

Metabolic Regulation of the Pancreatic β -Cell ATP-Sensitive K^+ Channel

A Pas de Deux

Andrei Tarasov, Julien Dusonchet, and Frances Ashcroft

Closure of ATP-sensitive K^+ channels (K_{ATP} channels) is a key step in glucose-stimulated insulin secretion. The precise mechanism(s) by which glucose metabolism regulates K_{ATP} channel activity, however, remains controversial. It is widely believed that the principal determinants are the intracellular concentrations of the metabolic ligands, ATP and ADP, which have opposing actions on K_{ATP} channels, with ATP closing and MgADP opening the channel. However, the sensitivity of the channel to these nucleotides in the intact cell, and their relative contribution to the regulation of channel activity, remains unclear. The precise role of phosphoinositides and long-chain acyl-CoA esters, which are capable of modulating the channel ATP sensitivity, is also uncertain. Furthermore, it is still a matter of debate whether it is changes in the concentration of ATP, of MgADP, or of other agents, which couples glucose metabolism to K_{ATP} channel activity. In this article, we review current knowledge of the metabolic regulation of the K_{ATP} channel and provide evidence that MgADP (or MgATP hydrolysis), acting at the regulatory subunit of the channel, shifts the ATP concentration-response curve into a range in which the channel pore can respond to dynamic changes in cytosolic ATP. This metabolic pas de deux orchestrates the pivotal role of ATP in metabolic regulation of the K_{ATP} channel. *Diabetes* 53 (Suppl. 3): S113–S122, 2004

The ATP-sensitive K^+ channel (K_{ATP} channel) activity plays a crucial role in glucose-stimulated insulin secretion by coupling β -cell metabolism to calcium entry (1). Insulin secretion is triggered by an increase in the cytoplasmic Ca^{2+} concentration, which results from Ca^{2+} influx through voltage-gated

Ca^{2+} channels in the plasma membrane. Opening of these channels is controlled by the membrane potential, which, in turn, is determined largely by the activity of the K_{ATP} channel (Fig. 1). At substimulatory glucose concentrations, K_{ATP} channels are open, and K^+ efflux through these channels serves to maintain the resting membrane potential at a hyperpolarized level of around -70 mV. A small inward current (which has not yet been identified) must also be present because the resting potential is less negative than the K-equilibrium potential. Elevation of the blood glucose concentration increases glucose uptake and metabolism by the β -cell, resulting in closure of the K_{ATP} channels. The reduction in K^+ efflux means that the contribution of the inward current to the membrane potential increases and the membrane depolarizes. If this depolarization is sufficient, voltage-gated Ca^{2+} channels open, triggering electrical activity, Ca^{2+} influx, and insulin secretion. Closure of K_{ATP} channels therefore initiates insulin release, while K_{ATP} channel opening inhibits secretion.

As might be expected, drugs that inhibit K_{ATP} channels, like sulfonylureas, stimulate insulin secretion even in the absence of glucose, whereas drugs that open K_{ATP} channels (e.g., diazoxide) reduce insulin secretion even in the presence of glucose (2). Furthermore, loss-of-function mutations in K_{ATP} channel genes result in congenital hyperinsulinism of infancy, a serious disorder characterized by excessive and unregulated insulin secretion (3,4). In contrast, gain-of-function mutations in the pore-forming subunit of the channel impair insulin secretion and produce permanent neonatal diabetes (5). Defective metabolic regulation of the K_{ATP} channel, as a consequence of impaired metabolism, also results in diabetes (3,6,7).

MOLECULAR BIOLOGY OF THE K_{ATP} CHANNEL

Opening and closing of the K_{ATP} channel is influenced by the intracellular concentrations of nucleotides (particularly ATP and ADP [8]), lipids such as phosphatidylinositides (9,10), and long-chain acyl-CoA esters (11–13). Our understanding of how they exert their functional effects has been illuminated by an increasing knowledge of the molecular structure of the K_{ATP} channel.

The β -cell K_{ATP} channel is an octameric complex of two different types of protein subunit that coassemble in a 4:4 stoichiometry. The pore is a tetramer of inwardly rectifying K^+ channel (Kir6.2) subunits (14,15). Although all four subunits possess a binding site for ATP, ligand binding to a single Kir6.2 subunit is sufficient to

From the University Laboratory of Physiology, University of Oxford, Oxford, U.K.

Address correspondence and reprint requests to Frances M. Ashcroft, University Laboratory of Physiology, Parks Rd., Oxford OX1 3PT, U.K. E-mail: frances.ashcroft@physiol.ox.ac.uk

Received for publication 17 March 2004 and accepted in revised form 26 May 2004.

A.T. and J.D. contributed equally to this work.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

ABC, ATP-binding cassette; AK, adenylate kinase; $[ATP]_i$, intracellular ATP concentration; CK, creatine kinase; FFA, free fatty acid; K_{ATP} channel, ATP-sensitive K^+ channel; LC-CoA, long-chain acyl-CoA ester; NBD, nucleotide-binding domain; PCr, phosphocreatine; PI, phosphatidylinositol; PPI, phosphoinositide; SUR, sulfonylurea receptor; UCP, uncoupling protein.

© 2004 by the American Diabetes Association.

