Evidence for a Unique Long Chain Acyl-CoA Ester Binding Site on the ATP-regulated Potassium Channel in Mouse Pancreatic Beta Cells*

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The mechanism by which long chain acyl-CoA (LC-CoA) esters affect the ATP-regulated potassium channel (K\textsubscript{ATP} channel) was studied in inside-out patches isolated from mouse pancreatic beta cells. Addition of LC-CoA esters dramatically increased K\textsubscript{ATP} channel activity. The stimulatory effect of the esters could be explained by the induction of a prolonged open state of the channel and did not involve alterations in single channel unitary conductance. Under control conditions, absence of adenine nucleotides, the distribution of K\textsubscript{ATP} channel unitary conductance. Under control conditions, the channel and did not involve alterations in single

Potassium channels that are ATP-sensitive (K\textsubscript{ATP})

are found in many types of cells and serve to couple metabolic state to electrical activity. In the pancreatic beta cell the K\textsubscript{ATP} channel provide a critical link between changes in blood glucose concentration and insulin secretion (1, 2). The initial step in the

stimulus-secretion-coupling in the beta cell is closure of the K\textsubscript{ATP} channel subsequent to a rise in the ATP/ADP ratio, resulting in depolarization, activation of voltage-dependent Ca\textsuperscript{2+} channels and thereby triggering of insulin secretion (3). Stimulation of the beta cell with intermediate glucose concentrations results in a characteristic pattern of slow oscillations in membrane potential on which bursts of action potentials are superimposed (4). Intracellular free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) oscillates in synchrony with electrical activity (5). We recently showed that fluctuations in the activity of the K\textsubscript{ATP} channel underlie the oscillations in electrical activity and [Ca\textsuperscript{2+}]\textsubscript{i}, in single pancreatic beta cells (6). A possible mechanism underlying such oscillations in K\textsubscript{ATP} channel activity could be metabolism-driven oscillations in the ATP/ADP ratio (7). Because of the close relation between K\textsubscript{ATP} channel activity and beta cell electrical activity, it is essential to study mechanisms which control or modulate the activity of this channel.

We have recently shown that long-term exposure to free fatty acid increases cellular levels of LC-CoA esters in the beta cell and that these esters are able to directly stimulate K\textsubscript{ATP} channel activity (8). This indicates that increased steady-state content of cytosolic LC-CoA esters could affect glucose-induced closure of the K\textsubscript{ATP} channel. In the present study, we have investigated in detail the mechanisms by which LC-CoA esters exert their stimulatory action and to what extent they interact with ATP and ADP in modulating K\textsubscript{ATP} channel activity in the pancreatic beta cell. These findings show that LC-CoA induces a distinct open state leading to increased channel activity, characterized by openings of long duration which does not require the presence of Mg\textsuperscript{2+}.

Thus, binding of LC-CoA induces a conformational change of the K\textsubscript{ATP} channel protein. A potent stimulatory effect of LC-CoA esters also occurred in the presence of different ratios of ATP/ADP, indicating that the esters may play an important role in modulating the channel under physiological conditions.

EXPERIMENTAL PROCEDURES

Animals and Preparation of Cells—Adult obese mice (gene ob/ob) of both sexes were obtained from a local noninbred colony (9). The mice were fasted for 24 h and then killed by decapitation. The islets of these mice contain more than 90% beta cells (10). Dispersed islets were isolated by a collagenase technique (11). Collagenase was obtained from Boehringer Mannheim GmbH, Germany. A cell suspension was prepared and washed essentially as described previously (12). The cells were resuspended in RPMI 1640 culture medium (Flow Laboratories, Scotland, UK), containing 11 m\textsubscript{g} glucose, supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 \mu\textsubscript{g}/ml streptomycin, and 60 \mu\textsubscript{g}/ml gentamycin. The cell suspension was seeded into Petri dishes (Corning Glass Works, Corning, NY) and incubated at 37 °C in 5% CO\textsubscript{2} for 1–3 days.

