Stimulus-Response Coupling in Insulin-secreting HIT Cells

EFFECTS OF SECRETAGOGUES ON CYTOSOLIC Ca\(^{2+}\), DIACYLGLYCEROL, AND PROTEIN KINASE C ACTIVITY

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The hamster islet B cell line HIT retains the ability to secrete insulin in response to glucose and several receptor agonists. We used HIT cells to study the initial signaling events in glucose or receptor agonist-stimulated insulin secretion. Glucose stimulated insulin release from HIT cells in a dose-dependent manner with a half-maximal effect seen already at 1 mM. Insulin release was also stimulated by carbachol in a glucose-dependent manner. Glucose depolarized the HIT cell membrane potential as assessed with the fluorescent probe bisoxonol and raised intracellular Ca\(^{2+}\) as revealed by fura-2 measurements. Using a Mn\(^{3+}\)-fura-2 quenching technique, we could show that the rise in intracellular Ca\(^{2+}\) was due to Ca\(^{2+}\) influx following opening of voltage-gated Ca\(^{2+}\) channels.

Glucose is thought to increase the diacylglycerol (DAG) content of insulin-secreting cells. However, although HIT cells respond to glucose in terms of insulin secretion, membrane depolarization, and Ca\(^{2+}\) rise, the hexose was unable to increase the proportion of protein kinase C activity associated with membranes. In contrast, the membrane-associated protein kinase C activity increased in HIT cells exposed to the two receptor agonists carbachol and bombesin. Bombesin was shown to generate DAG with the expected fatty acid composition of activators of phospholipase C. Glucose, in contrast, only caused minor increases in DAG containing myristic and palmitic acid without affecting total DAG mass. The failure to detect stimulation of protein kinase C by glucose could be due to both the limited amount and to the different fatty acid composition of the metabolically generated DAG. The latter was in part supported by experiments performed on protein kinase C partially purified from HIT cells. Indeed, 1,2-dipalmitoylglycerol, presumed to be the main DAG species generated by glucose, was only one-third as active as 1,2-dioleoylglycerol and 1-stearoyl-2-arachidonoylglycerol in stimulating the isolated enzyme at physiological Ca\(^{2+}\) concentration. It is therefore unlikely that DAG and protein kinase C play a major role in glucose-stimulated insulin secretion.

Insulin secretion from the pancreatic B cell is influenced by a variety of physiological factors. These include the elevation of glucose concentration in the circulation and the release of acetylcholine from nerve endings within the pancreatic islets (1). It is well established that glucose depolarizes the B cell membrane potential by closing ATP-sensitive K⁺ channels, in turn causing the gating of voltage-dependent Ca\(^{2+}\) channels (2). The resulting rise of cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) can be explained entirely by Ca\(^{2+}\) influx and precedes the stimulation of insulin secretion (3). In contrast, receptor agonists such as cholinergic substances and bombesin lead to a [Ca\(^{2+}\)]\(_{i}\), rise mainly due to mobilization of Ca\(^{2+}\) from intracellular stores (4, 5). This effect is mediated by the second messenger inositol 1,4,5-trisphosphate, which is generated, together with 1,2-diacylglycerol (DAG), by the hydrolysis of phosphatidylinositol 4,5-bisphosphate (6). The increase of DAG in the plasma membrane is thought to activate protein kinase C, a Ca\(^{2+}\)- and phospholipid-dependent enzyme (7, 8). Stimulation of pancreatic islets with glucose resulted in a similar increase of total mass of DAG as that evoked by carbachol in two studies (9, 10), whereas no increase was found in a third (11). Although glucose promotes phosphatidylinositol 4,5-bisphosphate hydrolysis (12, 13), the DAG was believed to be formed mainly by de novo synthesis via glycolytic intermediates and phosphatidic acid (9, 11, 14-16). However, a recent study measuring total islet mass of DAG by an enzymatic method did not find any change due to glucose, despite stimulation of the de novo synthesis pathway (11).

