhibitory effect of 3-deazaadenosine and/or L-homocysteine upon functional events is not necessarily related, however, to a change in phospholipid methylation and may be due, instead, to impaired methylation of endogenous proteins [20].

Little is known of the relevance of lipid or protein methylation to the process of insulin release. Using pig skin gelatin or bovine brain calmodulin as the methyl acceptor protein, Campillo and Ashcroft [17] first reported the presence in islet homogenates of a protein carboxymethylase. However, neither the activity of the enzyme, as measured in islet homogenates, nor the carboxymethylation of endogenous methyl acceptor proteins, as measured in intact islets, were affected by exposure of the islets to D-glucose (3.3 and 20.0 mM). In a more recent study, Saceda *et al.* [21] reported the presence in islets of a phospholipid methyltransferase and observed that glucose (16.7 mM) causes a modest and transient stimulation of ^{3}H-methyl incorporation from L-[methyl-^{3}H] methionine into islet lipids.

The present work indicates that 3-deazaadenosine and DL-homocysteine indeed inhibit methylation of

Table 4. Effect of methylation inhibitors (0.1 mM 3-deazaadenosine and 0.2 mM DL-homocysteine) upon the ^{32}P content of islet phospholipids

Secretagogues (mM)	Inhibitors†	PI/PA	PC	LC	PE	(N)
D-Glucose (2.8)	–	278 ± 27*	268 ± 19	33 ± 3	53 ± 5	(4)
D-Glucose (22.2)	–	543 ± 2	373 ± 45	39 ± 5	67 ± 5	(3)
D-Glucose (22.2)	+	530 ± 2	433 ± 39	37 ± 2	74 ± 5	(3)
Carbamylcholine (2.0)	–	744 ± 68	239 ± 18	22 ± 2	53 ± 3	(3)
Carbamylcholine (2.0)	+	808 ± 49	270 ± 33	28 ± 11	49 ± 3	(3)

* Mean values (± S.E.M.) are expressed as cpm/islet.

† The islets were preincubated for a first period of 60 min in 0.8 ml of medium containing D-glucose (2.8 mM) and $^{32}PO_4^{3-}$ (100 μCi; carrier free). A second preincubation period of 60 min was performed after addition of a small volume (0.1 ml) of medium containing, when required, the methylation inhibitors to provide a final concentration of 0.1 mM 3-deazaadenosine and 0.2 mM DL-homocysteine. At the onset of the final incubation (30 min), the volume of incubation medium was raised from 0.9 to 1.0 ml by addition of a solution containing, as required, D-glucose or carbamylcholine in order to reach the final concentrations of secretagogues indicated in the Table.

PI/PA: phosphatidylinositol/phosphaditate; PC: phosphatidylcholine; LC: lysophosphatidylcholine; PE: phosphatidylethanolamine.

endogenous proteins in intact islets. The tritiation of endogenous proteins in islets exposed to L-[methyl-^{3}H]methionine did not reflect, to any significant extent, incorporation of this amino acid in newly synthesized proteins. The methylation inhibitors also decreased the labelling of endogenous phospholipids. In this respect, the data illustrated in Table 1 probably reflect inhibition by 3-deazaadenosine and DL-homocysteine of ^{3}H-methyl incorporation not solely into phosphatidylcholine and lysophosphatidylcholine but also into phosphatidyl-*N*-methylethanolamine and phosphatidyl-*N*,*N*-dimethylethanolamine (and the corresponding lysophospholipids), which in the chromatography system employed probably migrate in the vicinity of phosphatidylethanolamine (and lysophosphatidylethanolamine) thus accounting for the label associated with the latter. The nature of the radioactivity associated with phosphatidate and phosphatidylinositol is unknown, though it may be related to the amphiphilic labelled material which remained at the origin in the lipid chromatography system. In any case, the levels of these materials were not altered by methylation inhibitors, again suggesting that the former were non-specific contaminants rather than products of lipid methylation. The effect of the methylation inhibitors appeared rather specific, as they failed to adversely affect such variables as glucose oxidation, ^{86}Rb and ^{45}Ca fractional outflow rates, ^{45}Ca net uptake, cyclic AMP production and turnover of phospholipids in the phosphatidylinositol cycle.

