Mechanism of Cloned ATP-sensitive Potassium Channel Activation by Oleoyl-CoA*

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Fiona M. Gribble‡, Peter Proks, Barbara E. Corkey, and Frances M. Ashcroft§

From the University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, United Kingdom and Boston University Medical Center, Boston, Massachusetts 02118

Insulin secretion from pancreatic beta cells is coupled to cell metabolism through closure of ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels, which comprise Kir6.2 and sulfonylurea receptor (SUR1) subunits. Although metabolic regulation of K\(_{\text{ATP}}\) channel activity is believed to be mediated principally by the adenine nucleotides, other metabolic intermediates, including long chain acyl-CoA esters, may also be involved. We recorded macroscopic and single-channel currents from Xenopus oocytes expressing either Kir6.2/SUR1 or Kir6.2ΔC36 (which forms channels in the absence of SUR1). Oleoyl-CoA (1 μM) activated both wild-type Kir6.2/SUR1 and Kir6.2ΔC36 macroscopic currents, ~2-fold, by increasing the number and open probability of Kir6.2/SUR1 and Kir6.2ΔC36 channels. It was ineffective on the related Kir subunit Kir1.1a. Oleoyl-CoA also impaired channel inhibition by ATP, increasing the \( K_i \) values for both Kir6.2/SUR1 and Kir6.2ΔC36 currents by ~3-fold. Our results indicate that activation of K\(_{\text{ATP}}\) channels by oleoyl-CoA results from an interaction with the Kir6.2 subunit, unlike the stimulatory effects of MgADP and diazoxide which are mediated through SUR1. The increased activity and reduced ATP sensitivity of K\(_{\text{ATP}}\) channels by oleoyl-CoA might contribute to the impaired insulin secretion observed in non-insulin-dependent diabetes mellitus.

Potassium channels that are inhibited by ATP (K\(_{\text{ATP}}\) channels) are found in many tissues, where they serve to couple the metabolic state of the cell to its electrical activity (1). In the pancreatic beta cell, for example, they provide the link between changes in blood glucose concentration and insulin secretion. The K\(_{\text{ATP}}\) channel sets the beta cell resting membrane potential, and its closure in response to glucose metabolism elicits membrane depolarization, activation of voltage-gated Ca\(^{2+}\) channels, and a rise in Ca\(^{2+}\) influx that stimulates insulin release (2). In cardiac muscle, and in brain neurons, K\(_{\text{ATP}}\) channels may be involved in the response to ischemia, whereas in smooth muscle they are important for the regulation of vascular tone (3, 4).

K\(_{\text{ATP}}\) channels are formed by the physical association of four inwardly rectifying K\(^+\) channel (Kir6.2) subunits with four sulfonylurea receptor (SUR) subunits (5–7). Kir6.2 serves as an ATP-sensitive pore that is common to many types of K\(_{\text{ATP}}\) channels. SUR is a regulatory subunit that modulates the channel gating properties, enhances the apparent ATP-sensitivity, and acts as the target for sulfonylurea drugs, K-channel openers and intracellular Mg-nucleotides, which modulate K\(_{\text{ATP}}\) channel activity (8–12). It is currently believed that Kir6.2/SUR1 forms the beta cell K\(_{\text{ATP}}\) channel, Kir6.2/SUR2A forms the cardiac K\(_{\text{ATP}}\) channel, and Kir6.2/SUR2B forms the smooth muscle K\(_{\text{ATP}}\) channel (13–17).

