Acute Stimulation with Long Chain Acyl-CoA Enhances Exocytosis in Insulin-secreting Cells (HIT T-15 and NMRI β-Cells)*

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Non-insulin-dependent diabetes mellitus is associated with, in addition to impaired insulin release, elevated levels of free fatty acids (FFA) in the blood. Insulin release is stimulated when β-cells are acutely exposed to FFA, whereas chronic exposure may inhibit glucose-induced insulin secretion. In the present study we investigated the direct effects of long chain acyl-CoA (LC-CoA), the active intracellular form of FFA, on insulin exocytosis. Palmitoyl-CoA stimulated both insulin release from streptolysin-O-permeabilized HIT cells and fusion of secretory granules to the plasma membrane of mouse pancreatic β-cells, as measured by cell capacitance. The LC-CoA effect was chain length-dependent, requiring chain lengths of at least 14 carbons. LC-CoA needed to be present to stimulate insulin release, and consequently there was no effect following its removal. The stimulatory effect was observed after inhibition of protein kinase activity and in the absence of ATP, even though both kinases and ATP, themselves, modulate exocytosis. The effect of LC-CoA was inhibited by cerulenin, which has been shown to block protein acylation. The data suggest that altered LC-CoA levels, resulting from FFA or glucose metabolism, may act directly on the exocytotic machinery to stimulate insulin release by a mechanism involving LC-CoA protein binding.

Glucose-induced insulin secretion is associated with inhibition of FFA oxidation, increased FFA esterification, and complex lipid formation by pancreatic β-cells (4, 6, 7). Islets also contain high levels of triglycerides similar to liver (8, 9). In addition, exogenous fatty acids acutely potentiate glucose-stimulated insulin secretion (3, 7, 10), possibly by providing additional acyl groups for LC-CoA formation or complex lipid synthesis. Evidence that a rise in cytosolic LC-CoA plays a role in intracellular signaling is indirect and based on the following findings. First, addition of FFA increases total LC-CoA (3). Second, although glucose acutely lowers total LC-CoA, because of a decreased mitochondrial content as FFA oxidation is inhibited (3, 11), it appears to increase the cytosolic pool because complex lipid synthesis, regulated by LC-CoA availability, is stimulated (3). Third, stimulation of islets with glucose for 30 min increases total LC-CoA (11). Fourth, pharmacological inhibition of mitochondrial LC-CoA oxidation, which elevates cytosolic LC-CoA, enhances glucose-induced secretion (7, 12). Fifth, inhibition of malonyl-CoA production from glucose, which presumably prevents the rise in cytosolic LC-CoA, blocks glucose-induced insulin secretion (12). Sixth, specific antisense mRNA inhibition of expression of acetyl-CoA carboxylase, the enzyme that synthesizes malonyl-CoA, inhibits glucose-induced insulin secretion (13).

Exogenous FFA have been shown to readily traverse cellular membranes by non-carrier-mediated passive diffusion (flip-flop) (14–16). Upon entry into the cell FFA must undergo esterification to their intracellular LC-CoA esters to be further metabolized.

The roles of cytosolic LC-CoA esters are many and include synthesis of lipids for structural integrity and energy storage as well as second messengers involved in intracellular signaling. In recent years there has been increased interest in the intracellular effects of LC-CoA esters themselves. In a cell free system, LC-CoA esters have been shown to stimulate budding of vesicles from the cis surface of the Golgi apparatus and fusion of vesicles to the medial surface of the Golgi apparatus (17–20). In the β-cell, LC-CoA stimulates the endoplasmic reticulum Ca2⁺ ATPase (1), the ATP-sensitive K⁺ channel (21, 22), and protein kinase C (PKC) isoforms (23).

Non-insulin-dependent diabetes mellitus results in elevated levels of FFA in the blood (24–26). The effects of FFA on isolated islets and clonal pancreatic insulin secreting cells (HIT) are dependent on the exposure time. β-Cells stimulated acutely with FFA exhibit an enhanced secretory response to glucose (3), whereas chronic exposure results in impaired secretion (1, 27). The mechanisms by which these effects are mediated have yet to be elucidated. Acute stimulation by non-

Lipids involved in intracellular signaling and stimulation of insulin release in the pancreatic β-cell are derived from glucose metabolism and endogenous lipids as well as from the extracellular supply of lipids delivered by the blood. Glucose metabolism results in increased levels of cytosolic long chain acyl-CoA (LC-CoA)1 compounds, as a consequence of increased malonyl-CoA production, and inhibition of carnitine palmitoyl transferase-1, leading to inhibition of β-oxidation of free fatty acids (FFA) (1–5).

