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Protein phosphorylation in pancreatic islets induced by 3-phosphoglycerate and 2-phosphoglycerate

(anaerobic glycolysis/insulin secretion/islets of Langerhans)

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ABSTRACT **We have shown previously that 3-phosphoglycerate, which is a glycolytic metabolite of glucose, induces protein phosphorylation in bovine and rat brain and in rat heart, kidney, liver, lung, and whole pancreas. Since glycolytic metabolism of glucose is of paramount importance in insulin release, we considered the possibility that 3-phosphoglycerate may act as a coupling factor, and we searched for evidence for the existence of 3-phosphoglycerate-dependent protein phosphorylation systems in freshly isolated normal rat pancreatic islets. Membrane and cytosol fractions were incubated with [γ-^{32}P]ATP and appropriate test substances and were subjected to NaDodSO$_4$/PAGE and autoradiography. As little as 0.005 mM 3-phosphoglycerate or 2-phosphoglycerate stimulated the phosphorylation of a 65-kDa cytosol protein by as early as 0.25 min. The phosphate bond of the 65-kDa phosphoprotein was sufficiently stable to withstand dialysis; the radioactivity could not be chased out by subsequent exposure to ATP, ADP, 3-phosphoglycerate, or 2,3-bisphosphoglycerate. Moreover, cAMP, cGMP, phorbol 12-myristate 13-acetate, or calcium failed to stimulate the phosphorylation of the 65-kDa protein. Phosphoglycerate-dependent protein phosphorylation in islets may have relevance to stimulation of insulin secretion.**

The metabolism of glucose in pancreatic islets, predominantly in the glycolytic pathway, is essential for glucose-induced insulin biosynthesis and release (1, 2). The processes that couple the metabolism of glucose to the secretion of insulin have not been defined conclusively; the involvement of glycolytic metabolites of glucose is likely. Protein phosphorylation plays a role in the regulation of insulin secretion; glucagon, cAMP, and calcium, all secretagogues of insulin, induce phosphorylation of specific islet proteins through involvement of various protein kinases (3–5).

The classical concepts in regard to protein kinases are that they are activated by "second messengers," such as cAMP, or directly by "first messengers," such as insulin. Can a metabolic intermediate of a nutrient function as a messenger by activating a protein kinase, which then activates certain functionally important proteins, thus rendering the nutrient capable of initiating major processes in the cell? More specifically, can a metabolite of glucose evoke protein phosphorylation in a cell in which glucose plays a dominant regulatory role? One of us (T.U.) explored this possibility in the bovine brain and demonstrated that 3-phosphoglycerate (3PG) evoked marked stimulation of the phosphorylation of specific proteins (6). This phosphorylation phenomenon was shown not to be mediated by any of the conventional protein kinases and to be operative in rat brain, heart, liver, lung, kidney, and whole pancreas. The goal of the present study was to find out whether 3PG-dependent protein phosphorylation systems exist in normal rat pancreatic islets. In this report, we present evidence that the phosphorylation of an islet cytosol protein is stimulated by 3PG and 2-phosphoglycerate (2PG) and that an unconventional protein kinase is likely to be involved in this process.

MATERIALS AND METHODS

Preparation of Islet Tissue. Pathogen-free young male adult Wistar rats (Charles River Breeding Laboratories) weighing 250–300 g were used. They were kept in an air-conditioned room equipped with an automatic 12-hr light/12-hr dark cycle. They had free access to standard rat chow (Purina) and water until the experiments commenced. Pancreatic islets were isolated according to the method of Lacy and Kostianovsky (7), as modified by Gotoh *et al.* (8) to improve the consistency of the yield. The excised pancreatic tissue was digested with collagenase (Sigma; type V), filtered through an 860-μm metal screen, applied to a discontinuous Ficoll (Sigma) density gradient, and the harvested islets were sorted out under a dissecting microscope. Frequently, the integrity of the islet cells was ascertained by the trypan blue dye exclusion technique.

