Palmitate Stimulation of Glucagon Secretion in Mouse Pancreatic α-Cells Results From Activation of L-Type Calcium Channels and Elevation of Cytoplasmic Calcium

Charlotta S. Olofsson,[1] Albert Salehi,[1] Sven O. Göpel,[1] Cecilia Holm,[2] and Patrik Rorsman[1,3]

We have investigated the short-term effects of the saturated free fatty acid (FFA) palmitate on pancreatic α-cells. Palmitate (0.5 or 1 mmol/l bound to fatty acid–free albumin) stimulated glucagon secretion from intact mouse islets 1.5- to 2-fold when added in the presence of 1–15 mmol/l glucose. Palmitate remained stimulatory in islets depolarized with 30 mmol/l extracellular K^+ or exposed to forskolin, but it did not remain stimulatory after treatment with isradipine or triacsin C. The stimulatory action of palmitate on secretion correlated with a 3.5-fold elevation of intracellular free Ca^{2+} when applied in the presence of 15 mmol/l glucose, a 40% stimulation of exocytosis (measured as increases in cell capacitance), and a 25% increase in whole-cell Ca^{2+} current. The latter effect was abolished by isradipine, suggesting that palmitate selectively modulates L-type Ca^{2+} channels. The effect of palmitate on exocytosis was not mediated by palmitoyl-CoA, and intracellular application of this FFA metabolite decreased rather than enhanced Ca^{2+}-induced exocytosis. The stimulatory effects of palmitate on glucagon secretion were paralleled by a ~50% inhibition of somatostatin release. We conclude that palmitate increases α-cell exocytosis principally by enhanced Ca^{2+} entry via L-type Ca^{2+} channels and, possibly, relief from paracrine inhibition by somatostatin released by neighboring δ-cells.
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Type 2 diabetes is associated with increased plasma free fatty acid (FFA) levels, and this correlates with abrogated insulin action in peripheral tissue as well as impaired glucose-stimulated insulin release (1). However, the disease is characterized not only by diminished insulin secretion but also by surplus release of glucagon, despite the hyperglycemia (2). Furthermore, diabetic patients fail to respond with increased glucagon secretion upon lowering of the blood glucose concentration, possibly leading to fatal hypoglycemia. It is unclear whether these abnormalities cause or are secondary to the hyperlipidemia. Indeed, the role of lipids in the control of glucagon secretion remains obscure. Previous studies on the effects of FFAs on glucagon secretion have led to conflicting results, and they have variably been reported to either stimulate or inhibit glucagon release (3,4).

It is well established that long-term exposure to elevated plasma glucose and FFA levels compromise β-cell function (5). Nevertheless, a lipid-derived metabolite has been reported to be a prerequisite for a normal secretory β-cell response (6,7). Given the similarities in stimulus-secretion coupling of the different islet cells (8,9), a lipid messenger may also be important for the maintenance of the secretory capacity of the α-cell.

Here we have investigated the short-term effects of the saturated FFA palmitate on mouse pancreatic α-cells. Measurements of glucagon secretion in intact islets were combined with high-resolution single-cell measurements of α-cell exocytosis, whole-cell Ca^{2+} currents, and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). We demonstrate that palmitate enhances glucagon secretion and that the stimulatory action is both comparable to that obtained in response to hypoglycemia and dependent on increased Ca^{2+} influx. The functional and pathophysiological implications are discussed.

RESEARCH DESIGN AND METHODS

Islet and cell preparation. Pancreatic islets were isolated from female NMRI mice by collagenase P digestion (Roche, Bromma, Sweden). Single cells were prepared and cultured as previously described (10).

Preparation of palmitate-BSA solutions. Palmitate was prepared in a solution bound to fatty acid–free BSA (Roche), as outlined previously (10). The final concentration of BSA in the control and test solutions was 1% (wt/vol). Palmitate was added at total concentrations of either 0.5 or 1 mmol/l as indicated. The resulting free concentrations amount to 26 nmol/l and >10 μmol/l, respectively (10).

Electrophysiology. Whole-cell currents and exocytosis were recorded using an EPC-9 patch-clamp amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany) and Pulse software (version 8.50). α-Cells were functionally identified by their small size and Na^+ current inactivation properties (11). All experiments involving application of palmitate were performed using the perforated patch whole-cell technique, in which cell metabolism is maintained. The extracellular medium (EC-1) contained (in mmol/l) 118 NaCl, 20 TEA-Cl (tetraethylammonium chloride), 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES (pH 7.4 with NaOH), and 15 mmol/l glucose. In a few experiments, an equimolar amount of $BaCl_2$ substituted for $CaCl_2$ (see Figs. 3 and 4*A*). The pipette solution contained (in mmol/l) 76 Cs_2SO_4, 10 NaCl, 10 KCl, 1 $MgCl_2$, and 5 HEPES (pH 7.35 with CsOH). Exocytosis was monitored as changes in α-cell capacitance (12). These measurements were carried out in EC-1 supplemented with 10 μmol/l forskolin and 500 μmol/l of the ATP-sensitive K^+ (K_{ATP}) channel opener diazoxide. The latter was included to repolarize the α-cell and to prevent spontaneous exocytosis before stimulation. The BSA-containing solution was present for 4 min before the first train of depolariza-

From the [1]Department of Physiological Sciences, Lund University, Lund, Sweden; the [2]Department of Cell and Molecular Biology, Lund University, Lund, Sweden; and the [3]Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, U.K.