Activators of protein kinase C, such as phorbol esters and synthetic diacylglycerols, have been shown to stimulate insulin release (17-19), but the involvement of this enzyme during the stimulation of intact B cells with physiological secretagogues remains controversial. As expected, the muscarinic agonist carbachol activates protein kinase C in islets as measured either by the association of the enzyme with membranes (20) or by phosphorylation of a protein substrate (21). Translocation also occurred in the insulin-secreting cell line RINm5F (22). With respect to carbohydrate stimuli, the situation is less clear. In islets, glucose failed to elicit protein kinase C translocation (20, 23), while causing a small phosphorylation of a protein kinase C substrate (21). In RINm5F cells, which do not recognize glucose as a secretagogue (24), D-glyceraldehyde caused a marked redistribution of the enzyme (25).

The purpose of the present study was to examine whether both Ca\(^{2+}\)-mobilizing receptor agonists and glucose activate protein kinase C as a step in the initiation of insulin secretion. We employed the hamster B cell line HIT-T15, which has been shown to respond to various secretagogues including glucose (26, 27). Here, we demonstrate that glucose, in addi-
tion to stimulating insulin release, depolarizes the cells and raises [Ca^{2+}], suggesting that HIT cells are a valid model for the study of stimulus-response coupling in insulin secretion. However, while carbachol and bombesin caused protein kinase C redistribution, reflecting the activation of the enzyme, such was not the case for glucose. This is probably due to only a minute increase of DAG of a fatty acid composition not favoring protein kinase C activation following glucose stimulation.

**EXPERIMENTAL PROCEDURES**

**Materials**—The fluorescent probes fura-2 acetoxyethyl ester and bisoxonol were obtained from Molecular Probes, Eugene, OR. Carbachol, phosphatidylserine, and all diacylglycerols were purchased from Sigma. Synthetic bombesin was a generous gift of Dr. R. Castiglione (Farmitalia, Milan, Italy). All other reagents were of analytical grade.

**Cell Culture**—HIT cells, clone T15, were obtained from Dr. Matchinsky's laboratory (University of Pennsylvania) and from Dr. Boyd's laboratory (Dartmouth College). The cells were cultured in monolayers in RPMI 1640 medium supplemented with 2 mM glutamine, 100 mM sodium, and 10% fetal calf serum as published previously (26, 27). The experiments were performed during passages 69-80. For part of the protein kinase C measurements, the cells were plated in 10-cm-diameter Petri dishes and used when confluent. All other experiments were performed in cell suspensions following a 3-h spinner culture period under conditions identical with those described for RINm5F islets (1). In contrast, the sensitivity to glucose alone is exaggerated in HIT cells.

**Insulin Secretion**—Insulin secretion was measured during 15-min static incubations of cell suspensions in a modified Krebs-Ringer bicarbonate buffer. The cells were kept at room temperature to reduce fura-2 leakage until the addition of the appropriate amount of CaCl2 to a buffer containing 1 mM EGTA. Phosphatidylserine and the different mono- and diacylglycerol species were mixed in a small volume of chloroform, dried under nitrogen, and reconstituted in 20 mM Tris-HCl buffer, pH 7.4, by sonication. The concentration of phosphatidylinerine, when present in the assay, was 16.4 μg/ml and the mono- and diacylglycerols were added at a final concentration of 0.8 μg/ml. The reaction was carried out and stopped as described above.

**Diacylglycerol Measurements and Fatty Acid Analysis**—After incubation of the cells, the lipids were extracted and separated on thin-layer chromatography plates as described previously (9). The spots containing DAG were scraped and extracted with chloroform/methanol (1:1, v/v). A known amount of heptadecanoic acid was added to the extracts as an internal standard and the extracts were dried under an argon stream. Conversion to methyl esters was performed by boiling the lipids for 10 min at 60°C in 300 μl of dry methanol containing 15% (w/v) boron trifluoride. After addition of water, the fatty acid methyl esters were extracted in 2 ml of pentane. The pentane solution was dried over anhydrous Na2SO4, before concentration. The methyl esters were chromatographed on a 200-cm glass column (3 mm internal diameter) packed with 3% OV-1 on 100/120 Chromosorb (H, AW-DMS). Analyses were performed on a Perkin-Elmer gas chromatograph equipped with a flame ionization detector. The column temperature was 210°C while injector and detector were maintained at 220°C. Nitrogen was used as a carrier at a flow rate of 95 ml/min. Elution times were compared to authentic standards. The mass of fatty acids in the samples was calculated by comparing with the internal standard added. The content of heptadecanoic acid in DAG was lower than that of pentadecanoic acid and was the same with unstimulated and stimulated cells. The recovery of fatty acids from a DAG standard, 1-stearoyl-2-arachidonoylglycerol was 94 ± 1 and 78 ± 2% (n = 4) for stearic acid and arachidonic acid, respectively.

