Augmentation of Ca^{2+}-Stimulated Insulin Release by Glucose and Long-Chain Fatty Acids in Rat Pancreatic Islets

Free Fatty Acids Mimic ATP-Sensitive K^+ Channel–Independent Insulinotropic Action of Glucose

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Glucose augments Ca^{2+}-stimulated insulin release from the pancreatic β-cell in an ATP-sensitive K^+ channel (K_{ATP} channel)–independent manner. In studying the mechanisms underlying this action, we used rat pancreatic islets and examined the effects of exogenous free fatty acids (FFAs), which are precursors of long-chain acyl-CoA (LC-CoA), on KCl-induced Ca^{2+}-stimulated insulin release. Myristate, palmitate, and stearate augmented insulin release induced by 50 mmol/l KCl in the presence of 2.8 mmol/l glucose. Added acutely, their potency was weak compared with that of glucose-induced augmentation. The FFA-induced augmentation became much greater, however, when islets were preincubated with FFAs under stringent Ca^{2+}-free conditions (with 1 mmol/l EGTA) before the KCl stimulation. Under these conditions, 16.7 mmol/l glucose augmented 13-fold insulin release induced by 50 mmol/l KCl, whereas palmitate or myristate (both at a free concentration of 10 μmol/l) produced 5.8- and 5.2-fold augmentations. Effects of FFAs and glucose were concentration-dependent. The temporal profiles of augmentation induced by 11.1 mmol/l glucose and 10 μmol/l palmitate were similar. Glucose and palmitate caused almost identical augmentation patterns for the initial 10 min of stimulation; subsequently, glucose augmentation was better sustained than palmitate augmentation. This suggests the existence of a longer-term glucose-specific signaling moiety that cannot be mimicked by FFAs. Our results provide direct evidence that FFAs can mimic the K_{ATP} channel–independent action of glucose. Taking these results together with previous results, we conclude that glucose augments Ca^{2+}-stimulated insulin release, at least in part, by increasing malonyl-CoA and cytosolic LC-CoA. However, one or more other glucose-specific signaling molecules are required for the full expression of augmentation.* ***Diabetes* 48:1543–1549, 1999**

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BSA, bovine serum albumin; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; FFA, free fatty acid; K_{ATP} channel, ATP-sensitive K^+ channel; KRBB, Krebs-Ringer bicarbonate buffer; LC-CoA, long-chain acyl-CoA.

Glucose is one of the most important secretagogues for insulin. It has intricate actions on pancreatic β-cells and stimulates insulin release through multiple interdependent signaling pathways. Among them, the ATP-sensitive K^+ channel (K_{ATP} channel)–dependent pathway has been extensively examined and relatively well characterized. This signaling pathway is envisaged as follows. Glucose is metabolized in pancreatic β-cells, giving rise to an elevation of ATP or the ATP/ADP ratio, which closes the K_{ATP} channels, leading to membrane depolarization (1–4). The membrane depolarization opens L-type voltage–dependent Ca^{2+} channels, and Ca^{2+} influx and elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) ensue (5,6). Finally, Ca^{2+}-stimulated insulin release takes place, although the mechanistic coupling between $[Ca^{2+}]_i$ elevation and insulin exocytosis remains to be established (7).

This pathway, however, can explain only a part of glucose stimulation of insulin release. In particular, in rat as well as human pancreatic islet β-cells, stimulation with a high concentration of glucose produces far greater insulin responses than those with a high concentration of KCl or sulfonylurea, a K_{ATP} channel closer, which cause simple membrane depolarization. This fact implies the existence of additional mechanisms by which glucose enhances insulin exocytosis. In 1992, two laboratories independently described experimental conditions in which at least one of the additional mechanisms of glucose could be unequivocally demonstrated (8,9). Namely, glucose was shown to strongly augment KCl-induced insulin release even if the K_{ATP} channels were kept open by inclusion of diazoxide. This is now generally referred to as the K_{ATP} channel–independent action of glucose (10–12).

Little information is available as to the mechanisms of the K_{ATP} channel–independent action of glucose. We consider it likely that several mutually dependent mechanisms are operating to stimulate insulin exocytosis. Previously raised possibilities include activation of protein kinase C (13) and phospholipase A_2 (14), mobilization of intracellular Ca^{2+} (15), acti-

vation of ATP-dependent exocytotic mechanisms (16), and increase in the level of cytosolic long-chain acyl-CoA (LC-CoA) esters (17).