Address correspondence and reprint requests to Charlotta S. Olofsson, Dept. of Physiological Sciences, BMC B11, SE-221 84 Lund, Sweden. E-mail: charlotta.olofsson@mphy.lu.se.

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$[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; FFA, free fatty acid; K_{ATP} channel, ATP-sensitive K^+ channel.

TABLE 1
Glucagon secretion from intact islets

Glucose (mmol/l)	Substance tested (mmol/l)	Controls	Palmitate 1 mmol/l	Palmitate 0.5 mmol/l
1	—	36.0 ± 1.8	53.6 ± 3.9*	—
3	—	24.1 ± 2.7	33.7 ± 2.2*	—
7.5	—	14.9 ± 1.7	31.0 ± 3.3*	—
15	—	17.9 ± 0.9	40.1 ± 3.3*	34.1 ± 3.0*
15	Diazoxide (0.5); KCI (30)	66.1 ± 5.0	141.2 ± 6.6*	—
15	Forskolin (0.01)	37.6 ± 5.0	89.5 ± 14.5†	—
15	Triacsin C (0.01)	18.7 ± 1.2	21.8 ± 1.3‡	—
1	Isradipine (0.01)	33.3 ± 4.5	31.4 ± 3.0§	—

Data are means ± SE of indicated number of experiments (n = 6–35). Glucagon secretion is in picograms per islet per hour from intact islets after 60 min static incubation with or without addition of palmitate. *$P < 0.001$, †$P < 0.01$, for comparisons between the palmitate groups with respective control groups; ‡$P < 0.001$ vs. 15 mmol/l glucose + 1 mmol/l palmitate; § $P < 0.01$ vs. 1 mmol/l glucose + 1 mmol/l palmitate.

tions (control). Palmitate-BSA was then added, and the responses were measured 4 min later. When investigating the effects of triacsin C on exocytosis, cells were first preincubated with the inhibitor for 1–2 h. After establishment of a giga-seal, the BSA-containing control solution without triacsin C was perfused into the dish, and the experiment continued as outlined above.

Infusion experiments with palmitoyl-CoA were performed using the standard whole-cell configuration and an extracellular medium containing (in mmol/l) 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES (pH 7.4 with NaOH, EC-2), and 5 mmol/l glucose. The pipette solution consisted of (in mmol/l) 125 K-glutamate, 10 KCl, 10 NaCl, 1 $MgCl_2$, 5 $CaCl_2$, 10 EGTA (free Ca^{2+} ~0.2 μmol/l), 5 HEPES (pH 7.1 with KOH), 3 Mg-ATP, and 0.1 cAMP. The pipette solution was supplemented with 0 (control), 1, or 10 μmol/l palmitoyl-CoA (dissolved in water). Effects of palmitoyl-CoA on depolarization-evoked exocytosis and Ca^{2+} currents were studied using EC-2, and the pipette solution consisted of (in mmol/l) 125 CsOH, 125 glutamate, 10 CsCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES (pH 7.15 with KOH), 3 Mg-ATP, 0.1 cAMP, 25 μmol/l EGTA (measured resting free Ca^{2+} ~0.2 μmol/l) (13), and 0 or 10 μmol/l palmitoyl-CoA.

Measurements of $[Ca^{2+}]_i$. Changes in $[Ca^{2+}]_i$ were recorded by dual-wavelength microfluorimetry (10) in dispersed α-cells loaded with 2 μmol/l fura-2 AM (acetoxymethyl) in the presence of 0.007% wt/vol pluronic acid (Molecular Probes, Leiden, the Netherlands). The bath medium consisted of EC-2 supplemented with glucose as indicated. α-Cells were identified by their responses to low and high glucose concentrations and the ability of adrenaline (5 μmol/l) to selectively increase $[Ca^{2+}]_i$ in α-cells (14).

Measurements of hormone release. Hormone release was studied using intact islets as described previously (10). Glucagon and somatostatin concentrations in the incubation media were subsequently assayed using in-house radioimmunoassays (15,16). Diazoxide and forskolin (both from Sigma), triacsin C (Biomol Research Laboratories), isradipine (kind gift from Dr. J. Striessing), and glucose were included as indicated in Tables 1 and 2. When KCl was increased (Table 1), NaCl was equimolarly reduced to maintain iso-osmolarity.

Data analysis. Data are presented as mean values ± SE for the indicated number of experiments. The statistical significance of difference between two means was evaluated using Student's t test, paired or unpaired as appropriate.

RESULTS

The effect of palmitate on glucagon release. We investigated the effect of palmitate (0.5 or 1 mmol/l) on glucagon secretion (Table 1). Palmitate augmented glucagon release from intact islets exposed to 1–15 mmol/l glucose. Depolarization with 30 mmol/l K^+ in the presence of glucose (15 mmol/l) and the K_{ATP} channel opener diazoxide (500 μmol/l)—to clamp the membrane potential and to suppress action potential firing—enhanced glucagon release fourfold. Again, the addition of palmitate more than doubled glucagon release. This effect must have occurred independently of K_{ATP} channel modulation because this pathway is bypassed under these conditions. Palmitate likewise remained stimulatory in the presence of 10 μmol/l of the adenylate cyclase activator forskolin. This concentration of forskolin elevates the intracellular cAMP concentration to >100 μmol/l (X. Ma, L. Eliasson, and P.R., unpublished observations), a concentration sufficient to maximally activate cAMP-dependent pathways involved in exocytosis (17). Thus, the effect of palmitate is unlikely to be mediated by an increase in intracellular cAMP with resultant stimulation of glucagon secretion (18,19).