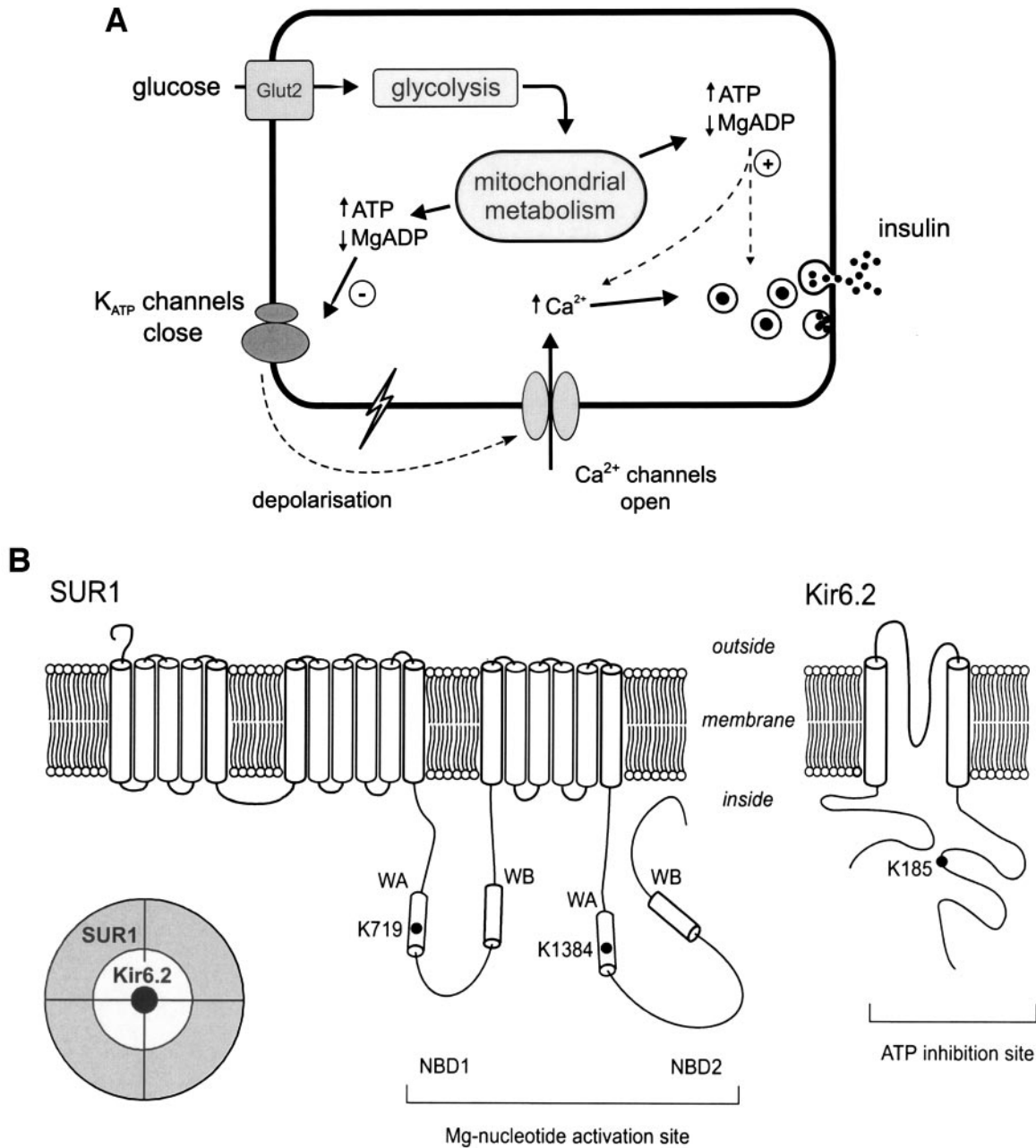


FIG. 1. A: Glucose enters the cell via the GLUT2 transporter. Subsequently, glycolytic and mitochondrial metabolism of the sugar leads to an increase in ATP and fall in MgADP. This results in K_{ATP} channel closure, membrane depolarization, opening of voltage-gated Ca^{2+} channels, Ca^{2+} influx, and exocytosis of insulin granules. **B:** Membrane topology of SUR1 and Kir6.2 subunits and their arrangement in the K_{ATP} channel. Residues K719 and K1384 of SUR1 and K185 of Kir6.2 are mentioned in the text. WA (WB), Walker A (Walker B) motifs.

close the channel (16). Each Kir6.2 subunit is associated with a regulatory sulfonylurea receptor (SUR)-1 subunit, which endows the channel with sensitivity to drugs such as sulfonylureas and K^+ channel openers, as well as to the stimulatory action of MgADP (17). SUR1 is a member of the ATP-binding cassette (ABC) transporter family, and like other ABC proteins, it has two cytosolic nucleotide-binding domains (NBDs). Interaction of Mg-nucleotides (e.g., MgATP, MgADP) with the NBDs leads to opening of the channel (18–21). The nucleotide regulation of the K_{ATP} channel is therefore complex, as channel activity is inhibited by nucleotide binding to

Kir6.2 and activated by Mg-nucleotide interaction with SUR1.

Much of the published literature states that changes in the ATP/ADP ratio regulate K_{ATP} channel activity. It is worth emphasizing, however, that this idea is misleading, as it is the *absolute* concentrations of ATP and ADP that are critical. Studies on inside-out patches demonstrate very clearly that for the *same* ATP/ADP ratio, high concentrations of ATP and ADP result in channel inhibition, whereas low concentrations of nucleotides support channel activity (8). This is a reflection of the fact that nucleotides exert both inhibitory and stimulatory

effects on channel activity, and that the inhibitory action of ADP dominates at concentrations >1 mmol/l (8).

PROPERTIES OF THE NUCLEOTIDE-BINDING SITES ON KIR6.2 AND SUR1

Both Kir6.2 and SUR1 subunits are required to form a functional channel. However, truncation of the last 26–36 amino acids from Kir6.2 (Kir6.2 Δ C) allows this subunit to reach the surface membrane in the absence of SUR (18). This construct provides a useful tool to dissect the effects of nucleotides on Kir6.2 and SUR1. The discovery that Kir6.2 Δ C channels are inhibited by ATP, for example, revealed that ATP (and ADP) interact with Kir6.2 to cause channel closure (18,22). Site-directed mutagenesis has identified a number of residues in Kir6.2 that, when mutated, reduce the channel ATP sensitivity (22–24), and photoaffinity labeling with radiolabeled ATP analogs confirmed that Kir6.2 indeed binds ATP (25,26). Nucleotide binding does not require Mg^{2+} , is strongly selective for the adenine base, and both ATP and ADP can mediate channel inhibition (22,27).

There is good evidence that Mg nucleotides stimulate K_{ATP} channel activity via interaction with SUR1. First, MgADP only stimulates K_{ATP} channels that contain SUR subunits: indeed, when Kir6.2 Δ C is expressed in the absence of SUR1, MgADP blocks channel activity (18). This is because ADP also interacts with Kir6.2 to produce channel inhibition (22). Because Mg^{2+} is required for nucleotide binding to SUR1, ADP also blocks the channel in the absence of the cation. Second, mutations within the NBDs of SUR1 abolish the stimulatory effects of Mg nucleotides and unmask their inhibitory action on Kir6.2 (19,21).

In most ABC transporters, MgATP is the major ligand, and its hydrolysis to MgADP provides the energy required for substrate transport. Because ATP produces a potent block of the K_{ATP} channel via Kir6.2, the role of MgATP at the NBDs of SUR1 was investigated by coexpressing SUR1 with an ATP-insensitive pore mutant, Kir6.2-R50G (27). These channels were stimulated by MgATP, an effect that was abolished by mutations within the NBDs of SUR1. Thus, like MgADP, MgATP stimulates K_{ATP} channel activity by interaction with SUR1. It seems probable, however, that MgATP must be hydrolyzed to MgADP before it is able to enhance K_{ATP} channel activity, although this has only been shown explicitly for Kir6.2/SUR2A (the cardiac type of K_{ATP} channel) to date (28).

Photoaffinity labeling with 8-azido-[^{32}P]ATP has been used to explore the nucleotide-binding properties of the NBDs of SUR1 (29). These studies showed that NBD1 of SUR1 binds 8-azido-ATP strongly in a Mg^{2+} -independent manner, and that NBD2 binds 8-azido-ATP in a Mg^{2+} -dependent manner. Because K_{ATP} channels are activated by ADP only in the presence of Mg^{2+} (20), this suggests that NBD2 is primarily responsible for channel activation by MgADP. NBD1 binds both ATP and ADP more tightly than NBD2 (as measured by their ability to displace 8-azido-ATP); however, there was no significant difference in the ability of NBD2 to bind ATP or ADP (29). The NBDs also differ in their ability to hydrolyze ATP. In particular, NBD2 of SUR1 appears to be more efficient at hydrolyzing ATP than NBD1 (29).

The ability of MgADP to stimulate K_{ATP} channel activity is also observed in the presence of ATP (20,21,30). Indeed, MgADP shifts the ATP concentration-inhibition curve to a higher intracellular ATP concentration ($[ATP]_i$) (30). This suggests that the ATP sensitivity in the cell will be less than that measured in inside-out patches, because of the presence of MgADP. It also explains why the ATP sensitivity measured in inside-out patches is affected by the intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$), the half-maximal inhibitory concentration (IC_{50}) for channel inhibition by ATP being 6–10 μ mol/l in Mg^{2+} -free solution (27) and 10–30 μ mol/l in the presence of Mg^{2+} (18,27,31). This difference presumably reflects ATP hydrolysis by the NBDs of SUR1 in the presence of Mg^{2+} , which leads to occupation of NBD2 by MgADP (28) and causes a small amount of channel activation. Because the ability of MgADP to stimulate channel activity in excised patches is labile (32), the extent to which the ATP sensitivity is reduced is variable.

