Inositol Glycan Phosphate Derived from Human Erythrocyte Acetylcholinesterase Glycolipid Anchor and Inositol Cyclic 1,2-Phosphate Antagonize Glucagon Activation of Glycogen Phosphorylase

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In this study we examine the hypothesis that an inositol glycan phosphate can act similarly to insulin on intact cells. The inositol glycan phosphate used in this study (glycan α) was isolated previously from the glycoinositol phospholipid anchor of human erythrocyte acetylcholinesterase and was shown to have the structure glycine-ethanolamine-PO_4-Man-Man-(*N,N*-dimethylethanolamine-PO_4)Man-(*N,N*-dimethyl)GlcN-inositol-PO_4. The cellular response investigated was the glucagon-stimulated activation of glycogen phosphorylase in rat hepatocytes. When hepatocytes were incubated with 20 nM glucagon for 4 min, the ratio of phosphorylase *a* activity to total phosphorylase increased from a basal value of 0.49 ± 0.02 to 0.82 ± 0.03 (means ± SE, $n = 15$). Inclusion of either 100 nM insulin or 3–10 μM glycan α during the glucagon incubation significantly decreased the glucagon-stimulated activity ratio to 0.74 ± 0.03 for either agent. Furthermore, hepatocyte preparations differed in their response to insulin and were divided into insulin-responsive and -resistant groups. Glycan α had a significant effect only in the insulin-responsive group for which the observed activity ratio for 10 μM glycan α plus glucagon (0.68 ± 0.05) compared closely with that for insulin plus glucagon (0.70 ± 0.04). For the insulin-resistant group, the activity ratio in the presence of 10 μM glycan α was 0.81 ± 0.03, unchanged from the control with glucagon alone. Because glycan α contains an inositol phosphate group, the effect of inositol cyclic 1,2-phosphate on the glucagon-stimulated activity ratio was determined. Inositol cyclic 1,2-phosphate at 1–10 μM significantly decreased the activity ratio to the same extent as insulin in seven insulin-responsive preparations. Glycan α and inositol cyclic 1,2-phosphate differed from insulin in eliciting no decrease in the 8-bromoadenosine cyclic 3′-5′-monophosphate–stimulated phosphorylase activity ratio in hepatocytes. The results indicate that glycan α and inositol cyclic 1,2-phosphate can mimic some effects of insulin on intact hepatocytes. *Diabetes* 42:1318–23, 1993

Saltiel and Cuatrecasas (1) first offered the hypothesis that insulin binding to its receptor promotes phospholipase C cleavage of a GPI to generate two intracellular signals, diacylglycerol and an inositol glycan phosphate. This glycan also has been proposed to mediate actions of several other hormones, including nerve growth factor (2), interleukin-2 (3), and thyroid-stimulating hormone (4). GPIs were originally identified as membrane anchors of several extracellularly oriented proteins (5). The core glycan structure of these GPI anchors is conserved from protozoa to mammals and corresponds to ethanolamine-PO_4-6Manα1–2Manα1–6Manα1–4GlcNH_2α1–6-*myo*-inositol-1-PO_4. Purified bacterial PIPLC cleaves many GPI anchors, and the observation that PIPLC also generated insulinmimetic activity from a hepatic plasma membrane lipid fraction was a key element in the Saltiel and Cuatrecasas hypothesis. The putative glycan mediator has been partially purified from several sources, including liver membranes (6,7), T-cells (8), and BC_3H1 cells (9). When added to intact cells, these glycan preparations have effects similar to insulin, as they increase glucose oxidation (10,11), increase pyruvate dehydrogenase activity (12,13), and antagonize glucagon activation of glycogen phosphorylase (14).