For each experiment, 1500–2000 islets (cumulatively containing 750–1000 μg of protein) were isolated from six to eight rats concurrently and pooled. The islets were centrifuged gently; the supernatant was discarded. The islets were resuspended in 0.2 ml of ice-cold hypotonic lysis solution consisting of 6 mM Tris·HCl (pH 8.1) (6). In the early experiments, the lysis solution did not contain any proteinase inhibitors; subsequently, aprotinin (1000 units/ml) purified from bovine lung (Sigma) was added routinely to the solution, although in experiments conducted in parallel, the substrate protein patterns had not been much different in the presence or absence of aprotinin. After ≈15 min in an ice bath, the islets suspended in the lysis solution were disrupted with a Teflon pestle homogenizer (12 strokes at 1200 rpm). The disrupted islets were kept in the lysis solution in an ice bath for 45 min more. On many occasions, the completeness of cell lysis was ascertained by microscopic examination of the homogenate. To separate the membrane fraction from the cytosol fraction, the homogenate was ultracentrifuged for 1 hr at 150,000 × *g* at 4°C. The supernatant (0.2 ml) containing the cytosol fraction was aspirated and, to remove endogenous glycolytic metabolites and nucleotides, dialyzed for 15 hr at 4°C across a semipermeable membrane (Visking, Spec-

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Abbreviations: 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; 2,3BPG, 2,3-bisphosphoglycerate.

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trum Medical Industries) against 1000 vol of 10 mM Tris·HCl (pH 7.4). The pellet containing the membrane fraction was resuspended in 0.2 ml of 10 mM Tris·HCl (pH 7.4) and stored at −20°C or −80°C while the dialysis was in progress.

Phosphorylation Assay. For the phosphorylation assay, the cytosol fraction was used as soon as the dialysis was complete. The membrane fraction was thawed, further diluted to ≈0.3 ml, and homogenized again by five to seven strokes at 1200 rpm. The protein content was determined in 10-μl aliquots of each fraction by the method of Lowry *et al.* (9). Aliquots (20–30 μl) of each fraction containing 50 μg of protein were assayed for endogenous phosphorylation, as described by Ueda and Plagens (6). Each aliquot was preincubated for 2 min at 37°C in a mixture containing 50 mM Tris·HCl (pH 7.4) and 10 mM $MgSO_4$ without or with 3PG or other test agents. Then, 20 μM [γ-^{32}P]ATP (specific activity, 16–20 Ci/mmol; 1 Ci = 37 GBq; ICN) was added to start the phosphorylation reaction; the final vol was 100 μl. The entire mixture was incubated for various time periods at 37°C. The reaction was terminated by the addition of 50 μl of a "stop solution" containing 186 mM Tris·HCl (pH 6.7), 15% (vol/vol) glycerol, 9% $NaDodSO_4$, bromphenol blue (0.5 mg/ml), and 6% (vol/vol) 2-mercaptoethanol. The sample was boiled for 2 min. The entire volume of each sample was subjected to $NaDodSO_4$/PAGE for 18 hr at room temperature (10). The acrylamide concentration in the gels was 7.5% or 11%. The gels were submitted to autoradiography for various periods of time at −80°C with Kodak RP x-ray film.

Data Acquisition and Analysis. Each type of experiment was repeated at least three times, and each time a different batch of islets was used. The autoradiographs were scrutinized individually by three or more of the authors and frequently by an independent expert. The results of a series of experiments were considered conclusive only if unequivocal concordance existed among the individual experiments included in that series.

RESULTS

Phosphorylation of Islet Membrane and Cytosol Proteins in Response to Exogenous 3PG. In preliminary experiments, the cytosol and membrane fractions obtained from rat islets were submitted to the phosphorylation assay for 2, 5, or 10 min, in the absence and presence of 5 mM 3PG. The autoradiography revealed nonspecific phosphorylation of numerous proteins, which occurred in the presence or absence of exogenous 3PG. 3PG stimulated the phosphorylation of a 65-kDa protein consistently and reproducibly in the cytosol fraction; selective phosphorylation of a membrane protein was not evident. Subsequent experiments were carried out only with the cytosol fraction.