Triacsin C is an inhibitor of acyl-CoA synthetase (20) and possibly of FFA uptake (21). Whereas glucagon secretion in the presence of 15 mmol/l glucose alone was unaffected by inclusion of 10 μmol/l triacsin C in the extracellular solution, the palmitate stimulation was strongly attenuated by this inhibitor and only amounted to +16% ($P = 0.08$).

Consistent with earlier observations (22), glucagon secretion in the presence of 1 mmol/l glucose was likewise unaffected by 10 μmol/l of the L-type Ca^{2+} channel blocker isradipine. Palmitate failed to stimulate glucagon release in the presence of isradipine, suggesting that activation of L-type Ca^{2+} channels is essential for the stimulatory effect on secretion.

Palmitate stimulates α-cell exocytosis. To address the mechanisms by which palmitate enhances glucagon release, we applied high-resolution capacitance measurements to individual α-cells (23). Figure 1*A* shows the exocytotic responses elicited by a train of four 500-ms depolarizations from −70 to 0 mV. The total capacitance increase elicited by the train increased by >40% in response to palmitate stimulation and averaged 208 ± 46 and 297 ± 48 fF ($n = 8$, $P < 0.001$) in the absence and presence of the FFA, respectively. The average capacitance increase elicited by the first pulse increased from a control value of 146 ± 40 to 230 ± 35 fF after the addition of palmitate (+70%, $P < 0.01$) (Fig. 1*B*). To ascertain that the observed effects were not secondary to prolonged exposure to BSA, experiments were conducted in which cells were exposed to the BSA-containing control solution throughout without the addition of palmitate (Fig. 1*C*). The total capacitance increase elicited by the train of depolarizations after an additional 4-min period in BSA (to mimic the experimental protocol used for the FFA addition) was 102 ± 13% of the control ($n = 5$).

A multitude of experimental data indicate that secretory vesicles in neurons and in a variety of (neuro-)endocrine cells can be functionally defined according to their release competence (23). A small fraction (1–5%) of the granules is

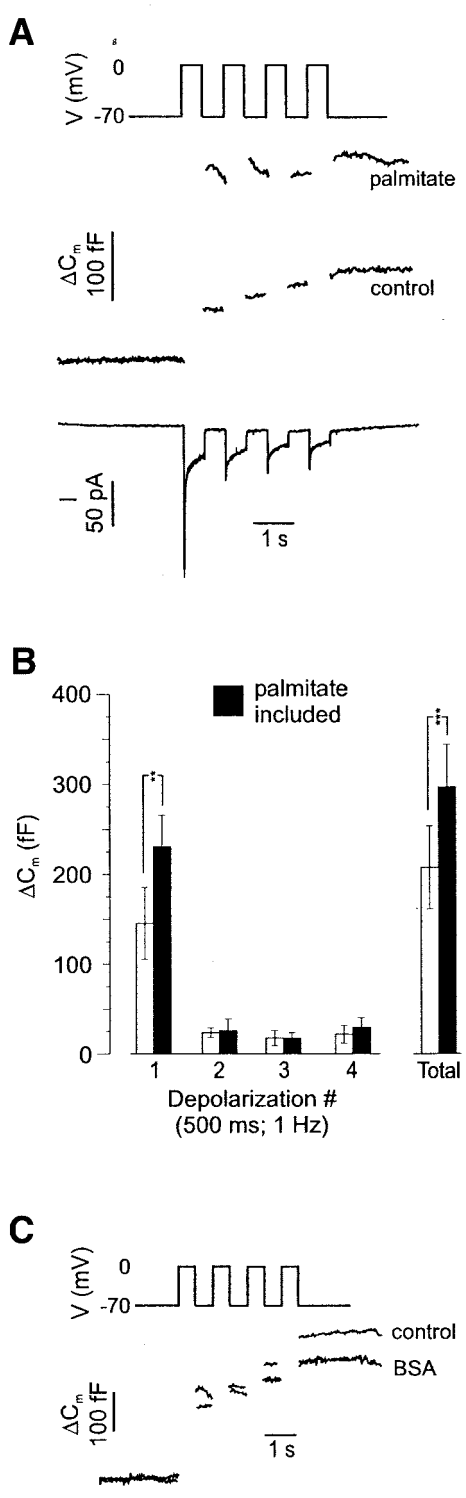

FIG. 1. Regulation of α-cell exocytosis by palmitate. *A*: Increase in cell capacitance (ΔC_m) (*middle*) and Ca^{2+} currents (I) elicited by a train of depolarizations (*top*) before and after stimulation with 0.5 mmol/l palmitate. *B*: Histogram of mean increases in cell capacitance (ΔC_m) plotted against depolarization number. *C*: As in *A* but in the continuous presence of BSA.

accordingly referred to as the readily releasable pool, whereas the remainder belongs to a larger reserve pool. The latter granules may be recruited from the reserve pool into the readily releasable pool and thereby attain release competence. Although there are alternative ways of interpreting phasic hormone secretion (24,25), the pool concept has turned out to be an instructive way of interpreting capacitance measurements and is now well established. We have therefore analyzed our capacitance measurements according to this paradigm.