Among these possibilities, glucose-induced increase in cytosolic LC-CoA has received intense scrutiny in recent years. A high concentration of glucose increases cytosolic LC-CoA levels both in insulinoma cell lines (17) and in normal rat pancreatic islets (18). This effect is due to an increase in cytosolic malonyl-CoA, which inhibits the mitochondrial enzyme carnitine palmitoyltransferase I, leading to decreased fatty acid oxidation and increased cytosolic LC-CoA levels. Based on these findings, Prentki et al. (19) proposed that cytosolic LC-CoA esters are functioning as signaling molecules in glucose-induced insulin release. This hypothesis has come to be known as the “LC-CoA hypothesis” (20–25). Recent work in INS-1 cells, however, casts doubt on the validity of the hypothesis (26). INS-1 cells with an overexpression of malonyl-CoA decarboxylase had lowered intracellular malonyl-CoA levels, under which condition the LC-CoA pathway was largely disturbed. Nevertheless, glucose-stimulated insulin release was intact in these cells. The finding led the authors to conclude that the LC-CoA pathway is playing little role in glucose-stimulated insulin release. However, we interpret their data in a different way. In the INS-1 cells, the maximum effect of glucose on insulin release is not significantly greater than simple depolarization by KCl (27), suggesting that “non-ionic” insulinotropic action of the nutrient is very weak in this cell line. Furthermore, it should be noted that the fold stimulation of insulin secretion in response to high glucose in the INS-1 cells was very poor (less than twofold). These issues were appropriately discussed in a recent review article (28). Thus, the results obtained in the INS-1 cells have to be interpreted with some caution. It was also found that triacsin C, an inhibitor of LC-CoA synthetase, markedly attenuated palmitate oxidation and decreased total LC-CoA content without affecting glucose-stimulated insulin release in normal rat pancreatic islets. It is possible, however, that there is a separate pool of acyl-CoA that is not affected by triacsin C (29). Furthermore, these data are in contrast with previous work using hydroxycitrate (21).

In view of the controversy about the LC-CoA hypothesis for glucose-induced insulin release, we examined the effects of exogenous free fatty acids (FFAs) in rat pancreatic islets, in which the K_{ATP} channel–independent nutrient action is prominent. Exogenous FFAs are converted to acyl-CoA by acyl-CoA synthetase in the β-cell. Therefore, we believed exogenous FFAs should mimic glucose effects in rat islets, if the LC-CoA is a functionally important component of the pathway. In this study, using freshly isolated normal rat pancreatic islets, we demonstrate that FFAs do mimic the K_{ATP} channel–independent action of glucose.

RESEARCH DESIGN AND METHODS

Isolation of pancreatic islets. Male Wistar rats weighing 250–450 g were killed by CO_2 asphyxiation. Immediately after death, the pancreases were surgically removed, and the islets were isolated by collagenase dispersion (30). Krebs-Ringer bicarbonate buffer (KRBB) containing 129 mmol/l NaCl, 5 mmol/l $NaHCO_3$, 4.8 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$, 5.6 mmol/l glucose, 0.1% bovine serum albumin (BSA), and 10 mmol/l HEPES at pH 7.4 was used for isolation and pooling of the islets.

Measurements of insulin release. Insulin release was measured in static incubation and perifusion experiments. In static incubation, batches of five size-matched islets per tube were used. In most of the static incubation experiments, the islets were washed with Ca^{2+}-free KRBB containing 129 mmol/l NaCl, 5 mmol/l $NaHCO_3$, 4.8 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 1 mmol/l EGTA, 2.8 mmol/l glucose, 100 µmol/l (0.68%) BSA (essentially FFA-free), and 10 mmol/l HEPES at pH 7.4 (Ca^{2+}-free KRB/EGTA buffer). Then, the islets were incubated in 1 ml of the same buffer for 60 min at 37°C (preincubation). After the preincubation, buffer was removed by aspiration, and 1 ml of fresh regular KRBB (no EGTA) containing 2.5 mmol/l Ca^{2+} and test substance was introduced. Test incubation was then continued for 30 min at 37°C. In some experiments, regular KRBB containing 2.5 mmol/l Ca^{2+} or Ca^{2+}-free KRBB without EGTA was used in washing and preincubation periods. When effects of FFAs were tested, they were present in both preincubation and test incubation periods. At the end of the incubations, the medium was aspirated and kept at –20°C until it was radioimmunoassayed for insulin. Rat insulin was used as standard for the radioimmunoassay. For perifusion experiments, 40–50 size-matched islets were placed in 10 columns, and all columns were perifused in parallel at a flow rate of 1 ml/min at 37°C (8). The islets were first perifused for 60 min with Ca^{2+}-free KRB/EGTA buffer with test substances (2.8 mmol/l glucose, 2.8 mmol/l glucose plus 10 µmol/l palmitate in free concentration, or 11.1 mmol/l glucose). After that, the islets were stimulated with a depolarizing concentration of KCl in the continuous presence of palmitate or a high concentration of glucose. Samples were collected every 1, 2, or 5 min, and insulin in the perifusate was measured by radioimmunoassay. Because most FFA molecules are bound to albumin in the solution, the effects of FFAs on pancreatic β-cells depend on free concentrations of FFAs (19,31). Therefore, FFA-free BSA was used in this study. The concentrations of free FFAs were estimated from the molar ratio of FFA and BSA as reported in the literature (32). To do this, a fixed concentration of BSA, 100 µmol/l (0.68%), was used in all experiments, and total concentrations of FFAs were varied to obtain the desired concentrations of free FFAs. (In the figure legends, the total concentrations of FFAs are also indicated.) For solubilization of FFAs, immediately before use, FFA was prepared in an Eppendorf tube with a small volume of water (100×). Then it was warmed up by putting it in a boiling water bath for 15–20 s with occasional vortexing. Immediately after dissolving, a small volume of the FFA solution was added to the buffer.