Dose–Response Studies with 3PG and 2PG. To determine the optimal concentration of 3PG at which the 65-kDa protein was phosphorylated, the cytosol fraction was incubated for 5 min with 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mM 3PG; the closely related metabolite 2PG was also tested in the same manner (Fig. 1). Either phosphoglycerate stimulated the phosphorylation of the 65-kDa protein band at the lowest concentration tested. The phosphorylation was maximal at 0.5 mM 3PG or 2PG; it seemed to be inhibited at concentrations beyond 1 mM.

Time Course of 3PG-Induced Protein Phosphorylation. The cytosol fractions were incubated for 0.25, 0.5, 1, 2, and 5 min in the absence and presence of 0.8 mM 3PG. The stimulation of phosphorylation of the 65-kDa protein by 3PG was evident at the earliest time point tested; the maximal level was attained at 2 min (Fig. 2). At 5 min, the intensity of the phosphorylation of the 65-kDa band had decreased slightly below the level seen at 2 min.

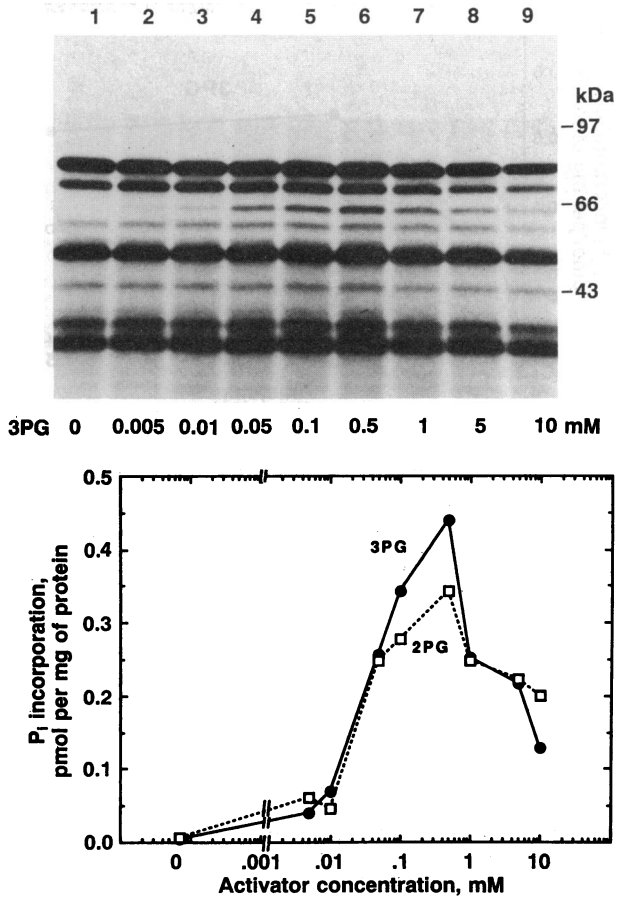

FIG. 1. Effect of various concentrations of 3PG or 2PG on the phosphorylation of the 65-kDa islet cytosol protein. The dialyzed cytosol fraction was incubated for 5 min with [γ-^{32}P]ATP for protein phosphorylation to occur, in the absence and presence of various concentrations of 3PG or 2PG, and then subjected to $NaDodSO_4$/PAGE (7.5% acrylamide) and autoradiography. (*Upper*) Autoradiograph showing the responses to 3PG. Lane 1, control without 3PG. Lanes 2–9, the concentrations of 3PG were 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mM, respectively. (*Lower*) With the exposed film used as a guide, the portion of the gel corresponding to the 65-kDa protein band was cut out and its radioactivity was quantified by liquid scintillation counting. The counts were transformed to reflect [^{32}P]phosphate incorporation into protein.

Activator Specificity Among Glucose Metabolites for 3PG-Stimulated Protein Phosphorylation. To determine whether the 65-kDa protein phosphorylation is specifically stimulated by 3PG, islet cytosol preparations were incubated individually with glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, fructose 2,6-bisphosphate, dihydroxyacetone phosphate, glyceraldehyde, glyceraldehyde 3-phosphate, 3PG, 2PG, 2,3-bisphosphoglycerate (2,3BPG), glycerate, phospho*enol*pyruvate, pyruvate, or lactate, each at a concentration of 0.8 mM. Results of some of these experiments are shown in Fig. 3. Strong phosphorylation of the 65-kDa protein was observed with 3PG. In addition to 3PG, 2PG and, to a lesser extent, phospho*enol*pyruvate also stimulated the phosphorylation of the substrate protein; the other substrates had no consistent effect.

