Glucose-induced Phosphorylation of Myristoylated Alanine-rich C Kinase Substrate (MARCKS) in Isolated Rat Pancreatic Islets*

(Received for publication, April 28, 1992)

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In order to further evaluate the role of protein kinase C activation in glucose-induced insulin secretion, the extent of phosphorylation of the myristoylated alanine-rich C kinase substrate (MARCKS) was examined in freshly isolated rat pancreatic islets prelabeled with [32P]orthophosphate. The islets were incubated with either 2.75 mM glucose alone, 2.75 mM glucose + 1 µM phorbol myristate acetate, 20 mM glucose, or 20 mM glucose + 50 mM staurosporine. After stimulation, the homogenized islets were processed by immunoprecipitation with a specific polyclonal anti-MARCKS antibody, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Densitometric analysis of autoradiograms revealed that phorbol myristate acetate caused a 3.78 ± 0.97-fold increase in MARCKS phosphorylation over control. In the islets exposed to 20 mM glucose, an increase of 3.43 ± 0.46-fold over control was observed. In islets exposed to 20 mM glucose, MARCKS phosphorylation was inhibited by 90 ± 4% compared with control islets exposed to 20 mM glucose alone. Islets similarly treated (but incubated without 32P) were examined by immunocytochemistry using an α-PKC-specific monoclonal antibody and visualized by confocal immunofluorescence microscopy. The α-PKC redistributed from the cytosol to the plasma membrane in the β-cells of islets exposed to 20 mM glucose. In separate experiments, unlabeled islets similarly treated were shown to respond with a 5–7-fold increase in insulin secretion in static incubation. Thus, when freshly isolated rat pancreatic islets are exposed to stimulatory glucose concentrations, they exhibit both a translocation of α-PKC and a significant increase in the extent of phosphorylation of MARCKS protein. These data suggest that α-PKC is activated during glucose-induced insulin secretion.

Glucose-induced insulin secretion has been shown to depend on the activation of several signal transduction pathways, including increased calcium influx, increased intracellular cAMP (1–4), and increased phosphoinositide hydrolysis (5). More recently, the involvement of yet another regulatory molecule, protein kinase C (PKC),1 in glucose-induced insulin secretion has been proposed. For instance, it is well known that phorbol esters (pharmacologic activators of PKC) like phorbol 12-myristate 13-acetate (PMA) can induce insulin secretion (6). When PMA is combined with agents that cause the activation of calcium influx (sulfonylureas) and cAMP production (forskolin), a normal, biphasic pattern of insulin secretion can be induced in isolated pancreatic islets (7). In addition, we have recently reported that α-PKC is present in pancreatic islets and that it translocates to the membrane fraction of islet homogenates in response to glucose stimulation (5). Although translocation of PKC to the membrane fraction has been regarded as indicative of PKC activation in many tissues, it has become evident that there is no simple correlation between the extent of PKC translocation and the magnitude of the cellular response (8). Hence, additional means for assessing PKC activation in intact cells are required. One such means is that of measuring the extent of phosphorylation of specific PKC substrates (9).

Myristoylated alanine-rich C kinase substrate (MARCKS) is a specific PKC substrate initially described in brain tissue, and later found to be present in a variety of tissues, including the rat pancreas (10, 11). Measurement of the extent of MARCKS phosphorylation has been used to specifically detect PKC activation in several tissues (12, 13). Using specific anti-MARCKS antibodies to immunoprecipitate 32P-labeled MARCKS, others have reported the absence of increased phosphorylation of MARCKS in glucose-stimulated cultured rat pancreatic islets (9). From these observations, these workers concluded that PKC activation is probably not an important feature in the glucose-induced signaling events in β-cells. This result differs from our observation showing glucose-induced α-PKC translocation in freshly isolated rat pancreatic islets (8). The contradictory results of these two studies may imply that glucose induces PKC translocation but not MARCKS phosphorylation or that differences in the experimental techniques account for the apparent discrepancy. In order to clarify this issue, we examined the effect of glucose on the extent of MARCKS phosphorylation using freshly isolated rat pancreatic islets prelabeled with [32P]orthophosphate. Using this approach, we show that glucose-stimulated freshly isolated islets exhibit a significant increase in the phosphorylation of the PKC-specific substrate MARCKS and that translocation of α-PKC and insulin secretion also occur under the same experimental conditions.