Measurement of incorporation of ^{14}C-palmitate into the islet cells in the presence or total absence of extracellular free Ca^{2+}. The batch of 60–70 islets in an Eppendorf tube was incubated in 0.5 ml regular KRBB (2.5 mmol/l Ca^{2+}) or 0.5 ml Ca^{2+}-free EGTA buffer at 37°C for 60 min. The buffer contained 100 µmol/l BSA, 600 µmol/l palmitate (10 µmol/l palmitate in free form), and 0.5 µCi [^{14}C]palmitate. After the incubation, the supernatant was carefully removed and the islets were washed three times with 1 ml ice-cold phosphate-buffered saline containing 0.1% BSA. Then the islets were lysed by adding 100 µl formic acid, and ^{14}C, which reflected primarily the labeled lipids, was quantitated by liquid scintillation spectrometry.

Materials. Myristate, palmitate, stearate, FFA-free BSA, and EGTA were obtained from Sigma (St. Louis, MO). [$^{14}C(U)$]palmitate (specific activity 500 mCi/mmol) was purchased from New England Nuclear (Boston, MA).

Data analysis. Data are presented as means ± SE, and statistical significance was evaluated using one-way analysis of variance with pairwise comparison by Bonferroni/Dunn or Fisher methods. Differences were considered significant at $P < 0.05$.

RESULTS

Effects of glucose, palmitate, and myristate on insulin release induced by 50 mmol/l KCl in the presence of 250 µmol/l diazoxide. In preliminary experiments, we found that inclusion of palmitate or myristate only during the test incubation had no effect on basal and KCl-induced (Ca^{2+}-stimulated) insulin release (data not shown). Accordingly, effects of FFAs were examined by exposing the islets to FFAs throughout preincubation and test incubation periods. In Fig. 1 is shown a comparison of the effects of 16.7 mmol/l glucose, 10 µmol/l palmitate, and 10 µmol/l myristate on basal insulin release and insulin release induced by 50 mmol/l KCl. Under these conditions, 16.7 mmol/l glucose has no effect on basal insulin release because of the presence of diazoxide. Palmitate and myristate caused a marginal (insignificant) increase in insulin release. When Ca^{2+}-stimulated insulin release was evoked by a depolarizing concentration of KCl (50 mmol/l), it was ~200% of the basal during 30-min incubation (see bars 1 and 5 in Fig. 1). Presence of 16.7 mmol/l glucose throughout the preincubation and test incubation periods dramatically (8.6-fold) augmented Ca^{2+}-stimulated insulin

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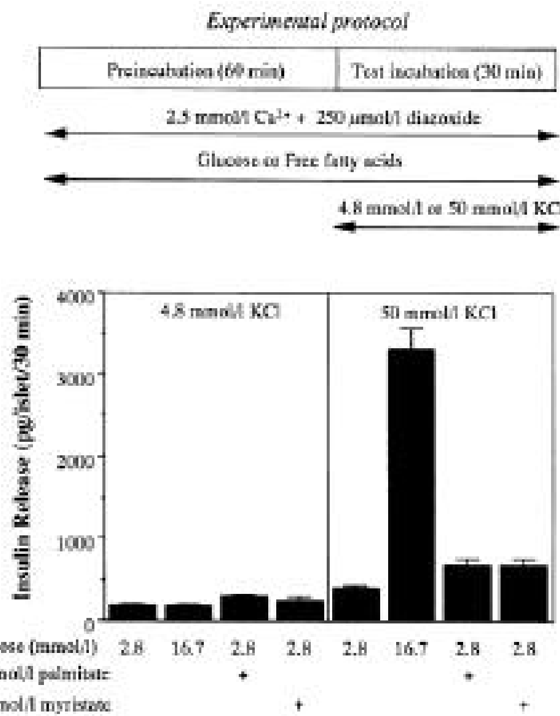

FIG. 1. Effects of glucose, palmitate, and myristate on basal insulin release (4.8 mmol/l KCl) or insulin release induced by 50 mmol/l KCl. Insulin release was measured in static incubation conditions as described in METHODS. Experimental protocol is illustrated above the figure. The amounts of insulin released during the test incubation are indicated. The experiment was performed in the presence of 0.68% (100 µmol/l) BSA. The total concentrations of palmitate and myristate were 0.6 and 0.56 mmol/l, respectively. Resulting estimated free concentration of palmitate and myristate is 10 µmol/l. Values are means ± SE from 10 determinations.

