Long Chain Coenzyme A Esters Activate the Pore-forming Subunit (Kir6.2) of the ATP-regulated Potassium Channel*

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The ATP-dependent potassium (K\textsubscript{ATP}) channel in the pancreatic β-cell is a complex of two proteins, the pore-forming Kir6.2 and the sulfonylurea receptor type 1 (SUR1). Both subunits are required for functional K\textsubscript{ATP} channels because expression of Kir6.2 alone does not result in measurable currents. However, truncation of the last 26 or 36 amino acids of the C terminus of Kir6.2 enables functional expression of the pore-forming protein in the absence of SUR1. Thus, by using the truncated form of Kir6.2, expressed in the absence and presence of SUR1, it has been shown that the site at which ATP mediates channel inhibition is likely to be situated on Kir6.2. We have now examined the effects of long chain acyl-CoA (LC-CoA) esters on the C-terminally truncated mouse Kir2.3Δ365–390 (Kir6.2ΔC26) in inside-out patches isolated from Xenopus laevis oocytes. LC-CoA esters, saturated (C14:0, C16:0) and unsaturated (C18:1), increased Kir6.2ΔC26 currents, whereas short and medium chain CoA esters (C5:0, C8:0, C12:0) were unable to affect channel activity. The LC-CoA esters were also able to counteract the blocking effect of ATP on Kir6.2ΔC26. The stimulatory effect of the esters could be explained by the induction of a prolonged open state of Kir6.2ΔC26. In the presence of the esters, channel open time was increased approximately 3-fold, which is identical to what was obtained in the native mouse K\textsubscript{ATP} channel. Coexpression of SUR1 together with Kir6.2ΔC26 did not further increase the ability of LC-CoA esters to stimulate channel activity. We conclude that Kir6.2 is the primary target for LC-CoA esters to activate the K\textsubscript{ATP} channel and that the esters are likely to induce a conformational change by a direct interference with the pore-forming subunit, leading to openings of long duration.

The ATP-sensitive potassium channel (K\textsubscript{ATP}) channel couples glucose metabolism to electrical activity and insulin secretion in the pancreatic β-cell. The metabolic regulation of the channel is thought to be mediated by changes in intracellular concentrations of ATP and ADP, which inhibit and stimulate channel activity, respectively (1). Thus, stimulation of the β-cell with glucose leads to an increased ATP/ADP ratio resulting in inhibition of K\textsubscript{ATP} channel activity, an effect which induces depolarization and activation of voltage-dependent Ca\textsuperscript{2+} channels, thereby triggering insulin secretion. This implies that agents capable of modulating the activity of the channel may also affect fuel-induced insulin secretion. Recently, we have discovered a new group of substances acting as K\textsubscript{ATP} channel openers (2). It consists of long chain acyl-CoA (LC-CoA) esters that are endogenous substances found in many cells, including the pancreatic β-cell. The LC-CoA esters are very potent activators of the β-cell K\textsubscript{ATP} channel, inducing a severalfold increase in channel activity (2). Both saturated and unsaturated LC-CoA esters induce a rapid and reversible action on the K\textsubscript{ATP} channel, whereas no stimulatory effect could be obtained with short chain CoA esters. We could not obtain an increased channel activity by administration of the fatty acid alone, nor following addition of the CoA-SH moiety. We have also shown that the LC-CoA esters are able to counteract the blocking effect of ATP. Thus, at a concentration of ATP that fully blocks channel activity, addition of LC-CoA ester reverses the nucleotide-induced inhibition (2, 3). The effects of the esters also appear to be specific for the K\textsubscript{ATP} channel, in that at least two other potassium channels are unaffected by the esters, the large-conductance K\textsuperscript{+} channel and the 8 pS K\textsuperscript{+} channel described in the β-cell (2). This indicates that the K\textsubscript{ATP} channel is a sensitive target for LC-CoA esters, and because of their ability to reverse the blocking effect of ATP, the esters may counteract glucose-induced depolarization of the β-cell membrane potential.

The β-cell K\textsubscript{ATP} channel is a complex of two proteins, the pore-forming protein Kir6.2 and the sulfonylurea receptor SUR1 (4). The SUR1 acts as a regulator of K\textsubscript{ATP} channel activity, conferring sensitivity to sulfonylureas, diazoxide, and ADP (5). Kir6.2 does not appear to express functional K\textsubscript{ATP} currents alone. However, the C-terminally truncated form of Kir6.2, in which the last 26 or 36 amino acids have been deleted, forms functional channels (6). By using the truncated form of Kir6.2, it was recently shown that the primary site at which ATP acts to mediate K\textsubscript{ATP} channel inhibition is located on Kir6.2 (6). In the present study, we have examined the effects of LC-CoA esters on the C-terminally truncated mouse Kir6.2ΔC26 (Kir6.2ΔC26). We show that LC-CoA esters activate the K\textsubscript{ATP} channel by interacting at a binding site situated on the pore-forming subunit Kir6.2. The stimulatory effect was achieved by saturated and unsaturated LC-CoA esters and characterized by openings of long duration.