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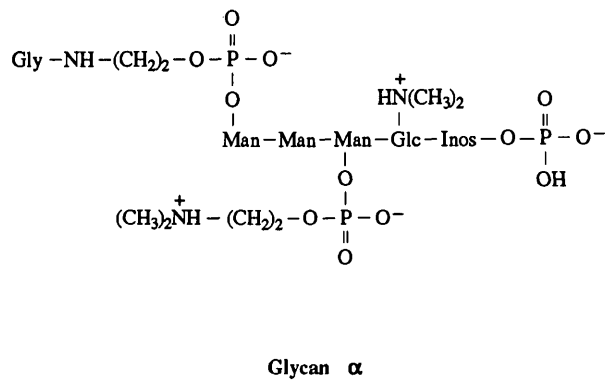
GPI, glycoinositol phospholipid; PIPLC, phosphatidylinositol-specific phospholipase C; AChE, acetylcholinesterase; ESI-MS, electrospray ionization-mass spectrometry; V_a, phosphorylase *a* activity; V_{tot}, total phosphorylase activity; HPLC, high-performance liquid chromatography; DTT, dithiothreitol; BSA, bovine serum albumin; TLC, thin-layer chromatography; 8-Br-cAMP, 8-bromo-cAMP; PI, phosphatidylinositol; VSG, variant surface glycoprotein; type II diabetes, non-insulin-dependent diabetes mellitus; K_i, inhibition constant.



Glycan α

FIG. 1. Structure of glycan α deduced from carbohydrate analysis and ESI-MS (15).

Despite extensive investigation of the cellular effects of these putative glycan preparations, the structure of a component that produces the insulin-like activities has not been determined. Indeed, the hypothesis itself has been questioned because an active glycan component has not been isolated and structurally defined. In this study, we provide support for one tenet of the hypothesis by showing that a highly purified inositol glycan phosphate derived from the GPI anchor of human erythrocyte AChE can mimic insulin in antagonizing glucagon activation of glycogen phosphorylase in intact hepatocytes. The AChE glycan, or glycan α (Fig. 1), contains a core structure consistent with that noted above for GPI anchors on proteins, but it also contains three additional modifications: the COOH-terminal Gly residue to which the anchor is attached in the intact protein; an extra phosphoethanolamine substitutent branching from the mannose adjacent to glucosamine that is a common feature of several mammalian GPI anchors; and two methyl groups attached to each of the two free amino groups that arise from reductive radiomethylation during our isolation procedure. Structural characterization of glycan α by ESI-MS indicates a terminal inositol monophosphate (15).

RESEARCH DESIGN AND METHODS

Preparation of glycan α. Glycan α was prepared from the GPI anchor of human erythrocyte AChE as described previously (15). AChE was purified by affinity chromatography on acridinium resin, and free amine groups were radiolabeled by reductive methylation. After complete digestion of AChE protein with proteinase K, the GPI anchor was isolated by elution in the void volume of a Sephacryl S-200 column equilibrated in 0.05% Triton X-100, 1 mM NaN_3, and 50 mM HEPES (pH 7.0) and deacylated with methanolic KOH. After adsorption of the deacylated GPI to phenyl-sepharose, the radiolabeled inositol glycan phosphates were released by cleavage with *Bacillus thuringiensis* PIPLC, desalted by chromatography on BioGel P2 in water, and separated on a Dionex CarboPac PA-1 anion exchange HPLC at pH 13. Fractions containing glycan α were neutralized with acetic acid, pooled, desalted on BioGel P2, dried, and resuspended at 100 μM in water for use in hepatocyte assays.

As a control for the BioGel P2 column solvent in which glycan α was desalted, an equal number of fractions eluting immediately before the glycan were pooled, dried, and resuspended in the same volume of water as glycan α. The glycogen phosphorylase activity ratio from hepatocytes treated with glucagon plus this column eluent blank did not differ from that with glucagon alone.

Modulation of glycogen phosphorylase activity in intact hepatocytes. Hepatocytes were prepared from fed 250–500 g male Sprague-Dawley rats (16), suspended at 1–3 × 10^7 cells/ml (>95% Trypan blue exclusive) in buffer (117 mM NaCl, 4.7 mM KCl, 2.4 mM $MgSO_4$, 1.1 mM $CaCl_2$, 1 mM glucose, 25 mM $NaHCO_3$, 1.2 mM Na_2HPO_4, and 10 mM Tris at pH 7.4), and stored briefly on ice. An aliquot (80 μl) was subjected to a stream of gas (95% O_2-5% CO_2) for 20 s, capped in a 2 ml polypropylene tube, and preincubated for 15 min at 37°C. Addition of 10 μl insulin (Sigma, St. Louis, MO), inositol phosphate, glycan α, or the solvent control was made 15 s before the addition of 10 μl buffered glucagon (a gift of Lilly, Indianapolis, IN) to 20 nM.