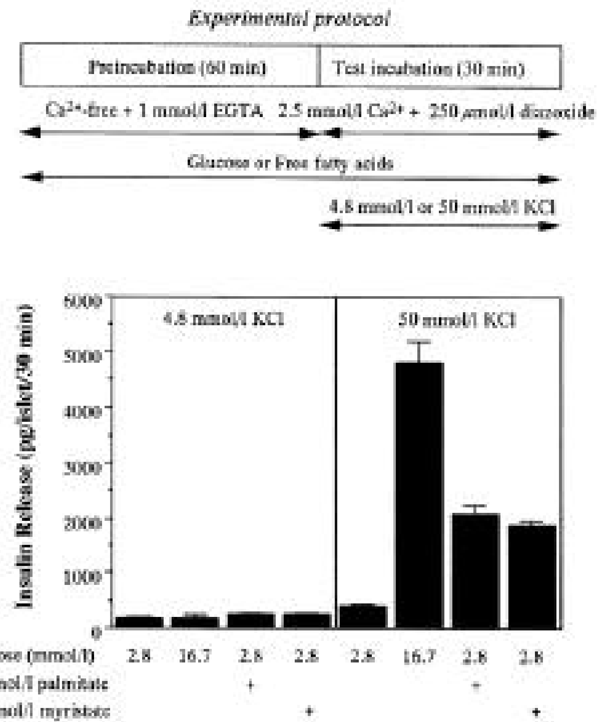

FIG. 2. Effects of glucose, palmitate, and myristate on basal insulin release (4.8 mmol/l KCl) or insulin release induced by 50 mmol/l KCl. Insulin release was measured in static incubation conditions as described in METHODS. Experimental protocol is illustrated above the figure. Note that 60-min preincubation was performed under stringent Ca^{2+}-free conditions. The amounts of insulin released during the test incubation are indicated. The experiment was performed in the presence of 0.68% (100 µmol/l) BSA. The total concentrations of palmitate and myristate are 0.6 and 0.56 mmol/l, respectively. Resulting estimated free concentration of palmitate and myristate is 10 µmol/l. Values are means ± SE from seven determinations.

release. In contrast, 10 µmol/l palmitate and 10 µmol/l myristate caused a minimum, albeit significant, augmentation of Ca^{2+}-stimulated insulin release (see bars 7 and 8 in Fig. 1).

From the previous studies, we knew that preincubation of the islets with palmitate or myristate under stringent Ca^{2+}-deprived conditions potently enhances subsequent Ca^{2+}-independent insulin release (33). We therefore compared the effects of pretreatment with glucose, palmitate, and myristate in the absence of extracellular free Ca^{2+}. As shown in Fig. 2, the islets were first treated with glucose, palmitate, or myristate in Ca^{2+}-free KRB/EGTA buffer. None of these three agents caused increase in insulin release under nondepolarized conditions. Depolarization of the β-cell with 50 mmol/l KCl doubled the release in the presence of 2.8 mmol/l glucose. Presence of 16.7 mmol/l glucose profoundly (13.3-fold) augmented the Ca^{2+}-stimulated insulin release. Quite interestingly, under this experimental condition, the presence of palmitate or myristate produced much stronger augmentation of the Ca^{2+}-stimulated insulin release, the magnitudes being 5.8- and 5.2-fold, respectively. To confirm the different effects of pretreatment on the augmentation of Ca^{2+}-stimulated insulin release, we next directly compared three different Ca^{2+} conditions during preincubation conditions using the same batch of islets. As shown in Fig. 3, palmitate augmentation of KCl-induced insulin release was most prominent when the islets were preexposed to palmitate in Ca^{2+}-free KRB/EGTA buffer. The pretreatment with palmitate in Ca^{2+}-containing or in simple Ca^{2+} omission (non-EGTA) buffer only slightly augmented the KCl-evoked release.

Incorporation of palmitate into the islet cells in the presence or absence of extracellular Ca^{2+}. Stringent Ca^{2+}-free conditions allow FFAs to cause stronger augmentation—what is the underlying reason for this? As the initial step toward answering this question, we examined an incorporation rate of FFAs into the islet cells under these conditions. To this end, we measured ^{14}C counts in the cells after 60-min incubation with 10 µmol/l palmitate and trace amount of [^{14}C]palmitate in the presence of 2.5 mmol/l Ca^{2+} and in the total absence of free Ca^{2+} (Ca^{2+}-free KRB/EGTA buffer). In the presence and absence of Ca^{2+}, ^{14}C counts were 255 ± 103 and 209 ± 38 dpm/50 islets, respectively (n = 5). Thus, presence or absence of extracellular free Ca^{2+} did not affect the incorporation rate of palmitate into the islet cells.