MATERIALS AND METHODS

Animals and Preparation of Cells—Electrophysiological studies on the native K\textsubscript{ATP} channel were performed on excised patches from β-cells...
isolated from adult obese mice (ob/ob). The animals were obtained from a local colony (7) and were fasted for 24-h before decapitation. Islets were isolated by a collagenase technique (8). Collagenase was obtained from Boehringer Mannheim (Mannheim, Germany). A cell suspension was prepared as described earlier (9). The cells were maintained for 1–3 days at 37 °C in DMEM–1640 culture medium (Flow Laboratories, Irvine, UK), containing 11 mM glucose, supplemented with 10% fetal bovine calf serum and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin, and 60 μg/ml gentamycin). The cells were seeded into Petri dishes (Nunc, Roshilde, Denmark) and incubated at 37 °C and 5% CO₂.

For collection of oocytes, extra large female Xenopus laevis were anaesthetized with 3-aminobenzoic acid methyl ester (1.5 gl; gl laboratory water, Sigma). Oocytes were removed from one ovary by laparotomy, the incision was sutured, and the animal was allowed to recover. Oocytes, stage V–VI, were defolliculated using collagenase A and injected using an Eppendorf transjector (Eppendorf, Hamburg, Germany) with 0.5–5 ng of mRNA/50 nl of sterile RNase-free water, encoding Kir6.2C26 or Kir6.2C26 together with SUR1. Control oocytes were injected with 50 nl of sterile water alone. Oocytes were maintained in culture at a temperature of 19 °C, and experiments were performed 2–5 days after injection.

**Molecular Biology—** The cDNAs of mouse Kir6.2 (GenBank accession number D50581) and hamster SUR1 (GenBank accession number L40623) were subcloned into pBlueScript II SK (Stratagene, La Jolla, CA), creating pBM.Kir6.2 and pBSUR1, respectively. Plasmid pBM.Kir6.2Δ365–390 was generated by introducing a stop codon (R365Stop) into pBM.Kir6.2 by site-directed mutagenesis using the QuikChange Mutagenesis kit (Stratagene). Capped mRNA was synthesized by in vitro transcription from linearized plasmids employing the mMESSAGE mMACHINE kit (Ambion, Austin, TX). The purified mRNA was dissolved in 10 mM Tris-HCl (pH 7.4) and stored in aliquots at −80 °C until use.

**Electrophysiology—** The standard extracellular solution contained (in mM) 138 NaCl, 5.6 KCl, 1.2 MgCl₂, 2.6 CaCl₂, and 5 HEPES-NaOH at pH 7.4. The intracellular solution (i.e. the bath solution) consisted of (in mM) 125 KCl, 1 MgCl₂, 10 EGTA, 25 KOH, and 5 HEPES-KOH at pH 7.4. Recordings of channel activity in symmetrical K⁺ solutions were done with a pipette solution containing (in mM) 140 KCl, 1.2 MgCl₂, 2.6 CaCl₂, 5 HEPES-KOH at pH 7.4, and an internal (bath) solution consisting of (in mM) 110 KCl, 1 MgCl₂, 30 KOH, 10 EGTA, 5 HEPES-KOH at pH 7.15. ATP was added as the Mg²⁺ salt to the “intracellular” solution as indicated in text and figures. ADP was added as Na⁺ salt, and Mg²⁺ was added to maintain excess of Mg²⁺. cis-9-Monounsaturated oleoyl-CoA (C18:1), palmitoyl-CoA (C16:0), myristoyl-CoA (C14:0), lauroyl-CoA (C12:0), octanoyl-CoA (C8:0), and malonyl-CoA (C3:0) were prepared as stock solutions in deionized water (Millipore) and then added to the intracellular solution at the final concentrations indicated in the figures. Nucleotides and CoA esters were from Sigma.