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1 The abbreviations used are: LC-CoA, long chain acyl-CoA; FFA, free fatty acids; PKC, protein kinase C; PKA, cAMP-dependent kinase; SNARE, SNAP receptor.
cell-permeant LC-CoA on insulin secretion was demonstrated in streptolysin-O-permeabilized clonal insulin-secreting cells (HIT T-15). Acute stimulatory effects of LC-CoA on exocytosis, as measured by changes in β-cell membrane capacitance (28, 29), were also documented using NMRI mouse β-cells in the standard whole cell configuration.

**EXPERIMENTAL PROCEDURES**

**Islet and Cell Culture**—Clonal insulin-secreting cells (HIT-T15) were cultured in RPMI 1640 medium supplemented with 50 units/ml penicillin and 50 μg/ml of streptomycin, 10% fetal calf serum, 10−7 M selenous acid, and 10 μg/ml glutathione (1). Cells were grown in 48-well plates (Costar) and were used between passages 67 and 85. NMRI mouse islets were prepared by collagenase digestion and dispersed in Ca2+-free media (29). Single β-cells were cultured in RPMI 1640 medium prior to capacitance measurements.

**Streptolysin-O Permeabilization of Cells**—Cells were washed two times in an intracellular buffer containing 140 mM K+ glutamate, 5 mM MgCl2, 5 mM NaCl, 5 mM EGTA, titrated with Ca2+ to a final free Ca2+ concentration of 100 mM, and 20 mM HEPES, pH 7.1. Streptolysin-O (Difco, Detroit, MI) was dissolved (4 ml/bottle) in intracellular buffer with 1 mM dithiothreitol. Cells were then incubated with streptolysin-O (3.2 units/ml, 150 μl/well) for 30 min at 0 °C in an ice water bath. Streptolysin was removed, and the cells were further incubated with intracellular buffer for 10 min at 37 °C and then cooled for 5 min in ice water before media were exchanged for the Ca2+/EGTA test solution. To measure insulin release from streptolysin-O-permeabilized cells in the absence of ATP, cells were permeabilized at 37 °C for 10 min in the absence of 2 mM ATP. The cells were then cooled, and the permeabilizing solution was exchanged for the Ca2+/EGTA test solution described below, modified by the substitution of creatine for ATP and creatine phosphate.

**Insulin Release from Permeabilized Cells**—Permeabilized cells were incubated in Ca2+/EGTA buffers containing 140 mM K+ glutamate, 1 mM MgCl2, 5 mM NaCl, 10 mM EGTA, 2 mM MgATP, 2 mM creatine phosphate, 10 units/ml creatine phosphokinase, and 25 mM HEPES, pH 7.0 with CaCl2 titrated to the designated free [Ca2+]1. To measure insulin release from permeabilized cells, in the absence of ATP, Ca2+/EGTA buffers were modified by the substitution of 2 mM creatine for ATP and creatine phosphate. The substitution of creatine for ATP and creatine phosphate was made to create a system that would generate ADP from residual ATP (30). Free [Ca2+]1 was determined using a Ca2+-sensitive electrode from Orion (Bosten, MA.) and Ca2+/EGTA standards from World Precision Instruments (Sarasota, FL). Acyl-CoA compounds were dissolved in water and added to the Ca2+/EGTA buffers prior to the addition of cells. Free [Ca2+]1 did not change subsequent to the addition of acyl-CoA esters. Following a 15-min incubation period at 37 °C, media were removed and samples were analyzed for insulin using a radioimmunoassay kit purchased from Linco Research Inc. (St. Louis, MO).