Effect of simultaneous addition of glucose and palmitate on insulin release induced by 50 mmol/l KCl. As both high glucose and palmitate augmented the Ca^{2+}-stimulated insulin release, we next examined the additivity of the augmentation. The results of such experiments are shown in Fig. 4. Glucose or palmitate augmented the Ca^{2+}-stimulated insulin release as expected from the data in Fig. 2. The simultaneous addition of glucose and palmitate caused significantly higher augmentation than the addition of either glucose or palmitate alone (glucose vs. glucose plus palmitate,

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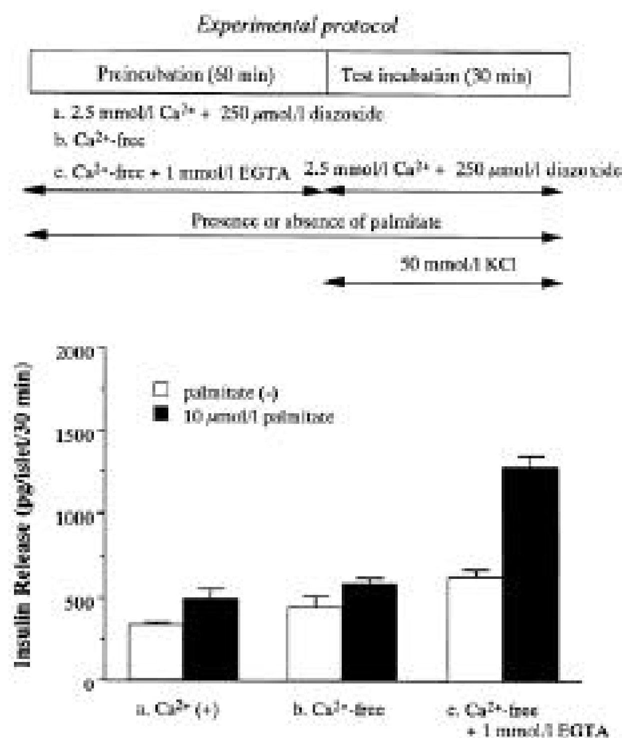

FIG. 3. Effects of palmitate on insulin release induced by 50 mmol/l KCl under three different preincubation conditions. Insulin release was measured in static incubation condition as described in METHODS. Experimental protocol is illustrated above the figure. Glucose concentration was 2.8 mmol/l throughout the experiments. The amounts of insulin released during the test incubation are indicated. The experiment was performed in the presence of 0.68% (100 μmol/l) BSA. The total concentration of palmitate was 0.6 mmol/l, resulting in the estimated free concentration of 10 μmol/l. Values are means ± SE from 10 determinations.

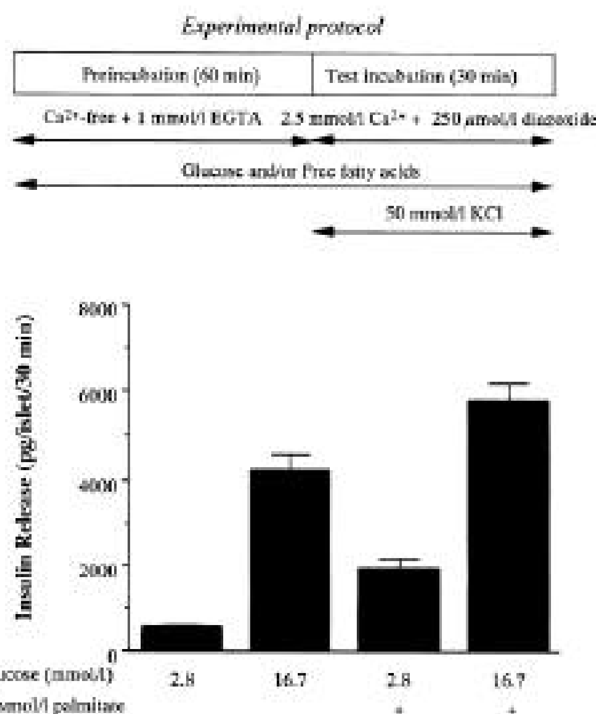

FIG. 4. Additive effect of glucose and palmitate on insulin release induced by 50 mmol/l KCl. Insulin release was measured in static incubation experiments as described in METHODS. Experimental protocol is illustrated above the figure. The amounts of insulin released during the test incubation are indicated. The experiments were performed in the presence of 0.68% (100 μmol/l) BSA. The total concentration of palmitate was 0.6 mmol/l, resulting in the estimated free concentration of 10 μmol/l. Values are means ± SE from 18 determinations.