Exocytosis induced by the first depolarization is thought to primarily represent the content of the readily releasable pool (26). The size of the readily releasable pool can be determined applying the two-pulse protocol of Gillis et al. (26). Using the responses to the first two depolarizations of the train, we estimated the size of the readily releasable pool to be 179 ± 40 fF under control conditions and 270 ± 45 fF after stimulation with palmitate ($P < 0.01$). Thus, exocytosis elicited by the train can almost entirely be accounted for by release of readily releasable pool granules, and mobilization of new granules does not appear to contribute much to secretion in the short term. The above capacitance values correspond to a readily releasable pool size of 90 and 135 granules (using a conversion factor of 2 fF/granule in α-cells) (22,27) in the absence and presence of palmitate, respectively.

We investigated the ability of palmitate to augment exocytosis in cells pretreated with triacsin C. In accordance with the glucagon release measurements, the stimulatory effect of palmitate was abolished under these conditions, and the total capacitance increase 4 min after inclusion of palmitate was 102 ± 14% of the control ($n = 4$) (not shown).

We investigated the involvement of palmitoyl-CoA in α-cell exocytosis by application of 1 or 10 μmol/l of this FFA metabolite into the cell interior, using the standard whole-cell configuration, in which the pipette solution replaces the cytosol. This recording mode thus clamps the intracellular milieu to the composition of the pipette solution, and the observed effects will therefore reflect the effects of palmitoyl-CoA itself rather than downstream metabolites. As shown in Fig. 2*A* and *B*, palmitoyl-CoA in fact inhibited exocytosis. The rate of capacitance increase ($\Delta C/\Delta t$), measured 30–80 s after establishment of the whole-cell configuration to permit wash-in of palmitoyl-CoA, amounted to 24 ± 5 fF/s under control conditions and 11 ± 3 fF/s ($P < 0.05$) and 10 ± 2 fF/s ($P < 0.05$) in the presence of 1 and 10 μmol/l palmitoyl-CoA, respectively.

When exocytosis was instead elicited by trains consisting of four 500-ms depolarizing pulses from −70 to 0 mV, palmitoyl-CoA was without effect (Fig. 2*C*). The capacitance increase elicited by the first pulse and the total increase at the end of the train averaged 510 ± 130 and 1,070 ± 300 fF under control conditions and 485 ± 120 and 865 ± 225 fF upon inclusion of palmitoyl-CoA. The 20% decrease in total capacitance increase did not reach statistical significance ($P = 0.6$). Collectively, these observations argue that palmitoyl-CoA does not represent the intracellular metabolite mediating the effects on exocytosis.

Palmitate increases α-cell Ca^{2+} currents. Glucagon secretion is a Ca^{2+}-dependent process mediated by Ca^{2+} influx through voltage-gated Ca^{2+} channels (27–29). We first measured whole-cell Ca^{2+} currents using Ba^{2+} as the charge carrier during depolarizations from a holding potential of −70 mV to membrane potentials between −50 and +60 mV before and after addition of 0.5 mmol/l palmitate (Fig. 3*A*). The current amplitude was analyzed