All reagents were of analytical grade. Excised inside-out patches from transfected oocytes were used to study the effects of the CoA esters on Kir6.2C26 activity. This type of recording mode allows free access to the cytoplasmic side of the plasma membrane, making it easy to vary the intracellular composition. Pipettes were pulled from alumina or borosilicate glass (Hilgenberg, Malsfeld, Germany). Filled with standard extracellular solution, the electrodes had resistances between 1–5 mΩ meqohm. Recordings were made using an Axon patch-clamp amplifier (Axopatch 200, Axon Instrument, CA). During the experiment, the current signal was stored on magnetic tape using a VCR (Sony-200, Sony, Tokyo, Japan). Channel records are displayed and stored according to the convention that upward deflections denote outward currents and vice versa. The experiments were carried out at 20–22 °C.

**Data Analysis—** Records were filtered at 1 kHz (−3 dB value, 8 pole Bessel filter, Frequency Devices, Haverhill, MA) digitized at 5 kHz using an Axon instrument analogue digital converter (TL-1), and stored in a computer. A filter frequency of 1 kHz, used for single channel analysis, appeared optimal because an increased filter setting (5 kHz) did not significantly affect mean open time (data not shown). Digitized segments of current records (30 s) were also used to determine channel activity using in-house software. The mean current (iᵢ) was calculated according to the following equation,

\[ iₓ = \sum \frac{N}{N} \left( Iₓ - Iₓ/I_N \right) \quad (Eq. 1) \]

where \( N \) is the number of samples, \( Iₓ \) is the current registered in sample \( x \) and \( Iₓ \) is the value of a user-defined baseline (10). The analysis of channel open time was restricted to segments of the experimental records containing a maximum of three active channels. Using the method of maximum likelihood (11), the kinetic constants were derived by approximation of the data to exponential functions. Data are presented as means ± S.E. Effects on channel activity were compared using Student’s t-test or ANOVA for multiple groups, and p values less than 0.05 were considered significant.

**RESULTS AND DISCUSSION**

LC-CoA esters activate the KATP channel in a similar way as Mg-ADP (3, 10). Thus, the ability to counteract the blocking effect of ATP, prevent channel rundown, and increase channel open time without affecting single channel conductance are characteristics shared by the two compounds (2, 3). The stimulatory action of Mg-ADP has been shown to be conferred to the nucleotide-binding folds of SUR1 (12) because mutations in these domains fully abolish the effect of Mg-ADP in cells coexpressing Kir6.2 and SUR1 (13). Accordingly, in cells expressing the truncated Kir6.2, in the absence of SUR1, no stimulatory effect of ADP is obtained (6).

**LC-CoA Esters Activate Kir6.2C26 Currents—** As seen in Fig. 1A, 1 μM palmitoyl-CoA increased native KATP channel activity. While our earlier studies on the native KATP channel have shown that LC-CoA interacts with the channel complex at a binding site separate from that of ADP (3), we tested whether the stimulatory action of the esters was mediated by an effect on Kir6.2C26. Interestingly, upon addition of 1 μM palmitoyl-CoA, a significant increase in Kir6.2C26 channel activity was observed (Fig. 1B). The presence of the LC-CoA ester induced an approximately 3.5-fold increase in mean Kir6.2C26 (p < 0.05; n = 9). In oocytes coexpressing Kir6.2C26 and SUR1, a similar extent of increased channel activity was obtained upon addition of 1 μM palmitoyl-CoA (Fig. 1C). This strongly indicates that the site where LC-CoA esters bind to exert their stimulatory action on KATP channel activity is conferred to Kir6.2. The stimulatory effect was relatively slow in onset, corresponding to what was seen in the native KATP channel and in oocytes coexpressing SUR1 and Kir6.2C26. In oocytes injected with 50 nl H₂O, no effect of LC-CoA esters on channel activity was obtained (Fig. 1D), showing that the stimulatory effect of the esters cannot be explained by activation of endogenous channels present in the oocyte.

We have previously reported that the stimulatory effect of LC-CoA esters is dependent on chain length (2). In accordance with these findings, the effects of the esters on Kir6.2C26 channel activity were also chain-length-dependent. Thus, whereas addition of oleoyl-CoA induced a significant increase in channel activity (Fig. 2A), no effect of malonyl-CoA (C3:0), octanoyl-CoA (C8:0), or lauroyl-CoA (C12:0) ester on Kir6.2C26 currents could be obtained (Fig. 2B–D). Thus, ATP blocked Kir6.2C26 currents, whereas both saturated (palmitoyl-CoA; C16:0), myristoyl-CoA; C14:0), and unsaturated (oleoyl-CoA; C18:1) LC-CoA esters stimulated the activity of Kir6.2C26 (Fig. 2E). Taken together, these actions of the different acyl-CoA esters on Kir6.2C26 currents are virtually identical to what we have reported on KATP channel activity from the mouse pancreatic β-cell (2). In agreement with earlier studies, inclusion of 0.1 mM ADP, known to stimulate native channel activity, did not affect the activity of Kir6.2C26 (Fig. 2E).