$P = 0.003$; palmitate vs. glucose plus palmitate, $P < 0.0001$). It should be noted, however, that there is no synergistic interaction between glucose and palmitate. In fact, the simultaneous addition of glucose and palmitate simply caused an additive effect.

Concentration-dependent effects of FFAs on insulin release induced by 50 mmol/l KCl. We next compared concentration dependence of myristate (C14:0), palmitate (C16:0), and stearate (C18:0) on Ca^{2+}-stimulated insulin release. All FFAs were included throughout the experiments, and a 60-min preincubation was performed in the Ca^{2+}-free KRB/EGTA buffer as described above. As shown in Fig. 5, all of the FFAs augmented Ca^{2+}-stimulated insulin release in a concentration-dependent manner. Calculated free concentrations of FFAs are indicated on the abscissa of Fig. 5 and total concentration of FFAs in the legend. Augmentation effects of these FFAs on Ca^{2+}-stimulated insulin release were detectable at 1 μmol/l, which was the lowest concentration tested. Maximum effects of myristate, palmitate, and stearate were found at 20, 10, and 5 μmol/l, respectively.

Temporal profiles of glucose and palmitate augmentation of insulin release induced by 50 mmol/l KCl. The temporal profiles of glucose and palmitate effects on insulin release induced by 50 mmol/l KCl were examined in perifusion experiments. The islets were first perifused with Ca^{2+}-free KRB/EGTA buffer containing 2.8 mmol/l glucose (control), 11.1 mmol/l glucose, or 2.8 mmol/l glucose plus 10 μmol/l palmitate for 60 min. After this 60-min prewash, the solution was changed to regular KRBB (2.5 mmol/l Ca^{2+}) containing 50 mmol/l KCl to elicit Ca^{2+}-stimulated insulin release. Respective concentrations of glucose and palmitate were present continuously during the KCl stimulation period. As shown in Fig. 6*A*, in the control islets, 50 mmol/l KCl produced a rapid increase in the rate of insulin release, which peaked at 2 min after stimulation. It was followed by a gradually decreasing level of insulin release. The amplitude of response to 50 mmol/l KCl was slightly lower than in our previous study, in which we performed entire experiments in the presence of regular Ca^{2+} concentration (6). Basal insulin release in the Ca^{2+}-free KRB/EGTA buffer in the presence of 11.1 mmol/l glucose was similar to that in control islets. However, the Ca^{2+}-stimulated insulin release produced by 50 mmol/l KCl was dramatically augmented by the presence of 11.1 mmol/l glucose. Namely, in the presence of high glucose, the rate of insulin release markedly increased upon addition of 50 mmol/l KCl and peaked at 3 min, followed by a rapid drop of the rates during the next 2 min and a slow diminution toward the end of the 30-min observation period. The presence of 10 μmol/l palmitate also dramatically augmented Ca^{2+}-stimulated insulin release, but the temporal profile of the Ca^{2+}-stimulated insulin release in the presence of 10 μmol/l palmitate is different from that in the presence of 11.1 mmol/l glucose. Basal insulin release in the Ca^{2+}-free KRB/EGTA buffer in the presence of 10 μmol/l palmitate and 2.8 mmol/l glucose was slightly but significantly lower than that in the presence of 2.8 mmol/l glucose alone or 11.1 mmol/l glu-

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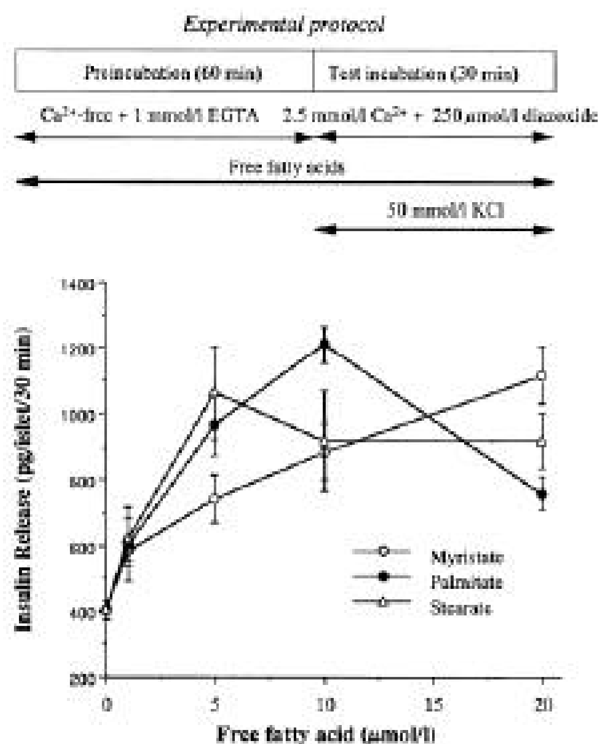