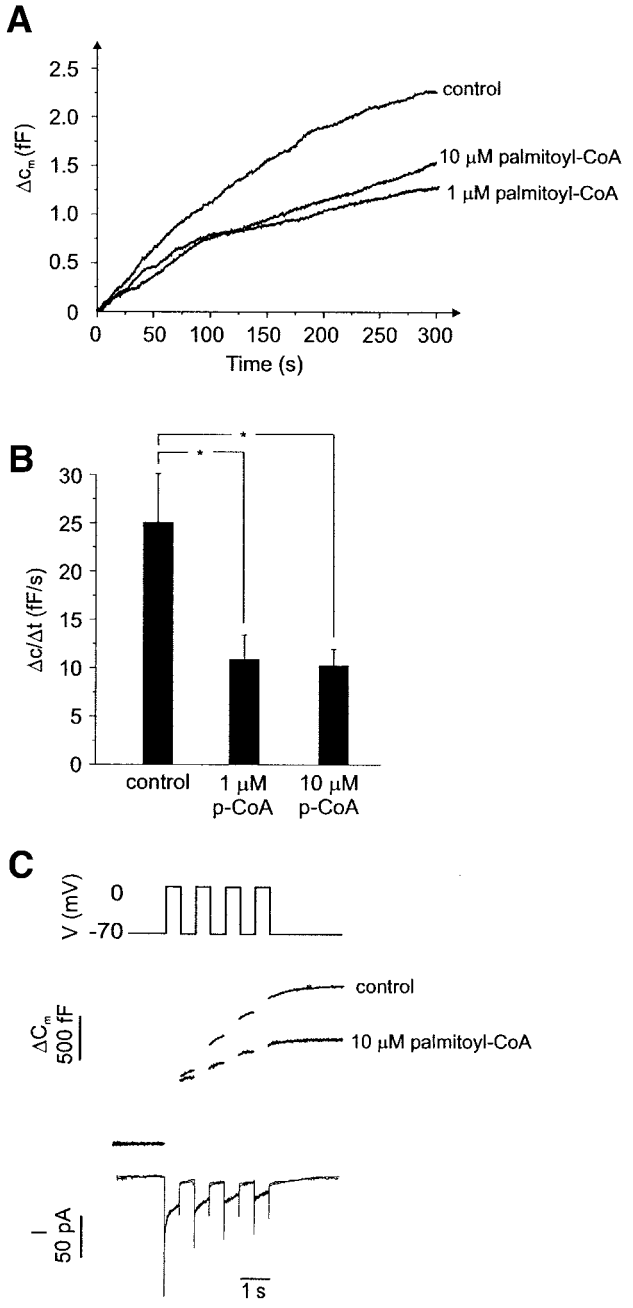

FIG. 2. Failure of palmitoyl-CoA to stimulate exocytosis. *A*: Increase in cell capacitance (ΔC_m) measured with 0, 1, or 10 μmol/l palmitoyl-CoA (p-CoA) in the pipette solution. *B*: Histogram summarizing the mean rate of capacitance increase (ΔC/Δt) measured at steady-state. Data are the mean values ± SE of 8–11 experiments. *C*: As in Fig. 1*A* but using the standard whole-cell configuration and a pipette solution containing 0 or 10 μmol/l palmitoyl-CoA. Stimulation commenced >3 min after establishment of the whole-cell configuration to allow complete wash-in of the pipette solution. Recording is representative of 8–11 individual experiments. V, voltage.

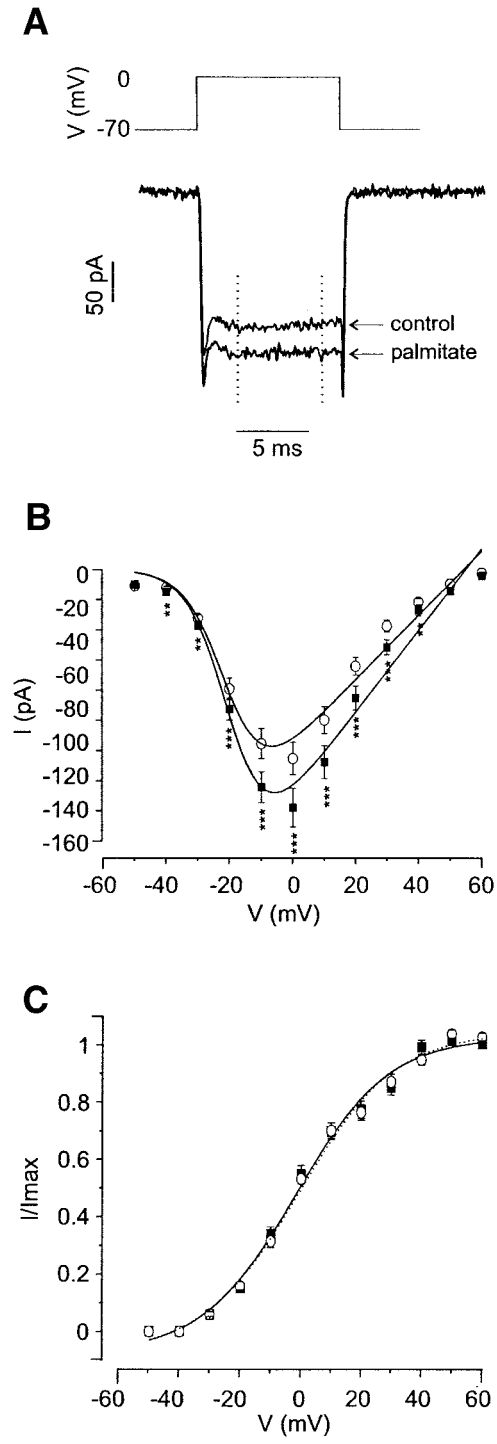

FIG. 3. Effects of palmitate on voltage-dependent α-cell Ca^{2+} currents. *A*: Depolarization-evoked Ca^{2+} currents before and 4 min after addition of 0.5 mmol/l palmitate. *B*: Ca^{2+} current (I)-voltage (V) relationship for Ca^{2+} currents evoked by depolarizations to voltages between −50 and +60 mV from −70 mV before and 4 min after introduction of 0.5 mmol/l palmitate. The curve was drawn by approximating Eq. 1 (in 27) to the data points. Current amplitudes were measured during the period indicated by the dotted lines and arrows in *A*. *C*: Normalized tail current amplitudes recorded in the absence (○) and presence (■) of 0.5 mmol/l palmitate. The curves were obtained by approximating the Boltzmann equation to the data points. Values in *B* and *C* are mean values ± SE for 10 experiments. **$P < 0.01$; ***$P < 0.001$.

>3 ms after the onset of the pulse to avoid contribution by Na^+ current (the transient spike observed initially during the depolarization). As shown in Fig. 3*B*, the magnitude of the current increased by ~25% at all voltages between −40 and +40 mV. Similar results were obtained using 1 mmol/l palmitate (not shown).

We also explored whether the increased Ca^{2+} current amplitude was associated with a shift in the gating by