Next, we recorded Kir6.2C26 currents from giant inside-out patches excised from Xenopus oocytes in symmetrical concentrations of potassium. We then applied a series of voltage ramps from −80 mV to +80 mV, in the absence and presence of LC-CoA ester. As seen in Fig. 3A, left, application of 1 μM palmitoyl-CoA to the intracellular solution increased Kir6.2C26 currents. The associated current-voltage relationship of the macroscopic currents shows an increase in current at both positive and negative potentials after addition of the
Kir6.2 in excised oocyte membrane patches. Channel activity was studied using symmetrical K\textsubscript{1}Na\textsubscript{3}DC26 mRNA. Addition of 5 mM ATP inhibits channel activity, whereas addition of palmitoyl-CoA induces a four-fold increase, from −10.4 pA to −48.2 pA, in channel activity. C, channel currents from an inside-out patch from an oocyte injected with mRNA encoding Kir6.2C26 and SUR1. Addition of 1 μM palmitoyl-CoA increased mean current from −18.1 pA to −68.9 pA. D, in control oocytes microinjected with 50 nl of water, palmitoyl-CoA did not induce channel activity.

Our data strongly suggest that not only the inhibitory action of ATP is conferred to Kir6.2, but that Kir6.2 also possesses an intrinsic LC-CoA stimulatory site. We therefore examined whether ATP-induced inhibition of Kir6.2C26 currents could be counteracted by applying LC-CoA esters. As shown in Fig. 4A, addition of 1 mM ATP almost fully blocked currents through Kir6.2C26. Addition of 1 μM LC-CoA was able to reverse the ATP-induced block of Kir6.2C26 activity. Thus, following addition of 1 μM LC-CoA esters, Kir6.2C26 currents increased to 350 ± 80% in the continued presence of 1 mM ATP, as compared with the activity observed in the presence of only ATP (n = 4; p < 0.01). These results cannot be explained by ATP losing its inhibitory effect with time because in control experiments, ATP was able to suppress Kir6.2C26 currents for several minutes (Fig. 4B). Compiled data of the stimulatory effect of LC-CoA in
the presence of ATP are shown in Fig. 4C. It is clear that LC-CoA esters are unable to fully neutralize the inhibitory effect of ATP.

Effects of LC-CoA Esters on Kir6.2 Channel Kinetics—We have previously reported that LC-CoA esters induce a distinct open state of the Kir6.2 channel, characterized by openings of long duration (3). To investigate whether this effect is conferred to Kir6.2, we made a detailed study on Kir6.2 open time kinetics for outward currents and compared the obtained results to the kinetics observed in the native Kir6.2 channel. Fig. 5 shows the effects on open time distribution of Kir6.2 and native Kir6.2 currents during control and following exposure of the patches to Mg-ADP and LC-CoA esters, respectively. In control solution, absence of adenine nucleotides and acyl-CoA esters, Kir6.2 channel activity consisted of very short openings (Fig. 5A, inset). The distribution of openings was best
described by a single exponential with a time constant ($t_o$) of 5.1 ms. In comparison, the native K\textsubscript{ATP} channel exhibited slightly longer openings, well fitted by a single exponential function with a time constant ($\tau$) of 17.1 ms for Kir6.2\Delta C26 currents and 17.1 ms for the native K\textsubscript{ATP} channel. The total number of events analyzed were 1540 and 1306, respectively. In the presence of 0.1 mM Mg-ADP, the open time distribution in native K\textsubscript{ATP} channel was best fitted as the sum of two exponentials, with $\tau_1 = 17.7$ and $\tau_2 = 161.2$ ms (27%), respectively. The number of events analyzed was 2644. Following exposure of Kir6.2\Delta C26 to 0.1 mM Mg-ADP, the distribution was best described by a single exponential function with a resulting $\tau$ of 17.1 ms (2053 events). When exposing the Kir6.2\Delta C26 to 1 \mu M LC-CoA esters, the distribution of channel open time was best fitted by a two-exponential function with $\tau_1 = 5.9$ and $\tau_2 = 17.2$ ms. Number of events analyzed was 3121, where 29% belonged to the slow component. Native K\textsubscript{ATP} channel open time distribution, in the presence of 1 \mu M LC-CoA esters, was also best fitted using a two-exponential function with $\tau_1 = 22.5$ and $\tau_2 = 149.5$ ms, respectively. A total number of 2432 events were analyzed, 14.1% belonging to the fast component. In the analysis, bin widths were set to 2 ms for Kir6.2\Delta C26 and 10 ms for native K\textsubscript{ATP} channel. The bar at the far right indicates the sum of events exceeding 50 ms and 200 ms, respectively. Insets show typical channel activity during the different experimental conditions. Arrowheads indicate current level when the channel is closed. Membrane potential was clamped at 0 mV.
and native $K_{ATP}$ channel currents, probably by promoting a conformational change of the channel protein that results in the induction of a long lasting open state.