**Cell Capacitance Measurements**—Exocytosis was measured as increases in cell capacitance (28, 29) using an EPC-9 patch clamp amplifier and the Pulse software (v. 8.30; HEKA Elektronik, Lambrecht/Pfalz, Germany). The interval between two successive points was 0.2 s, and the measurements of cell capacitance were initiated <10 s after establishment of the whole cell configuration. The extracellular medium consisted of 138 mM NaCl, 5.6 mM KCl, 2.6 mM CaCl2, 1.2 mM MgCl2, 50 mM HEPES (pH 7.4 with NaOH), and 5 mM glucose. The volume of the recording chamber was 0.4 ml, and the solution entering the bath (1.5–2 ml/min) was maintained at +33 °C. Pipettes were pulled from borosilicate glass, coated with Sylgard near their tips, and fire-polished. When filled with pipette solutions, the electrodes had a resistance of 3–4 MW. The pipette solution consisted of 125 mM K+ glutamate, 10 mM KCl, 10 mM NaCl, 1 mM MgCl2, 5 mM CaCl2, 3 mM Mg-ATP, 10 mM EGTA, 5 mM HEPES (pH 7.15 with KOH). The free Ca2+ concentration of the resulting buffer was 0.22 mM using the binding constants of Martell and Smith (31). Rp-cAMPs was from BIOLOG (Hamburg, Germany). Bisindolylmaleimide and calphostin C were obtained from Calbiochem (La Jolla, CA). All other chemicals were purchased from Sigma.

**Statistical Analysis**—Statistical analysis was performed using Student’s t test for unpaired data.

**RESULTS**

Palmitoyl-CoA stimulated insulin release from streptolysin-O-permeabilized insulin-secreting cells (HIT), as shown in Fig. 1. Raising the free Ca2+ concentration from 0.1 μM to 10 μM resulted in a 5-fold increase in insulin release from permeabilized HIT cells. To normalize and compare results from eight separate experiments, the data in Fig. 1 were expressed as percentages of release obtained in control cells at 0.01 μM Ca2+. Palmitoyl-CoA (10 μM) increased exocytosis of insulin from permeabilized cells at each free [Ca2+]1 tested. At 1 and 10 μM free [Ca2+], LC-CoA increased exocytosis by 59 and 50%, respectively. The enhancement by LC-CoA was less, 28 and 39%, respectively, at 0.01 and 0.1 μM free [Ca2+].

The effect of LC-CoA in stimulating insulin release from permeabilized insulin-secreting cells was concentration-dependent with a significant increase obtained at 1 μM and a nearly maximum stimulation obtained at 10 μM palmitoyl-CoA (Fig. 2A). There was some variability in the concentration at which the stimulatory effect of LC-CoA on exocytosis was observed, which could be explained by the high affinity of LC-CoA esters for membranes, resulting in differences in free and bound LC-CoA (32). Thus, as a result of variations in cell number, different amounts of free LC-CoA are delivered to the cells because of partitioning into cell membranes. The effect of LC-CoA was also dependent on chain length, with carbon chains of 16 or more causing large increases of about 60% in insulin release from permeabilized cells (Fig. 2B). Myristoyl-CoA (C14) had an intermediate effect of 30%, whereas shorter chain lengths such as hexanoyl-CoA (C6) were ineffective in stimulating insulin exocytosis from permeabilized insulin-secreting cells. Palmitoyl-carnitine could not substitute for the CoA ester in stimulating exocytosis (data not shown).

To explore the possibility that stimulation of exocytosis by LC-CoA was mediated by a classical PKC isofrom (cPKC), we examined the effect of LC-CoA in cells in which these isoforms had been inhibited using the kinase inhibitor staurosporine (33). Staurosporine, once presumed to be a specific PKC inhibitor (34), is now used as a general kinase inhibitor because of its rather nonspecific inhibitory action on a number of protein kinases (35). The presence of 200 nM staurosporine did not have a significant effect on basal insulin release but did not change subsequent to the addition of acyl-CoA esters, suggesting that the effect of LC-CoA was not dependent on the high affinity of LC-CoA esters for membranes. Thus, LC-CoA increased exocytosis by 59 and 50%, respectively. The enhancement by LC-CoA was less, 28 and 39%, respectively, at 0.01 and 0.1 μM free [Ca2+]1. The effect of LC-CoA in stimulating insulin release from permeabilized insulin-secreting cells was concentration-dependent with a significant increase obtained at 1 μM and a nearly maximum stimulation obtained at 10 μM palmitoyl-CoA (Fig. 2A). There was some variability in the concentration at which the stimulatory effect of LC-CoA on exocytosis was observed, which could be explained by the high affinity of LC-CoA esters for membranes, resulting in differences in free and bound LC-CoA (32). Thus, as a result of variations in cell number, different amounts of free LC-CoA are delivered to the cells because of partitioning into cell membranes. The effect of LC-CoA was also dependent on chain length, with carbon chains of 16 or more causing large increases of about 60% in insulin release from permeabilized cells (Fig. 2B). Myristoyl-CoA (C14) had an intermediate effect of 30%, whereas shorter chain lengths such as hexanoyl-CoA (C6) were ineffective in stimulating insulin exocytosis from permeabilized insulin-secreting cells. Palmitoyl-carnitine could not substitute for the CoA ester in stimulating exocytosis (data not shown).

