Long-Chain Acyl–Coenzyme A Esters and Fatty Acids Directly Link Metabolism to K\textsubscript{ATP} Channels in the Heart

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Abstract—ATP-sensitive K (K\textsubscript{ATP}) channels are inhibited by cytosolic ATP, a defining property that implicitly links these channels to cellular metabolism. Here we report a direct link between fatty acid metabolism and K\textsubscript{ATP} channels in cardiac muscle cells. Long-chain (LC) acyl–coenzyme A (CoA) esters are synthesized from fatty acids and serve as the principal metabolic substrates of the heart. We have studied the effects of LC acyl-CoA esters and LC fatty acids on K\textsubscript{ATP} channels of isolated guinea pig ventricular myocytes and compared them with the effects of phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2}). Application of oleoyl-CoA (0.2 or 1 μmol/L), a naturally occurring acyl-CoA ester, to the cytosolic side of excised patches completely prevented rundown of K\textsubscript{ATP} channels, but not of Kir2 channels. The open probability of K\textsubscript{ATP} channels measured in the presence of oleoyl-CoA or PIP\textsubscript{2} was voltage dependent, increasing with depolarization. Oleoyl-CoA greatly reduced the ATP sensitivity of K\textsubscript{ATP} channels. At a concentration of 2 μmol/L, oleoyl-CoA increased the half-maximal inhibitory concentration of ATP >200-fold. The time course of the decrease in ATP sensitivity was much faster during application of oleoyl-CoA than during application of PIP\textsubscript{2}. The effects of PIP\textsubscript{2}, but not of oleoyl-CoA, were inhibited by increasing Ca\textsuperscript{2+} to 1 mmol/L. Oleate (C\textsubscript{18:1}; 10 μmol/L), the precursor of oleoyl-CoA, inhibited K\textsubscript{ATP} channels activated by oleoyl-CoA. Palmitoleoyl-CoA and palmitoleate (C\textsubscript{16:1}) exerted similar reciprocal effects. These findings indicate that LC fatty acids and their CoA-linked derivatives may be key physiological modulators of K\textsubscript{ATP} channel activity in the heart. (Circ Res. 2001;88:918-924.)

Key Words: free fatty acids ■ acyl-CoA esters ■ PIP\textsubscript{2} ■ K\textsubscript{ATP} channels
sequently metabolized via β-oxidation. Any impairment of oxidative phosphorylation, for example during cardiac ischemia, is likely to affect the cytosolic concentrations of free fatty acids and the corresponding acyl-CoA esters and thus may modulate the activity of K_ATP channels in cardiac muscle cells.

Materials and Methods

Ventricular myocytes were isolated from guinea pig hearts using standard enzymatic techniques. For single-channel recording, high-resistance pipettes (16 to 20 MΩ) were used, and only patches containing one K_ATP channel were analyzed. The pipette solution contained (in mmol/L) KCl 145, CaCl2 1, MgCl2 1, and HEPES 5 (pH 7.4). The “intracellular” solution contained (in mmol/L) KCl 150, EGTA 10, and HEPES 10 (pH 7.2). Single-channel currents were recorded using an Axon Instruments 200B amplifier and pClamp8 software (sampling rate, 10 kHz; filter, −3 dB at 2 kHz). Stock solutions of LC acyl-CoA esters (1 mmol/L in water), fatty acids (40 mmol/L in DMSO), and PIP2 (1 mmol/L in water) were stored at −80°C. Diluted solutions of LC fatty acids and PIP2 were sonicated on ice for 15 minutes immediately before experiments.

For whole-cell recording, pipette resistances were 2 to 4 MΩ. The pipette solution contained (in mmol/L) KCl 50, potassium glutamate 65, MgCl2 7.9, KH2PO4 10, EDTA 5, HEPES 5, K2-ATP 1.9, and Na3-GTP 0.2 (pH 7.2). The extracellular bath solution contained (in mmol/L) NaCl 140, KCl 5.4, CaCl2 1, MgCl2 1, NaH2PO4 0.33, glucose 10, and HEPES 5 (pH 7.4). Oleate was solubilized using methyl-β-cyclodextrin (Sigma) at a 1:6 molar ratio. Control experiments were carried out using fatty acid–free methyl-β-cyclodextrin (Sigma). All experiments were performed at room temperature (20°C to 23°C). When appropriate, data are reported as mean±SEM.