We further analyzed mean open time in Kir6.2ΔC26 and native channels, respectively (Fig. 6). In the absence of nucleotides and acyl-CoA, mean open time for Kir6.2ΔC26 was estimated to 5.4 ± 0.9 ms ($n = 4$). The corresponding value for the native channel was 22.8 ± 3.1 ms ($n = 5$). Thus, Kir6.2ΔC26 currents exhibited a mean open time approximately four times faster than what we observed in the native channel ($p < 0.01$). No effect of Mg-ADP on Kir6.2ΔC26 mean open time could be observed, whereas the nucleotide increased native $K_{ATP}$ mean open time approximately 3-fold, to 68.0 ± 4.2 ms ($n = 3$; $p < 0.01$), well in agreement with earlier reports (3, 10). As expected from the results obtained on open time kinetics, Kir6.2ΔC26 mean open time increased from 5.4 ± 0.9 ms in the absence to 14.3 ± 2.6 ms ($n = 3$; $p < 0.01$) in the presence of LC-CoA. This relative increase is similar to what we found in native channels, from 22.8 ± 3.1 ms to 61.6 ± 4.5 ms ($n = 3$; $p < 0.01$) (3). Thus, LC-CoA esters induce an approximately 3-fold increase in mean open time in both Kir6.2ΔC26 and native $K_{ATP}$ channel currents.

The molecular background to the faster kinetic properties of Kir6.2ΔC26, as compared with the native $K_{ATP}$ channel, is not clear. In preliminary studies, we found that coexpression of Kir6.2ΔC26 with SUR1 normalizes open time kinetics and thus, that the SUR moiety affects channel gating. Indeed, differences in burst duration and interburst interval observed in the β-cell $K_{ATP}$ (Kir6.2 and SUR1) channel and following combination of Kir6.2 with SUR2, expressed in heart and skeletal muscle, point to the influence of the SUR moiety on channel gating properties. However, single channel conductance, inward rectification, and mean channel open and closed times within bursts are similar in Kir6.2/SUR1 and Kir6.2/SUR2 (15). It is, therefore, reasonable to assume that these properties of ion conductance are determined by the pore-forming subunit. Taken together, we speculate that the difference in kinetics between the truncated Kir6.2 and native $K_{ATP}$ channel activity is caused by the C-terminal truncation of Kir6.2 in combination with the absence of SUR1. It is important to note that the relative increase in channel activity, following addition of LC-CoA ester, is virtually identical in Kir6.2ΔC26 and native channels. This strongly supports the notion that the stimulatory effects of the esters on $K_{ATP}$ channel activity is conferred to Kir6.2.

**Conclusion**—Our earlier studies have shown that LC-CoA esters are potent activators of $K_{ATP}$ channel activity in the mouse pancreatic β-cell and that CoA esters interact at a binding site different from that of ADP. We have earlier proposed that this binding site could be conferred to SUR1 (3), based on the fact that the site of action of all other channel openers at that time were acting at SUR1 and that changes in channel open time kinetics after inclusion of ADP, diazoxide, or LC-CoA esters exhibited a striking similarity. We now show that the esters directly activate Kir6.2ΔC26 and, thus, that the binding site of LC-CoA is conferred to the pore-forming subunit. Our data also show that the stimulatory effect of LC-CoA esters does not involve alterations in inward rectification of Kir6.2ΔC26 currents nor affects single channel conductance. The relative increase in channel activity, induced by LC-CoA, is identical to what was found in the native channel although Kir6.2ΔC26 channel kinetics are significantly faster compared with native mouse $K_{ATP}$ channels. We therefore suggest that the site where LC-CoA esters exert their stimulatory action on $K_{ATP}$ channel activity is located on Kir6.2. Because Kir6.2 is thought to constitute the pore-forming subunit of $K_{ATP}$ channels found in brain, smooth-, cardiac-, and skeletal muscle (16), as well as in pancreatic α- and δ-cells (17), we postulate that LC-CoA esters also activate $K_{ATP}$ channels present in these cells. It is therefore plausible that the stimulatory effect of LC-CoA esters on the inward rectifier Kir6.2 constitutes an important mechanism by which $K_{ATP}$ channel activity is increased in several cell types.

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**REFERENCES**

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