Demonstration That 3PG-Responsive Phosphoprotein Is Not Phosphoglycerate Mutase Transferring [^{32}P]Phosphate. Three commercial preparations of rabbit muscle phosphoglycerate mutase were obtained from Boehringer Mannheim and Sigma, and their purity was tested by $NaDodSO_4$/PAGE, followed by protein staining. All three preparations displayed a major band corresponding to 30 kDa. In addition, the two

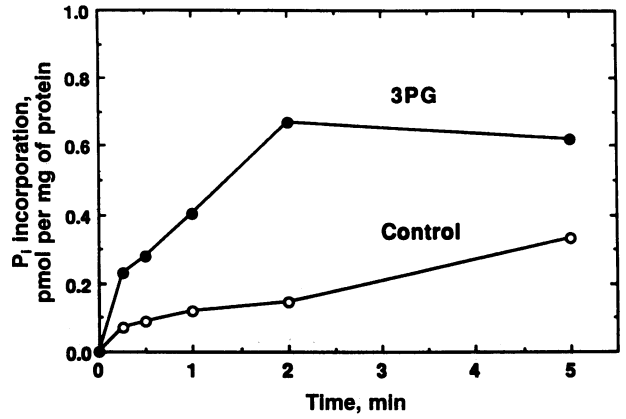

FIG. 2. Time course of phosphorylation of the 65-kDa protein stimulated by 3PG. The phosphorylation reaction was allowed to occur in the cytosol fraction for 0.25, 0.5, 1, 2, and 5 min in the absence and presence of 0.8 mM 3PG; the phosphorylated proteins were analyzed by $NaDodSO_4$/PAGE and autoradiography. [^{32}P]Phosphate incorporation into protein in the presence and absence of 3PG (3PG and control, respectively) was determined as described for Fig. 1.

Sigma preparations exhibited a minor band of ≈65 kDa, and the Boehringer preparation exhibited a faint band of ≈37 kDa plus a negligible smudging in the range of 50–65 kDa. Each preparation was submitted to the standard phosphorylation assay in the absence and presence of 3PG; the results with two of these preparations are depicted in Fig. 4. No radioactivity was discernible with the Boehringer preparation. With the two Sigma preparations, a radioactive band was observed in the 65-kDa region, which increased in intensity in the presence of 3PG.

The physicochemical characteristics of the 65-kDa protein detected in the two Sigma phosphoglycerate mutase preparations were contrasted with those of the dialyzed cytosol fraction. A Sigma lot that had displayed radioactivity in the 65-kDa region upon exposure to [γ-^{32}P]ATP and 3PG and the cytosol fraction were submitted separately to the standard phosphorylation assay in the presence of 3PG; then, the reaction mixtures were dialyzed for 15 hr at 4°C against 1000 vol of 10 mM Tris·HCl (pH 7.4) and 10 mM $MgSO_4$ and were then subjected to $NaDodSO_4$/PAGE. Upon dialysis, the