After an additional 10-s exposure to gas and a 4-min incubation in the capped tube, the sample was centrifuged for 3 s at 16,000 *g*; the cell pellet was lysed by the addition of 300 μl of 100 mM MOPS buffer, 400 mM sucrose, 20 mM EDTA, 150 mM NaF, 5 mM DTT, and 0.03% Triton X-100 at pH 7.0 (17); and the lysate was stored at −20°C. Thawed samples were vortexed and centrifuged for 2 min at 16,000 *g*, and phosphorylase *a* activity or V_a and total phosphorylase activity or V_{tot} (estimated by activation with AMP) in the supernatant were assayed (18,19).

Inositol phosphates (Sigma) were resuspended in water for use in hepatocyte assays. Analysis of the stock inositol cyclic 1,2-phosphate by Dionex anion exchange HPLC (15) revealed that inositol 1- or 2-phosphate accounted for <5% of the total inositol phosphate.

Assay of cAMP-dependent protein kinase. Assays (20) contained glycan α or inositol phosphate at the indicated concentrations, 0.3 μg/ml catalytic subunit of cAMP-dependent protein kinase (a gift of Dr. S. Taylor, University of California, San Diego), 1 μM kemptide, 10 μM ATP, 1 × 10^6 cpm of [γ-^{32}P]ATP, 1 mM $MgCl_2$, 0.25 mg/ml BSA, and 50 mM MOPS buffer (pH 7.0) in 25 μl at 30°C. Reactions were initiated by addition of kinase (5 μl) after 5-min preincubation of the other components. After 5-min of incubation, 20 μl was spotted onto Whatman P81 filter paper and immersed in 75 mM H_3PO_4 to terminate the reaction. The filter papers were washed 3 times in the same solvent and dried, and the scintillation counted. In an alternative assay, the reaction was terminated by freezing at −70°C and analyzed by TLC after thawing (21). Results were identical with either assay.

Statistical analysis. Data sets were analyzed by the paired, one-tailed Student's *t* test. A *P* value <0.05 was considered statistically significant.

RESULTS

Glucagon increases glycogen phosphorylase activity through a cAMP-dependent pathway (22). Glucagon interaction with its receptor activates adenylyl cyclase through G_s. The consequent increase in cAMP concentration stimulates cAMP-dependent protein kinase to initiate a phosphorylation cascade involving phosphorylase kinase and glycogen phosphorylase. The result is an increase in both absolute phosphorylase *a* activity and the fraction of total phosphorylase in the active phosphorylase *a* form. In Fig. 2*A*, treatment of hepatocytes with 20 nM glucagon for 4 min increased the ratio of phosphorylase *a* to total phosphorylase from a basal value of 0.49 ± 0.02 to 0.82 ± 0.03 (n = 15). Inclusion of 100 nM insulin during the glucagon incubation decreased this activity ratio to 0.74 ± 0.03 (n = 15), 90% of the ratio with glucagon alone. Hepatocyte phosphorylase *a* activity in the presence of glucagon plus insulin was reported previously to be ~75% of that observed with glucagon alone (23). Addition of the purified AChE glycan α to freshly isolated rat hepatocytes decreased the glucagon activation of phosphorylase in a concentration-dependent manner to an activity ratio of 0.74 ± 0.02 (n = 17, 3 μM, and 10 μM glycan α in Fig. 2*A*). Thus the maximal inhibition of glucagon-stimulated phosphorylase activity by glycan α and insulin was comparable.

In several hepatocyte preparations, insulin had no effect on glucagon-stimulated phosphorylase activity. Insulin insensitivity of glucagon-stimulated glycogen phosphorylase activity has been reported previously in freshly isolated hepatocytes (24,17), although the feature that distinguishes insulin-responsive from insulin-resistant preparations is unknown and probably arises from a subtle variation in the hepatocyte isolation procedure. When only insulin-responsive hepatocyte preparations were analyzed (8 of 15 total preparations), the extent of inhibition by insulin and glycan α increased (Fig. 2*B*). Observed activity ratios in this subset of preparations were 0.70 ± 0.04 (n = 8) and 0.68 ± 0.05 (n = 5) when insulin and 10 μM glycan α, respectively, were included with glucagon. These ratios corresponded to 81.8 ± 2.7 and 78.9 ± 7.6% of the respective glucagon-only controls. However, in insulin-resistant preparations, glycan α failed to antagonize glucagon activation of glycogen phosphorylase (Fig. 2*B*). Observed activity ratios (0.79 ± 0.03 [n = 7] for insulin plus glucagon and 0.81 ± 0.03 [n = 4] for 10 μM glycan α plus glucagon) were 102.7 ± 1.1 and 101.1 ± 4.2% of the glucagon-only controls. Note that glycan α antagonized glucagon-stimulated phosphorylase activity only in hepatocyte preparations that showed insulin inhibition of glucagon-stimulated phosphorylase activity. This concordance supports further the proposal that insulin and glycan α affect a common cell-signaling pathway.