Solutions—The standard extracellular solution contained (in m\textsubscript{M}): 138 NaCl, 5.6 KCl, 1.2 MgCl\textsubscript{2}, 2.6 CaCl\textsubscript{2}, and 5 HEPES-NaOH at pH 7.4. The intracellular solution (i.e. the bath solution) consisted of (in
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Effects of LC-CoA Esters on K<sub>ATP</sub> Channel Kinetics—Fig. 1, A and B, show channel activity following administration of a 3-carbon (malonyl), 14-carbon (myristoyl), and 18-carbon (oleoyl) CoA ester to inside-out patches shortly after isolation. It is clear that K<sub>ATP</sub> channel activity, in the presence of myristoyl-CoA and oleoyl-CoA, was increased compared with the activity under control conditions. In Fig. 1, C–F, we have quantified this effect by analyzing the distribution of channel open time during exposure of the patches to oleoyl-, myristoyl-, and malonyl-CoA. In control solution, channel activity consisted of short openings (Fig. 1, C, inset). The distribution of the openings was best described by a single exponential with a time constant (τ) of 19.8 ms. Six different patches and a total number of 4314 events were analyzed, of which 27.6% of the integrated events belonged to the slow component. The bar at the far right indicates the sum of events exceeding 1000 ms. E, when exposing patches to 1 μM myristoyl-CoA, similar results to those with oleoyl-CoA were obtained. Time constants of τ<sub>fast</sub> = 48.1 ms and τ<sub>slow</sub> = 219.1 ms were obtained with 19.4% of the total number of events (3902; n = 4) belonging to the slow component. F, distribution of open times in the presence of 1 μM malonyl-CoA was not significantly different from what was obtained during control conditions. A total of 2379 events were analyzed from three different patches, and the distribution of open times could be described by a single exponential with a time constant of 28.9 ms. Insets show typical channel activity during the different experimental conditions. Arrowheads show current level when the channel is closed.
open time was estimated to be 29.3 ± 4.5 ms (n = 5). In the presence of oleoyl-CoA, there were two types of channel openings, short openings, similar to those observed under control conditions, and long openings, occasionally lasting several hundred milliseconds (Fig. 1D). The distribution of the openings was best described as the sum of two exponentials with τ values of 49.6 and 260.4 ms, respectively. The slow component comprised 27.6% of the events and the mean open time was increased approximately 3-fold to 84.6 ± 29.0 ms (n = 5). Similar results were obtained when adding myristoyl-CoA to the patch (Fig. 1E), resulting in τ values of 46.1 and 219.1 ms, respectively, with 19.4% belonging to the slow component. However, when applying malonyl-CoA (Fig. 1F), channel activity did not differ from control conditions with a mean open time of 34.0 ± 9.4 ms (n = 5). The distribution could be described by a single exponential with a time constant of 28.9 ms.

We have previously reported comparable effects on K\textsubscript{ATP} channel open time induced by ADP and diazoxide (17). Thus, ADP and diazoxide increase channel activity by promoting a similar long lasting open state. We now demonstrate that, LC-CoA esters also induce a conformational change of the K\textsubscript{ATP} channel, leading to a prolonged open state. Another possibility could be that LC-CoA esters affect single channel unitary conductance. In this context it should be noted that the CoA moiety has a close structural resemblance to ADP, suggesting the possibility of competition for a common binding site. However, on a molar basis, LC-CoA esters are considerably more potent than ADP, and the esters also induce a significantly higher degree of channel stimulation. To what extent this difference between ADP and LC-CoA esters can be accounted for by an additional 3'-phosphate group on the CoA moiety is not clear. Nevertheless, this may indicate that LC-CoA esters interact at a site different from that of ADP on the K\textsubscript{ATP} channel complex. In Fig. 2A, perfusions with 1 μM oleoyl-CoA enhanced channel activity 5-fold. An addition of 0.1 mM MgADP, in the continuous presence of oleoyl-CoA, further increased mean current by 720 ± 220% (n = 4; p < 0.001). Noteworthy is that channel openings were characterized by long openings. As MgADP was withdrawn, the long openings of the channel disappeared. In trace B, addition of 0.1 mM MgADP led to an increase in mean current of 320 ± 100% (n = 3). Channel activity declined significantly as the MgADP concentration was increased to 0.5 mM. Inhibition of K\textsubscript{ATP} channel activity at higher concentrations (>0.3 mM) of MgADP is a well documented effect (18–20). Exposing the same patch to oleoyl-CoA, in the continuous presence of MgADP, induced a dramatic augmentation in channel activity with a mean current increase of 690 ± 140% (n = 4). In C, we have quantified the effects by making amplitude histograms under the different conditions tested in the recording of trace B. Compiled data on mean open time clearly show that a combination of MgADP and LC-CoA ester led to a supra additive effect (Fig. 3).