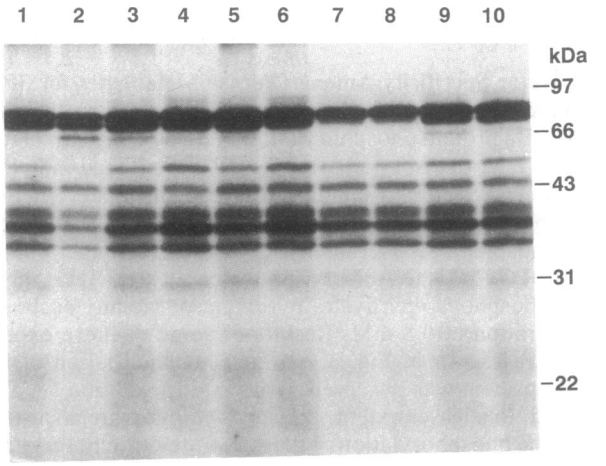

FIG. 3. Effect of glycolytic metabolites on the phosphorylation of the 65-kDa protein. The cytosol fraction was incubated for 5 min in the absence and presence of individual glycolytic intermediates (0.8 mM), and the phosphorylated proteins were analyzed by $NaDodSO_4$/PAGE (11% acrylamide) and autoradiography. Lanes 1–10, control without metabolite, 3PG, 2PG, 2,3BPG, glyceric acid, glyceraldehyde, glyceraldehyde 3-phosphate, dihydroxyacetone phosphate, phospho*enol*pyruvate, and pyruvate, respectively.

radioactivity in the 65-kDa band of the mutase preparation diminished almost completely; on the other hand, the radioactivity of the cytosolic 65-kDa protein band withstood the dialysis unaltered and was not removed upon reincubation for 1 min with 0.8 mM 3PG (data not shown).

The islet cytosol fraction was submitted to the phosphorylation assay as a mixture with either the Boehringer phosphoglycerate mutase preparation or one of the Sigma preparations (Fig. 4). With the Boehringer preparation, the intensity of radioactivity in the 65-kDa cytosol protein band was unaltered; with the Sigma preparation, the observed increase in the intensity represented the sum of the amounts detected in these bands when the cytosol fraction and the mutase preparation had been assayed separately. When cytosol had been mixed with either mutase preparation, the phosphorylation of several other bands increased, particularly in the 30-kDa region; however, these findings were inconsistent.

The cytosol fraction, alone and as a mixture with the Sigma phosphoglycerate mutase preparation used in the preceding experiments, was submitted to the standard phosphorylation assay for 4 min; at the end of the 3 min of incubation, unlabeled ATP or ADP was added. In the presence of 3PG, the intensity of the 65-kDa band of the mixture was much greater than that of the cytosol fraction alone; the addition of ATP or ADP did not reduce the radioactivity appreciably (Fig. 5 *Upper*). To ascertain that the failure of ATP or ADP to remove the radioactivity from the 65-kDa band was not due to the continued presence of [γ-^{32}P]ATP, the cytosol fraction was first submitted to the standard phosphorylation assay in the presence of 3PG, then dialyzed for 15 hr, and then incubated for 5 min in the presence of 2–200 μM unlabeled ATP or ADP; the radioactivity of the band remained unaltered after this treatment (Fig. 5 *Lower*).

The ability of 2,3BPG, which is the phosphate donor to phosphoglycerate mutase (11), to remove the phosphate from the 65-kDa islet cytosol phosphoprotein was tested. The cytosol fraction was submitted to the standard phosphorylation assay for 3 min in the presence of 3PG. Then, 0.8 mM 2,3BPG was added, and the reaction continued for 1, 5, or 10 min. Exposure to 2,3BPG had no appreciable effect on 3PG-stimulated phosphorylation of the 65-kDa protein band (data not shown).

Effects of Activators of Conventional Protein Kinases on Phosphorylation of the 65-kDa Cytosol Protein. The possible involvement of one of the conventional protein kinases in 3PG-induced phosphorylation of the 65-kDa protein rat islets was investigated. The effects of activators of various protein kinases are shown in Fig. 6. As compared to the controls without any protein kinase activator, 3PG enhanced the phosphorylation of the 65-kDa protein band. The 65-kDa protein was not phosphorylated in the presence of cAMP, cGMP, calcium, or the combination of the activators of protein kinase C. The phosphorylation of at least two protein bands, corresponding to 35 and 31 kDa, was noted to be enhanced by cAMP, and to a lesser extent by cAMP. Under the incubation conditions used, stimulation of protein phosphorylation was not discernible with calcium alone or with the protein kinase C activators.

DISCUSSION

The results of our study provide evidence that 3PG and 2PG stimulate the phosphorylation of a distinct cytosolic protein in rat pancreatic islets.