EXPERIMENTAL PROCEDURES

[32P]Orthophosphate was purchased from Amersham Corp. Aprotinin was obtained from Calbiochem. Other protease inhibitors and

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1 The abbreviations used are: PKC, protein kinase C; MARCKS, myristoylated alanine-rich C kinase substrate; PMA, phorbol 12-myristate 13-acetate; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; PKA, protein kinase A.
collagenase were purchased from Boehringer Mannheim. The monoclonal α-PKC antibody was obtained from Seikugaku America. Other reagents were purchased from Sigma unless otherwise indicated.

The anti-MARCKS antibody was a generous gift from Drs. Paul Greengard and Angus Nairn from The Rockefeller University. The antibody was raised in rabbits against purified rat brain MARCKS and shown to recognize MARCKS in Western blot and immunocytochemical studies (14). Statistical analysis was performed using the two-tailed Student’s t test for comparison of paired data and the Bonferroni test for comparison of multiple means.

Preparation of beta cells—The pancreas was dissected from anesthetized male Sprague-Dawley rats fed ad libitum and weighing between 260 and 300 g. The pancreas was collagenase-digested, and islets were picked manually and placed in a vial with 2 ml of buffer (bicarbonate-buffered media containing 115 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2.2 mM CaCl₂, 0.17% bovine serum albumin, 24 mM NaHCO₃, 10% FCS, 5% O₂, 5% CO₂, 90% N₂). After centrifugation at 2,000 rpm for 4 min to dispose of the pellet twice with 200 µl of PBS, each sample was resuspended in the incubation buffer (21 islets/variable) and without Triton X-100 for 3 h at room temperature. Again, the samples were centrifuged at 2,000 rpm for 4 min to dispose of the supernatant. After washing with 20 µl of PBS, the samples were used and the supernatant discarded. After washing, the pellets were incubated with CL-4B-protein A beads (1 mg/20 µl) for 3 h at room temperature and with gentle shaking, followed by incubation with CL-4B-protein A beads (1 mg/20 µl) for 3 h. The samples were then centrifuged for 2 min at 2,000 rpm in an VS-15 Sheltone centrifuge. The supernatant was saved for further processing. The pellets from each sample were suspended overnight, G₂₀, with 10 µl of anti-MARCKS antibody in 300 µl of PBS, 1% Triton X-100 and 100 mM NaCl. After overnight incubation, 20 µl of CL-4B-protein A was added to each sample and incubated for 3 h at room temperature. Again, the samples were centrifuged at 2,000 rpm for 2 min; this time the CL-4B-protein A pellet with the bound MARCKS was saved and the supernatant discarded. After washing the pellet twice with 200 µl of PBS, each sample was resuspended in 40 µl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (0.25 M Tris pH 6.8, 2 mM EGTA, 4% SDS, 20% glycerol, 0.001% bromophenol blue, 10% β-mercaptoethanol) and boiled for 5 min. The samples were processed by SDS-PAGE (15% acrylamide gel, 24 mM NaHCO₃, and 2.75 mM glucose, gassed with 95% O₂ and 5% CO₂) (15). The capacity of the islet preparation to respond with a biphasic insulin secretion to a glucose stimulus was also assessed. Filters with 20–40 islets were first placed in vials with 200 µl of incubation/perfusion buffer, sealed, and incubated for 90 min at 37 °C as described above to simulate the ³²P-labeling conditions. The filters containing the islets were then transferred to a perfusion chamber (variable) and incubated for 30 min with G₂₀ to establish a stable basal insulin secretory rate. The islets were then perfused with G₂₀ for maximal stimulation. Samples of the perfusate were collected at timed intervals for insulin analysis by radioimmunoassay (17).