The fact that a combination of ADP and LC-CoA esters activate the channel to a larger extent than administration of either of the two substances alone, suggests that they interact

FIG. 2. Simultaneous presence of MgADP and oleoyl-CoA results in long channel openings. A, inside-out patch exposed to 1 μM oleoyl-CoA led to a mean current of 3.3 pA. Further addition of 0.1 mM MgADP to the patch resulted in a channel activity characterized by long openings and a mean current of 4.4 pA. B, exposure of a patch to 0.1 mM MgADP increased mean current from 0.7 to 1.7 pA. As the MgADP concentration was further increased to 0.5 mM, K\textsubscript{ATP} channel mean current decreased to 0.8 pA. Adding 1 μM oleoyl-CoA to the 0.5 mM MgADP-containing solution induced a mean current of 4.9 pA. C, amplitude histograms of data shown in panel B. Currents were filtered at 0.5 kHz and sampled at 1 kHz. A total number of about 48 × 10\textsuperscript{3} events were obtained for each experimental condition. C denotes closed channel, and the z axis indicates the order of changes of solutions.

FIG. 3. The effects of ADP and LC-CoA on channel open time. Compiled data on the effects of ADP and oleoyl-CoA, alone and in combination, on K\textsubscript{ATP} channel open time. Each value represents mean ± S.E. for control (n = 15), 100 μM MgADP (n = 4), 1 μM oleoyl-CoA (n = 6) and the combination of MgADP + oleoyl-CoA (n = 6). ***p < 0.001.
at distinct binding sites. In an attempt to obtain more information on this matter, we have tried modifying the K_{ATP} channel by applying a short pulse of trypsin. This approach was used earlier in studies of various types of ion channels including Na^{+} (21), Ca^{2+} (22), and K^{+} channels (23). Although the technique seems crude, it modifies the K_{ATP} channel in very specific ways (24). There is a resulting complete loss of the stimulatory effect of ADP as well as of the inhibitory effect of sulfonylurea on channel activity. However, inhibition of channel activity by ATP remains intact, although with slightly decreased sensitivity (24). One interpretation is that the binding sites for ADP and sulfonylurea are lost due to alterations of the channel proteins as a result of proteolytic effects of trypsin, which has a primary affinity for arginine and lysine residues (25). Interestingly, in trypsin modified patches, where ADP was totally ineffective in altering channel activity, addition of LC-CoA esters induced a pronounced increase in channel activity. LC-CoA esters were also able to potently counteract ATP-induced inhibition of channel activity in modified patches (Fig. 4). A possible explanation for these results is that trypsin alters or removes the site to which ADP binds to exert activation, whereas the site involved in LC-CoA-induced stimulation remains. These data further support the notion that LC-CoA esters interact at a unique binding site, separate from that of ADP.