In islets, the estimated molecular mass of the cytosolic protein, which was consistently phosphorylated in response to 3PG, was 65 kDa. In the previous study, the dominant protein substrates for 3PG-dependent phosphorylation in the bovine brain cytosol had an estimated size of 72 and 155 kDa

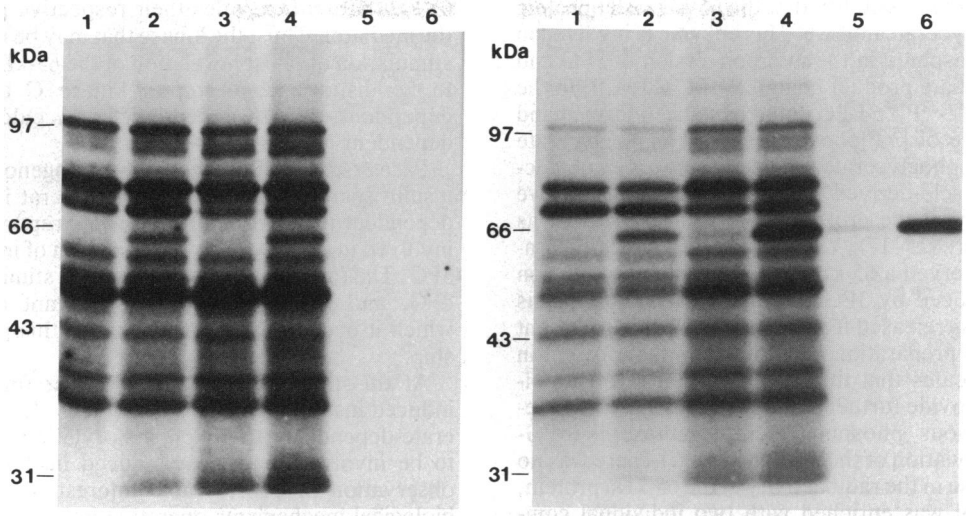

FIG. 4. Phosphorylation of the 65-kDa islet cytosol protein in the presence of exogenous phosphoglycerate mutase. The commercial phosphoglycerate mutase preparations used were Boehringer Mannheim lot 11221425-30 (*Left*) and Sigma lot 107F-9670 (*Right*). Three-minute incubations with [γ-^{32}P]ATP were followed by NaDodSO$_4$/PAGE on 7.5% acrylamide slab gel and autoradiography. Lanes: 1 and 2, islet cytosol; 3 and 4, islet cytosol plus 2 μg of phosphoglycerate mutase; 5 and 6, 2 μg of phosphoglycerate mutase preparation alone; 1, 3, and 5, in the absence of 3PG; 2, 4, and 6, in the presence of 0.8 mM 3PG.

(3PG-PP$_{72}$ and 3PG-PP$_{155}$, respectively), but several other cytosol and membrane phosphoproteins were observed in the rat brain, heart, liver, lung, kidney, and whole pancreas (6). Thus, the phenomenon of 3PG-dependent phosphorylation involves proteins with a broad range of molecular mass, is observed both in the cytosol and the membrane fractions, and occurs in a wide variety of tissues in more than one species.