RESULTS

Phosphorylation of MARCKS in Situ—Groups of 70–90 islets were isolated for each variable tested. Within any one experiment all variables were exactly matched for the number of islets. The islets were incubated in 200 µl of incubation buffer containing 100 µCi of [³²P]orthophosphate for 90 min in order to label the ATP pools. The islets were then stimulated with the cholesterol/solvent (0.25% ethanol) (G₂₀), 1 µM phorbol 12-myristate 13-acetate (2.7 µg/ml) and staurosporine. At the end of the incubation, the incubation buffer was removed and the islets placed on 100 µl of homogenization buffer (20 mM Tris pH 7.4, 5 mM EGTA, 1% Triton X-100, 50 µM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 25 µg/ml aprotinin, 10 µM pepstatin A, and 0.1% β-mercaptoethanol). The islets were homogenized by bath sonication in ice-cold water. Parallel experiments were performed using the same protocol but without [³²P]orthophosphate in the incubation buffer (21 islets/variable) and without Triton X-100 or β-mercaptoethanol in the homogenization buffer. These samples were assayed for protein content using the Bio-Rad DC protein assay method.

MARCKS Immunoprecipitation—The homogenized samples were loaded onto BM-Quick Spin G50 Sephadex Columns (Boehringer Mannheim) and centrifuged at 1,100 × g for 4 min to dispose of unincorporated label. The eluate was recovered and processed for immunoprecipitation. Each sample was first preclared by incubation with 20 µl of rabbit preimmune serum in 300 µl of PBS, 1% Triton X-100 for 3 h at room temperature and with gentle shaking, followed by incubation with CL-4B-protein A beads (1 mg/20 µl) for 3 h. The samples were then centrifuged for 2 min at 2,000 rpm in an VS-15 Sheltone centrifuge. The supernatant was saved for further processing. The pellets from each sample were incubated in 300 µl of incubation buffer (21 islets/variable) with 10 µl of anti-MARCKS antibody in 300 µl of PBS, 1% Triton X-100 and adjusted to 100 mM NaCl. After overnight incubation, 20 µl of CL-4B-protein A was added to each sample and incubated for 3 h at room temperature. Again, the samples were centrifuged at 2,000 rpm for 2 min; this time the CL-4B-protein A pellet with the bound MARCKS was saved and the supernatant discarded. After washing the pellet twice with 200 µl of PBS, each sample was resuspended in 40 µl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (0.25 M Tris pH 6.8, 2 mM EGTA, 4% SDS, 20% glycerol, 0.001% bromophenol blue, 10% β-mercaptoethanol) and boiled for 5 min. The samples were processed by SDS-PAGE (15% acrylamide gel, 24 mM NaHCO₃, and 2.75 mM glucose, gassed with 95% O₂ and 5% CO₂) (15). The samples were processed for immunoprecipitation to assess the localization of α-PKC, as described below.

Translocation—When the autoradiograms were analyzed by densitometry we found that the increase in phosphorylation after 50 nM staurosporine in the presence of low glucose (G₂₀) was only 10% of a positive control of islets stimulated with G₂₀. After bleaching and drying in a Hoeffer SE 540 slab gel dryer. The labeled bands were visualized by autoradiography. Quantification of the extent of MARCKS phosphorylation was accomplished by densitometric analysis of the 86.5-kDa band corresponding to MARCKS, using a Bioimaging Visage 2000 densitometer, and expressed as optical density (O.D.). MARCKS phosphorylation was expressed as the fold-increase of each sample’s O.D. over control O.D. (the control is a sample of islets that were only exposed to G₂₀).}