METABOLIC CHANGES IN ATP AND ADP CONCENTRATION

In pancreatic β -cells, an increase in plasma glucose concentration results in enhanced glycolytic and mitochondrial metabolism and the generation of ATP at the expense of ADP (33). More than 95% of ATP in the β -cell is produced by the mitochondria, and mitochondrially generated ATP is of primary importance for insulin release. Indeed, depletion of mitochondria from β -cells results in the complete loss of insulin secretion (34). Uncoupling proteins induce a mitochondrial proton leak that leads to impaired ATP production (35). Uncoupling protein (UCP)-2 is expressed in islets, and mice in which UCP2 was knocked out had higher islet ATP levels and increased insulin secretion; conversely, rodent islets overexpressing UCP2 had impaired insulin secretion (35).

A substantial fraction of the ATP (68%) and ADP (45%) within the β -cell is nondiffusible and contained in intracellular organelles (especially the insulin secretory granules) (36). Measurements of $[ATP]_i$ in purified rat β -cells suggest that $[ATP]_i$ is 2 pmol/ 10^3 cells in the absence of glucose and increases to >4 pmol/ 10^3 cells when glucose is raised to 10 mmol/l (37). Because the β -cell has a volume of roughly 1 pl, this suggests $[ATP]_i$ is ~ 2 mmol/l at rest and increases to 4 mmol/l on glucose stimulation. These concentrations are in broad agreement with other studies in which β -cell $[ATP]_i$ is estimated to lie between 1 and 5 mmol/l, even during metabolic inhibition (38–40). However, a change in $[ATP]_i$ on exposure to glucose is not universally observed (39), and in some studies the increase in $[ATP]_i$ saturated at 10 mmol/l glucose (37). Total $[ADP]_i$ in purified β -cells was 750 μ mol/l in the absence of glucose and fell to 250 μ mol/l in response to 10 mmol/l glucose (37). Like in muscle, much ADP is probably bound to plasma proteins as the free concentrations are much lower—between 30 and 50 μ mol/l (39). Glucose also causes a marked increase in phosphocreatine (PCr) and a fall in phosphate (41,42).

It is important to remember that an increase in metabolic flux consequent on an increased substrate supply need not necessarily be accompanied by an increase in $[ATP]_i$ (or fall in $[ADP]_i$). This will depend on the relative

rates of nucleotide generation and consumption and is discussed further below.

CAN CHANGES IN ADENINE NUCLEOTIDES REGULATE K_{ATP} CHANNEL ACTIVITY?

There is little doubt that changes in intracellular adenine nucleotide concentrations influence K_{ATP} channel activity in intact β -cells (43). Nutrients that elevate ATP, like glucose, produce K_{ATP} channel inhibition, as does supply of reducing equivalents directly to cytochrome C, the final step in the electron transport chain (44). Conversely, agents that inhibit mitochondrial metabolism and reduce ATP generation, like rotenone, DNP, and azide, activate K_{ATP} channels in pancreatic β -cells (45). Similarly, such agents open cloned β -cell K_{ATP} channels expressed in *Xenopus* oocytes or mammalian cell lines (18,31,46). Inhibition of the mitochondrial ATP/ADP translocase by bongkrekic acid, which prevents ATP export from the mitochondrion, also activates K_{ATP} channels (45).

But there are also considerable problems with the idea that ATP is the sole regulator of K_{ATP} channel activity (43). The principal argument against such a role for ATP is the clear discrepancy between the ATP sensitivity of the channel measured in the inside-out patch ($IC_{50} = 10\text{--}30 \mu\text{mol/l}$; [22]) and that in the intact cell ($IC_{50} = 0.8 \text{ mmol/l}$; [47]). Furthermore, significant channel activity can be recorded from on-cell patches on β -cells exposed to glucose-free solutions (1), despite the fact that the $[ATP]_i$ measured under similar conditions suggests the channels should be almost completely closed (37). A number of different explanations can be put forward to account for this phenomenon. First, the submembrane ATP may not be the same as the bulk concentration, owing to the activity of membrane ATPases. Second, changes in $[ATP]_i$ could serve to mediate metabolic effects on channel activity if the ATP sensitivity is shifted, by another agent, into the range over which physiological changes in $[ATP]_i$ occur. Third, ATP could provide a tonic level of inhibition against which metabolically generated changes in some other substance regulate channel activity.

Theoretically, it is possible that ATP consumption by membrane ATPases (e.g., Na^+/K^+ ATPase) could lower the submembrane ATP concentration. In practice, however, this does not seem to account for the different ATP sensitivities of the K_{ATP} channel in excised patches and intact cells. Measurements using targeted luciferin indicate that $[ATP]$ immediately beneath the β -cell plasma membrane is similar to that in the bulk cytosol, $\sim 1 \text{ mmol/l}$ (38). Even when Kir6.2 ΔC itself was used to sense submembrane ATP, values of $\sim 1.5 \text{ mmol/l}$ in COSm6 cells and 5 mmol/l in oocytes were observed in control solution, and values of $\sim 1 \text{ mmol/l}$ were observed following metabolic poisoning (46).

At least three agents are known to modulate the ATP sensitivity of the K_{ATP} channel: PIP₂ (phosphatidylinositol-4,5-bisphosphate) and related phosphoinositides (PPIs) (9,10), long-chain acyl-CoA esters (LC-CoAs) (11–13), and MgADP (30). All decrease the ability of ATP to close the K_{ATP} channel. A key question is whether these agents simply shift the ATP concentration-inhibition curve to higher ATP concentrations, so that the channel is now sensitive to physiological changes in $[ATP]_i$, or whether

metabolically generated changes in PIP₂, LC-CoA, or MgADP serve to couple metabolism to K_{ATP} channel inhibition.

REGULATION BY PHOSPHOLIPIDS

PPIs such as PIP₂ and PIP₃ interact with K_{ATP} channels to increase their open probability and reduce their ATP sensitivity. This has been shown by direct application of PPIs to K_{ATP} channels in excised patches (9,10). In addition, overexpression of phosphatidylinositol (PI) 5-kinase, which enhances PIP₂ levels, reduces the ATP sensitivity of the K_{ATP} channel (48), whereas breakdown of PIP₂ by phospholipase C increases the ATP sensitivity (49).

These effects are mediated principally through the Kir6.2 subunit, because Kir6.2 ΔC expressed in the absence of SUR shows a reduced ATP sensitivity to applied PIP₂ (10), and direct binding of PIP₂ and other PPIs to Kir6.2 ΔC has been demonstrated (26). The binding site for PPIs is distinct from that of ATP (50), but the two interact allosterically, with ATP reducing the binding of PPIs, and PPIs decreasing ATP binding (26). Two mechanisms have been proposed to account for the PPI-induced decrease in K_{ATP} channel ATP sensitivity (26): 1) compromised ATP binding and 2) changes in the intrinsic gating of the channel that indirectly influence ATP sensitivity (9) (briefly, PPIs reduce the time spent in a long closed state that is stabilized by ATP). Both are probably involved.

It appears that PPIs account, in part, for the difference in the ATP sensitivity of the K_{ATP} channel in the isolated patch and intact cell. Although in heterologous expression systems hormones and transmitters may modulate K_{ATP} channel activity via changes in PIP₂ levels (49), this may not be the case in β -cells. For example, acetylcholine mediates PIP₂ breakdown in β -cells but does not influence K_{ATP} currents (51). There is no evidence that glucose influences channel activity by modulating PIP₂ levels. For example, wortmannin, which blocks PIP₂ production by inhibiting PI 3-kinase, had no effect on glucose-stimulated insulin secretion, and glucose had no effect on either PI 3-kinase activity or PIP₃ production (52).