The present work suggests that the integrity of methylation reactions is required for maintenance of a normal secretory activity. Thus, 3-deazaadenosine and/or DL-homocysteine inhibited, slightly but significantly, glucose-induced insulin secretion. The combination of these two inhibitors also impaired insulin release in response to other nutrient secretagogues, to gliclazide and to the combination of Ba^{2+} and theophylline.

As already mentioned, the inhibition of insulin release could not be attributed to alteration of either glucose metabolism or the capacity of glucose to inhibit K^+ conductance (as judged from the decrease in ^{86}Rb fractional outflow rate), stimulate Ca^{2+} inflow (as judged from the increase in ^{45}Ca fractional outflow rate) and to provoke Ca^{2+} accumulation in the islet cells (as judged from the increase in $^{45}Ca^{2+}$ net uptake). Moreover, the combination of 3-deazaadenosine and DL-homocysteine failed to significantly affect the production of cyclic AMP by glucose-stimulated islets, whether in the absence or presence of 3-isobutyl-1-methylxanthine. Since the effect of glucose to stimulate cyclic AMP synthesis in islet cells is currently ascribed to activation of adenylate cyclase by Ca-calmodulin [10, 22], the latter finding suggests that methylation inhibitors do not act specifically through alteration of calmodulin action.

An alternative explanation for the present findings could be that methylation inhibitors affect insulin release by altering the methylation of either an endogenous methyl acceptor protein yet to be identified or endogenous phospholipids, these methyl acceptors participating in a late event in the stimulus-secretion sequence. For instance, methylation inhibitors, by preventing the conversion of phosphatidylethanolamine to phosphatidylcholine, could suppress the glucose-induced fluidification of islet cell membranes [4]. Such an hypothesis would also account for the fact that methylation inhibitors alter the secretory response to a great variety of secretagogues.

Our results clearly indicate that the participation of methylation reactions in the process of glucose-induced insulin release differs in several respects from that ascribed by Axelrod and his colleagues to phospholipid methylation in the receptor-induced release of histamine from mast cells. In the latter system phospholipid methylation is thought to trigger a cascade of events including the translocation of methylated phospholipid from the inside to the outside of the plasma membrane, facilitated influx of Ca^{2+}, activation by Ca^{2+} of phospholipase A_2, hydrolysis of phosphatidylcholine rich in arachidonic acid, generation of physiologically-active metabolites of arachidonate such as prostaglandins and leukotrienes, and eventual release of histamine [23]. This cascade of events is impaired in the presence of methylation inhibitors [18, 23]. Such inhibitors failed, however, to affect the capacity of glucose to facilitate Ca^{2+} inflow into the islet cells. Even when glucose was used at an intermediate concentration (8.3 mM), no impairment of the glucose-stimulated exchange between influent ^{40}Ca and effluent ^{45}Ca was observed in islets exposed to 3-deazaadenosine and DL-homocysteine. At this intermediate glucose concentration the stimulated entry of Ca^{2+} into the islet cells is apparently mediated to a large extent by a pathway poorly sensitive to verapamil and distinct from the voltage-sensitive Ca^{2+} channels [24]. It is also remarkable that the methylation inhibitors exerted the most marked inhibitory effect upon insulin release when the latter process was stimulated by agents, such as barium and theophylline, which mobilize Ca^{2+} from intracellular sequestration sites rather then by facilitating the inflow of Ca^{2+} into the B-cell [25, 26]. Moreover, our data suggest that an extensive impairment of methylation reactions, such as that seen after preincubation in the presence of 3-deazaadenosine and DL-homocysteine, is required to cause a partial reduction in insulin output. This behaviour could account for the failure of these inhibitors to alter insulin release when administered to islets during sustained stimulation by D-glucose. Incidentally, there are a number of other tissues in which, like in islets, methylation reactions do not appear to play an essential role in the early steps of the stimulus-secretion coupling process [20, 27].

In conclusion, the present work suggests that an endogenous methyl acceptor protein and/or phospholipid participates in the process of insulin release at a distal site in the secretory sequence. For instance, such a methyl acceptor may play a limited role in the coupling between Ca^{2+} accumulation and granule exocytosis in the B-cell.

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