It has recently been shown that long chain (LC) acyl-CoA esters are able to activate native K\(_{\text{ATP}}\) channels in inside-out membrane patches excised from pancreatic beta cells (18, 19). The intracellular concentrations of LC acyl-CoA esters are predicted to vary with the metabolic state of the cell. Because long-term exposure to nonesterified fatty acids increases cellular levels of LC acyl-CoA esters in the beta cell (18, 20), this finding may have implications for the regulation of insulin secretion under conditions in which nonesterified fatty acids are increased, as in obese subjects (21, 22). The elevated intracellular LC acyl-CoA ester concentration would be expected to activate K\(_{\text{ATP}}\) channels, thereby hyperpolarizing the beta cell and inhibiting insulin secretion. It is possible, therefore, that increased K\(_{\text{ATP}}\) channel activity, induced by LC acyl-CoA esters, may contribute to the impaired insulin secretion observed in obese non-insulin-dependent diabetics.

The mechanism of beta cell K\(_{\text{ATP}}\) channel activation by oleoyl-CoA differs from that of the classical potassium channel opener diazoxide in several ways. In particular, the effects of oleoyl-CoA do not require Mg\(^{2+}\), nor are they abolished following mild proteolysis of the inner membrane surface (19). This suggests that oleoyl-CoA and diazoxide may act by different mechanisms. In this paper, we show that oleoyl-CoA stimulates the activity of the cloned beta cell K\(_{\text{ATP}}\) channel (Kir6.2/SUR1). We further show this effect is mediated by interaction of the acyl-CoA ester with the Kir6.2 subunit of the channel and that it is associated with a reduced sensitivity to the inhibitory effects of ATP and a decreased rate of channel rundown. Activation by oleoyl-CoA is not observed for a related member of the inward rectifier K-channel family (Kir1.1a).

EXPERIMENTAL PROCEDURES

Molecular Biology—Mouse Kir6.2 (GenBank\(^\text{TM}\) D50581, Refs. 13 and 15), rat Kir1.1a (GenBank\(^\text{TM}\) X72341, Ref. 23; kindly supplied by Dr. S. Hebert, Vanderbilt University), and rat SUR1 (GenBank\(^\text{TM}\) L40624, Ref. 24; kindly supplied by Dr. G. Bell, University of Chicago) were used in this study. A 36-amino acid C-terminal deletion of mouse Kir6.2 (Kir6.2ΔC36) was made by introduction of a stop codon at the appropriate residue using site-directed mutagenesis (12). For oocyte expression studies, Kir6.2 and SUR1 constructs were subcloned into the pX vector that provides the S' and 3' untranslated regions of the Xenopus \( \beta \) globin gene. Synthesis of capped mRNA was carried out using the mMessage mMachine large scale in vitro transcription kit (Ambion, Austin, TX).

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§ To whom correspondence should be addressed. Tel.: 44-1865-272478; Fax: 44-1865-272469; E-mail: frances.ashcroft@physiol.ox.ac.uk.
**RESULTS**

**Effect of Oleoyl-CoA on Wild-type Kir6.2-SUR1 Currents**—Wild-type $K_{ATP}$ currents were measured in giant macropatches from oocytes co-injected with mRNAs encoding Kir6.2 and SUR1. In cell-attached patches, the Kir6.2/SUR1 conductance was very small and similar in magnitude to that of uninjected oocytes. A marked increase in current was observed, however, following excision of the patch into a nucleotide-free solution as reported previously (25). At $-100$ mV, the mean current amplitude in the inside-out patch immediately after patch excision was $-3.7 \pm 1.0$ nA ($n = 11$). Subsequently, the current magnitude slowly declined, but it could be restored following exposure to $1 \mu M$ MgATP (Fig. 1A, panel a). A similar rundown and MgATP-dependent refreshment of channel activity has also been observed for native beta cell $K_{ATP}$ channels following patch excision (26, 27).