Results

Effects of Oleoyl-CoA on Rundown of Cardiac K_ATP Channels

K_ATP channels in cardiomyocytes isolated from guinea pig heart were studied using the patch-clamp technique. Inside-out membrane patches were excised from the sarcolemma, and oleoyl-CoA was applied to the intracellular face of the membrane. High-resistance pipettes were used to obtain patches containing only one K_ATP channel. A typical recording of K_ATP channel activity after isolating a membrane patch into divalent-free solution containing 150 mmol/L K⁺ is shown in Figure 1A. In this example, the open probability (P_o) immediately after excision was 0.87 and then progressively decreased to 0.035 within 34 minutes, a recognized phenomenon termed rundown. Superfusion of the intracellular side of the patch with solution containing 1 μmol/L oleoyl-CoA evoked rapid and complete recovery of channel activity (Figure 1A). To assess whether oleoyl-CoA could prevent channel rundown, we excised patches directly into solutions containing oleoyl-CoA. With 0.2 μmol/L oleoyl-CoA at the intracellular face of the patch, a constant and sustained level of channel activation (P_o, 0.85 to 0.89 at −60 mV) was observed for 30 minutes (Figure 1B, ▽). In one patch, no rundown was observed for 90 minutes during application of 1 μmol/L oleoyl-CoA. In accord with earlier work,11–14 we found that PIP2 (5 μmol/L) also prevented rundown (n=8; Figure 1B, △).

When K_ATP channels were allowed to partially rundown, we were able to fully restore channel activity by introducing either oleoyl-CoA (n=7) or PIP2 (n=6). However, once the K_ATP channels had completely run down (P_o=0 for at least 5 minutes), they could not be reactivated by either oleoyl-CoA (n=5) or PIP2 (n=6). The mechanism of this apparently irreversible rundown is unknown. It may involve degradation of cytoskeleton elements17 that are known to be included in the inside-out patch.18

Although both oleoyl-CoA and PIP2 rapidly activated K_ATP channels after partial rundown, their rate of washout was very slow. After a 15-minute period of exposure to either of these molecules, almost no rundown of K_ATP channels was observed during 30 minutes of washout with divalent-free solution (Figure 1B, arrow). Without prior exposure to oleoyl-CoA or PIP2, the time course of the rundown was widely variable from patch to patch (Figure 1B, inset). Considering the persistent effects of endogenous molecules such as oleoyl-CoA and PIP2, the scatter in the time course of the rundown
probably reflects variation in membrane-associated constituents of the particular excised patch.

Effects of Oleoyl-CoA on the Kinetics of $K_{\text{ATP}}$ Channels

The ability of oleoyl-CoA to prevent rundown facilitated detailed investigation of single-channel kinetics. In the presence of oleoyl-CoA, the $P_o$ of $K_{\text{ATP}}$ channels increased with depolarization (Figures 2A and 2B, □), the maximum of $P_o$ was reached at $-20$ mV. Both open time distribution and closed time distribution could be fitted with a single exponential (Figure 2C). The mean open time ($\bullet$) increased with depolarization; the mean closed time (■) decreased with depolarization (Figure 2D). The relation between $P_o$ and membrane potential measured during application of $5 \, \mu\text{mol/L} \, \text{PIP}_2$ (△) was virtually identical to that found during application of oleoyl-CoA (Figure 2B). Having established the relation between $P_o$ and membrane potential under conditions in which rundown was prevented (by oleoyl-CoA or PIP2), we tried to determine this relation in the first 2 minutes after excision of the patch in the absence of oleoyl-CoA (before rundown). Mean open time (Figure 2D, ○), mean closed time (Figure 2D, □), and $P_o$ (Figure 2B, ■) observed under these conditions were similar to the values observed in the presence of oleoyl-CoA. The additional long closed state described previously19–21 was not found in the first 2 minutes after excision of the patch (n = 9) but was clearly identifiable after partial rundown (Figure 1A). The conductance of $K_{\text{ATP}}$ channels determined by linear regression of single-channel currents measured between $-120$ and $-40$ mV (Figure 2E) was the same in the control group ($85.3 \pm 1.1 \, \text{pS}; \, n = 14$) and with $1 \, \mu\text{mol/L} \, \text{oleoyl-CoA} (85.7 \pm 0.94 \, \text{pS}; \, n = 21)$ or with $5 \, \mu\text{mol/L} \, \text{PIP}_2 (87.2 \pm 1.1 \, \text{pS}; \, n = 17)$.