REGULATION BY ACYL-CoAS

LC-CoAs enhance the open probability of the K_{ATP} channel and reduce its ATP sensitivity by interaction with the Kir6.2 subunit (11,12). They appear to bind to the same site as PIP₂ (13). Interestingly, other Kir channels are not activated by LC-CoA, and this property is correlated with the uniquely low specificity of Kir6.2 for PPIs.

Increases in cytosolic LC-CoA in the β -cell can result from glucose metabolism and from free fatty acids (FFAs) delivered by the blood or released from endogenous lipid stores (rev. in 33). Because K_{ATP} channels are opened by LC-CoA, they cannot be involved in coupling glucose metabolism to channel inhibition, although a role in modulating β -cell electrical activity and, thus, insulin secretion at elevated glucose has been proposed (33). However, it has been suggested that LC-CoA may contribute to the reduced glucose sensitivity of the β -cell observed in obese diabetic individuals because chronic exposure to FFA leads to increased levels of LC-CoA within β -cells (11). These not only enhance K_{ATP} channel activity directly, they also decrease ATP production by, for example, inhi-

bition of the ATP/ADP translocase and by upregulation of UCP2 (rev. in 35). LC-CoA may therefore exert a tonic effect on the K_{ATP} channel ATP sensitivity, shifting the concentration-inhibition curve into a range in which the channel opens more readily. As a consequence, glucose metabolism would not fully close K_{ATP} channels, and its ability to stimulate electrical activity, Ca^{2+} influx, and insulin secretion would be reduced.

REGULATION BY MgADP

Like PPIs and LC-CoAs, MgADP shifts the ATP dose-response curve to higher ATP concentrations (30,43). However, metabolically induced changes in MgADP have also been proposed to couple cell metabolism to channel activity. Nichols et al. (19) first reported that a mutation within NBD2 of SUR1 (G1479R) strongly reduced channel activation by MgADP. Importantly, K_{ATP} channels carrying this mutation were permanently closed even at low glucose levels and gave rise to congenital hyperinsulinism in humans. Subsequently, many other mutations within both SUR1 and Kir6.2 have been found to reduce MgADP activation and simultaneously prevent channel activation by metabolic inhibition (4,21). This raises the possibility that changes in MgADP couple cell metabolism to channel activity.

At first sight, this idea seems eminently reasonable. However, there are a number of problems. The first is that NBD2 of SUR1 binds ATP and ADP with similar affinities (see above), yet ATP is present at much greater concentration in the cell, even in the absence of glucose. This means that it is unlikely that metabolically induced changes in adenine nucleotides would lead to displacement of MgATP from SUR1 by MgADP.

One possibility is that an ATP hydrolysis cycle at NBD2 generates bound MgADP and that changes in cell metabolism influence K_{ATP} channel activity by modulating the length of time that NBD2 remains in the MgADP-bound (active) state (28). When metabolic activity is high, cytosolic ADP levels will be low, so that MgADP should dissociate rapidly from NBD2, causing channel activity to decrease. In contrast, when metabolic activity declines, the rise in $[ADP]_i$ will slow the off-rate of MgADP and promote channel opening. In this way, SUR1 could monitor changes in intracellular MgADP concentration. An alternative view, however, is that MgADP simply shifts the ATP dose-response curve into the physiological range and that changes in ATP constitute the means by which metabolism regulates K_{ATP} channel activity. Theoretical calculations suggest that 100 $\mu\text{mol/l}$ ADP is sufficient to shift the ATP concentration-inhibition curve (8). It is also possible that both explanations are correct and that both SUR1 and Kir6.2 serve as metabolic sensors.

IS KIR6.2 ABLE TO SERVE AS A METABOLIC SENSOR?

To reexamine these two hypotheses, we tested the effects of metabolic poisoning on wild-type and mutant K_{ATP} channels. When wild-type Kir6.2 is coexpressed with SUR1 in *Xenopus* oocytes, K_{ATP} currents are almost undetectable due to inhibition by high $[ATP]_i$. However, addition of 3 mmol/l azide, which blocks mitochondrial metabolism

and lowers cytosolic ATP, produces a large increase in current (Fig. 2A and E) (31).

Each of the NBDs of SUR1 contains a number of highly conserved motifs that are involved in Mg-nucleotide binding and hydrolysis and in K_{ATP} channel activation by MgATP and MgADP. These include the Walker A, Walker B, and linker motifs. Mutation of the highly conserved lysine in the Walker A motif of NBD1 (K719) or NBD2 (K1384) abrogates ATP binding (53) and abolishes the ability of MgADP or MgATP to stimulate channel activity (20). Indeed, MgADP now blocks the channel because the inhibitory effect of the nucleotide (mediated via Kir6.2) is unmasked (20). As previously reported (20), when Kir6.2 is coexpressed with SUR1 in which both K719 and K1384 have been mutated (to alanine and methionine, respectively, which we refer to as SUR1-KAKM), azide is no longer able to stimulate channel activity to any significant extent (Fig. 2B and E).

At first sight, this result is in agreement with the proposal that metabolically generated changes in MgADP modulate channel activity: in other words, the channel no longer responds to metabolism because it cannot detect changes in MgADP levels. However, it is important to remember that mutations that abolish MgADP activation will also prevent the shift in the ATP concentration-response curve to higher $[ATP]_i$ produced by MgADP. Thus, the ATP sensitivity of the mutant channel in the *cell* will be much less than that of wild-type channels (millimolar range) and closer to that measured in the excised patch (micromolar range). Thus, it is possible that the lack of metabolic activation of Kir6.2/SUR1-KAKM channels simply reflects a greater ATP sensitivity, which keeps the channel closed even when ATP falls on metabolic inhibition.

To test this idea, we coexpressed wild-type or mutant SUR1 with a mutant form of Kir6.2 with reduced ATP sensitivity. The aim was to shift the ATP sensitivity of Kir6.2 to higher ATP concentrations and then determine whether SUR1 mutations that abolish MgADP activation of the K_{ATP} channel still prevent metabolic activation. If so, then changes in Mg-nucleotides sensed by SUR1 are the sole link between β -cell metabolism and K_{ATP} channel activity. However, if metabolic inhibition is able to activate the channel, then changes in ATP mediated via Kir6.2 may also contribute to metabolic regulation.

Mutation of residue K185 in Kir6.2 to aspartate causes a profound decrease in the ability of ATP to block the K_{ATP} channel: half-maximal block increased from $\sim 10 \mu\text{mol/l}$ to 1.9 mmol/l ATP (24). Consistent with this finding, oocytes expressing Kir6.2-K185D/SUR1 channels had large currents in control solution, which were further activated by metabolic poisoning (Fig. 2C and E).

