Activation of the ATP-sensitive K⁺ Channel by Long Chain Acyl-CoA
A ROLE IN MODULATION OF PANCREATIC β-CELL GLUCOSE SENSIVITY*

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Long term exposure to elevated levels of long chain free fatty acids decreases glucose-induced insulin secretion from pancreatic islets and clonal pancreatic β-cells. The mechanism for this loss of glucose sensitivity is at present not known. In this study, we evaluated the possibility that increases in long chain acyl-CoA esters (LC-CoA), the metabolically active form of free fatty acids, might mediate the loss of glucose sensitivity. We observed that cellular levels of LC-CoA increased more than 100% in response to overnight incubation with 0.5 mM palmitic acid complexed to albumin. In the same studies, the total CoA pool increased by about 40%. Patch-clamp studies demonstrated that saturated and unsaturated LC-CoA, but not malonyl-CoA or free CoASH, induced a rapid and slowly reversible opening of ATP-sensitive K⁺ channels. The effect was concentration-dependent between 10 nM and 1 μM. These findings indicate that the ATP-regulated K⁺ channel is a sensitive target for LC-CoA and suggest that high levels of LC-CoA, which accumulate in response to hyperglycemia or prolonged exposure to free fatty acids, may prevent channel closure and contribute to the development of β-cell glucose insensitivity.

Exposure to elevations in free fatty acids (FFA) decreases glucose-induced insulin secretion. The concept of gluco-lipotoxicity is increasingly being invoked to explain such loss of glucose-induced insulin secretion, but the identity of putative effector molecules that might mediate these effects is unknown. Normal glucose-induced insulin secretion is associated with inhibition of FFA oxidation and increased lipid synthesis in pancreatic β-cells (1–4). In addition, exogenous FFA acutely potentiates glucose-stimulated insulin secretion (4, 5). We have demonstrated that glucose causes marked alterations in the acyl-CoA profile of clonal pancreatic β-cells, with the largest (5-fold) and earliest (by 2 min) change occurring in malonyl-CoA (1, 5). However, we did not obtain a good correlation between secretion and malonyl-CoA levels but rather between secretion and decreases in long chain acyl-CoA (LC-CoA) levels (5).

The total CoA pool is fixed over short intervals and distributed between mitochondrial and cytosolic compartments that are not interchangeable (6, 7). Thus, during an acute response, the maximum LC-CoA concentration is limited by the total CoA pool and its distribution between cytosol and mitochondria. In some cell types, high fat and certain drugs or steroids have the potential to increase the total CoA pool over a period of hours to days and also lead to increases in the LC-CoA pool (7).

Our studies were undertaken to determine whether increased levels of LC-CoA occur in response to exposure of β-cells to FFA and to assess the effect of physiological concentrations of LC-CoA on the ATP-sensitive K⁺ channel (KATP channel). We demonstrate, for the first time, that both total CoA and LC-CoA levels increase in cells cultured in elevated FFA and document dramatic increases in KATP channel activity in response to LC-CoA.

EXPERIMENTAL PROCEDURES

Growth and Incubation of Cells and Islets—Ad libitum fed obese hyperglycemic mice (gene symbol ob/ob) of both sexes were obtained from a local non-inbred colony. The mice were starved for 24 h, and islets were isolated by collagenase digestion and dispersed into single cells as described previously (8). The cells were resuspended in RPMI 1640 medium, supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 60 μg/ml gentamycin, and 10% fetal calf serum. The cell suspension was plated in Petri dishes and incubated at 37 °C in 5% CO₂ for 1–3 days. Clonal pancreatic β-cells (HIT-T15) were cultured in RPMI 1640 medium supplemented with 50 units/ml penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum, used between passages 64 and 80, with harvested phosphate-buffered saline containing 0.02% EDTA, and washed in Hanks' Ca⁺⁺-Mg⁺⁺ free buffer, pH 7.4 (9).

Electrophysiology—The electrophysiology experiments were carried out using the inside-out configuration of the patch-clamp technique (10) on dispersed mouse β-cells. This type of recording mode allows access to the cytoplasmic side of the plasma membrane. The electrodes had resistances between 3 and 4 megohms when filled with an extracellular buffer containing (in mM): 138 NaCl, 5.6 KCl, 1.2 MgCl₂, 2.6 CaCl₂, and 5 Heps-NaOH; pH 7.4. The bath solution contained (in mM): 125 KCl, 1 MgCl₂, 10 EGTa, 30 KÖH, 5 Heps, pH 7.15. Channel activity was measured at 0 mV membrane potential. With the solutions used, ion currents are outward (i.e. into the pipette). Channel records are displayed with upward deflections denoting outward currents. The KATP channel activity is identified based on the unitary amplitude (1.5–2 pA) and its sensitivity to ATP. All experiments were carried out at room temperature (22–24 °C). ATP was added as the Mg⁺⁺ salt, CoASH as the Na⁺ salt; CoA esters were dissolved in water and the Na⁺ salt of oleic acid was dissolved in 95% ethanol. The final concentration of ethanol was 0.1%. Patches were excised into nucleotide-free solution, and 0.1 mM ATP was first applied to test for channel inhibition. After inhibition had been demonstrated, ATP was removed and the patch was subsequently exposed to the test solutions indicated in figures.