Protein assays of the unlabeled islets that were incubated and homogenized under conditions that permit accurate pro-
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Fig. 1. Phosphorylation of MARCKS in rat pancreatic islets. Freshly isolated rat pancreatic islets were prelabeled with \[^{32}P\]orthophosphate. This was followed by stimulation for 15 min with G2.75, 1 \(\mu\)M PMA, or G20. After stimulation, the islets were homogenized and MARCKS protein immunoprecipitated with a specific anti-MARCKS antibody, as described under “Experimental Procedures.” The phosphorylated MARCKS was visualized by autoradiography. A, the extent of MARCKS phosphorylation was quantified by densitometric analysis of the autoradiograms. The absorbance changes were expressed as fold over control. Islets exposed to PMA (n = 5) had an increase in MARCKS phosphorylation 3.98 \(\pm\) 0.97-fold over control (p < 0.01). Islets exposed to G20 (n = 9) had an increase in MARCKS phosphorylation of 4.93 \(\pm\) 0.40-fold over control (p < 0.01). The difference between PMA-treated and G20-treated islets was not statistically significant. B, a typical autoradiogram is shown.

Fig. 2. Inhibition of glucose-induced MARCKS phosphorylation by staurosporine. Freshly isolated rat pancreatic islets were prelabeled with \[^{32}P\]orthophosphate. This was followed by stimulation for 15 min with G2.75, G20, or G20 and 50 nM staurosporine (STR). The islets were then homogenized and immunoprecipitated with the anti-MARCKS antibody as described under “Experimental Procedures.” Changes in phosphorylation were visualized by autoradiography and quantified by densitometry. A, a typical autoradiogram is shown. B, the results are expressed as the percent stimulation of MARCKS phosphorylation compared with a G20-positive control (n = 4). Staurosporine inhibited glucose-induced MARCKS phosphorylation by 90 \(\pm\) 4% (p < 0.002).

Fig. 3. Insulin secretion in response to glucose. A, groups of 20–40 islets were laid on a nylon filter and incubated for 90 min at 37 °C to mimic the \[^{32}P\]orthophosphate labeling conditions. The number of islets was exactly matched for all variables within an experiment. The islets were then exposed to either G2.75 or G20 for 15 min. Samples for insulin secretion were obtained as described under “Experimental Procedures.” The results are expressed as fold increase over basal. The islets exposed to G20 exhibited a secretory response which was 5.6 \(\pm\) 1.5-fold greater than basal. B, in other experiments, groups of 20–40 islets were incubated for 90 min at 37 °C and then transferred to a perifusion chamber. The islets were perfused with buffer containing G2.75 for 30 min followed by G20 for 15 min. Timed samples were collected for insulin content determination. The results are expressed in pg/islet/min. The rate of insulin secretion 15 min after the addition of G20 was 13-fold over basal.

胞素测量 (no Triton X-100 or \(\beta\)-mercaptoethanol) revealed that there was no significant difference between the amount of protein in stimulated (G20 or PMA) versus unstimulated (G2.75) islets (not shown).

Insulin Secretion—As previously described, the islets were incubated for 90 min at 37 °C in the presence of G2.75 only, to mimic the \[^{32}P\]orthophosphate incubation. Subsequently the islets were either stimulated with G20 and insulin secretion assayed in a static incubation or transferred to a perfusion chamber and perfused with G20. Basal secretion in the presence of G2.75 was used as a control. When G20 was added to islets in static incubation, a 5.6 \(\pm\) 1.5-fold increase in the rate of insulin secretion was observed (Fig. 3A). When the islets were perfused, the insulin secretory response to glucose displayed a normal biphasic pattern (Fig. 3B). The rate of insulin secretion in the perfused islets 15 min after the addition of G20 was 13-fold over basal.