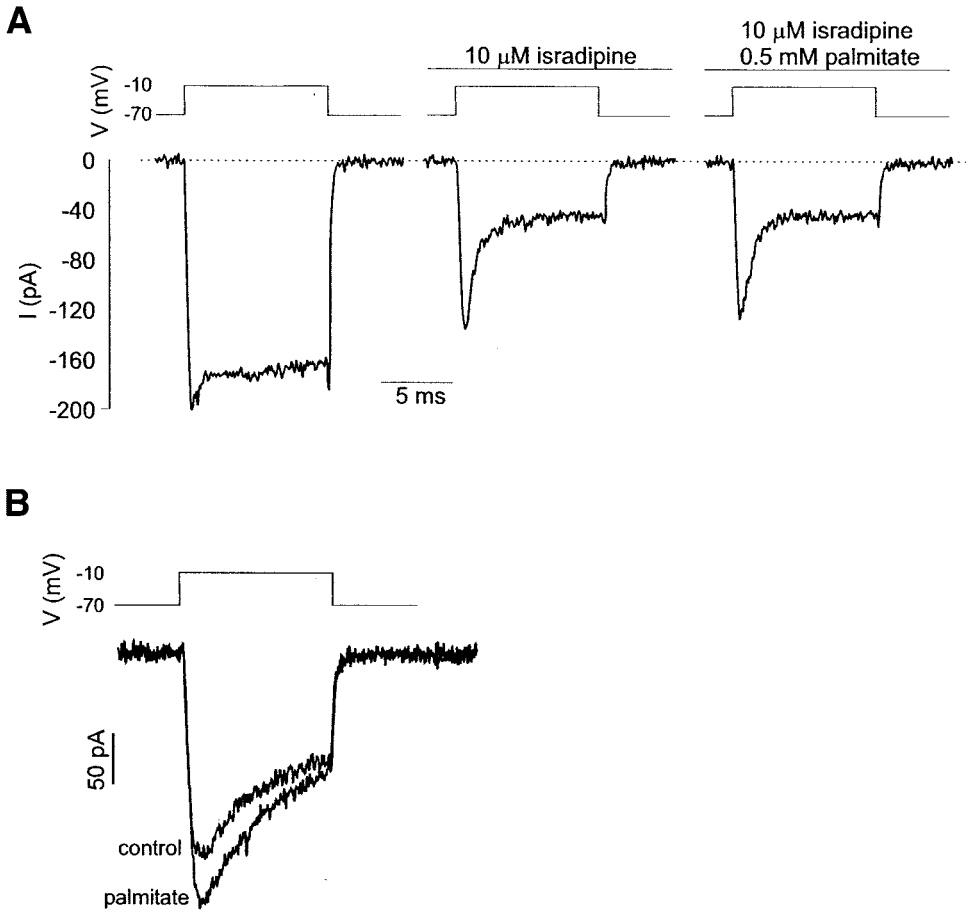

FIG. 4. *A*: Ca^{2+} currents (I) recorded in the presence of isradipine before and 4 min after addition of palmitate. *B*: Palmitate augmentation of Ca^{2+} currents using Ca^{2+} as the charge carrier. V, voltage.

measuring the instantaneous current-voltage relation ("tail current" analysis). The currents observed upon stepping back the voltage to −70 mV provide an estimate of the relative Ca^{2+} channel open probability that is independent of the membrane potential (30). The data points were approximated to

$$h_\infty(V_m) = 1/\{1 + \exp[(V_h - V_m)/k_h]\} \quad (1)$$

where h_∞ (V_m) is the relative current amplitude measured during a depolarization to the membrane potential V_m, V_h is the membrane potential at which h equals 0.5, and k_h is the steepness coefficient. The average current amplitude elicited by depolarizations between +40 mV and +60 mV was taken as unity (I_{max}) and the currents expressed as the fraction hereof (i.e., $h_\infty = I/I_{max}$). No shift in the gating was detected, and V_h averaged −1 ± 2 and −2 ± 2 mV before and after inclusion of 0.5 mmol/l palmitate, respectively (Fig. 3*C*).

Pancreatic α-cells contain several types of voltage-gated Ca^{2+} channel (9,27). As shown in Fig. 4*A*, the addition of 10 μmol/l isradipine decreased the Ca^{2+} current at −10 mV by 60 ± 6% ($n = 4$, $P < 0.01$). Palmitate was unable to increase α-cell Ca^{2+} currents in the presence of isradipine, and the current at −10 mV averaged −62 ± 10 pA in the presence of isradipine alone vs. −63 ± 12 pA at 4 min after addition of 0.5 mmol/l palmitate ($n = 4$). We therefore conclude that palmitate acts selectively on L-type Ca^{2+} channels.

To rule out the possibility that palmitate was only capable of increasing currents through voltage-gated Ca^{2+} channels when Ba^{2+} was used as the charge carrier, we measured Ca^{2+} currents elicited by depolarizations from −70 to −10 mV in the presence of a solution containing 2.6 mmol/l $CaCl_2$ and 400 nmol/l tetrodoxin to block Na^+ currents (Fig. 4*B*). Palmitate remained capable of increasing the Ca^{2+} current (~23%) under these conditions, and the average peak current rose from a control value of −123 ± 29 to −151 ± 36pA at 4 min after the addition of 0.5 mmol/l palmitate ($n = 4$, $P < 0.05$). We also measured the Ca^{2+} current generated by depolarizations from −70 to −10 mV with or without inclusion of palmitoyl-CoA in the patch pipette. However, palmitoyl-CoA had no effect on the Ca^{2+} current amplitude (not shown).