To examine the structural specificity of glycan antagonism in this assay, the effects of inositol cyclic 1,2-phosphate and inositol 2-phosphate were investigated. Both inositol monophosphates antagonized glucagon activation of glycogen phosphorylase (Table 1). Because phospholipase C cleavage of inositol phospholipids often generates inositol cyclic 1,2- phosphate (25,26), this compound was examined in greater detail. When only insulin-responsive preparations were analyzed, inositol cyclic 1,2-phosphate inhibited the glucagon activation in a concentration-dependent manner with an apparent EC_{50} of <1 μM (Fig. 3). Observed activity ratios were 89.0 ± 2.8 (n = 7) and 89.2 ± 1.6% (n = 5) of the control with glucagon alone when insulin and 1 μM inositol cyclic 1,2-phosphate, respectively, were included. In contrast, in insulin-resistant preparations, inositol cyclic 1,2-phosphate, like glycan α, had no effect on glucagon

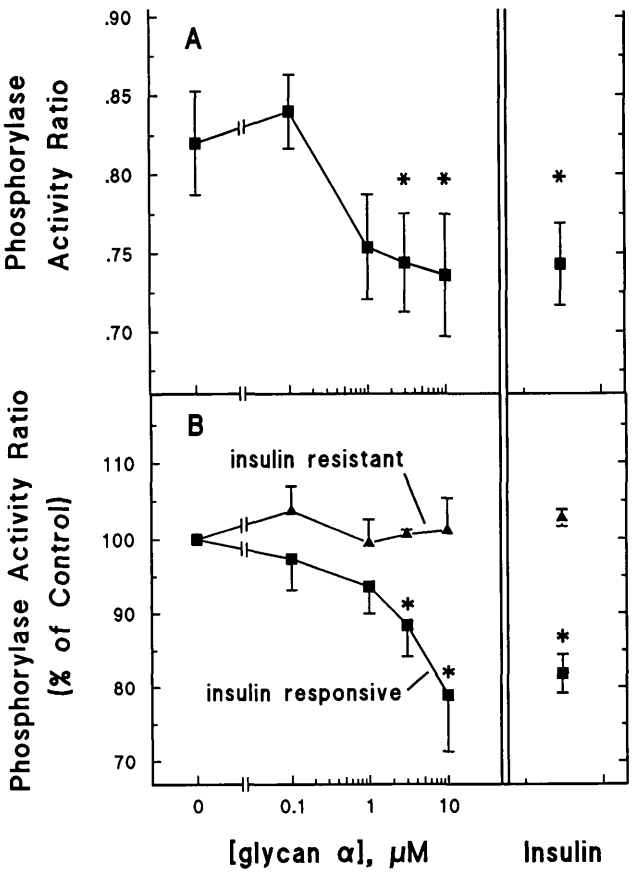


FIG. 2. Effect of glycan α on glucagon activation of glycogen phosphorylase. *A*: Freshly isolated rat hepatocytes were incubated with glucagon for 4 min in the presence of the indicated concentration of glycan α, a solvent control, or 100 nM insulin; cells were lysed; and phosphorylase *a* activity and total phosphorylase activity in lysate supernatants were measured as outlined in METHODS. Data are expressed as mean activity ratios (V_a/V_{tot}) ± SE. Basal activity ratios in the absence of glucagon and glucagon-stimulated activity ratios were measured in 15 hepatocyte preparations, and 5 different preparations of glycan α were used. Mean basal value (0.49 ± 0.02) was not significantly altered by 0.1–10 μM glycan alone in two preparations where this was checked. (*), Means obtained with glucagon plus glycan α that differ significantly from mean obtained with glucagon alone (0.82 ± 0.03). Specific activity of total phosphorylase under basal conditions averaged 42 ± 17 (means ± SD) nmol · min^{-1} · mg $protein^{-1}$ among hepatocyte preparations. The response to insulin is shown on the right. *B*: Insulin-responsive preparations were defined as those in which addition of insulin decreased the phosphorylase activity ratio with glucagon by >2%. Data from *A* were analyzed separately for 8 insulin-responsive and 7 insulin-resistant hepatocyte preparations. Data points indicate the activity ratio with glucagon plus insulin or glycan α expressed as a percentage of the control activity ratio (20 nM glucagon in the absence of glycan) and are means of 2–8 measurements. (*), Percentages that differ significantly from 100. No significant difference existed in V_{tot} between insulin-responsive and -resistant preparations. The responses to insulin are shown on the right.