We next coexpressed Kir6.2-K185D with SUR1-KAKM. Mutation of the Walker A lysines in both NBDs of SUR1 prevented channel activation by MgADP (Fig. 3A and B). These channels also exhibited *smaller* whole-cell currents in control solution than Kir6.2-K185D/SUR1 channels ($P < 0.05$) (Fig. 2D and E). The ATP sensitivity of Kir6.2-K185D/SUR1-KAKM channels, measured in the excised patch, was $1.99 \pm 0.43 \text{ mmol/l}$ ($n = 4$) (Fig. 3C), similar to that of Kir6.2-K185D/SUR1 (1.9 mmol/l) (24) (Fig. 3A and B). Thus, the difference in resting currents (*i*, Fig. 2E) must

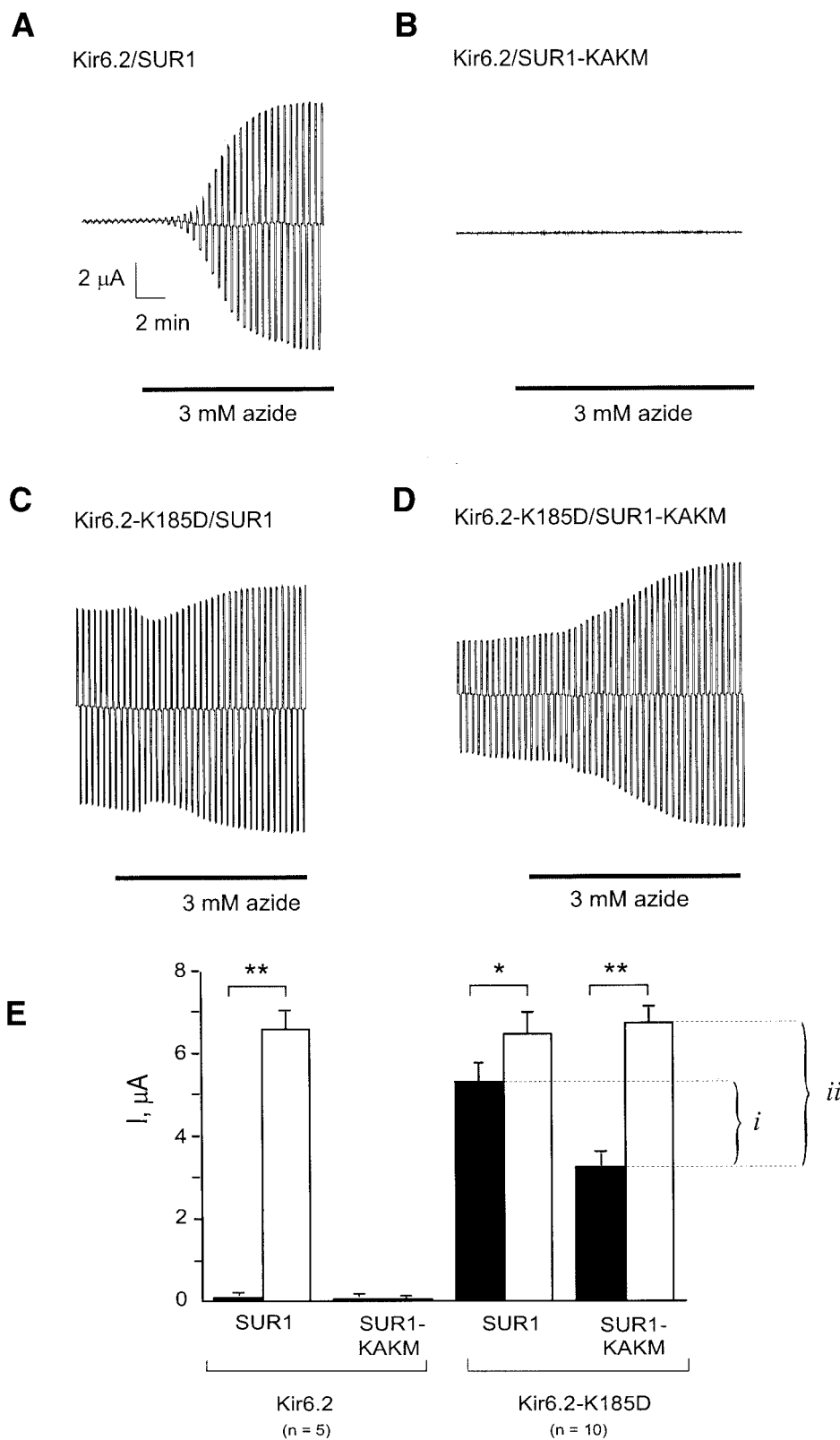


FIG. 2. A-D: Whole-cell currents recorded from oocytes expressing Kir6.2/SUR1 (A), Kir6.2/SUR1-KAKM (B), Kir6.2-K185D/SUR1 (C), or Kir6.2-K185D/SUR1-KAKM (D) channels. Currents were elicited by ± 10 mV voltage steps from a holding potential of -10 mV. Azide (3 mmol/l) was added to the bath as indicated by the bars. For methods, see Gribble et al. (20). The initial decrease in Kir6.2-K185D/SUR1 current produced by azide is not observed when the NBDs of SUR1 are mutated, suggesting that azide blocks nucleotide hydrolysis at the NBDs, as observed for other ABC proteins. **E:** Mean K_{ATP} current (\pm SEM) recorded from oocytes expressing wild-type or mutant channels, as indicated, before (■) and after (□) addition of 3 mmol/l azide. Currents were elicited by -10 mV voltage steps from a holding potential of -10 mV. The number of oocytes is given below the bars. *i*, difference between the mean control Kir6.2-K185D/SUR1 and Kir6.2-K185D/SUR1-KAKM currents; *ii*, difference between control and azide-activated Kir6.2-K185D/SUR1-KAKM currents; * $P < 0.05$; ** $P < 0.01$.

reflect the contribution of MgADP/MgATP to the regulation of K_{ATP} channel activity in control solution, and it is consistent with the idea that cytosolic Mg-nucleotides induce a significant decrease in the apparent ATP sensitivity of the channel in the cell.

If K_{ATP} channel activation on metabolic inhibition

were to be mediated entirely by Mg-nucleotide interaction with SUR1, Kir6.2-K185D/SUR1-KAKM channels should not be activated by azide (because they are insensitive to MgADP). But as Fig. 2D and E shows, Kir6.2-K185D/SUR1-KAKM channels were in fact activated by azide. Thus, this increase in current (*ii*, Fig. 2E) must be