Effects of Oleoyl-CoA on ATP Sensitivity

In the presence of $1 \, \mu\text{mol/L} \, \text{oleoyl-CoA}$, higher concentrations of ATP were required to inhibit $K_{\text{ATP}}$ channels (Figure 3A). ATP sensitivity reached a steady state within a few minutes after excision of the patch in solutions containing $0.2$, $1$, or $2 \, \text{mmol/L} \, \text{oleoyl-CoA}$. No further change in $P_o$ was observed after repeated application and washout of ATP (Figure 3B).

Figure 4A illustrates the ATP dependence in the absence of oleoyl-CoA determined immediately after excision of the patch. In the first 2 minutes after excision, the half-maximal inhibitory concentration ($IC_{50}$) for ATP was $0.015 \, \text{mmol/L}$ (Figure 4C), consistent with the $0.01$ to $0.1 \, \text{mmol/L}$ range reported in the literature.2–4 When oleoyl-CoA was present at a concentration of $0.2 \, \mu\text{mol/L}$, estimated to be the upper physiological free concentration of acyl-CoA esters,22 the $IC_{50}$ was shifted from $15$ to $244 \, \mu\text{mol/L}$ ATP (Figure 4C). Further increase of oleoyl-CoA concentration to $1$ and $2 \, \mu\text{mol/L}$ produced, respectively, $>60$- and $200$-fold increases in $IC_{50}$. Hence, at least in the native cardiac myocyte, oleoyl-CoA is capable of profoundly reducing the ATP sensitivity of sarcolemmal $K_{\text{ATP}}$ channels.

Because $K_{\text{ATP}}$ channels have been reported to become insensitive to sulfonylurea drugs in the presence of PIP2,23 we also tested the effects of glibenclamide ($10 \, \mu\text{mol/L}$). We found that application of oleoyl-CoA ($1 \, \mu\text{mol/L}$) rendered $K_{\text{ATP}}$ channels insensitive to the $K_{\text{ATP}}$ channel blocker glibenclamide ($n = 3$, not illustrated).

Differences Between the Effects of Oleoyl-CoA and PIP2

The time course of the equilibration of oleoyl-CoA with the membrane was studied using the protocol illustrated in Figure 5A. One minute after excision of the patch, ATP ($1 \, \text{mmol/L}$) and oleoyl-CoA ($1 \, \mu\text{mol/L}$) were applied and mean $P_o$ was recorded in 1-minute intervals (●, n = 5). The $P_o$ stabilized at values of $\approx 0.5$ within 3 minutes after addition of oleoyl-CoA.
The effects of oleoyl-CoA on the $P_o$ of cardiac $K_{ATP}$ channels were independent of divergent cations, as illustrated in Figure 5B. After activation by oleoyl-CoA, the $P_o$ of $K_{ATP}$ channels was unaffected by application of 1 mmol/L Ca$^{2+}$ (10 minutes observation; $n=7$). Application of 1 mmol/L Ca$^{2+}$ plus 1 mmol/L Mg$^{2+}$ also had no effect ($n=3$; not illustrated). Even after removal of oleoyl-CoA from the superfusate, when the $K_{ATP}$ channels still had a very high $P_o$ on application of Ca$^{2+}$ did not decrease $P_o$ ($n=4$; not illustrated). In contrast, the stimulatory effect of PIP$_2$ was completely blocked by 1 mmol/L Ca$^{2+}$ within 2 to 4 minutes ($n=4$; Figure 5C). Removal of Ca$^{2+}$ in the continued presence of PIP$_2$ restored channel activity within 20 seconds ($n=4$).