Translocation of \(\alpha\)-Protein Kinase C—We have previously shown by Western blot that \(\alpha\)-PKC translocates to the membrane fraction of pancreatic islets upon stimulation with G20 and that this translocation correlates with insulin secretion (8). We have now found that MARCKS phosphorylation also occurs upon stimulation with glucose. To show that \(\alpha\)-PKC translocation occurred under the same experimental conditions, we examined the localization of \(\alpha\)-PKC by immunocytochemistry in islets incubated for 90 min with G2.75 followed by stimulation with either 1 \(\mu\)M PMA or G20. Visualization by immunofluorescence confocal microscopy showed a diffuse cytosolic staining pattern in the control islets (Fig. 4A). Control islets that were not exposed to the primary antibody did not show detectable staining (not shown). The islets treated with either PMA or high glucose showed a redistribution of the \(\alpha\)-PKC with a significant increase in the staining in the periphery of the \(\beta\)-cell (Fig. 4B).

DISCUSSION

The possible role of protein kinase C in glucose-induced insulin secretion has been a matter of debate (18, 19). Several groups have reported negative results in experiments designed to detect PKC activation during glucose-induced insulin secretion by detecting PKC translocation (9, 20, 21). These studies used either measurements of PKC activity or measurements of \[^{3}H\]4-phorbol 12,13-dibutyrate binding in the cytosolic and membrane fractions of homogenized islets to detect translocation. Using isoenzyme-specific anti-PKC antibodies, we recently showed by Western blot that \(\alpha\)-PKC is present in pancreatic islets. We also demonstrated that \(\alpha\)-PKC translocates from the cytosolic fraction to the membrane fraction of isolated rat pancreatic islets stimulated with high
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**Fig. 4. Immunocytochemical localization of α-PKC in rat pancreatic islets.** Groups of 20–40 islets were exposed to G2.75, PMA, or G20 in a static incubation as described under "Experimental Procedures." The islets were then fixed and probed by immunocytochemistry with a monoclonal anti-α-PKC antibody, using fluorescein isothiocyanate anti-rabbit as a secondary antibody. The β-cells were visualized by immunofluorescence confocal microscopy. A, in the islets exposed to G2.75 alone the pattern of immunostaining was found to be diffuse and cytosolic. B, in the islets exposed to either PMA or G20 the immunostaining was observed to redistribute to the periphery of the β-cell. A photomicrograph of the G20 stimulated islets is shown. Control islets that were not exposed to the primary antibody had no detectable staining (not shown).

glucose as well as other fuel agonists. This translocation correlates temporally with insulin secretion and is blocked by inhibition of glucose metabolism with mannheptulose (8). Most recently we have confirmed and extended these observations by showing a glucose-induced translocation of α-PKC to the plasma membrane of the β-cell with the use of immunocytochemistry. In addition, we have shown that the PKC inhibitor staurosporine causes a marked inhibition of glucose-induced insulin secretion under conditions where staurosporine does not inhibit the rate of glucose metabolism (22).

In many systems, translocation of PKC alone has been accepted as evidence of its activation during a given cellular event (23, 24). However, this assumption has been challenged by some authors. For example, Trilivas and co-workers (25) found that, in 32D1N astrocytoma cells, stimulation with carbachol induces a translocation of PKC much earlier than a rise in diacylglycerol. Since PKC is thought to require both Ca2+ and diacylglycerol for its activation, these data would argue that in the case of the 32D1N astrocytoma cells PKC translocates to the membrane but without activation of its kinase activity. In another example, Salari and co-workers (26) reported that platelet-activating factor, thrombin and prostacyclin induce an increase in the PKC activity (measured by histone 1 phosphorylation in vitro) found in the cytosolic and particulate fractions of rabbit platelets, without appreciable change in the relative distribution of the total PKC activity (i.e. without evidence of translocation).

Several groups have also studied the phosphorylation pattern of phosphoproteins during glucose-induced insulin secretion using PAGE of crude extracts of 32P-labeled pancreatic islets (27) or two-dimensional gel electrophoresis and immunoprecipitation of selected phosphoproteins with specific antibodies (9). The phosphorylation of several phosphoproteins of different molecular weights were induced by phorbol esters, suggesting that they may be PKC substrates (27). To address the issue of PKC activation during glucose-induced insulin secretion we have now examined the phosphorylation of MARCKS, a specific substrate for PKC. The extent of MARCKS phosphorylation in islets was markedly stimulated with either PMA or glucose. Preliminary data seems to indicate that this is detectable as early as 5 min after the initiation of the glucose stimulus, which correlates with our previous data in perfused islets showing α-PKC translocation after 5 min in response to G20 (8). These results strongly suggest that glucose stimulates PKC-dependent phosphorylation of proteins in pancreatic islets and argue for the activation of PKC during glucose-induced insulin secretion.