Application of $1 \mu M$ oleoyl-CoA to the intracellular surface of excised patches, caused an increase in conductance, which started after a delay of $10-30$ s and reached a peak $1-2$ min later (Fig. 1B, panel a). The mean activation of Kir6.2/SUR1 currents by $1 \mu M$ oleoyl-CoA was $2.4 \pm 0.3$-fold ($n = 10; p = 0.0005$). The response to oleoyl-CoA was apparent at membrane potentials from $-110$ mV to $+100$ mV, demonstrating that the effect is not voltage-dependent. Following removal of the acyl-CoA, the patch conductance remained high for more than $5$ min unless $0.1\%$ BSA was added to the solution, in which case the effect of oleoyl-CoA was rapidly reversed (Fig. 1B, panel b). BSA did not affect Kir6.2/SUR1 currents when applied to the patch prior to activation with oleoyl-CoA.

No effect of $1 \mu M$ oleoyl-CoA was observed in patches excised from uninjected oocytes ($n = 4$). This indicates that the current activated by oleoyl-CoA results from activation of a recombinant $K_{ATP}$ channel rather than a current endogenous to the oocyte.

**Effects of Oleoyl-CoA on Kir6.2ΔC36 Currents**—Previous...
studies have suggested that the mechanism of activation of native beta cell K\textsubscript{ATP} channels by oleoyl-CoA differs from that produced by MgADP or diazoxide; unlike diazoxide and MgADP, oleoyl-CoA is effective in the absence of Mg\textsuperscript{2+} ions or following mild trypsinization of the intracellular membrane surface (19). Channel activation by MgADP or diazoxide is mediated by the SUR1 subunit of the K\textsubscript{ATP} channel complex (8–10). The different behavior of oleoyl-CoA might be explained, therefore, if this compound were to interact with the Kir6.2 subunit.

To investigate this possibility, we studied the effect of oleoyl-CoA on a truncated form of Kir6.2 (Kir6.2\text{ΔC36}), which expresses functional channels in the absence of the sulfonylurea receptor subunit (12). Macroscopic currents in cell-attached patches on oocytes expressing Kir6.2\text{ΔC36} were small in the cell-attached configuration, increased on patch excision into nucleotide-free solution, and subsequently slowly ran down (Fig. 1A, panel b). As reported previously (12), Kir6.2\text{ΔC36} currents were inhibited by ATP and were refreshed following ATP removal. Application of 1 \textmu M oleoyl-CoA to the inner membrane surface enhanced Kir6.2\text{ΔC36} currents by 2.6 ± 0.6-fold (n = 9; p = 0.03), with a time course similar to that observed for Kir6.2/SUR1 currents (Fig. 1B, panel b). As observed for Kir6.2/SUR1 currents, this effect was maintained on return to control solution but reversed following addition of 0.1% BSA. These results suggest that K\textsubscript{ATP} channel activation by oleoyl-CoA is mediated either by a direct interaction with the Kir6.2 subunit or, possibly, via a third subunit endogenously expressed in Xenopus oocytes. In the latter case, a similar protein must also be expressed in pancreatic beta cells because native K\textsubscript{ATP} channels are also sensitive to oleoyl-CoA.

Interaction between Oleoyl-CoA and ATP—Since inhibition of K\textsubscript{ATP} channels by ATP results also from an interaction with the Kir6.2 subunit, we explored whether the inhibitory effect of the nucleotide is modified by oleoyl-CoA. We compared the ATP-sensitivity of Kir6.2\text{ΔC36} currents measured in the absence of oleoyl-CoA with that obtained for patches in which Kir6.2\text{ΔC36} channels were first activated by pretreatment with the acyl-CoA at 1 \mu M. Fig. 2 shows that the inhibitory effect of ATP on both Kir6.2/SUR1 and Kir6.2\text{ΔC36} currents was impaired following exposure to oleoyl-CoA. Half-maximal inhibitory (K\textsubscript{i}) of Kir6.2\text{ΔC36} currents increased from a mean value of 130 ± 8 \mu M (n = 7) in control solution to 381 ± 37 \mu M (n = 6; p = 0.0001) after application of 1 \mu M oleoyl-CoA. The Hill coefficient was unaffected, being 1.0 ± 0.1 and 1.2 ± 0.1 in the absence and presence of oleoyl-CoA, respectively. Similar results were obtained for wild-type K\textsubscript{ATP} channels (Kir6.2/SUR1), where the mean K\textsubscript{i} for ATP increased from 31 ± 5 \mu M (n = 9) to 76 ± 17 \mu M (n = 8; p = 0.0005). The Hill coefficient was 1.1 ± 0.1 before and 1.0 ± 0.1 after addition of oleoyl-CoA. A reduction in ATP sensitivity in the presence of oleoyl-CoA has also been reported for native K\textsubscript{ATP} channels although the effect was not quantified (18).