TABLE 1
Effects of inositol monophosphates on glucagon activation of glycogen phosphorylase

	Activity ratio with glucagon alone (%)
Inositol cyclic 1,2-phosphate (100 μM)	91 ± 3 (6,5)*
Inositol 2-phosphate (100 μM)	94 ± 3 (6,3)*
Insulin (100 nM)	93 ± 3 (8,5)*

Data are means ± SE for the total number of preparations examined; (Total number of preparations studied, number of preparations that were insulin responsive). Data indicate the activity ratio with glucagon plus insulin or inositol phosphate expressed as a percentage of the activity ratio with glucagon alone. Basal and glucagon-stimulated activity ratios were 0.53 ± 0.03 and 0.81 ± 0.04, respectively. Experiments were conducted and data were calculated as described in Fig. 2.
*Significantly different from control (100%).

TABLE 2
Activity of the catalytic subunit of cAMP-dependent protein kinase in the presence of the indicated concentrations of glycan α and inositol phosphates

	Concentration (μM)	Control activity (%)
Glycan α	20	103 ± 6 (5)
Inositol cyclic 1,2-phosphate	50	103 ± 2 (4)
	500	95 ± 3 (4)
Inositol 1-phosphate	50	103 ± 1 (2)
	500	106 ± 1 (2)
Inositol 2-phosphate	50	96 ± 3 (3)
	500	98 ± 9 (3)
Inositol 1,2,3,4,5,6-phosphate	50	103 ± 7 (2)
	500	66 ± 3 (3)*

Data are means ± SE (number of experiments). Control kinase activity for glycan α was obtained with the eluent control in the absence of the glycan. Control kinase activity for the inositol phosphates was obtained with water in place of the inositol phosphate stock and averaged 0.5 ± 0.1 pmol/min. Kinase activity with the eluent blank averaged 86 ± 6% of the kinase activity obtained with water. Duplicate assays were conducted in each experiment. Assays were conducted as outlined in METHODS. Glycan α data are compiled from 3 different glycan preparations, each of which antagonized glucagon action in hepatocyte preparations.
*The inhibition observed with inositol hexaphosphate was competitive with respect to ATP and demonstrated a K_i of ~700 μM.

activation of phosphorylase (data not shown). Neither glycan α (20 μM) nor inositol monophosphates (up to 100 μM) had any effect on total phosphorylase or phosphorylase *a* activity when added to hepatocyte extracts in vitro (data not shown), excluding a direct effect on phosphorylase or interference with the assay and supporting the hypothesis that these agents antagonize the signal transduction system through which glucagon activates phosphorylase. Furthermore, the rapid effects of exogenously added glycan α or inositol monophosphates suggest that a binding site for these agents exists on the external surface of the plasma membrane as part of a transport system or a receptor.

Previous reports have suggested several mechanisms by which glycans derived from GPIs could inhibit the effects of glucagon. Because glucagon elevates cAMP concentration and increases cAMP-dependent protein kinase activity, one proposal is that these glycans inhibit cAMP-dependent protein kinase (27), as glycans have been reported to inhibit the catalytic subunit of this kinase without altering cAMP binding to the regulatory subunit (27). However, we observed no change in the activity of purified catalytic subunit of cAMP-dependent protein kinase with glycan α (20 μM) or inositol monophosphates (up to 500 μM), although slight inhibition was seen with inositol hexaphosphate (Table 2). A second proposed mechanism involves a glycan-induced alteration in cAMP steady-state levels (14) arising from inhibition of adenylyl cyclase (6) and/or activation of cAMP phosphodiesterase (28,6). To explore the applicability of this mechanism, we examined the effects of insulin, glycan α, and inositol cyclic 1,2-phosphate on the activation of glycogen phosphorylase by 100 μM 8-Br-cAMP. Consistent with previous studies (29), insulin inhibited 8-Br-cAMP activation of hepatocyte glycogen phosphorylase. In contrast, neither glycan α (10 μM) nor inositol cyclic 1,2-phosphate had any effect on 8-Br-cAMP–stimulated phosphorylase activation (Table 3). The data in Table 3 suggest that glycan α and inositol monophosphates act at a signal transduction step proximal to cAMP activation of cAMP-dependent protein kinase. Thus, these results could be consistent with a glycan-induced alteration in cellular cAMP concentrations. Clearly, these agents are not simply glucagon receptor antagonists, because the insulin-resistant hepatocyte preparations on which glycan α and inositol monophosphates had no effect showed the same extent of glucagon activation of phosphorylase as the insulin-responsive preparations.