The glucose-induced increase in MARCKS phosphorylation was blocked by inhibiting the action of PKC with staurosporine. Staurosporine is a very effective inhibitor of PKC activity as well as a potent inhibitor of insulin secretion (22, 28). However, staurosporine is not a completely specific inhibitor of PKC since it can also inhibit protein kinase A (PKA), with a higher Kᵢ, but still within the same order of magnitude (29). The phosphorylation domain of MARCKS contains, in addition to the four PKC phosphorylation sites, a 3-amino acid sequence that has been identified as a phosphorylation site in PKA substrates. Although there is evidence for weak phosphorylation of all of the above mentioned sites by PKA in vitro, PKA activation in intact cells has been shown to have no effect on MARCKS phosphorylation (30, 31). In a previous study, we showed that concentrations of staurosporine between 20 and 100 nM inhibit the second phase of glucose-induced insulin secretion by 70–80%. However, staurosporine did not affect the glucose-induced increase in phosphoinositide hydrolysis and glucose usage and actually enhanced the first phase of insulin secretion (22). These results make it unlikely that staurosporine's effect on insulin secretion or MARCKS phosphorylation is due to nonspecific toxic effects on the β-cell. Thus, the inhibition of glucose-induced MARCKS phosphorylation by staurosporine in intact pancreatic islets argues that this is a PKC-mediated event. The observation that staurosporine does not affect the basal phosphorylation of MARCKS at the concentration and time of exposure used for our experiments may indicate that at least one of MARCKS phosphorylation sites has a slow turnover.

Easom and co-workers have also studied the effect of glucose on MARCKS phosphorylation in rat pancreatic islets (9). They found that stimulation of cultured islets with 500 μM carbachol induces a statistically significant increase in MARCKS phosphorylation after 5 min of stimulation and that this is potentiated by concomitant stimulation with 500 μM carbachol and 10 mM glucose. However, they did not observe a significant increase in MARCKS phosphorylation when islets were exposed to high glucose alone. They could find no evidence of glucose-induced PKC translocation either (9). The exact reason for the difference between their results and the present results are not clear. The main difference in experimental protocols is that Easom's group used islets cultured overnight in medium containing 5.5 mM glucose, whereas our group used freshly isolated islets. It has been shown that overnight culture of pancreatic islets in medium containing low glucose suppresses glucokinase activity and that these islets are less sensitive to glucose stimulation (32, 33). The latter observation may translate into an impairment in signal transduction in islets cultured overnight, as suggested by the absence of glucose-induced PKC translocation and MARCKS phosphorylation in cultured islets.

In order to correlate MARCKS phosphorylation with the insulin secretory response we measured insulin secretion under conditions similar to those used for the phosphorylation experiments. Measurement of insulin secretory rates of islets in static incubation after stimulation with G20 showed a 5–7-fold increase over basal. Because of the wide differences in

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the incubation protocols (different glucose concentration, time of incubation, volume of incubation, etc.) it is difficult to make direct comparisons of the secretory capacity of the islets employed by different investigators. However, the secretory rates we obtained seem to be comparable to those reported by others using islets stimulated in static incubation (21, 34). However, this rate of secretion is less than half of the usual secretory rates obtained when freshly isolated islets are perfused (22). Because of this difference, the issue of islet responsiveness was analyzed further by taking islets which were preincubated under conditions similar to those used for the phosphorylation experiments, and then perfused. The perfused islets responded to glucose stimulation with a normal, biphasic secretory pattern and with a second phase of insulin secretion as high as 13-fold above basal. The lower secretory response of the islets under static incubation conditions may be related to a lower oxygen tension in the incubation buffer than that achieved in perfusion, where the buffer is gassed constantly (35). It could also be due to a negative feedback of insulin on its own secretion, a phenomenon that has been described by others (36). Nonetheless, we have shown that insulin secretion occurs under the experimental conditions under which glucose-induced MARCKS phosphorylation is observed.