Palmitate increases $[Ca^{2+}]_i$ in dispersed α-cells. The pancreatic α-cell is electrically excitable, and action potential firing mediates the Ca^{2+} influx, triggering exocytosis of glucagon-containing secretory granules (27,29). Measurements of $[Ca^{2+}]_i$ in isolated α-cells therefore provide an indirect but noninvasive means of monitoring electrical activity. In the presence of 1 mmol/l glucose, a concentration associated with stimulated glucagon secretion, the average $[Ca^{2+}]_i$ (calculated time average) amounted to 207 ± 23 nmol/l ($n = 8$). Consistent with the inhibitory effects of glucose on glucagon secretion, application of 15 mmol/l glucose decreased $[Ca^{2+}]_i$ to 75 ± 13 nmol/l. Subsequent addition of 0.5 mmol/l palmitate in the

TABLE 2
Somatostatin secretion from intact islets

Glucose (mmol/l)	Substance tested (mmol/l)	Controls	Palmitate 1 mmol/l	Palmitate 0.5 mmol/l
1	—	1.0 ± 0.1	0.8 ± 0.1	—
15	—	4.8 ± 0.3	2.2 ± 0.2*	2.9 ± 0.3*
15	Triacsin C (0.01)	4.5 ± 0.2	2.9 ± 0.2*	—

Data are means ± SE for 5–12 experiments. Palmitate inhibition of somatostatin secretion in picomoles per islet per hour in intact islets after 60-min static incubation. *$P < 0.001$ vs. control values on same line.

continued presence of 15 mmol/l glucose promptly and reversibly increased $[Ca^{2+}]_i$ to 266 ± 33 nmol/l ($P < 0.001$). In the presence of 15 mmol/l glucose and palmitate, $[Ca^{2+}]_i$ in fact exceeded that observed in the presence of 1 mmol/l glucose alone by ~30% ($P < 0.05$). This effect cannot simply be accounted for by increased Ca^{2+} entry because the α-cells are likely to be electrically silent under these conditions.

Palmitate inhibits somatostatin secretion. Somatostatin is a powerful inhibitor of glucagon secretion (31,32). We therefore considered the possibility that FFAs influence glucagon secretion via modulation of somatostatin release from neighboring somatostatin-producing δ-cells (Table 2). Somatostatin secretion was low in the absence of a stimulatory glucose concentration (1 mmol/l) and increased 4.8-fold in response to stimulation with 15 mmol/l glucose. Palmitate (0.5–1 mmol/l) was without effect at low glucose, but it inhibited secretion by ~50% at the higher glucose concentration. Somatostatin secretion in the presence of 15 mmol/l glucose alone was unaffected by triacsin C, but the inhibitor partially counteracted the inhibitory action of palmitate on somatostatin secretion.

DISCUSSION

Increased plasma FFA levels is a hallmark of type 2 diabetes, but the acute and long-term effects of FFAs on islet cell function have only partially been elucidated, and studies have principally focused on the β-cell (33). Here, we report that short-term exposure to palmitate stimulates glucagon secretion at both low and high glucose concentrations and that somatostatin release is inhibited by this FFA.

The ability of triacsin C to counteract the effects of palmitate on α-cell exocytosis and glucagon release (Table 1) suggests that the palmitate-induced stimulation of glucagon secretion requires metabolic conversion of the FFA (20) and/or intracellular uptake of palmitate (21). This finding is difficult to reconcile with the involvement of a membrane-bound FFA receptor (34–36). The failure of palmitoyl-CoA, the activated form of palmitate, to mimic the effect of palmitate on exocytosis (Fig. 2) indicates that this it is not the mediator of the effect and that the stimulation is more likely caused by downstream FFA metabolites.

The pancreatic α-cell is electrically excitable (27,29), and the presence of K_{ATP} channels in α-cells is well documented (9,37). It has been proposed that glucose-induced closure of K_{ATP} channels leads to inhibition of glucagon release in mouse α-cells via voltage-dependent inactivation of membrane currents involved in action potential generation (9). Long-chain acyl-CoAs (38,39) and phospholipids (40) have been shown to activate K_{ATP} channels via interaction with Kir6.2. It is therefore conceivable that a lipid metabolite derived from palmitate stimulates glucagon release by exerting a diazoxide-like action reactivating K_{ATP} channels previously closed by glucose. Alternatively, transport of negatively charged long-chain fatty acids across the plasma membrane can be envisaged to produce membrane repolarization independently of K_{ATP} channel regulation. Long-chain fatty acids exist, at a physiological pH, mainly in their anionic form and are thus negatively charged. Previous studies demonstrate that ionized lipophilic molecules and long-chain FFAs can translocate rapidly and spontaneously across membranes (41,42). The latter scenario is suggested by analogy to the effects of the positively charged amino acid arginine, the stimulatory action of which on glucagon secretion and electrical activity is caused at least in part by its electrogenic entry with resultant membrane depolarization (43).

Irrespective of the mechanism and given the observations that palmitate increases $[Ca^{2+}]_i$ (Fig. 5) while not affecting Ca^{2+} channel gating (Fig. 3*C*), it seems clear that the FFA must be capable of reestablishing action potential firing in α-cells in which electrical activity has been suppressed by glucose. However, palmitate must also influence glucagon secretion at a level distal to changes in membrane potential. Indeed, palmitate stimulated exocytosis elicited by short (0.5 s) voltage-clamp depolarizations by 43% (Fig. 1). This extent of stimulation is in close agreement with the ~45% stimulation of glucagon secretion observed in 1 or 3 mmol/l glucose (Table 1), i.e., when the α-cells are likely to be electrically active. The finding that palmitate enhanced glucagon secretion at 1 mmol/l glucose also argues that it does not simply act by counteracting the inhibitory action of glucose but exerts a true stimulatory effect.