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In an attempt to determine the temporal relationship between elevated free LC-CoA concentration and stimulation of
Acyl-CoA Enhanced Exocytosis

FIG. 2. Stimulation of insulin exocytosis from streptolysin-O-permeabilized insulin-secreting cells is dependent on concentration (A) and chain length (B) of LC-CoA. Experiments were performed as described in the legend to Fig. 1. Data in A represent the percentages of increase over that obtained with 10 μM free [Ca^{2+}] alone, at 1 μM (n = 3) and 10 μM (n = 9) palmitoyl-CoA, respectively. The effects of palmitoyl-CoA (C16, n = 9), oleoyl-CoA (C18:1, n = 3), arachidonyl-CoA (C20:4, n = 3), myristoyl-CoA (C14, n = 3), and hexanoyl-CoA (C6, n = 3) are shown in B. Each separate experiment represents the mean of at least three samples, and n designates the number of separate experiments. *, p < 0.01 as compared with control.

FIG. 3. Role of PKC and other protein kinases in stimulation of insulin exocytosis from streptolysin-O-permeabilized insulin-secreting cells. Effect of inhibition with staurosporine. Experiments were performed as described in the legend to Fig. 1. Data show the stimulatory effect of 10 μM palmitoyl-CoA in the presence of 100 nM free [Ca^{2+}] in the presence and absence of 200 μM staurosporine (n = 3). Each separate experiment represents the mean of at least three samples, and n designates the number of separate experiments. *, p < 0.05 as compared with control.

FIG. 4. Effect of pretreatment with LC-CoA on stimulation of insulin exocytosis from streptolysin-O-permeabilized insulin-secreting cells. Experiments were performed as described in the legend to Fig. 1 except that the fourth bar represents cells that were preincubated for 15 min in the presence of 10 μM palmitoyl-CoA. Incubations were performed in the presence of indicated concentrations of free [Ca^{2+}] plus 10 μM palmitoyl-CoA (in the third bar only), (n = 4). Each separate experiment represents the mean of at least three samples, and n designates the number of separate experiments. *, p < 0.05 as compared with control. #, p < 0.05 as compared with preincubation conditions.

Exocytosis from permeabilized insulin-secreting cells, insulin release from cells incubated with LC-CoA was compared with insulin release from cells preincubated with palmitoyl-CoA (Fig. 4). Enhanced insulin release was obtained only from cells in which LC-CoA had been present during the incubation period. Permeabilized cells, in which LC-CoA was removed following a 15-min preincubation, responded similarly to control cells that were not exposed to LC-CoA. This indicates that the free LC-CoA indeed has to be present to stimulate exocytosis of insulin.

To further evaluate the site of action of LC-CoA on the exocytotic machinery, the effect of replacing ATP and phosphocreatine with creatine in the incubation medium was examined. This effectively replaces ATP and the ATP regenerating system with a buffer that converts any ATP formed to ADP (30). Although the removal of ATP from the permeabilized cells decreased exocytosis in response to 100 nM free [Ca^{2+}], this did not diminish the ability of LC-CoA to enhance insulin release as shown in Fig. 5. These data suggest that the effect of LC-CoA occurs late in the exocytotic process, after ATP-dependent docking of secretory granules.

To investigate this possibility further, we employed the patch-clamp technique. The effect of LC-CoA on the cell capacitance of mouse pancreatic β-cells was measured using the standard whole cell configuration to allow exchange of the pipette solution for the cell cytosol. Under these conditions, LC-CoA (1–10 μM) increased the β-cell whole cell capacitance, suggesting that LC-CoA increased fusion of secretory granules to the plasma membrane (Fig. 6) (28, 29). The kinetics of the capacitance changes revealed that the rapid initial phase of exocytosis was significantly increased by LC-CoA. The initial rate of exocytosis was increased from 29 ± 3 fF/s to 51 ± 9 fF/s with LC-CoA (p < 0.05; n = 4 for control and n = 5 for LC-CoA), whereas the subsequent slower rate increased from 7 ± 3 fF/s to 13 ± 4 fF/s (not significant; n = 4 for control and n = 5 for LC-CoA). It is proposed that distinct functional pools of secretory granules are responsible for the different kinetic phases of exocytosis observed by cell capacitance measurements (36). Thus, the rapid kinetics have been proposed to be due to exocytosis of already docked granules, comprising a readily releasable pool (37), whereas the final slower kinetic rate has been proposed to reflect the mobilization of secretory granules from a reserve pool (38). Based on this hypothesis, LC-CoA significantly enhanced release from the docked pool of secretory granules.