To further study the interaction between LC-CoA esters and ATP, we performed a series of experiments under Mg^{2+}-free conditions, since the ability of ADP to open channels requires Mg^{2+}. As shown in Fig. 5A, LC-CoA activated the K_{ATP} channel in the absence of Mg^{2+}, whereas ADP^{3-} not only failed to activate but had an inhibitory effect on K_{ATP} channel activity (Fig. 5B) (17). This blocking effect of ADP^{3-} is well documented and it has been proposed that ADP^{3-} binds to the ATP site thereby explaining the inhibitory effect on the channel (20). Addition of oleoyl-CoA to the patch, in the continuous presence of ADP^{3-}, still evoked a dramatic elevation in channel activity (Fig. 5B). Analyzing the effects of simultaneous additions of oleoyl-CoA and ADP^{3-} in the absence of Mg^{2+} in five patches showed an increase of 590 ± 280% in mean currents (p < 0.01). Inclusion of Mg^{2+} in the perfusion medium caused the K_{ATP} channel activity to display openings of long duration. Together these observations lend strong support to the idea that simultaneous exposure of the K_{ATP} channel to a combination of LC-CoA esters and MgADP, results in a unique activity pattern with extremely long open times, not previously observed with either substance alone. All effects of the LC-CoA esters were fully reversible upon withdrawal. Due to the extreme pattern and the resulting high number of open channels in the patches, a precise determination of channel open time was not possible.

Earlier studies suggested that the physiological regulation of the K_{ATP} channel results from changes in the ATP/ADP ratio (3), changes in ADP exerting the major influence (1). This implies that ATP-induced blockade of the channel is potently counteracted by intracellular ADP. We therefore assessed the extent to which LC-CoA esters were able to further activate channel activity in the presence of fixed ATP/ADP ratios. Fig. 6A shows the effect of 100 μM ATP and ADP on channel activity, a ratio which has been reported to give maximal stimulation of K_{ATP} currents. Subsequent addition of 1 μM oleoyl-CoA, in the continuous presence of nucleotides, resulted in an augmentation of the K_{ATP} currents. In six out of six patches we found that addition of oleoyl-CoA, in the presence of 100 μM ATP and ADP, increased mean currents significantly (380 ± 140%; p < 0.01; n = 5). Adding LC-CoA esters to 500 μM ATP and ADP also induced an increase in K_{ATP} channel activity (Fig. 6B). It should, however, be pointed out that the most
The K<sub>ATP</sub> channel is encoded by the SUR1 and an inwardly rectified K<sup>+</sup> channel, which is no longer able to counteract the blocking effect of ATP and occasional openings were observed. Addition of 1 μM oleoyl-CoA at their binding sites. Even at an ATP/ADP ratio of 10, partially prevented the inhibitory effect of ATP and occasional openings were observed. Addition of 1 μM oleoyl-CoA esters in the continuous presence of nucleotides, potently increased K<sub>ATP</sub> currents. The vertical calibration bar represents 5 pA in A and C and 10 pA in B.

dramatic effects were seen when the nucleotides and CoA ester were washed out. Thus, just after withdrawal of the substances, we repeatedly observed the same channel activity pattern as following the combination of MgADP and LC-CoA esters (see Fig. 3), characterized by channel activity with very long openings. A possible explanation for this phenomena is that ADP is washed-out more slowly than ATP (26), leaving MgADP and LC-CoA at their binding sites. Even at an ATP/ADP ratio of 10, administration of 1 μM oleoyl-CoA potently increased channel activity (Fig. 6C).

Concluding Remarks—The recent cloning of the rat sulfonylurea receptor (SUR1) (27) combined with the reconstitution of the K<sub>ATP</sub> channel (28) has elegantly shown that the beta cell K<sub>ATP</sub> channel is encoded by the SUR1 and an inwardly rectified K<sup>+</sup> channel (Kir6.2) (Fig. 7) with small intrinsic activity. The SUR1 belongs to a superfamily of ATP-binding cassette proteins and their interaction with ATP, ADP, and LC-CoA esters strongly suggest that ADP and LC-CoA esters do not bind to the same site. Thus, LC-CoA esters form a class of substances which with high potency activates the beta cell K<sub>ATP</sub> channel. The fact that most of our results were obtained by CoA esters derived from oleate, which is one of the predominant free fatty acid components in rodent and man plasma, supports the notion that these esters may serve the function of important modulators of beta cell electrical activity and thereby insulin release under physiological conditions.