The capacitance measurements revealed that the enhancement of exocytosis by palmitate can almost entirely be accounted for by release of readily releasable pool granules, and recruitment of new granules does not appear to contribute much to secretion in the short term. The FFA-induced stimulation of exocytosis was associated with a ~25% enhancement of the Ca^{2+} current (Fig. 3), an effect selective for the L-type Ca^{2+} channels (Fig. 4). The findings that inclusion of the L-type Ca^{2+} channel antagonist isradipine eliminated the FFA-induced stimulation of both the Ca^{2+} current and glucagon secretion indicate that increased Ca^{2+} entry is a key event. This conclusion is further underpinned by the observation that the stimulatory action of palmitate on glucagon release was abolished even though 40% of the Ca^{2+} current was resistant to the antagonist (Table 1 and Fig. 4). Glucagon secretion depends differentially on Ca^{2+} influx through N- and L-type

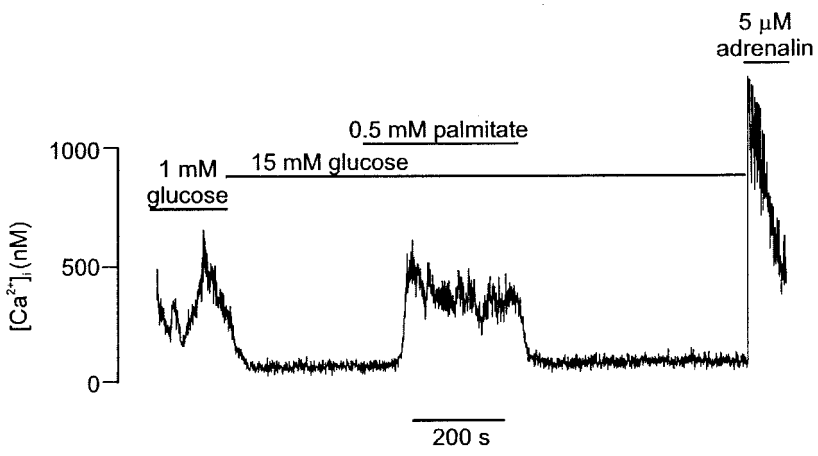

FIG. 5. Increase in $[Ca^{2+}]_i$ in an α-cell upon addition of 0.5 mmol/l palmitate in the presence of 15 mmol/l glucose. The recording selected for display is representative of a total of eight separate experiments.

Ca^{2+} channels. Whereas ω-conotoxin–sensitive N-type Ca^{2+} channels are particularly important under basal conditions (hypoglycemia alone), dihydropyridine-sensitive L-type Ca^{2+} channels become more important when glucagon secretion is stimulated. For example, agents that elevate intracellular cAMP and activate protein kinase A result in a selective stimulation of L-type Ca^{2+} channel–dependent exocytosis (29). The same appears to apply to glucagon secretion evoked by palmitate. It may appear that the action on the Ca^{2+} current is too small to be functionally important. However, L-type Ca^{2+} channels only account for ~60% of the whole-cell Ca^{2+}current. Thus, the L-type Ca^{2+}channel activity must have increased by >40% to produce the observed 25% stimulation of the whole-cell current, which includes components apparently not affected by FFAs. Indeed, the 40% stimulation of the L-type Ca^{2+} current component is in good agreement with the observed enhancement of exocytosis (45%). Our data suggest that the effects of palmitate are principally attributable to enhanced Ca^{2+} entry through L-type Ca^{2+} channels. Such a concept accounts for the ability of isradipine to abolish the stimulatory effect on glucagon secretion. On the other hand, the ability of palmitate to stimulate exocytosis by >100% under certain conditions (e.g., high-K^+ stimulation in the presence of diazoxide) (Table 1) may indicate the involvement of additional mechanisms, such as a direct effect on exocytosis, as has previously been documented in β-cells (10).

Somatostatin is a potent inhibitor of glucagon secretion (19,44). The finding that palmitate is a strong inhibitor of somatostatin release therefore suggests that palmitate may augment glucagon secretion by relief from paracrine somatostatin–induced inhibition. Indeed, previous work has established that somatostatin antagonists stimulate glucagon release (44,45). However, the fact that palmitate accelerated depolarization-evoked exocytosis in isolated α-cells (Fig. 1), an experimental situation that minimizes paracrine interactions, suggests that palmitate must also enhance glucagon secretion by a direct effect on the α-cell.

It may seem surprising that the inhibitory action of palmitate on somatostatin release was only partially counteracted by triacsin C. However, this does not necessarily suggest that δ-cells contain isoforms of acyl-CoA synthetase that are not inhibited by triacsin C (46). It should be noted that whereas the α-cells preferentially locate to the islet periphery, the δ-cells occur throughout the islet (47). Thus, the difference may simply be attributable to the presence of diffusion barriers for triacsin C within the islet. The observed partial reversal of the palmitate-induced inhibition may accordingly be attributed to an effect of triacsin C on peripheral δ-cells.

It is tempting to consider the pathophysiological implications of these observations. Both hyperlipidemia and hyperglycemia are characteristics of type 2 diabetes (1). Our observation that palmitate stimulates glucagon secretion at high glucose concentrations raises the interesting possibility that hyperlipidemia contributes to oversecretion of glucagon, which in turn aggravates the hyperglycemia. In this context it is also worthy of note that release of somatostatin, which normally inhibits glucagon secretion, is impaired in diabetic subjects (48). Collectively, these considerations reinforce the notion that type 2 diabetes is best characterized as a bihormonal (49) or even trihormonal disorder. We acknowledge that so far we have only explored the effects of palmitate during short-term exposure. Given the present findings, it is now essential to investigate long-term effects of FFAs on glucagon secretion and whether there is an α-cell equivalent of the lipotoxic action previously documented in β-cells (5).

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