The cellular function of MARCKS has not been yet established. It is known that MARCKS is a calmodulin-binding protein and that its affinity for calmodulin is dependent on its phosphorylation state (37, 38). PKC phosphorylation of MARCKS results in the rapid release of calmodulin making it available for activation of calmodulin-dependent enzymes (38). This kind of interaction could allow for mechanisms for cross-talk between the PKC and the calmodulin-dependent signal transduction pathways. There is also evidence that MARCKS is localized to cytoskeletal structures in its unphosphorylated state and that upon PKC phosphorylation it detaches and redistributes to the cytosol (39). This observation raises the possibility that the phosphorylation-dependent regulation of MARCKS' membrane binding might locally modify the interaction between the cytoskeleton and the membrane and in this manner influence membrane associated cellular events, like the exocytotic secretion of peptide hormones such as insulin.

Our previous published work with freshly isolated perfused islets shows translocation of aPKC from the cytosolic to the membrane fraction of islets stimulated with glucose as demonstrated by Western blot analysis of the fractions (8). In a set of experiments to be published separately aPKC's localization within the β-cell was studied by immunocytochemistry using an aPKC specific monoclonal antibody and visualized by confocal immunofluorescence microscopy. These experiments showed that aPKC co-localizes with insulin to the β-cells of pancreatic islets. In the basal state the aPKC has a diffuse cytosolic distribution; after perfusion in the presence of high glucose the aPKC translocates to the periphery of the β-cell, suggesting that aPKC is translocating to the plasma membrane of the β-cells or to a closely associated domain. Similar results were observed in fixed sections of pancreata of rats infused intravenously with glucose and sacrificed after 15 min. Our present study confirms that this pattern of change in aPKC localization can also be observed in islets stimulated with glucose in a static incubation, within the same time frame that MARCKS phosphorylation is detected.

In summary, we have established that high glucose by itself is capable of inducing the phosphorylation of MARCKS in isolated rat pancreatic islets, and that this change in MARCKS phosphorylation correlates with aPKC translocation from the cytosol to the plasma membrane of the β cell or to a compartment closely associated with the plasma membrane, as assessed by immunocytochemistry. These two separate lines of evidence strongly support the hypothesis that PKC is activated in islets exposed to glucose. The fact that insulin secretion is also observed within the same time frame suggests that aPKC may be actively involved in mediating glucose-induced insulin secretion. A better understanding of the physiological role of MARCKS may eventually allow us to more fully establish a relationship of cause and effect between PKC activation and insulin secretion.

Acknowledgments—We thank Drs. Katherine Albert, James Wang, and Paul Greengard for their generous gift of anti-MARCKS antibody and Drs. Angus Nairn and Walter W. Zawalich for helpful discussion. We are also indebted to Kathy Zawalich for technical assistance with the insulin assay.