To further characterize the effect of LC-CoA on the β-cell membrane capacitance we examined the effects of chain length, protein kinase inhibition, removal of ATP, and addition of cerulenin, an inhibitor of protein acylation (Fig. 7). Consistent

Effect of inhibition with staurosporine. Experiments were performed as described in the legend to Fig. 1. Data show the stimulatory effect of 10 μM palmitoyl-CoA in the presence of 100 nM free [Ca^{2+}] in the presence and absence of 200 μM staurosporine (n = 3). Each separate experiment represents the mean of at least three samples, and n designates the number of separate experiments. *, p < 0.05 as compared with control.

Effect of pretreatment with LC-CoA on stimulation of insulin exocytosis from streptolysin-O-permeabilized insulin-secreting cells. Experiments were performed as described in the legend to Fig. 1 except that the fourth bar represents cells that were preincubated for 15 min in the presence of 10 μM palmitoyl-CoA. Incubations were performed in the presence of indicated concentrations of free [Ca^{2+}] plus 10 μM palmitoyl-CoA (in the third bar only), (n = 4). Each separate experiment represents the mean of at least three samples, and n designates the number of separate experiments. *, p < 0.05 as compared with control. #, p < 0.05 as compared with preincubation conditions.
Experiments were performed as described under “Experimental Procedures.” Incubations were performed in the presence of 100 nM free 
[Ca2+] plus 10 μM palmitoyl-CoA, as indicated (n = 3). The two left bars represent the standard incubation condition with an ATP regenerating system present as described under “Experimental Procedures.” The two right bars represent data obtained in the absence of ATP and in the presence of creatine to convert any ATP formed to ADP. Each separate experiment represents the mean of at least three samples, and n designates the number of separate experiments. * , p < 0.05 as compared with control. #, p < 0.05 as compared with −ATP condition.

**DISCUSSION**

It is well established that hormone responsiveness is altered by chronic hyperglycemia or hyperlipidemia. Although such metabolic alterations are often accompanied by changes in LC-CoA levels, the underlying mechanisms responsive for changes in signal transduction have not been established. LC-CoA esters and products formed from them are potent regulators of enzymes and channels. It has been hypothesized that elevations in LC-CoA, phosphatidylinositol, and diacylglycerol resulting from glucose stimulation (43) directly modulate the activity of enzymes including PKC isofoms (44, 45) or modify the acylation state of key proteins involved in regulation of ion channel activity and exocytosis (46–48). In this study we provide evidence that LC-CoA can directly modulate exocytosis of insulin from the pancreatic β-cell as determined by radioimmunoassay of insulin released from permeabilized HIT cells and capacitance measurements of mouse pancreatic β-cells. The membrane capacitance measurements performed in mouse β-cells suggest that LC-CoA enhances fusion of secretory granules with the β-cell plasma membrane and that this effect is physiologically relevant and not limited to clonal pancreatic cells.

The concentration and chain length dependence of LC-CoA-mediated stimulation of exocytosis appear to occur in a physiologically relevant range. Previous studies from our laboratory have estimated that the total cytosolic LC-CoA pool in HIT cells is about 90 μM and that 0.5 μM of this is free (1). Thus, the effective concentrations (1–10 μM) that stimulated exocytosis in the present study were within this range. The chain length requirements for exocytosis were fairly nonspecific. However, the general requirement for longer chain length compounds may be physiologically important based on their higher degree of partitioning into lipid bilayers (32) and the high prevalence of these chain length FFA in cells (49).