FIG. 5. Effects of various concentrations of myristate, palmitate, and stearate on insulin release induced by 50 mmol/l KCl. Insulin release was measured in static incubation experiments as described in METHODS. Experimental protocol is illustrated above the figure. The amounts of insulin released during the test incubation are indicated. The experiment was performed in the presence of 0.68% (100 μmol/l) BSA. Total concentrations of FFAs were varied to obtain respective free concentrations. The total concentrations of myristate were 0.3, 0.48, 0.56, and 0.64 mmol/l for the estimated free concentrations of 1, 5, 10, and 20 μmol/l, respectively; those of palmitate were 0.34, 0.54, 0.60, and 0.71 mmol/l; and those of stearate were 0.2, 0.46, 0.56, and 0.68 mmol/l. Values are means ± SE from eight determinations.

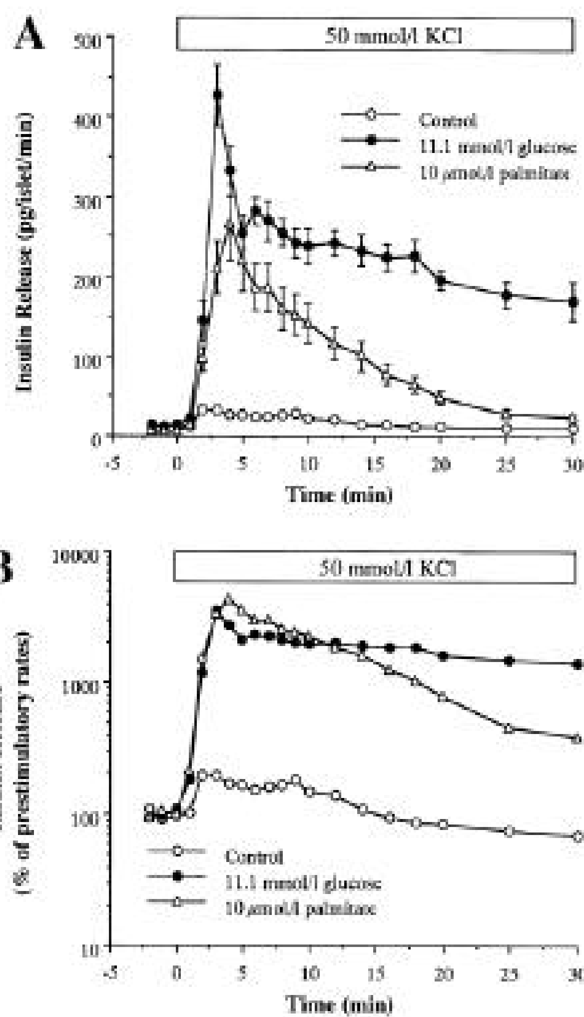

FIG. 6. Temporal profile of glucose- and palmitate-induced augmentation of insulin release induced by 50 mmol/l KCl. Perifusion experiments were performed as described in METHODS. The islets were first perifused with Ca^{2+}-free KRB/EGTA buffer. Control condition and 10 μmol/l palmitate condition include 2.8 mmol/l glucose. At time 0, the solution was changed to regular KRBB (2.5 mmol/l Ca^{2+}) containing 50 mmol/l KCl. *A*: The rates of insulin release were plotted with absolute values. *B*: The rates of insulin release were replotted as percent of prestimulatory levels. Values are means ± SE from 10 determinations.

cose. Stimulation with 50 mmol/l KCl in the presence of palmitate produced a large increase in insulin release, indicating an augmentation of Ca^{2+}-stimulated insulin release by palmitate. After the peak at 4 min, the rate of insulin release sharply declined during the rest of the observation period. Because the prestimulatory level of insulin release in palmitate-treated islets was lower than that in the control or glucose-treated islets, the data shown in Fig. 6*A* were replotted with percent of prestimulatory levels in log scale instead of actual rates of insulin release (Fig. 6*B*). This plot revealed that 11.1 mmol/l glucose and 10 μmol/l palmitate caused almost identical augmentation during the initial 10 min of stimulation with KCl. After 10 min, the palmitate effect significantly faded away with time compared with the glucose effect.

DISCUSSION

In stimulus-secretion coupling for glucose-induced insulin release, the K_{ATP} channel–dependent signaling pathway constitutes one part and the K_{ATP} channel–independent glucose actions constitute the rest (11,12,34). In this study, we demonstrate that glucose augments Ca^{2+}-stimulated insulin release from rat pancreatic β-cells. Of particular importance, it was also found that FFAs such as myristate, palmitate, and stearate similarly time-dependently increased Ca^{2+}-stimulated insulin release.