Effect of Oleoyl-CoA on the Single-channel Kinetics—To investigate further the mechanism by which oleoyl-CoA potentiates the macroscopic current, we recorded single-channel currents from oocytes expressing either Kir6.2\text{ΔC36} or wild-type K\textsubscript{ATP} channels (Kir6.2/SUR1). Application of the acyl-CoA had no effect on the single-channel current amplitude at −60 mV (Fig. 3), which was 4.3 ± 0.3 pA before and 4.3 ± 0.3 pA after addition of 1 \mu M oleoyl-CoA (n = 4) for Kir6.2/SUR1 currents and was 4.1 ± 0.2 pA before and 4.1 ± 0.2 pA after addition of 1 \mu M oleoyl-CoA (n = 4) for Kir6.2\text{ΔC36} currents. However, the acyl-CoA enhanced the channel activity (N\textsubscript{Po}) by 2.3-fold and 4.2-fold for Kir6.2/SUR1 and Kir6.2\text{ΔC36} currents, respectively (Table I). This was a consequence of an increase in both the number of active channels and in the channel open probability. The effect on the open probability resulted principally from a reduction in the duration and frequency of the long closed time (τ\textsubscript{Cz}); the mean open time (τ\textsubscript{O}) and short closed time (τ\textsubscript{C1}) were unaffected (Table I).

The value of P\textsubscript{o}, measured for Kir6.2/SUR1 currents in control solution (0.2; Table I) was similar to that previously reported for the recombinant channel (0.11; Ref. 12) and for the native K\textsubscript{ATP} channel in beta cells (0.1; Ref. 28). Likewise, the mean open and closed times were close to those found for recombinant and native K\textsubscript{ATP} channels (29, 30).

A lack of effect of oleoyl-CoA on the single-channel current amplitude has also been reported for the native beta cell K\textsubscript{ATP} channel (19). Interestingly, however, the effect of oleoyl-CoA on the single-channel kinetics of native K\textsubscript{ATP} channels was different: a 2-fold increase in τ\textsubscript{o} was reported at 0 mV. It is possible that this reflects the different membrane potentials at which the experiments were carried out. We conducted our experiments at −60 mV because this is close to the resting potential of the pancreatic beta cell (2).

Effect of Oleoyl-CoA on Kir1.1a—Finally, we explored
Whether oleoyl-CoA is able to inhibit Kir1.1a, a related Kir channel that shares ~45% sequence identity with Kir6.2. As shown in Fig. 4, 1 μM oleoyl-CoA did not cause a significant activation of Kir1.1a. Rather, the acyl-CoA induced a slow rundown of Kir1.1a currents. This is in marked contrast to the lack of rundown found for Kir6.2A/C36 currents after exposure to oleoyl-CoA. The mean macroscopic conductance in the presence of the drug was 30.3 ± 6.2% (n = 4) of that in control solution after 3 min of exposure to 1 μM oleoyl-CoA.

**DISCUSSION**

We report here that oleoyl-CoA activates the cloned beta cell K<sub>ATP</sub> channel (Kir6.2/SUR1) expressed in Xenopus oocytes. This suggests that the acyl-CoA interacts directly with the K<sub>ATP</sub> channel, or a channel regulator that is expressed endogenously in the Xenopus oocyte, rather than with a beta cell-specific membrane component.