Oleoyl-CoA did not affect cardiac inward-rectifier channels, which are most likely members of the Kir2 subfamily. When oleoyl-CoA was applied to a patch containing a single $K_{ATP}$ channel together with a single Kir channel, activity of the latter channel ceased within 1 to 5 minutes, whereas the activity of the $K_{ATP}$ channel persisted ($n=11$). In contrast, 5 mmol/L PIP$_2$ prevented rundown of both inward-rectifier and $K_{ATP}$ channels ($n=5$).

**Effects of Free Fatty Acids on Cardiac $K_{ATP}$ Channels**

Under physiological conditions, oleoyl-CoA is synthesized from oleate. We therefore tested whether this fatty acid affected channel function. $K_{ATP}$ channels activated by 1 mmol/L oleoyl-CoA were blocked by intracellular application of 10 mmol/L oleate within 30 seconds (Figure 6A; $n=13$). The trans-isoform of oleate, elaidate (10 to 20 mmol/L), exerted no inhibitory effect ($n=4$; not shown). When a patch contained both a single $K_{ATP}$ and a single Kir channel (of which the rundown was prevented by PIP$_2$), oleate selectively blocked the $K_{ATP}$ channel ($n=4$). Once complete oleate-induced block was attained, reversal of inhibition was not observed during a 10- to 20-minute washout period. The channel block also could not be reversed by application of 5 mmol/L PIP$_2$, in addition to 1 mmol/L oleoyl-CoA. The poor reversibility of oleate was probably secondary to its slow rate of washout from the patch lipid bilayer.

Modulation of the cardiac $K_{ATP}$ channel is not restricted to oleoyl-CoA and oleate. We found that 1 mmol/L palmitoleoyl-CoA (C16:1 acyl group) also activated single $K_{ATP}$ channels after partial rundown and reduced ATP sensitivity ($n=4$; not shown). In another series of experiments, we compared the inhibitory effects of oleate (C18:1) with those of palmitoleate (C16:1). Palmitoleate (10 to 20 mmol/L) was less potent than oleate at inhibiting acyl-CoA ester-activated channels. At a concentration of 20 mmol/L, palmitoleate initially induced partial channel block, which converted to full block after 4 to 8 minutes of exposure ($n=4$; not shown).
Figure 5. A, Time course of effects of oleoyl-CoA and PIP$_2$. Mean $P_o$ of 1-minute intervals is plotted against time after excision of the patch. The patch was always excised in control intracellular solution (without ATP) to ascertain that it contained only 1 functional K$_{ATP}$ channel. After 1 minute, 1 mmol/L ATP plus 1 μmol/L oleoyl-CoA (●) or 1 mmol/L ATP plus 2 μmol/L PIP$_2$ (▲) was applied. In control experiments (n=3), only ATP (1 mmol/L) was applied 1 minute after excision of the patch (◼). B, Typical recording illustrating the effects of Ca$^{2+}$ on $P_o$ of K$_{ATP}$ channels activated by oleoyl-CoA (1 μmol/L). Upper trace was recorded 5 minutes after excision of patch in Ca$^{2+}$-free solution containing 1 μmol/L oleoyl-CoA; lower trace was recorded after patch had been exposed to 1 mmol/L Ca$^{2+}$ for 5 minutes. Even after 10 or 15 minutes, no change in $P_o$ was induced by 1 mmol/L Ca$^{2+}$. C, Typical recording illustrating effects of Ca$^{2+}$ on $P_o$ of K$_{ATP}$ channels activated by PIP$_2$ (5 μmol/L). First trace, 15 minutes after excision of the patch in Ca$^{2+}$-free solution containing 5 μmol/L PIP$_2$. Second trace, 1 minute after addition of 1 mmol/L Ca$^{2+}$. Third trace, 2.5 minutes after addition of 1 mmol/L Ca$^{2+}$. Fourth trace, Immediately after removal of Ca$^{2+}$.