The role of LC-CoA in pancreatic β-cell physiology has been proposed in many aspects (22), including activation of certain types of protein kinase C, modulation of ion channels, protein acylation such as palmitoylation, and binding to nuclear transcriptional factors (19,35). Theoretically, all of these actions facilitate β-cell function and insulin release. Direct evidence is lacking, however, that elevated cytosolic LC-CoA augments insulin release in the presence of nonstimulatory concentrations of glucose, thus hampering the recognition of LC-CoA as a key regulatory molecule for glucose-induced insulin release (36). In this study, we demonstrate that long-chain FFAs, precursors of LC-CoA, strongly augment Ca^{2+}-stimulated insulin release in the presence of a nonstimulatory, basal concentration of glucose. The perifusion studies revealed similar degrees and patterns of augmentation by glucose and palmitate, at least for the initial 10 min of stimulation. In fact, when the data were plotted as percent basal in log scale (Fig. 6*B*), the temporal profiles of

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glucose- and palmitate-induced augmentation were almost identical during the initial 10 min of stimulation. During the later period of stimulation, glucose had a stronger effect. Furthermore, augmentation by simultaneous exposure of 16.7 mmol/l glucose and 10 µmol/l palmitate resulted in a simple additive, not a synergistic, interaction. Thus, we consider it likely that glucose enlarges the Ca^{2+}-responsive, releasable pool of insulin, at least in part, by virtue of elevated LC-CoA. A more sustained effect of glucose implies the existence of glucose-specific signals that cannot be generated by exogenous FFAs. It may be ATP or other metabolic signals originating in glycolysis or mitochondrial metabolism of glucose (16,37–39).

Effects of FFAs on pancreatic β-cells have been studied for many years. An acute exposure of the pancreatic β-cell to FFAs results in augmentation of insulin release, whereas a longer exposure results in desensitization and suppression of insulin (31,40,41). In addition, it was recently reported that reduction of plasma FFA levels in fasted rats or humans by administration of nicotinic acid severely impaired glucose-induced insulin release (28,42–44). The results imply that increased FFA is keeping β-cells glucose-competent under fasting conditions. Thus it is most likely that the FFA effect described here is important under physiologic conditions as well.

We do not know how soon extracellular FFAs enter the β-cell and what is the concentration of cytosolic LC-CoA under our experimental conditions. The physiologic concentration of cytosolic LC-CoA in various tissues is reported to be 1–20 µmol/l (45,46). Liang and Matschinsky (18) measured LC-CoA content in rat pancreatic islets. The LC-CoA concentration in the islets after 30-min perifusion with 2.5 mmol/l glucose is calculated to be 9.9 µmol/l on an assumption of the mean islet volume being 2 nl (47). Thirty-minute stimulation with 25 mmol/l glucose increases the LC-CoA concentration to 20 µmol/l. Interestingly, mixed fuels (25 mmol/l glucose, 10 mmol/l glutamine, 10 mmol/l lactate, and 1 mmol/l pyruvate) cause further increases in both insulin release and LC-CoA concentration compared with stimulation with 25 mmol/l glucose alone. This might be relevant to our finding that the simultaneous addition of glucose and palmitate caused additive augmentation. It should be noted that the actions of glucose and FFAs being additive increased the complexity of interpreting the data. Reasons could be different mechanisms or more LC-CoA in the presence of glucose and FFA. These data indicate that the micromole-per-liter range of unbound FFAs is a reasonable concentration to mimic glucose-induced increases in LC-CoA concentration in rat pancreatic islets.

A point we have to address in future studies is why the augmentation by FFAs occurs much more prominently in the Ca^{2+}-free KRB/EGTA buffer than in regular Ca^{2+}-containing buffer. Currently, we would suggest three possibilities. First, FFAs are more effectively incorporated into the β-cell under Ca^{2+}-free conditions. This possibility seems unlikely, however, because the measurement of palmitate incorporation revealed no difference irrespective of the presence and absence of extracellular Ca^{2+}. Second, a lower $[Ca^{2+}]_i$ milieu might facilitate production and/or distribution of LC-CoA, leading to increased effectiveness. Third, the augmentation process itself might be operating effectively under lower $[Ca^{2+}]_i$ milieu for unknown reasons. To further explore the third possibility, we performed a preliminary experiment in which glucose-induced augmentation was determined in the presence and absence of Ca^{2+} using the same experimental protocol as in Figs. 1 and 2. High glucose–induced augmentation of subsequent Ca^{2+}-stimulated insulin release was greater if glucose exposure was done in Ca^{2+}-free KRB/EGTA buffer (8.2-fold increase) than in regular Ca^{2+}-containing buffer (6.6-fold increase). The results suggest existence of negative permissive effects of Ca^{2+} on "augmentation process."

In summary, it was found that glucose and FFAs augmented Ca^{2+}-stimulated insulin release. These observations reinforce the hypothesis of a "malonyl-CoA and LC-CoA pathway" in glucose stimulus-secretion coupling in pancreatic β-cells.

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