**RESULTS**

Insulin secretion from HIT cells was assessed in static incubations following a period of glucose deprivation as described by Meglasson et al. (27). The results in Fig. 1 demonstrate that glucose stimulates insulin secretion from HIT cells in a dose-dependent manner with a half-maximal effect at approximately 1 mM. The preincubation in the absence of glucose in these experiments was 60 min, but similar results were obtained after 30 min. The addition of 100 μM carbachol enhanced insulin secretion in a glucose-dependent manner with an optimal effect seen at 3 mM glucose (Fig. 1). This glucose dependency is similar to that found in pancreatic islets (1). In contrast, the sensitivity to glucose alone is exaggerated in HIT cells.

Glucose depolarized the HIT cell membrane potential as assessed with the fluorescent probe bisoxonol. The effect was apparent at 1 mM and maximal at 10 mM (Fig. 2A). There was a 1-min lag and glucose depolarized the cells optimally.
added before glucose (Fig. 2E). This suggests that part of the depolarizing current in HIT cells stimulated with glucose is due to Ca^{2+} influx. Experiments were then performed to show Ca^{2+} entry. To this end, Mn^{2+} was added to the extracellular medium in order to enable the monitoring of Mn^{2+} influx into HIT cells. It has been shown previously that Mn^{2+} quenches the fluorescence of quin 2 and fura-2 and that the decrease of fluorescence in dye-loaded cells reflects Mn^{2+} influx through Ca^{2+} conductance channels (30–32). As can be seen in Fig. 3A, the addition of Mn^{2+} (300 μM) to fura-2-loaded HIT cells caused a rapid decrease in fluorescence due to the quenching of extracellular fura-2. Thereafter, the fluorescence steadily decreased, reflecting the slow uptake of Mn^{2+} by the cells. The addition of 10 mM glucose resulted in an accelerated uptake of Mn^{2+}, which continued until voltage-dependent Ca^{2+} channels were blocked with 20 μM verapamil. When verapamil was added prior to glucose, the rate of Mn^{2+} entry was not altered by the stimulus (data not shown). Theoretically, the increased dye quenching could be explained by increased leakage of fura-2 from the glucose-stimulated cells.

Experiments were then performed to show the anion channel blocker sulfinpyrazone (29). The results in Fig. 3B obtained in the presence of 200 μM sulfinpyrazone are qualitatively similar to those shown in Fig. 3A. This effect is also observed in nominally Ca^{2+}-free medium, where it is possible to reduce the Mn^{2+} concentration to 100 nM. As expected, the Ca^{2+} ionophore ionomycin still caused a [Ca^{2+}]_{i} rise in the presence of verapamil (Fig. 4B). The action of glucose on [Ca^{2+}]_{i} was then tested in the presence of 200 μM sulfinpyrazone, which was also present during the 30-min preincubation period at 37 °C. Again, glucose raised [Ca^{2+}]_{i} from 137 ± 9 nM to 222 ± 28 nM (n = 7). A representative trace of the seven experiments is shown in Fig. 4A. The rise in [Ca^{2+}]_{i} was attenuated by verapamil, added either after (Fig. 4A) or before (Fig. 4B) glucose. In the latter case, the values were 128 ± 8 and 139 ± 13 nM (n = 4) before and after the addition of glucose, respectively. Similar results were obtained when 5 μM nifedipine was used to block voltage-dependent Ca^{2+} channels (data not shown). As expected, the Ca^{2+} ionophore ionomycin still caused a [Ca^{2+}]_{i} rise in the presence of verapamil (Fig. 4B). The action of glucose on [Ca^{2+}]_{i} was then tested in the presence of 200 μM sulfinpyrazone, which was also present during the 30-min preincubation period at 37 °C. Again, glucose raised [Ca^{2+}]_{i} from 204 ± 9 to 318 ± 19 nM (n = 6), which is illustrated in Fig. 4C. The addition of 5 μM nifedipine caused an immediate return of [Ca^{2+}]_{i} to basal levels (Fig. 4C). In the absence of extracellular Ca^{2+}, glucose failed to raise [Ca^{2+}]_{i} (not shown). Taken together, these results demonstrate that glucose raises [Ca^{2+}]_{i} by stimulating Ca^{2+} influx following the opening of verapamil- and nifedipine-sensitive Ca^{2+} channels.