REFERENCES

1. Ashcroft, P. M., and Rorsman, P. (1989) Prog. Biophys. Mol. Biol. 54, 87–143
2. Henquin, J. C., and Meissner, H. P. (1984) Experientia 40, 1043–1052
3. Ashcroft, P. M., and Rorsman, P. (1990) Biochem. Soc. Trans. 18, 109–111
4. Dean, P. M., and Matthews, E. K. (1968) Nature 218, 389–390
5. Santos, R. M., Rosario, L. M., Nadal, A., Garcia-Sancho, J., Soria, B., and Valledemillos, M. (1991) Pflügers. Arch. 418, 417–422
6. Larsson, O., Kindmark, H., Branstrom, R., Fredholm, B., and Berggren, P. O. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5161–5165
7. Nilsson, T., Schultz, V., Berggren, P. O., Corkey, B. E., and Tornheim, K. (1996) Biochem. J. 314, 91–94
8. Larsson, O., Deeney, J. T., Braunstrom, R., Berggren, P. O., and Corkey, B. E. (1996) J. Biol. Chem. 271, 10625–10626
9. Hellman, B. (1965) Ann. N. Y. Acad. Sci. 131, 541–558
10. Nilsson, T., Arkhammar, P., Hallberg, A., Hellman, B., and Berggren, P. O. (1987) Biochem. J. 248, 329–336
11. Lacy, P. E., and Kostianovsky, M. (1967) Diabetes 16, 35–39
12. Lernmark, A. (1974) Diabetologia 10, 431–438
13. Boyd, J. G., and Hamilton, J. A. (1992) Biochemistry 31, 557–567
14. Ordway, R. W., Singer, J. J., and Walsh, J. V., Jr. (1991) Trends Neurosci. 14, 96–100
15. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) Pflügers. Arch. 391, 85–100
16. Colquhoun, D., and Sigworth, F. J. (1983) in Single-Channel Recording (Sakmann, B., and Neher, E., eds) pp. 191–263, Plenum Press, New York
17. Larsson, O., Ammalia, C., Bokvist, K., Fredholm, B., and Rorsman, P. (1993) J. Physiol. 463, 349–365
18. Ghosh, A., Ronner, P., Cheong, E., Khalid, P., and Matschinsky, F. M. (1991) J. Biol. Chem. 266, 22887–22892
19. Ohts, M., Nelson, D., Nelson, J., Meglasson, M. D., and Erecinska, M. (1991) Biochem. Pharmacol. 42, 595–598
20. Hopkins, W. F., Silverman, S., Peter-Riesch, B., Corkey, B. E., and Cook, D. L. (1992) J. Membr. Biol. 129, 287–295
21. Armstrong, C. M. (1981) Physiol. Rev. 61, 644–683
22. Hescheler, J., and Trautwein, W. (1988) J. Physiol. 404, 259–274
23. Hescheler, J., and Schroeter, K. (1989) in Secretion and Its Control pp. 83–95, The Rockefeller Press, New York
24. Proks, P., and Ashcroft, F. M. (1993) Pflügers Arch. 424, 63–72
25. Begen, R. J., and Boyd, J. S. (1989) in Proteolytic Enzymes—A Practical Approach, Oxford University Press, Oxford
26. Schwantesteher, M., Loser, S., Rietze, I., and Panten, U. (1991) Naunyn-Schmiedebergs. Arch. Pharmacol. 343, 83–89
27. Aguilar-Bryan, L., Nicholls, C. G., Wechsler, S. W., Clement, J. P., 4th, Boyd, A. E., 3rd, Gonzalez, G., Herrera-Sosa, H., Nguyen, B., Bryan, J., and Nelson, D. A. (1995) Science 268, 423–426
28. Inagaki, N., Conot, T., Clement, J. P., IV, Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., and Bryan, J. (1995) Science 270, 1166–1170
29. Nichols, C. G., Shyng, S. L., Nestorowicz, A., Glaser, B., Clement, J. P., IV, Gonzalez, G., Aguilar-Bryan, L., Permutt, M. A., and Bryan, J. (1996) Science 272, 1785–1787