REFERENCES

1. Sharp, G. W., G. W., Wiedenkeller, D. K., Kaelin, D. G., Siegel, E. G., and Wollheim, C. (1980) Diabetes 29, 74–77
2. Grill, V., and Cerasi, E. (1991) J. Biol. Chem. 266, 4196–4201
3. Gylfe, E. (1988) J. Biol. Chem. 263, 13750–13754
4. Draznin, B. (1980) Am. J. Med. 5, 44–56
5. Zawalich, W. S., and Zawalich, K. S. (1988) Diabetes 37, 1294–1300
6. Zawalich, W., Brown, C., and Raasmussen, H. (1983) Biochem. Biophys. Res. Commun. 117, 448–455
7. Zawalich, W., Zawalich, K., and Raasmussen, H. (1984) Cell Calcium 5, 551–558
8. Ganesan, S., Calle, R., Zawalich, K. C., Smallwood, J. L., Zawalich, W. S., and Raasmussen, H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9682–9687
9. Rosam, R. A., Landt, M., Coles, J. R., Hughes, J. H., Turk, J., and McDaniel, M. (1990) J. Biol. Chem. 265, 14905–14909
10. Albert, K. A., Wang, S. W., Jiang, K., and Greengard, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 89, 12538–12538
11. Blackshear, P. J., Wen, L., Glynn, B. F., and Witters, L. A. (1986) J. Biol. Chem. 261, 1459–1469
12. James, G., and Olson, E. N. (1989) J. Biol. Chem. 264, 29026–29033
13. Blackshear, G., Friedrich, B., and Gullberg, M. (1989) Scand. J. Immunol. 30, 233–240
14. Omi, C. T., Wang, J. K., Wang, S., Turner, K. A., and Greengard, P. (1990) J. Neurochem. 56, 1833–1839
15. Lacy, P. E., and Kostianovsky, M. (1997) Diabetes 16, 35–39
16. Laemmli, U. K. (1970) Nature 227, 680–685
17. Albano, J. D., Ekin, R. P., Moritz, G., and Turner, R. C. (1972) Acta Endocr. 70, 487–509
18. Metz, S. A. (1988) Diabetes 37, 3–7
19. Wollheim, C. B., and Regazzi, R. (1990) FEBS Lett. 268, 370–380
20. Rosam, R. A., Hughes, J. H., Landt, M., Wolf, M. A., and McDaniel, M. L. (1990) J. Biol. Chem. 265, 27–33
21. Persaud, S. J., Jones, P. M., Sugden, D., and Howell, S. L. (1989) FEBS Lett. 245, 80–84
22. Zawalich, W. S., Zawalich, K. C., Ganesan, S., Calle, R., and Raasmussen, H. (1991) Biochem. J. 279, 807–813
23. Neidell, J. E., and Blackshear, P. J. (1989) Biochim. Biophys. Acta 977, 92–96
24. Neidell, J. E., and Blackshear, P. J. (1989) Biochim. Biophys. Acta 977, 92–96
25. T Oliveira, M. E., and Larkins, R. G. (1986) Arch. Biochem. Biophys. 248, 542–549
26. Tamsaki, K., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, T. (1990) Biochem. Biophys. Res. Commun. 155, 397–402
27. Robbins, U. T., and Burgess, G. M. (1989) Trends Pharmacol. Sci. 10, 216–220
28. Blackshear, P. J., Winters, L. A., Girard, P. R., Kuo, J. P., and Quinno, S. N. (1988) J. Biol. Chem. 263, 13994–13997
29. Graff, J. M., Rajman, R. R., Randall, R. R., Nairn, A. C., and Blackshear, P. J. (1991) J. Biol. Chem. 266, 14900–14908
30. Strassmann, G., Walsh, J. P., and Welsh, M. (1991) Diabetes 40, 771–776
31. Lien, J., Najafi, H., and Matschinsky, F. M. (1990) J. Biol. Chem. 265, 16563–16566
32. Knaus, N., Corcos, A. P., Sarel, I., and Cerasi, E. (1991) Endocrinology 19, 2067–2070
33. Okha, M., Nelson, D., Nelson, J., Meglasson, M. D., and Erecinska, M. (1990) J. Biol. Chem. 265, 13984–13982
34. Draznin, B., Goodman, M., Leitner, J. W., and Sussman, K. E. (1991) Endocrinology 119, 1054–1064
35. McFarlane, B., Draznin, B., and Raasmussen, H. (1989) J. Biol. Chem. 264, 21816–21820
36. Rosen, A., Keenan, K. F., Thelen, M., Nairn, A. C., and Aderem, A. (1990) J. Exp. Med. 172, 1211–1215