As demonstrated previously in RINm5F cells (4), in mouse islet cells (38), in single HIT cells (37), and in B cells (38), carbachol enhanced [Ca^{2+}]_{i} in suspensions of HIT cells (Fig. 4D). The agonist increased [Ca^{2+}]_{i}, from 151 ± 24 to 225 ± 37 nM (n = 3). Thus, it appears that HIT cells respond to glucose and carbachol with both a [Ca^{2+}]_{i} rise and insulin secretion in a manner to be expected of these two secretagogues. In the following experiments, we examined whether both receptor agonists and glucose activate protein kinase C in HIT cells to estimate the involvement of this enzyme in insulin secretion. Mucaricin agonists lead to the activation of phospholipase C and the generation of DAG in pancreatic islets (9, 10). Protein kinase C attaches to cellular membranes
when it is activated, permitting the assessment of the degree of enzyme activation by measuring the subcellular distribution after exposure of intact cells to secretagogues. Using the homogenization protocol described under “Experimental Procedures,” about the same amount of protein was obtained in both cytosolic and microsomal fractions. Under resting conditions, 84 ± 1% (n = 5) of the protein kinase C activity was found to be soluble, while 10 ± 1% (n = 5) was recovered in the microsomal extract. As shown in Fig. 5, in cells exposed to 100 μM carbachol, there was a rapid increase in the amount of protein kinase C associated with the cellular membranes and a corresponding decrease in the cytosolic fraction. Maximal redistribution of protein kinase C was reached after 2 min of incubation in the presence of the agonist and was still seen after 10 min. In contrast, no changes in the subcellular localization of the enzyme were found in the controls even after 10 min of incubation (not shown).

In order to ascertain if exposure of HIT cells to glucose could evoke protein kinase C activation, these cells were preincubated for 30 min in the absence of glucose and then stimulated for 10 min with 10 mM of hexose. As seen in Table I, incubation of the cells in the presence of glucose did not change the subcellular localization of protein kinase C. Similar results were obtained when HIT cells were treated with glucose for 5 or 15 min (not shown). In contrast, in paired experiments, carbachol (100 μM) after 10 min of treatment caused an increase in protein kinase C activity associated with the microsomal fraction (Table I). In order to exclude that the failure to detect protein kinase C redistribution in glucose-stimulated cells was due to the method used, the protocol was varied in two ways. First, protein kinase C was assayed in the presence of phosphatidylserine and phorbolmyristate acetate and with histone H III-S as substrate instead of protamine (see “Experimental Procedures”). This approach yielded quantitatively similar results (data not shown). Second, the cells were incubated under conditions identical with

![Graph A](image1.png)

**Fig. 3.** Effect of glucose on Ca$^{2+}$ influx assessed by Mn$^{2+}$ quenching of intracellular fura-2. Suspensions of HIT cells were loaded with the fluorescent Ca$^{2+}$ indicator fura-2 and preincubated in the absence of glucose for 30-40 min (see “Experimental Procedures”). A and B were performed in Ca$^{2+}$-containing buffer and C in nominally Ca$^{2+}$-free buffer. In B and C, 200 μM sulfipyrazone was present in the cuvette. The initial rapid decrease in fluorescence is due to quenching of extracellular fura-2. The traces are representative of at least three separate experiments.