Given the potent effects of oleoyl-CoA and oleate on K$_{ATP}$ activity in inside-out patches, we also expected to see an effect in whole-cell recordings. After failing to activate K$_{ATP}$ channels in whole-cell configuration with oleoyl-CoA in the pipette solution, we superfused the cell with a water-soluble form of oleate. Application of 400 μmol/L oleate, solubilized with methyl-β-cyclodextrin, to 10 minutes had no effect on whole-cell currents (Figure 6B). However, when oleate was washed out, the current-voltage relation changed in a way typical for activation of K$_{ATP}$ channels (Figure 6B), an effect that we refer to as the “washout phenomenon.” The outward current at 0 mV increased from 78 ± 35 to 1688 ± 287 pA (n = 13). The delay to the maximal outward current was in the range 1.5 to 14 minutes; the median was 5.5 minutes. Because in most of the experiments the outward current continued to rise until the cell was damaged (unless oleate was reapplied), we do not know how long the increased outward current can be maintained.

Reintroduction of oleate abolished the increase in outward current and brought the current-voltage relation back to control (n = 3). The washout phenomenon was not observed when methyl-β-cyclodextrin alone was introduced and subsequently washed out (n = 6; not shown). The most likely explanation for these findings is that oleate diffused into the cytosol of the cardiomyocytes and served as substrate for acyl-CoA synthetase (Figure 7). On washout, the concentration gradient for oleate was reversed and it diffused out of the cell, allowing membrane-impermeable oleoyl-CoA synthesized in the cell to activate K$_{ATP}$ channels by decreasing their ATP sensitivity.

Discussion

Our findings show that the coupling between K$_{ATP}$ channels and energy metabolism in the heart may be more complex than previously realized. The LC acyl-CoA esters oleoyl-CoA and palmitoleoyl-CoA shift the relation between ATP concentration and $P_o$ of K$_{ATP}$ channels to higher ATP concentrations. This brings the half-maximal inhibitory concentrations of ATP closer to the physiological range and would facilitate the opening of K$_{ATP}$ channels during periods of metabolic impairment, for example, under conditions of myocardial ischemia or hypoxia.

Recently, LC acyl-CoA esters have been shown to activate native K$_{ATP}$ channels in inside-out patches excised from pancreatic β cells. Pancreatic and cardiac K$_{ATP}$ channels have the same α subunits (Kir6.2) but have different β subunits: SUR1 in pancreatic β cells and SUR2A in cardiomyocytes. The shift in the ATP-$P_o$ relation in cardiomyocytes reported here (Figure 4C) is dramatically greater than the 2- to 3-fold increase in IC$_{50}$ induced by 1 μmol/L oleoyl-CoA in pancreatic K$_{ATP}$ channels (Kir6.2/SUR1) expressed in Xeno-pus oocytes. Because truncated Kir6.2 channels (Kir6.2C36) were activated by oleoyl-CoA to the same extent as pancreatic K$_{ATP}$ channels, it was concluded that the Kir6.2 subunit was the site of action of oleoyl-CoA in pancreas. The much more dramatic action of oleoyl-CoA in native cardiomyocytes suggests that the β subunit SUR2A (but not SUR1), or some other membrane-associated regulatory protein, may also contribute to the effects of oleoyl-CoA on cardiac K$_{ATP}$ channels.

We also found that LC acyl-CoA esters prevented rundown (Figure 1B) and reactivated K$_{ATP}$ channels after partial rundown (Figure 1A) after excision of the patches. As long as micromolar concentrations of oleoyl-CoA were present, K$_{ATP}$ channels exhibited sustained activity (not interrupted by long closures) in the absence of ATP. Under these conditions, the closed-time distribution could be fitted by a single exponential, and the channels were insensitive to glibenclamide. These findings are consistent with the idea that oleoyl-CoA may induce a shift of the conformation of K$_{ATP}$ toward a ligand-insensitive state, similar to the effects of nucleoside diphosphates. Furthermore, our analysis of the kinetics of cardiac K$_{ATP}$ channels has revealed a pronounced voltage dependence of the $P_o$ observed immediately after.
excision of the patch (before rundown) and in the presence of oleoyl-CoA or PIP_2.