**Subunit Interaction**—We found that oleoyl-CoA activated both Kir6.2/SUR1 channels and Kir6.2A/C36 channels expressed in the absence of SUR1. This result indicates that the mechanism of acyl-CoA activation does not require the presence of the sulfonylurea receptor subunit and is thus very different from that of other K<sub>ATP</sub> channel activators, such as MgADP and diazoxide, which mediate their effects via SUR1 (8–12). Our results provide an explanation for the findings of other K-channels and lipid effectors—The anionic phospholipid PIP<sub>2</sub> interacts with native cardiac and beta cell K<sub>ATP</sub> channels to enhance their activity (38, 39). Activation of Kir6.2/SUR1 currents by PIP<sub>2</sub> has also been reported (39). However, the properties of PIP<sub>2</sub> activation are not identical to those of oleoyl-CoA, because the effect of PIP<sub>2</sub> is instantaneous and the compound also activated Kir1.1a (39). This makes it less likely that the two compounds interact with the channel in exactly the same way.

**Physiological implications**—Our results indicate that oleoyl-CoA interacts with the Kir6.2, rather than the SUR1, subunit of the K<sub>ATP</sub> channel. Kir6.2 is expressed in a number of tissues, including heart, skeletal muscle, and certain brain regions (13, 15, 41). It is believed to serve as the pore-forming subunit in Kir6.2/SUR1 channels to ATP. There are a number of possible explanations for this finding. First, the acyl-CoA might compete with ATP for its binding site. Second, oleoyl-CoA might allosterically affect the conformation, and thereby the affinity, of the ATP-binding site. Third, oleoyl-CoA might indirectly alter the apparent ATP sensitivity by affecting the single-channel kinetics; for example, if the ATP-inhibited state are only accessible when the channel were closed, an increase in the open probability would necessarily decrease the channel ATP sensitivity (36, 37). Because oleoyl-CoA does in fact alter the single-channel kinetics and shifts the gating of the channel toward the open state, the latter possibility may contribute, at least in part, to the reduced ATP-sensitivity.

![Graph showing effect of oleoyl-CoA on single-channel kinetics](image)

**Table I**

| Clone                | N<sub>o</sub> | N | P<sub>o</sub> | τ<sub>o</sub> | τ<sub>C1</sub> | τ<sub>C2</sub> | C<sub>2</sub> | %     |
|----------------------|--------------|---|-------------|-------------|-------------|-------------|-------------|------|
| Control (n = 6)      | 0.85 ± 0.13  | 4.5 ± 0.3 | 0.20 ± 0.03 | 1.66 ± 0.08 | 0.33 ± 0.02 | 38.7 ± 10.6 | 5.7 ± 2.1  |
| 1 μM oleoyl-CoA      | 2.45 ± 0.45  | 7.0 ± 0.4 | 0.31 ± 0.01 | 1.38 ± 0.14 | 0.32 ± 0.01 | 8.5 ± 1.1   | 3.3 ± 1    |
| Kir6.2A/C36 (n = 4)  | 0.29 ± 0.02  | 3.0 ± 0  | 0.10 ± 0.01 | 0.77 ± 0.01 | 0.30 ± 0.02 | 5.03 ± 0.32 | 44.8 ± 4.6 |
| 1 μM oleoyl-CoA      | 1.17 ± 0.11  | 5.3 ± 0.3 | 0.21 ± 0.01 | 0.73 ± 0.02 | 0.31 ± 0.04 | 2.06 ± 0.49 | 25.2 ± 4.7 |

**Fig. 4.** Macroscopic currents recorded from an inside-out patch excised from an oocyte injected with Kir1.1a mRNA in response to a series of voltage ramps from −110 mV to +100 mV. 1 μM oleoyl-CoA was added to the internal solution as indicated by the bar.
the reduced sensitivity of the pancreatic beta cell to glucose observed in non-insulin-dependent diabetes mellitus.

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