![Graph B](image2.png)

**Fig. 4.** Effect of glucose and carbachol on [Ca$^{2+}$]$_i$ in HIT cells. The cells were loaded with fura-2 and preincubated as indicated in the legend to Fig. 3. The Ca$^{2+}$ concentration of the buffer was 1 mM throughout. In C, the cuvette contained 200 μM sulfipyrazone (see “Experimental Procedures”). The traces are representative of at least three separate experiments.

![Graph C](image3.png)

**Fig. 5.** Time course of subcellular redistribution of protein kinase C in HIT cells stimulated by carbachol. HIT cells in monolayer culture were exposed to carbachol (100 μM) in the modified Krebs-Ringer bicarbonate buffer containing 2.8 mM glucose. The cell incubation was stopped and subcellular fractions prepared as described under “Experimental Procedures.” Protein kinase C (PKC) activity was measured in the cytosolic and microsomal fraction after partial purification of the enzyme by polyacrylamide gel electrophoresis. The results are given as mean ± S.E. of three to five independent observations.
those used to measure insulin secretion, i.e., in suspension rather than attached to a substratum (Table I). Both approaches failed to reveal an effect of glucose on protein kinase C subcellular distribution. Bombesin, another activator of phospholipase C, has been shown to raise [Ca\(^{2+}\)], increase DAG turnover, and stimulate insulin secretion in suspensions of HIT cells (5). In the present study, bombesin (100 nM) activated protein kinase C in suspensions of HIT cells, after both 2 and 10 min of stimulation (Table I). Thus, under conditions where glucose, like carbachol and bombesin, raises [Ca\(^{2+}\)], and stimulates insulin secretion, only the receptor agonists cause a detectable increase in protein kinase C activity associated with the membrane fraction.

The absence of protein kinase C activation by glucose could be due either to the failure to generate DAG in HIT cells, or, if indeed generated, the DAG species might be inefficient in stimulating protein kinase C. The fatty acid composition of DAG in bombesin- or glucose-stimulated HIT cells was measured by gas-liquid chromatography (see “Experimental Procedures”). After 30 min of preincubation in the absence of glucose, the cells were incubated either for 2 min with or without bombesin (100 nM) or for 10 min with or without glucose (10 mM). Under resting conditions, the main fatty acid species detected in DAG were palmitic, oleic, and stearic acids. These results are similar to our previous studies in rat islets except for a much lower content of arachidonic acid in HIT cells (9). Bombesin caused a small but significant increase in palmitic (10%), oleic (22%), stearic (22%), and arachidonic acids (50%) (see Fig. 6, top). Glucose stimulation, in contrast, caused a slight increase in DAG containing myristic (20%) and palmitic acids (15%) (Fig. 6, bottom).