The mechanism by which LC-CoA esters stimulated insulin release seems to involve a direct effect on exocytosis independent of known modulators of this process. The possibility that the effect of LC-CoA was dependent on cPKC, PKA, or a number of other kinases, was eliminated based on the failure of inhibition by calphostin C, bisindolylmaleimide, staurosporine and Rp-cAMPS to diminish the stimulatory effect of LC-CoA. In addition, the removal of ATP, although decreasing overall insulin release from the permeabilized cells, failed to influence LC-CoA-stimulated insulin release, suggesting that the effect of LC-CoA is not mediated by protein kinase-induced phosphorylation. The persistent effect of LC-CoA in the absence of ATP suggests that the LC-CoA effect is at a late stage of exocytosis, after ATP-dependent vesicle docking. The removal of ATP from the permeabilized system would limit further docking of secretory granules to the plasma membrane, but already docked vesicles would be unaffected and still capable of fusing with the membrane resulting in insulin release (36). The stimulatory effect of LC-CoA on the initial rate of exocytosis, as measured by changes in cell capacitance, supports a role for LC-CoA in stimulating fusion of already docked secretory vesicles with the plasma membrane. The stimulatory effect of LC-CoA on the slower subsequent phase of exocytosis, although not significant, may be due to increased turnover of docking sites at the plasma membrane. Thus, increased fusion of granules to the plasma membrane would lead to an increase in the number of available docking sites to which granules in the reserve pool can be bound.

Many of the SNAP receptor (SNARE) proteins believed to play a role in exocytosis are acylated, including SNAP-25 (50), vesicle associated membrane protein (VAMP) (51), and synaptotagmin (52). It has been demonstrated that the post-translational palmitoylation of SNAP-25 is responsible for anchoring of the protein to the plasma membrane (53). It is not known whether palmitoylation plays a functional role in the acute regulation of vesicle fusion by modulating the levels of SNARE.
proteins available at the membrane. Alternatively, binding of LC-CoA to a SNARE or associated protein at a LC-CoA-specific binding site, as has been shown for the ATP-sensitive K⁺ channel (21, 22), may be important in stimulating exocytosis. Binding of LC-CoA to an exocytotic protein such as synaptotagmin may increase the Ca²⁺ sensitivity of exocytosis, resulting in more of the docked secretory vesicles fusing with the membrane, even at low [Ca²⁺].

If the protein-bound form of LC-CoA is the active modulator stimulating exocytosis, then the binding of LC-CoA to its effector must be reversible, because preincubation followed by removal of LC-CoA did not enhance exocytosis. Interestingly, the agonist-induced palmitoylation of endothelial cell nitric-oxide synthase has been shown to regulate its association with cellular membranes and to be reversible within minutes (54). The effect of LC-CoA on the ATP-sensitive K⁺ channel of the β-cell has also been demonstrated to be readily reversible (21).

The ability of cerulenin to block both protein acylation and the stimulatory effect of LC-CoA on insulin exocytosis suggests that the effect is mediated through an association of LC-CoA with a protein. Our demonstration that LC-CoA stimulates exocytosis of vesicles from an already docked pool suggests that the SNARE proteins are prime targets for such a modification by LC-CoA. The reversibility of the LC-CoA effect suggests that the modulation of cellular LC-CoA levels may play a role in regulation of exocytosis in the β-cell. These findings are consistent with a role for LC-CoA as a prime signal molecule that acts with Ca²⁺ and ATP to stimulate exocytosis under a variety of conditions associated with increased levels of LC-CoA. The identification of the specific LC-CoA-binding protein responsible for acute stimulation of insulin release and whether this protein is acylated or noncovalently modified by LC-CoA have yet to be determined.

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FIG. 7. Characterization of palmitoyl-CoA effects on β-cell membrane capacitance. A shows the lack of long chain acyl-CoA specificity on the stimulation of exocytosis. (n = 5 for each CoA ester; p < 0.005). B shows that the stimulatory effect of acyl-CoA is independent of kinase activity including most isoforms of PKC and PKA. (n = 4; p < .01). PKC and PKA inhibitors were preincubated with cells for 20 min prior to capacitance measurements. Bisindolylmaleimide and Rp-CAMPS were also included in the pipette solution. The concentrations of inhibitors are as described under “Results.” C shows that the stimulatory effect of acyl-CoA is independent of ATP provided to the cell through the patch pipette (n = 4; p < 0.001). D shows that the stimulatory effect of acyl-CoA can be blocked by cerulenin (100 μg/ml), an inhibitor of protein acylation. Cerulenin was only added to the pipette solution (n = 5; p < 0.001).
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Acute Stimulation with Long Chain Acyl-CoA Enhances Exocytosis in Insulin-secreting Cells (HIT T-15 and NMRI β-Cells)

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