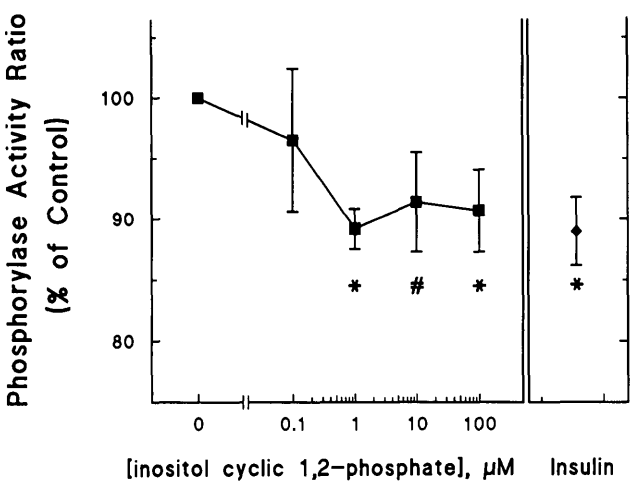


FIG. 3. Inositol cyclic 1,2-phosphate antagonizes glucagon activation of glycogen phosphorylase. Incubation, cell lysis, and assay of glycogen phosphorylase were conducted as in Fig. 2. Data points indicate the activity ratio with glucagon plus insulin or inositol cyclic 1,2-phosphate expressed as a percentage of the control activity ratio (20 nM glucagon in the absence of inositol monophosphate) and were obtained from 7 insulin-responsive preparations. Basal and glucagon-stimulated phosphorylase activity ratios were 0.54 ± 0.03 and 0.82 ± 0.05, respectively. The response to insulin for these preparations is shown on the right. (*), $P < 0.05$; (#), $0.05 < P < 0.1$ for percentages that differ significantly from 100.

TABLE 3
Effects of insulin, glycan α, and inositol cyclic 1,2-phosphate on 8-Br-cAMP activation of glycogen phosphorylase

	Activity ratio with 8-Br-cAMP alone (%)
Glycan α (10 μM)	98.0 ± 1.2 (3)
Inositol cyclic 1,2-phosphate (100 μM)	99.4 ± 0.6 (2)
Insulin (100 nm)	92.4 ± 2.2 (4)*

Data are means ± SE; (number of measurements). Experiments were conducted as described in Fig. 2 except that 8-Br-cAMP (Sigma) was added at a final concentration of 100 μM instead of glucagon. Data points indicate the activity ratio with 8-Br-cAMP plus insulin, glycan α, or inositol cyclic 1,2-phosphate expressed as a percentage of the activity ratio with 8-Br-cAMP alone and were obtained from 4 insulin-responsive preparations. Basal and 8-Br-CAMP-stimulated activity ratios were 0.50 ± 0.06 and 0.85 ± 0.l02, respectively.

DISCUSSION

The data in Fig. 2 and Table 3 indicate that glycan α can mimic some of the insulin effects related to activation of glycogen phosphorylase. The insulinmimetic effects obtained with the inositol monophosphates in Table 1 and Fig. 3 indicate that the inositol phosphate on glycan α may be a key structural requirement for the glycan effect in Fig. 2, if these compounds all have the same site of action. Acyclic inositol phosphates as well as inositol cyclic 1,2-phosphate can be generated by phospholipase C cleavage of inositol phospholipids (26), but we initially have focused on the cyclic 1,2-phosphate because it has been featured in investigations of the hypothesis of Saltiel and Cuatrecasas (1,31). However, structural analysis of glycan α showed that any cyclic phosphate structure initially produced by PIPLC had been opened (Fig. 1). Volwerk et al. (30) have reported that bacterial PIPLC can hydrolyze inositol cyclic 1,2-phosphate to inositol 1-phosphate at ~0.1% of the rate that it hydrolyzes PI to inositol cyclic 1,2-phosphate. Thus, the PIPLC used to isolate glycan α for the current studies (15) may have catalyzed the opening of an initial cyclic phosphate structure. It will be important to test whether the cyclic phosphate form of glycan α has a lower EC_{50} than that observed for glycan α in Fig. 2.