Thus, in HIT cells, a receptor agonist causes an increase of DAG with the composition expected for DAG originating from inositol-containing phospholipids (6, 9). Glucose, on the other hand, if at all, only generates DAG enriched in saturated fatty acids. Estimation of the total DAG mass from the data in Fig. 6 yields the following values (in picomoles/10^6 cells, mean ± S.E., n = 4): control 124 ± 6, bombesin 142 ± 6 (p < 0.025 Student’s paired t test); control 137 ± 8, glucose 146 ± 9 (p > 0.05 paired t test). The effect of the two types of stimulus on the total DAG mass was also examined using an enzymatic method (35). At 10 min, bombesin increased the DAG mass from 56 ± 6 to 70 ± 3 pmol/10^6 cells (p < 0.05), whereas the value for the cells incubated in parallel with 10 mM glucose was 57 ± 3. After 30 min of glucose stimulation, the DAG level was 58 ± 2 pmol/10^6 cells relative to 59 ± 8 in controls.
glucose (26, 27, 39, 40). In order to demonstrate that HIT cells behave like islet cells, it was important to ascertain that glucose depolarizes HIT cells and promotes the subsequent opening of voltage-dependent Ca\(^{2+}\) channels. Using the membrane potential probe biso-xonol, we could show that glucose indeed depolarizes these cells. The effect was already seen at 1 mM glucose, a concentration causing half-maximal insulin secretion (27). It should be noted that HIT cells display an exaggerated sensitivity to glucose compared to pancreatic islets, where the half-maximal glucose concentration is about 8 mM (1). The biso-xonol measurements suggest that part of the glucose effect is due to Ca\(^{2+}\) influx, since the depolarization was attenuated in Ca\(^{2+}\)-free medium. As the effect of K\(^{+}\) was also diminished by Ca\(^{2+}\) removal, it is possible that the depolarizing current is in part carried by Ca\(^{2+}\) because of the abundance of voltage-dependent Ca\(^{2+}\) channels in this cell line (41). By using the Mn\(^{2+}\)-quench technique previously described for platelets (30), neutrophils (31), and endothelial cells (32), it was possible to monitor Mn\(^{2+}\) influx through Ca\(^{2+}\)-conductance pathways. Glucose stimulated Mn\(^{2+}\) influx and the effect was blocked by the Ca\(^{2+}\) channel antagonists verapamil and nifedipine. This is a direct demonstration of the opening of Ca\(^{2+}\) channels by glucose. The effect was observed both in the presence and absence of extracellular Ca\(^{2+}\), suggesting that glucose-mediated gating of Ca\(^{2+}\) channels is similar under both conditions. As expected, glucose causes the rise in [Ca\(^{2+}\)], which, in this system, remained elevated for a long period of time. The effect was entirely due to Ca\(^{2+}\) influx since it was inhibited by Ca\(^{2+}\) channel blockers and was abolished in the absence of external Ca\(^{2+}\). At 37 °C, HIT cells display fura-2 leakage that, in other cell types, has been shown to be attenuated by the anion channel blockers probenecid and sulfinpyrazone (29, 42). Application of sulfinpyrazone largely inhibited the loss of fura-2 and confirms the conclusion that glucose raises [Ca\(^{2+}\)], by stimulating Ca\(^{2+}\) influx. Glucose and glyceroldehyde have been demonstrated to depolarize insulin-secreting cells by closing ATP-sensitive K\(^{+}\) channels (2). This has also been shown for glucose in HIT cells (43). Consequently, the HIT cells thus appeared to be a valid model for the investigation as to whether glucose stimulation is accompanied by protein kinase C activation.

We chose to assess protein kinase C activation by monitoring the association of the enzyme with cellular membranes. A similar methodology has been successfully applied to the investigation of agonist stimulation in many cell types (44, 45). Following stimulation of intact cells with receptor agonists, an increased proportion of the enzyme becomes associated with membranes. This intercalation depends on the receptor-mediated formation of DAG and on the rise of [Ca\(^{2+}\)] (1).

**Stimulation of HIT cells with the muscarinic agonist carbachol, which causes a transient [Ca\(^{2+}\)]\(_i\) rise, led to an approximately 50% increase of membrane-associated protein kinase C activity. The effect was first observed after 1 min and maintained for 10 min. Similar findings have been reported in other cell systems (47). Such an effect of carbachol has also been reported for RINr cells (22), another insulin-secreting cell line, and for isolated islets (20). Bombesin, another activator of phospholipase C in HIT cells (5), also promoted membrane association of protein kinase C (Table I). Thus, the binding of carbachol and bombesin to their respective receptors on insulin-secreting cells causes not only the formation of 1,4,5-triphosphate, the subsequent rise in Ca\(^{2+}\), and the generation of DAG, but also the activation of protein kinase C.