Assays and Materials—Acyl-CoA esters were extracted and meas-
**RESULTS AND DISCUSSION**

Culture of isolated rat islets or clonal β-cells in elevated concentrations of FFA decreases the ability of glucose to stimulate insulin secretion. We performed studies in which clonal β-cells (HIT-T15), incubated overnight in medium containing 0.5 mM palmitic acid, exhibited a 50% decrease in the ability of glucose to stimulate insulin secretion (data not shown). This observation is consistent with previously published findings in islets from starved animals or animals exposed to high circulating levels of FFA in which glucose-induced insulin release is diminished (12, 13). Analysis of soluble and insoluble pools of acyl-CoA esters showed that overnight exposure to 0.5 mM palmitic acid increased the pool of LC-CoA, the metabolically active form of FFA, as well as the total CoA pool without affecting the short chain acyl-CoA ester pool (Table I). The 100% increase in LC-CoA was opposite in direction and greater in magnitude than the change in secretion. These findings suggest that the accumulation of LC-CoA could be causally related to a loss of responsiveness to glucose.

It is well established that one of the initial events in the β-cell stimulus secretion coupling is closure of the KATP channel. The activity of this channel is also the main determinant of the β-cell resting potential (14). One possible explanation for the inhibition of glucose responsiveness following exposure to high FFA levels could therefore be modulation of KATP channel activity. It should also be noted that the molecular structure of the CoA moiety in the LC-CoA molecule bears a very close resemblance to ADP, a known stimulator of the KATP channel, we assessed regulation using single channel recordings of dispersed mouse β-cells with the inside-out configuration of the patch clamp technique (Fig. 1). Fig. 1A illustrates a reversible stimulatory effect of 1 μM oleoyl-CoA on KATP channel activity. Mean current increased 5-fold, from 0.8 to 4.4 pA in the presence of 10 μM oleoyl-CoA. The onset of the effect was considerably faster than the recovery. Channel activity is increased by the number of open channels and also by an increased mean open time (data not shown), similar to the effect exerted by low concentrations of ADP (17, 18). The second trace (Fig. 1B) shows a slight stimulatory effect of 100 nM oleoyl-CoA with a long lag and slow recovery in which the mean current increased approximately 2-fold from 0.6 to 1.5 pA. The kinetics of the oleoyl-CoA effects are probably related to the lipophilicity of the compound. Addition of 10 nM oleoyl-CoA (Fig. 1C) induced little effect except to possibly diminish run down of channel activity. In a previous study, we found cytosolic binding sites for LC-CoA with an estimated KD of approximately 1 μM (9), suggesting that the concentrations used are physiologically relevant.

Interaction between ATP and oleoyl-CoA was observed (Fig. 2). When 0.1 mM ATP was present continuously, a concentration that fully blocks KATP channel activity, 1 μM oleoyl-CoA induced a pronounced increase in channel activity (Fig. 2A). When 0.1 mM ATP was added to the bath solution one min after exposure of the patch to LC-CoA, ATP had a considerably less pronounced inhibitory effect on channel activity (Fig. 2B). This reduced blocking effect of ATP can be explained by the slow recovery of channel activity following exposure to the LC-CoA ester, due to its lipophilicity, since after approximately five min further washing, the normal sensitivity to ATP was regained (Fig. 2B). These experiments provide compelling evidence that LC-CoA esters have the ability to prevent ATP-induced closure of the KATP channel, in vitro. Interestingly, they may also be involved in the suppression of glucose-induced depolarization following exposure to high levels of lipids.