The effects of acyl-CoA esters and PIP_2 on K_{ATP} channels share some interesting properties. (1) They have no effect on P_o before rundown. (2) They nonetheless shift the ATP sensitivity under these conditions, suggesting a change in ATP binding affinity. (3) They prevent inhibition of K_{ATP} channels by sulfonylureas. On the other hand, the effects of acyl-CoA esters and PIP_2 differ in several important aspects. (1) The effects of oleoyl-CoA are not affected by changes in [Ca^{2+}]_i (Figure 5), in contrast to the effects of PIP_2. (2) The decrease in ATP sensitivity induced by oleoyl-CoA is relatively rapid (Figure 5), whereas PIP_2 equilibrates with K_{ATP} channels with a much slower time course. (3) The effects of oleoyl-CoA appear to be restricted to K_{ATP} channels, given that oleoyl-CoA did not prevent rundown of cardiac inward-rectifier channels; in contrast, PIP_2 activates various members of the inward-rectifier channel family and prevents their rundown. (4) It has been suggested that the effects of PIP_2 and other phosphatidylinositol phosphates on K_{ATP} channels are related to the number of negative charges of the head group. Such an electrostatic mechanism cannot fully explain the effect of oleoyl-CoA on ATP sensitivity. This is consistent with the observation that the effect was not antagonized by divalent cations, which would be expected to shield the negative charges. Taken together, these data suggest that LC acyl-CoA esters and PIP_2 modulate K_{ATP} channels via different mechanisms. Furthermore, because PIP_2 is mainly liberated via second-messenger cascades, whereas the cytosolic concentrations of LC acyl-CoA esters depend on cellular energy metabolism, the function of the two ion channel modulators in cardiac muscle cells may also be quite different.

During restriction of blood flow through the coronary arteries (myocardial ischemia), \(\beta\)-oxidation of fatty acids is inhibited and accumulation of fatty acids and their metabolic intermediates readily occurs. The mechanisms underlying the redistribution of free fatty acids between different cellular pools are incompletely understood and strongly depend on the experimental conditions. During low-flow ischemia, the total cellular concentration of acyl-CoA esters in cardiac muscle rises within 5 minutes. During this time, action potential duration decreases, K^+ efflux rises, and contractile function is disturbed without any concomitant change in bulk ATP concentration. It appears possible that the increase in acyl-CoA esters may contribute to the opening of K_{ATP} channels during the first 5 minutes of low-flow ischemia. The resulting shortening of the action potential would lead to a reduction in transsarcolemmal Ca^{2+} influx and to a decrease in both contractility and energy expenditure of cardiac muscle cells. Thus, the modulation of K_{ATP} channels by LC acyl-CoA esters may provide a mechanism to counterbalance energy supply and energy expenditure of the heart during the initial phase of low-flow ischemia.

We have found that K_{ATP} channels in guinea pig cardiomyocytes can be inhibited by the unsaturated fatty acids oleate and palmitoleate. These observations are consistent with a previous report describing inhibition of K_{ATP} channels in rat cardiomyocytes by the unsaturated fatty acids arachidonic, linoleic, and eicosatrienoic acid. Free fatty acids
also accumulate during cardiac ischemia, but with a much slower time course than acyl-CoA esters.\textsuperscript{30,31,34} Interestingly, action potential duration also shows a spontaneous recovery after prolonged ischemia,\textsuperscript{32,35} which might be related to changes in the electrical activity of the heart by modulating the activity of ATP-sensitive potassium channels. In conclusion, our results suggest that the principal metabolic substrates of the heart, acyl-CoA esters and free fatty acids, may link changes in myocardial energy metabolism to changes in the cardiovascular system and K\textsubscript{ATP} channels.

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