The receptor occupation is transduced by a G-protein that stimulates phospholipase C (48). In contrast, the mechanism by which carbohydrates stimulate insulin secretion is less well understood. Both glucose and glyceroldehyde depolarize the plasma membrane potential of insulin-secreting cells by closing ATP-sensitive K\(^{+}\) channels (2, 43). It has been proposed that channel closure is due to increases in ATP or in the ATP/ADP ratio following the metabolism of carbohydrates (2). Because of the high cytosolic ATP levels, additional coupling factors have been proposed for the control of the ATP-sensitive K\(^{+}\) channel. Activators of protein kinase C have been shown to depolarize insulin-secreting RINm5F cells by closing ATP-sensitive K\(^{+}\) channels, and to mimic the effect of glyceroldehyde in this cell system (19). As carbohydrates have been shown to promote de novo synthesis of DAG (9, 11, 14–16, 19), this mechanism provided an attractive alternative for the coupling of glucose metabolism to membrane depolarization and insulin secretion. In the present study, glucose only caused a minor increase in the DAG enriched in myristic and palmitic acid without significantly altering total DAG mass. This contrasts with our previous findings (9) and those of Wolf et al. (10) in rat islets demonstrating a sizeable increase in palmitic acid containing DAG following glucose stimulation. A rise in total DAG mass was also reported in HIT cells after 30 min of glucose stimulation (39). Recently, however, Wolf et al. (11) reported that glucose exposure up to 20 min failed to enhance DAG mass in both rat and human islets. The latter study (11) also provides evidence suggesting that de novo synthesis of DAG from glucose contributes only

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**TABLE II**

*Effects of various diacylglycerol species and Ca\(^{2+}\) on purified protein kinase C activity*

| Additions                        | Protein kinase C activity |
|----------------------------------|--------------------------|
|                                  | 2 μM Ca\(^{2+}\)         | 25 μM Ca\(^{2+}\) |
| None                             | 1,047 ± 157              | 2,096 ± 573   |
| Phosphatidylserine               | 1,261 ± 198 (100%)       | 14,552 ± 394 (100%) |
| PS + 1,2-dioleoylglycerol        | 8,877 ± 256 (704%)       | 43,796 ± 238 (301%) |
| PS + 1-stearyl-2-arachidonoylglycerol | 7,505 ± 952 (595%)   | 40,049 ± 275 (275%) |
| PS + 1,2-dipalmitoylglycerol     | 2,662 ± 181 (211%)       | 42,270 ± 172 (290%) |
| PS + 1,2-diaraethoxylglycerol    | 3,076 ± 221 (244%)       | 32,222 ± 11 (221%) |
| PS + 1-monooleooylglycerol      | 1,166 ± 155 (92%)        | 14,400 ± 541 (99%) |

We chose to assess protein kinase C activity by monitoring the association of the enzyme with cellular membranes. A similar methodology has been successfully applied to the investigation of agonist stimulation in many cell types (44, 45). Following stimulation of intact cells with receptor agonists, an increased proportion of the enzyme becomes associ-
slightly to islet DAG mass. The reason for these discrepant results is unclear. Nonetheless, our experiments demonstrate a slight increase in [Ca\(^{2+}\)], and subsequent insulin secretion in the face of minor changes in DAG.

Considering the main target for DAG, protein kinase C, it could be that the small amount of DAG generated still activates the enzyme. However, in contrast to the receptor agonists bombesin and carbachol, glucose was unable to cause membrane-association of protein kinase C. Similar results have been reported for islets (20, 23). In view of the moderate increase in DAG mass in bombesin-stimulated cells, the absence of detectable protein kinase C translocation after glucose exposure could be due not only to the small amount of DAG generated in response to carbohydrates, but also to its different fatty acid composition in receptor agonist- and glucose-stimulated cells (Fig. 6) (9, 10). Indeed, palmitic acid-enriched DAG (glucose) was found to be less efficient in activating protein kinase C than DAG containing unsaturated fatty acids in position 2 (receptor agonists). These findings agree with previous observations on the characteristics of protein kinase C from brain (49).

Thus, more and more evidence is accumulating against the involvement of protein kinase C in glucose-stimulated insulin secretion. In view of the present findings and those of Wolf et al. (11), DAG does not seem to be the coupling factor linking glucose metabolism to membrane ion fluxes and secretory events. Other lipidic compounds whose concentration increase in DAG mass in bombesin-stimulated cells, the absence of detectable protein kinase C translocation after glucose exposure could be due not only to the small amount of DAG generated in response to carbohydrates, but also to its different fatty acid composition in receptor agonist- and glucose-stimulated cells (Fig. 6) (9, 10). Indeed, palmitic acid-enriched DAG (glucose) was found to be less efficient in activating protein kinase C than DAG containing unsaturated fatty acids in position 2 (receptor agonists). These findings agree with previous observations on the characteristics of protein kinase C from brain (49).