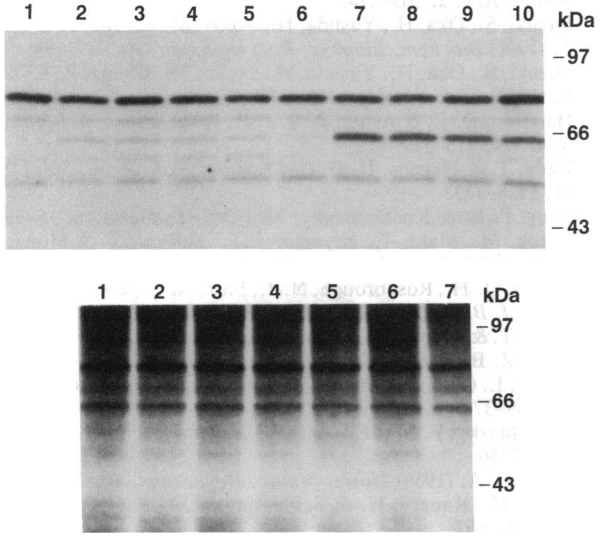

FIG. 5. Failure of ATP or ADP to displace radioactivity from the 65-kDa cytosol protein. Autoradiographs of NaDodSO$_4$/PAGE on 7.5% acrylamide slab gels. (*Upper*) The samples were incubated for 3 min in the standard phosphorylation assay. Lanes: 1–5, islet cytosol; 6–10, islet cytosol plus 2 μg of phosphoglycerate mutase Sigma preparation 107F-9670; 2–5 and 7–10, 0.8 mM 3PG was present. At the end of the 3 min of incubation, 10 μl of the following solutions was added and the incubation was extended for another minute: water (lanes 3 and 8), ATP (lanes 4 and 9), and ADP (lanes 5 and 10). The final concentration of ATP or ADP was 20 μM. (*Lower*) Samples of cytosol fraction were incubated for 3 min with 0.8 mM 3PG and then dialyzed for 15 hr at 4°C against 1000 vol of 10 mM Tris·HCl (pH 7.4) and 10 mM MgSO$_4$. The next day, ATP (lanes 2, 3, and 4; final concentrations, 2, 20, and 200 μM, respectively) or ADP (lanes 5, 6, and 7, final concentrations, 2, 20, and 200 μM, respectively) was added and the samples were incubated for 5 min at 37°C.

In inducing the phosphorylation of the 65-kDa islet cytosol protein, 2PG was as effective as 3PG. One possibility is that the mechanism involved in the phosphorylation of this protein, such as a protein kinase, does not discriminate between these phosphoglycerates. Alternatively, 2PG may have been converted by the cytosolic enzyme phosphoglycerate mutase to 3PG, and the phosphorylation may have been induced specifically by 3PG.

We observed that 0.005 mM 3PG or 2PG, the lowest concentration used, induced the phosphorylation of the 65-kDa protein. In erythrocytes, typical concentrations of 3PG and 2PG are 0.12 and 0.03 mM, respectively (12). In pancreatic islets, concentrations up to 0.1 mM are attainable during incubations with high concentrations of glucose (13, 14). Thus, our dose–response study demonstrates that protein phosphorylation induced in islets in response to exogenous 3PG or 2PG has potential physiological implications.

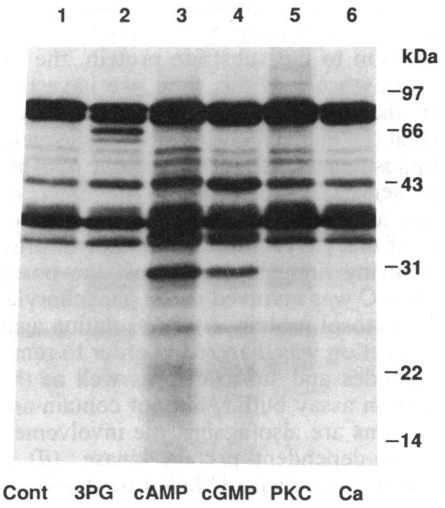

FIG. 6. Protein phosphorylation patterns in response to activators of various protein kinases. The cytosol fraction was incubated for 5 min in the absence and presence of various protein kinase activators and subjected to NaDodSO$_4$/PAGE (11% acrylamide) and autoradiography. Lanes 1–8, control without any activator, 0.8 mM 3PG, 10 μM cAMP, 10 μM cGMP, 0.2 mM Ca^{2+} plus phosphatidylserine (65 μg/ml) plus 0.1 μM phorbol 12-myristate 13-acetate, and 0.2 mM Ca^{2+}, respectively.