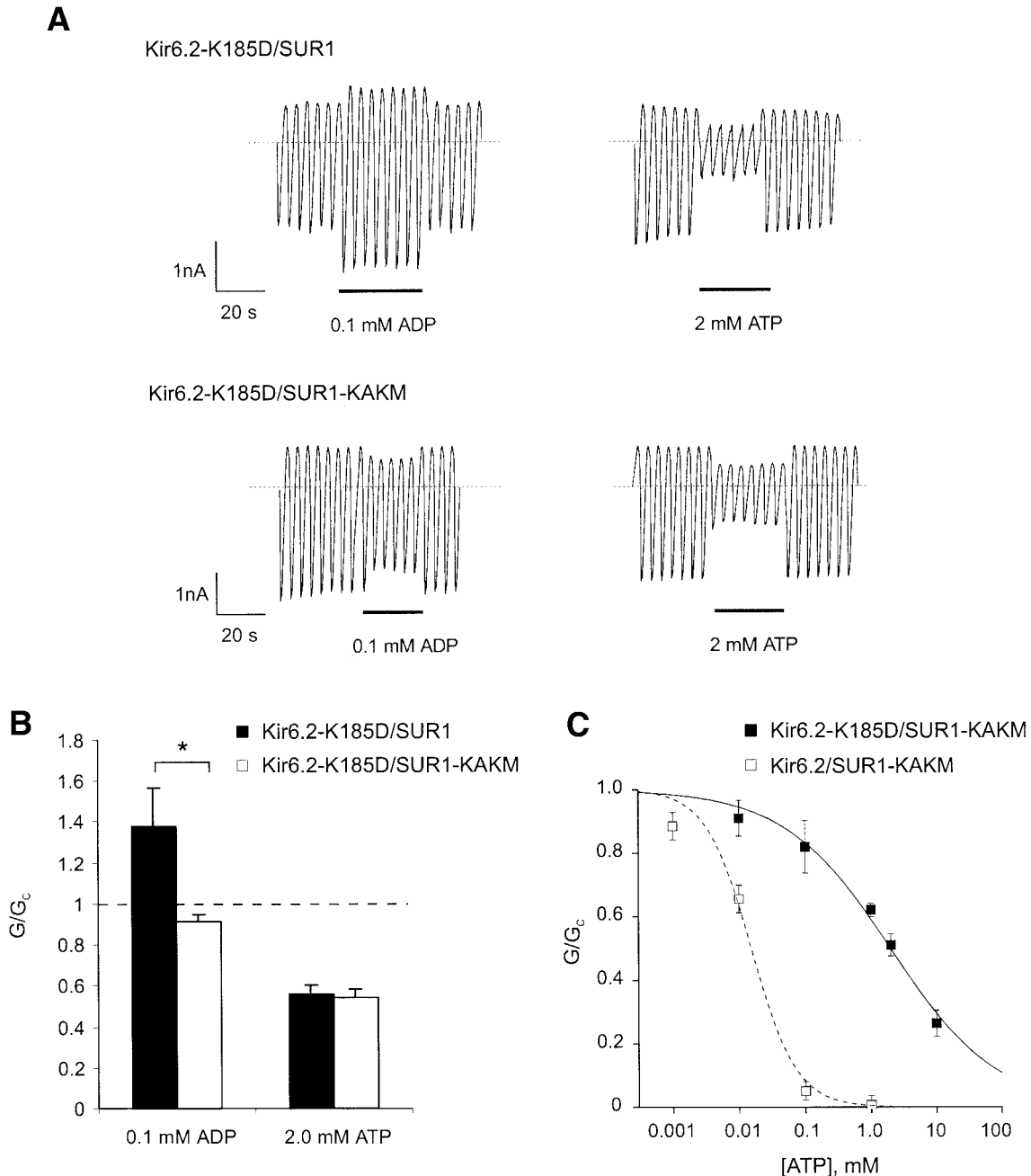


FIG. 3. A: Macroscopic currents recorded from inside-out patches excised from oocytes expressing Kir6.2-K185D/SUR1 or Kir6.2-K185D/SUR1-KAKM in response to 3-s voltage ramps from -110 to $+100$ mV (holding potential, 0 mV). ADP (0.1 mmol/l) or ATP (2.0 mmol/l) was applied to the intracellular solution as indicated. For methods, see Gribble et al. (20). **B:** Mean \pm SEM conductance of Kir6.2-K185D/SUR1 ($n = 4$) or Kir6.2-K185D/SUR1-KAKM ($n = 6$) channels in the presence of ADP (0.1 mmol/l) or ATP (2 mmol/l). Conductance (G) is expressed as a fraction of the mean of that (G_c) obtained in control solution before and after exposure to nucleotide. $*P < 0.05$. **C:** Mean ATP concentration-inhibition relationships for Kir6.2-K185D/SUR1-KAKM channel (■, solid line, $n = 4$) and Kir6.2/SUR1-KAKM (□, dashed line, $n = 6$). The solid line is drawn to the equation $G/G_c = 1/(1 + ([ATP]/IC_{50})^h)$ where $h = 0.57 \pm 0.15$ and $IC_{50} = 1.99 \pm 0.43$ mmol/l.

mediated by the fall in $[ATP]_i$, and sensed by Kir6.2. This is consistent with the observation that Kir6.2 Δ C expressed in the absence of SUR1 ($IC_{50} = 100$ – 200 μ mol/l) also shows a small activation on metabolic poisoning (18).

So why is activation of Kir6.2/SUR1-KAKM channels not observed on metabolic poisoning? We hypothesize that the enhanced ATP sensitivity of this channel in the cell, produced by the loss of Mg-nucleotide activation, is responsible and that the fall in $[ATP]_i$ produced by azide is no longer sufficient to activate the channel. In contrast, the much lower ATP sensitivity of Kir6.2-K185D ensures that

even when Mg-nucleotide activation is abolished by the SUR1-KAKM mutation, the channel is able to respond to metabolically generated changes in ATP. It is important to recognize that the reduction in cellular ATP sensitivity associated with the SUR1-KAKM mutation is much greater than that seen in the excised patch, because of the presence of cytosolic MgADP.

Our experiments indicate that it is not possible to exclude the possibility that changes in $[ATP]_i$ couple β -cell metabolism to K_{ATP} channel closure or that Kir6.2 serves as a metabolic sensor. They are also consistent with the idea

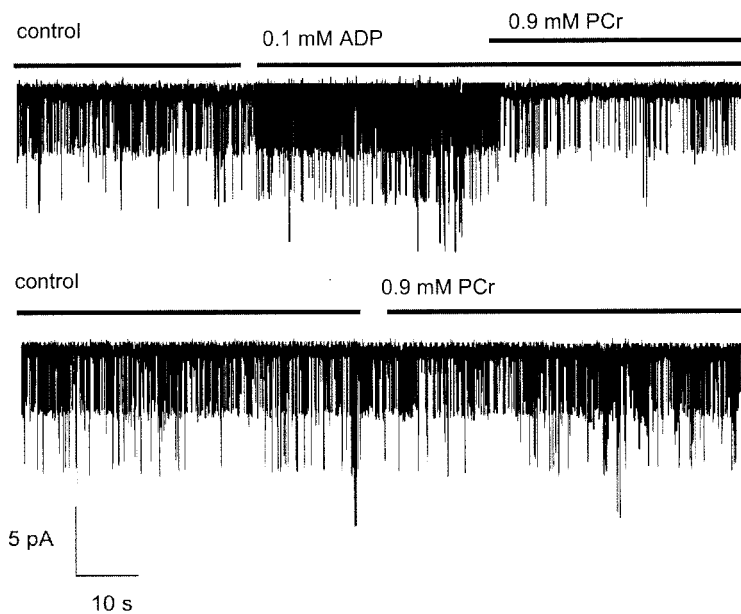
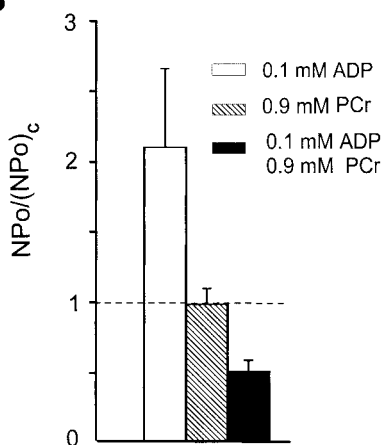
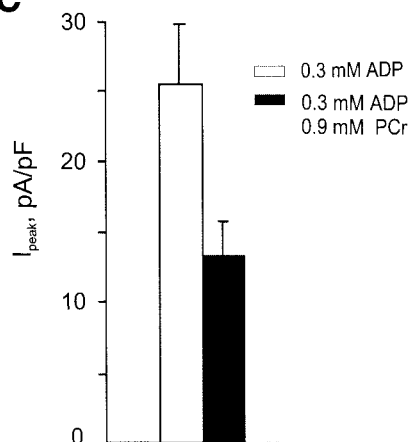
A

B

C


FIG. 4. *A:* Single-channel currents recorded at -60 mV from an inside-out membrane patch excised from a pancreatic β -cell. ADP and PCr were added as indicated by the bars. For methods, see Sakura et al. (15). *B:* Mean K_{ATP} channel activity (NPo), relative to that in control solution [(NPo)_c], recorded in the presence of ADP, PCr, and ADP plus PCr. The number of patches was eight. *C:* Mean peak whole-cell K_{ATP} currents recorded after dialysis with intracellular solution containing 0.3 mmol/l ADP ($n = 16$) or 0.3 mmol/l ADP plus 0.9 mmol/l PCr ($n = 15$). For methods and composition of intracellular solution, see Smith et al. (58).

that cytosolic MgADP (or MgATP hydrolysis) shifts the ATP dose-response curve into a range in which Kir6.2 can respond to physiologically relevant changes in ATP. Changes in MgADP consequent on metabolism are probably too small to influence channel activity by displacing MgATP binding to the NBDs of SUR1 (see above). However, changes in the rate of MgATP hydrolysis might also play a role in coupling metabolism to K_{ATP} channel inhibition, as demonstrated in heart (28).