Misek and Saltiel (31) recently compared the actions of a structurally characterized inositol glycan phosphate with those of insulin. That study involved a fragment with the structure Asp-ethanolamine-phosphate-6Manα1–2 Manα1–6Manα1–4GlcNH$_2$α1–6-*myo*-inositol-cyclic 1,2-phosphate produced by digestion of the GPI anchor of trypanosome VSG with PIPLC and Pronase. This structure differs from that of glycan α in Fig. 1 in containing Asp rather than Gly, lacking the extra phosphoethanolamine branching from the mannose adjacent to glucosamine and the two methyl groups attached to glucosamine, while apparently retaining the inositol cyclic 1,2-phosphate moiety. The VSG fragment showed insulinmimetic effects in inhibiting *1*) isoproterenol-stimulated lipolysis in isolated intact rat adipocytes; *2*) glucose-6-phosphatase activity in rat liver microsomes; *3*) fructose-1,6-bisphosphatase activity in rat liver cytosol; and *4*) glucose output in isolated rat hepatocytes. Inhibition was dose dependent with IC_{50} values of 60–140 μM, significantly higher than the effective concentration range for glycan α observed here. Inhibition was abolished by hydrolysis of the VSG fragment with acid, a treatment intended to be selective for opening of the inositol cyclic 1,2-phosphate to the acyclic 1- or 2-phosphate, although no posttreatment structural verification was undertaken (31). However, no inhibition was observed in any of the assays with inositol cyclic 1,2-phosphate at concentrations up to 800 μM, in striking contrast with our observations in Table 1 and Fig. 3. Our results are difficult to compare with those of Misek and Saltiel (31) because the cellular endpoints and assays used are quite different, but their data seem to imply that inositol glycan phosphates could have effects at both extracellular and intracellular sites.

Our experiments in this study address only limited features of the above-noted Saltiel and Cuatrecasas hypothesis. Whether insulin can activate a phospholipase C capable of cleaving GPIs and whether GPI cleavage is induced by insulin remains to be confirmed. Furthermore, the extent of the structural resemblance of any inositol glycan phosphates generated by insulin to glycan α from the GPI anchor of AChE is difficult to predict. Although the core glycan structure of GPI anchors on membrane proteins appears to be highly conserved, an alternative structure has been found in free GPIs from *Leishmania major* in which only the first mannose adjacent to glucosamine is retained and other residues of the GPI anchor core are replaced by sugars that include galactose (32). Preliminary results have suggested that the putative glycan mediator derived from BC_3H1 (9) and H35 hepatoma (7) cells contains galactose rather than mannose and that more than one glycan mediator may be identified in skeletal muscle (33) and activated T-cells (8). The existence of multiple glycan mediators might explain the divergence seen between glycan α and insulin in antagonizing 8-Br-cAMP activation of phosphorylase, as glycan α may lack structural features needed to inhibit an insulin-sensitive site distal to cAMP production (e.g., cAMP-dependent protein kinase). The absence of the GPI precursor for a glycan mediator has been postulated to account for the post–insulin receptor defect found in type II diabetes mellitus (34). However, structural definition of these previously reported mediators and any other endogenous glycans generated by insulin is necessary to examine these possibilities. Our observation of an insulinmimetic effect of inositol cyclic 1,2-phosphate and the suggestion of a similar effect of inositol 2-phosphate (Table 1) also indicate that future investigations should address production of inositol phosphates from endogenous PI in response to insulin exposure. The rapid action of exogenous inositol cyclic 1,2-phosphate suggests that this agent could be effective if produced from externally oriented PI, a pathway quite distinct from those involving the well-characterized intracellular phosphoinositides (26). A cell surface phospholipase C that could release inositol phosphates to the extracellular medium has been reported (35).

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