The specificity for oleoyl-CoA was tested by comparing the responses with the FFA and with free CoASH. As seen in Fig. 3, no effect was observed with 1 μM oleic acid in a patch that responded to ATP and oleoyl-CoA (Fig. 3A). Likewise, no effect...
on K<sub>ATP</sub> channel activity was observed when CoASH was administered, either at low (data not shown) or at high concentrations, to a patch where oleoyl-CoA induced a potent increase in mean current (Fig. 3B). Thus, the stimulatory effect of the LC acyl-CoA is dependent on both the acyl group and the CoA component. We also studied the effects of LC-CoA of different chain lengths on channel activity (Fig. 4). Stimulatory effects were seen with CoA esters of chain length 14 (Fig. 4C) and 16 carbons (Fig. 4C) but not with the 3-carbon malonyl-CoA (Fig. 4A). The effect of myristoyl-CoA (C14:0) was less pronounced than that obtained with palmitoyl-CoA (C16:0) or oleoyl-CoA (C18:1), both with regard to stimulatory ability and to recovery of the effect. These findings indicate that the K<sub>ATP</sub> channel is a target for LC-CoA with a responsiveness to both saturated and unsaturated LC-CoA esters from C-14 to C-18 and are consistent with the suggestion that high levels of LC-CoA, such as occur in response to elevated FFA, may prevent channel closure in response to glucose. This may explain the impair in K<sub>ATP</sub> channel closure that has been observed in islets from the diabetic Goto-Kakizaki rat (19). A previous report of FFA modulation of K<sup>+</sup> channel activity in clonal β-cells did not differentiate between the effects of the FFA and their metabolically active LC-CoA esters (20). Since added FFA can be rapidly converted to LC-CoA, it is necessary to compare the FFA and LC-CoA in an excised inside-out patch, as we have done. Such studies may also reveal a more general regulatory function of LC-CoA on K<sup>+</sup> channel activity in other cell types.

Fig. 5 compares the effect of oleoyl-CoA on different K<sup>+</sup> channels present in the β-cell. The upper trace shows that the big conductance K<sup>+</sup> channel (K<sub>BK</sub>), which is voltage- and Ca<sup>2+</sup>-dependent, is not influenced by 1 μM oleoyl-CoA in contrast to the K<sub>ATP</sub> channel, which is activated (compare the expanded regions in Fig. 5A). The addition of 100 μM CoASH had no effect on either the K<sub>ATP</sub> channel or the K<sub>BK</sub> channel. Fig. 5B shows that the 8-picosiemens K<sup>+</sup> channel, described previously in the β-cell (21), is also unaffected by the addition of oleoyl-CoA. Hence, the ability of LC-CoA to increase K<sup>+</sup> conductance seems to be specific for the K<sub>ATP</sub> channel. These findings identify a potent new regulator of the K<sub>ATP</sub> channel which causes up to fivefold increases in mean channel current in response to the
most common LC-CoA esters (7). This is observed at concentrations that are physiologically relevant and approximately equal to the $K_1$ for cytosolic binding sites for LC-CoA (9). On a molar basis, the LC-CoA esters are approximately 1000-10000 times more potent than ADP in stimulating channel activity.

LC-CoA esters and products formed from them have also been shown to be potent regulators of a variety of enzymes (22) and channels (20, 23). Thus, LC-CoA inhibits glucokinase activity, stimulates endoplasmic reticulum Ca$^{2+}$-ATPase, and inhibits acetyl-CoA carboxylase (9, 22, 24-27). The adenine nucleotide translocase, which plays an important role in controlling the cytosolic ATP/ADP ratio (26), is stimulated by LC-CoA in some cells. Based on these opposite effects on different established targets of LC-CoA and the ability of FFA to increase the total CoA pool in $\beta$-cells, as in other tissues (7, 11), the net effect of LC-CoA on glucose-induced insulin secretion can be either stimulatory or inhibitory and may depend on the LC-CoA level achieved.

We find it attractive to hypothesize that elevated circulating FFA, leading to increased cellular levels of LC-CoA, play a role in the development of glucose insensitivity and that elevated glucose levels, as in tissue culture or non-insulin-dependent diabetes mellitus, exacerbate the "toxic" action of lipids, because high glucose inhibits fatty acid oxidation and elevates cytosolic LC-CoA levels (1). Thus, isolated islets from 48-h lipid-infused rats display reduced glucose oxidation and insulin release in response to glucose (12), and the insulin secretory response of islets isolated from fat-fed mice is similar to the defective secretory pattern observed in human NIDDM (31). Furthermore, perfusion of islets for 3 h with palmitate causes impaired insulin release in response to glucose (32). This may also be compatible with the loss of insulin responsiveness to glucose seen during starvation, a condition that leads to increased levels of FFA (33). How long term increases in FFA impair glucose-induced insulin secretion is not known. It may be that LC-CoA inhibits the action of glucose by preventing the closure or promoting the opening of $K_{ATP}$ channels as we have shown in this study.

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J. Biol. Chem. 1996, 271:10623-10626. doi: 10.1074/jbc.271.18.10623

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