A role for Kir6.2 in metabolic sensing seems reasonable, given that the ATP sensitivity in the intact cell is considerably less than that of the excised patch (47). It also provides a rational explanation for the fact that a heterozygous Kir6.2 mutation (R201C), which produces a mere twofold decrease in ATP sensitivity in the inside-out patch, leads to loss of insulin secretion and permanent neonatal diabetes in humans (P. Proks, F.A., unpublished observations; see also ref. 5). It, too, is associated with an increase in resting current. Similar results have been reported for transgenic mice expressing Kir6.2 with reduced ATP sensitivity (7). A common polymorphism in Kir6.2 (E23K) is

associated with an increased risk of type 2 diabetes (54), which has been attributed to a small decrease in the channel ATP sensitivity (55) although the mechanism remains controversial (56). Nevertheless, the marked effects on insulin secretion of Kir6.2 mutations, which produce only small changes in ATP sensitivity, argue for a role of Kir6.2 in metabolic sensing.

ROLE OF CREATINE AND ADENYLATE KINASE SHUTTLES

How are the changes in mitochondrial ATP transferred to the plasma membrane? In cardiac muscle, this is mediated by an intracellular phosphotransfer network that shuttles high energy-rich phosphates from the mitochondria to the plasma membrane without much change in $[ATP]_i$ (57). It seems plausible that a similar system exists in β -cells, with creatine kinase (CK) linking ATP generation to K_{ATP} channel closure and adenylate kinase (AK) regulating K_{ATP} channel opening. As proposed for the heart (57), in the microenvironment of the channel AK could convert AMP

and ATP to ADP, promoting channel opening, whereas CK could catalyze the transfer of phosphate from PCr to ADP, producing creatine and ATP and, consequently, channel closure.

Recent studies indicate that the PCr concentration in islets increases on exposure to glucose (42). In addition, PCr reduces the ability of ADP to stimulate K_{ATP} channel activity in β -cells (42) (Fig. 4), suggesting that there may be a membrane-bound CK in the vicinity of the K_{ATP} channel. It will now be important to determine if CK is physically associated with the β -cell K_{ATP} channel, as is the case for cardiac channels (59). Likewise, the extent to which AK modulates K_{ATP} channel activity in β -cells requires further investigation.

CONCLUSIONS

It is apparent from this review that the metabolic regulation of the K_{ATP} channel is extremely complex. PPIs and LC-CoAs exert a tonic effect on the channel ATP, shifting the ATP dose-inhibition curve to higher ATP concentrations. Thus, they contribute to the difference in ATP sensitivity measured in excised patches and intact cells (40,43,47). However, changes in the concentrations of these compounds are probably not involved in modulating K_{ATP} channel activity in response to glucose. This role is played by adenine nucleotides. We propose that both Kir6.2 and SUR1 subunits participate in metabolic sensing, in a kind of molecular pas de deux. Their activity may be further modulated by additional factors that regulate the levels of nucleotides in the immediate environment of the channel. Thus, the K_{ATP} channel may be considered as part of a larger macromolecular complex that links metabolic events to electrical activity and ultimately insulin secretion.

ACKNOWLEDGMENTS

We thank the Wellcome Trust, the European Union (Grow-Beta), and the Royal Society for support. F.M.A. is the GlaxoSmithKline Research Professor of the Royal Society.

REFERENCES

- Ashcroft FM, Rorsman P: Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 54:87-143, 1989
- Gribble FM, Reimann F: Sulphonylurea action revisited: the post-cloning era. *Diabetologia* 46:875-891, 2003
- Seino S, Miki T: Physiological and pathophysiological roles of ATP-sensitive K⁺ channels. *Prog Biophys Mol Biol* 81:133-176, 2003
- Dunne MJ, Cosgrove KE, Shepherd RM, Aynsley-Green A, Lindley KJ: Hyperinsulinism in infancy: from basic science to clinical disease. *Physiol Rev* 84:239-275, 2004
- Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molnes J, Edghill EL, Frayling TM, Temple IK, Mackay D, Shield JP, Sumnik Z, van Rhijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Ellard S, Njolstad PR, Ashcroft FM, Hattersley AT: Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med* 350:1838-1849, 2004
- Ashcroft F, Rorsman P: Type 2 diabetes mellitus: not quite exciting enough? *Hum Mol Genet* 13 (Suppl. 1):R21-R31, 2004
- Koster JC, Marshall BA, Ensor N, Corbett JA, Nichols CG: Targeted overactivity of beta cell K(ATP) channels induces profound neonatal diabetes. *Cell* 100:645-654, 2000
- Hopkins WF, Fatherazi S, Peter-Riesch B, Corkey BE, Cook DL: Two sites for adenine-nucleotide regulation of ATP-sensitive potassium channels in mouse pancreatic beta-cells and HIT cells. *J Membr Biol* 129:287-295, 1992
- Fan Z, Makielski JC: Anionic phospholipids activate ATP-sensitive potassium channels. *J Biol Chem* 272:5388-5395, 1997
- Baukowitz T, Schulte U, Oliver D, Herlitz S, Krauter T, Tucker SJ, Ruppberg JP, Fakler B: PIP2 and PIP as determinants for ATP inhibition of KATP channels. *Science* 282:1141-1144, 1998
- Larsson O, Deeney JT, Branstrom R, Berggren PO, Corkey BE: Activation of the ATP-sensitive K⁺ channel by long chain acyl-CoA: a role in modulation of pancreatic beta-cell glucose sensitivity. *J Biol Chem* 271:10623-10626, 1996
- Gribble FM, Proks P, Corkey BE, Ashcroft FM: Mechanism of cloned ATP-sensitive potassium channel activation by oleoyl-CoA. *J Biol Chem* 273:26383-26387, 1998
- Schulze D, Rapedius M, Krauter T, Baukowitz T: Long-chain acyl-CoA esters and phosphatidylinositol phosphates modulate ATP inhibition of KATP channels by the same mechanism. *J Physiol* 552:357-367, 2003
- Inagaki N, Gono T, Clement JPt, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166-1170, 1995
- Sakura H, Ammala C, Smith PA, Gribble FM, Ashcroft FM: Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic beta-cells, brain, heart and skeletal muscle. *FEBS Lett* 377:338-344, 1995
- Markworth E, Schwanstecher C, Schwanstecher M: ATP4- mediates closure of pancreatic beta-cell ATP-sensitive potassium channels by interaction with 1 of 4 identical sites. *Diabetes* 49:1413-1418, 2000
- Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JPt, Boyd AE, 3rd, Gonzalez G, Herrera-Sosa H, Ngy K, Bryan J, Nelson DA: Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423-426, 1995
- Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM: Truncation of Kir6.2 produces ATP-sensitive K⁺ channels in the absence of the sulphonylurea receptor. *Nature* 387:179-183, 1997
- Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JPt, Gonzalez G, Aguilar-Bryan L, Permutt MA, Bryan J: Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* 272:1785-1787, 1996
- Gribble FM, Tucker SJ, Ashcroft FM: The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. *Embo J* 16:1145-1152, 1997
- Shyng S, Ferrigni T, Nichols CG: Regulation of KATP channel activity by diazoxide and MgADP: distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. *J Gen Physiol* 110:643-654, 1997
- Tucker SJ, Gribble FM, Proks P, Trapp S, Ryder TJ, Haug T, Reimann F, Ashcroft FM: Molecular determinants of KATP channel inhibition by ATP. *EMBO J* 17:3290-3296, 1998
- Drain P, Li L, Wang J: KATP channel inhibition by ATP requires distinct functional domains of the cytoplasmic C terminus of the pore-forming subunit. *Proc Natl Acad Sci U S A* 95:13953-13958, 1998
- John SA, Weiss JN, Xie LH, Ribalet B: Molecular mechanism for ATP-dependent closure of the K⁺ channel Kir6.2. *J Physiol* 552:23-34, 2003
- Tanabe K, Tucker SJ, Ashcroft FM, Proks P, Kioka N, Amachi T, Ueda K: Direct photoaffinity labeling of Kir6.2 by [γ -(32)P]ATP-[γ]-azidoanilide. *Biochem Biophys Res Commun* 272:316-319, 2000
- Wang C, Wang K, Wang W, Cui Y, Fan Z: Compromised ATP binding as a mechanism of phosphoinositide modulation of ATP-sensitive K⁺ channels. *FEBS Lett* 532:177-182, 2002
- Gribble FM, Tucker SJ, Haug T, Ashcroft FM: MgATP activates the beta cell KATP channel by interaction with its SUR1 subunit. *Proc Natl Acad Sci U S A* 95:7185-7190, 1998
- Zingman LV, Alekseev AE, Bienengraeber M, Hodgson D, Karger AB, Dzeja PP, Terzic A: Signaling in channel/enzyme multimers: ATPase transitions in SUR module gate ATP-sensitive K⁺ conductance. *Neuron* 31:233-245, 2001
- Matsuo M, Tanabe K, Kioka N, Amachi T, Ueda K: Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. *J Biol Chem* 275:28757-28763, 2000
- Kakei M, Kelly RP, Ashcroft SJ, Ashcroft FM: The ATP-sensitivity of K⁺ channels in rat pancreatic B-cells is modulated by ADP. *FEBS Lett* 208:63-66, 1996
- Gribble FM, Ashfield R, Ammala C, Ashcroft FM: Properties of cloned ATP-sensitive K⁺ currents expressed in *Xenopus* oocytes. *J Physiol* 498:87-98, 1997
- Bokvist K, Ammala C, Ashcroft FM, Berggren PO, Larsson O, Rorsman P: Separate processes mediate nucleotide-induced inhibition and stimulation of the ATP-regulated K(+) channels in mouse pancreatic beta-cells. *Proc R Soc Lond B Biol Sci* 243:139-144, 1991
- Deeney JT, Prentki M, Corkey BE: Metabolic control of beta-cell function. *Semin Cell Dev Biol* 11:267-275, 2000
- Silva JP, Kohler M, Graff C, Oldfors A, Magnuson MA, Berggren PO,