We considered the possibility that the 65-kDa islet protein may be phosphoglycerate mutase. This enzyme is involved in the transfer of phosphate in the interconversion of 3PG and 2PG, the intermediate product being 2,3BPG (11, 12). In the presence of 3PG, [2-^{32}P]2,3BPG could have been formed and served as a source of [^{32}P]phosphate for phosphoglycerate mutase. Islet phosphoglycerate mutase has not been characterized; in the muscle-derived commercial preparations, we found the majority of the proteins in the 30-kDa region, as reported previously (15–17). Yet, in the two Sigma preparations, we also observed a 65-kDa band, the phosphorylation of which was induced by 3PG. The fact that upon dialysis [^{32}P]phosphate was released from the 65-kDa protein present in the commercial preparations, but not from that present in islet cytosol, indicates that they are not identical. The following findings provide further evidence against the involvement of endogenous phosphoglycerate mutase in 3PG-induced phosphorylation of the islet protein. (*i*) There was no synergistic increase in the radioactivity in the 65-kDa protein, when islet cytosol was enriched with two individual commercial preparations. On the other hand, phosphoproteins in the 30-kDa region were revealed in these experiments involving mixtures of cytosol and exogenous mutase, which could represent the phosphorylation of the latter. (*ii*) Neither ATP nor ADP was capable of removing the [^{32}P]phosphate from the 65-kDa cytosolic phosphoprotein. (*iii*) Upon short exposure, neither 3PG nor 2,3BPG removed the [^{32}P]phosphate from the islet protein once the protein had been phosphorylated in response to 3PG.

Ueda and Plagens (6) have presented evidence that, in the brain, 3PG-dependent protein phosphorylation occurs on seryl residues. The physicochemical stability of the 65-kDa islet phosphoprotein would support the possibility that such a covalent bonding between the phosphate and the hydroxyl group of an amino acid may be involved. Deutscher and Saier (18) have shown that, in *Streptococcus pyogenes*, the phosphate transfer protein HPr is phosphorylated on a seryl residue in an ATP-dependent process catalyzed by a protein kinase, the activity of which is stimulated by glucose, glucose 6-phosphate, fructose 1,6-bisphosphate, and 2PG (3PG was not tested). These observations strengthen the possibility that a protein kinase is involved in phosphoglycerate-induced protein phosphorylation in islets.

If 3PG and 2PG were to induce the phosphorylation of the 65-kDa islet cytosol protein by activating a protein kinase, then, in addition to the substrate protein, the kinase must reside in the cytosol. At this time, we have no probes to identify or characterize such a kinase directly. One may speculate that the phosphoglycerates may be metabolized to glycerol 3-phosphate or dihydroxyacetone phosphate, which could then serve as substrates for *de novo* synthesis of phosphatidic acid (19, 20), eventually leading to increased availability of diacylglycerol, an activator of protein kinase C. The following findings are against the possibility that protein kinase C was involved in the phosphorylation of the 65-kDa islet cytosol protein. (*i*) The solution against which the cytosol fraction was dialyzed in order to remove endogenous nucleotides and substrates, as well as the standard phosphorylation assay buffer, did not contain any calcium. These conditions are also against the involvement of calcium/calmodulin-dependent protein kinase. (*ii*) Exogenous activators of protein kinase C failed to stimulate the phosphorylation of the 65-kDa protein. The failure of exogenous cAMP or cGMP to stimulate the phosphorylation of the 65-kDa protein excludes their respective protein kinases as the mediator. Thus, the kinase that may be responsible for the stimulation of phosphorylation of the 65-kDa protein appears to be distinct from protein kinase C and from cAMP-dependent, cGMP-dependent, and calcium/calmodulin-dependent protein kinases.

Pace *et al.* (21) have shown that exogenous 3PG stimulates insulin secretion from permeabilized rat islets. Thus, 3PG-dependent phosphorylation in islets reported here could be involved in some way in the activation of insulin secretion by 3PG. The rapidity of phosphorylation stimulated by 3PG and 2PG, and the physiologically relevant concentrations at which it occurs, are consistent with this proposed relationship.

At this time, the relevance of our findings to glucose-induced insulin release is not obvious. Even if phosphoglycerate-dependent protein phosphorylation in islets proves not to be involved in glucose-induced insulin release, it is an observation of considerable interest in terms of the cell-biological mechanisms operative in islet cells.

We conclude that protein phosphorylation induced by 3PG and 2PG in pancreatic islets may represent the activation by these glycolytic intermediates of a protein kinase distinct from the conventional protein kinases and may have relevance to stimulation of insulin secretion.

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