- Larsson NG: Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. *Nat Genet* 26:336–340, 2000
35. Chan CB, Saleh MC, Koshkin V, Wheeler MB: Uncoupling protein 2 and islet function. *Diabetes* 53 Suppl 1:S136–142, 2004
 36. Detimary P, Jonas JC, Henquin JC: Possible links between glucose-induced changes in the energy state of pancreatic B cells and insulin release: unmasking by decreasing a stable pool of adenine nucleotides in mouse islets. *J Clin Invest* 96:1738–1745, 1995
 37. Detimary P, Dejonghe S, Ling Z, Pipeleers D, Schuit F, Henquin JC: The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in beta cells but not in alpha cells and are also observed in human islets. *J Biol Chem* 273:33905–33908, 1998
 38. Kennedy HJ, Pouli AE, Ainscow EK, Jouaville LS, Rizzuto R, Rutter GA: Glucose generates sub-plasma membrane ATP microdomains in single islet beta-cells: potential role for strategically located mitochondria. *J Biol Chem* 274:13281–13291, 1999
 39. Ghosh A, Ronner P, Cheong E, Khalid P, Matschinsky FM: The role of ATP and free ADP in metabolic coupling during fuel-stimulated insulin release from islet beta-cells in the isolated perfused rat pancreas. *J Biol Chem* 266:22887–22892, 1991
 40. Niki I, Ashcroft FM, Ashcroft SJ: The dependence on intracellular ATP concentration of ATP-sensitive K-channels and of Na,K-ATPase in intact HIT-T15 beta-cells. *FEBS Lett* 257:361–364, 1989
 41. Doliba NM, Vatamaniuk MZ, Buettinger CW, Qin W, Collins HW, Wehrli SL, Carr RD, Matschinsky FM: Differential effects of glucose and glyburide on energetics and Na⁺ levels of betaHC9 cells: nuclear magnetic resonance spectroscopy and respirometry studies. *Diabetes* 52:394–402, 2003
 42. Krippeit-Drews P, Backer M, Dufer M, Drews G: Phosphocreatine as a determinant of K(ATP) channel activity in pancreatic beta-cells. *Pflugers Arch* 445:556–562, 2003
 43. Ashcroft FM: Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* 11:97–118, 1988
 44. Duchon MR, Smith PA, Ashcroft FM: Substrate-dependent changes in mitochondrial function, intracellular free calcium concentration and membrane channels in pancreatic beta-cells. *Biochem J* 294:35–42, 1993
 45. Kiranadi B, Bangham JA, Smith PA: Inhibition of electrical activity in mouse pancreatic beta-cells by the ATP/ADP translocase inhibitor, bongkrekic acid. *FEBS Lett* 283:93–96, 1991
 46. Gribble FM, Loussouarn G, Tucker SJ, Zhao C, Nichols CG, Ashcroft FM: A novel method for measurement of submembrane ATP concentration. *J Biol Chem* 275:30046–30049, 2000
 47. Schmid-Antomarchi H, de Weille J, Fosset M, Lazdunski M: The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K⁺ channel in insulin secreting cells. *J Biol Chem* 262:15840–15844, 1987
 48. Shyng SL, Barbieri A, Gumusboga A, Cukras C, Pike L, Davis JN, Stahl PD, Nichols CG: Modulation of nucleotide sensitivity of ATP-sensitive potassium channels by phosphatidylinositol-4-phosphate 5-kinase. *Proc Natl Acad Sci USA* 97:937–941, 2000
 49. Xie LH, Horie M, Takano M: Phospholipase C-linked receptors regulate the ATP-sensitive potassium channel by means of phosphatidylinositol 4,5-bisphosphate metabolism. *Proc Natl Acad Sci USA* 96:15292–15297, 1999
 50. Schulze D, Krauter T, Fritzenschaft H, Soom M, Baukrowitz T: Phosphatidylinositol 4,5-bisphosphate (PIP₂) modulation of ATP and pH sensitivity in Kir channels: a tale of an active and a silent PIP₂ site in the N terminus. *J Biol Chem* 278:10500–10505, 2003
 51. Gilon P, Henquin JC: Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev* 22:565–604, 2001
 52. Straub SG, Sharp GW: A wortmannin-sensitive signal transduction pathway is involved in the stimulation of insulin release by vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide. *J Biol Chem* 271:1660–1668, 1996
 53. Ueda K, Inagaki N, Seino S: MgADP antagonism to Mg²⁺-independent ATP binding of the sulfonylurea receptor SUR1. *J Biol Chem* 272:22983–22986, 1997
 54. Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM: Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572, 2003
 55. Schwanstecher C, Meyer U, Schwanstecher M: K(IR)6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic beta-cell ATP-sensitive K(+) channels. *Diabetes* 51:875–879, 2002
 56. Riedel MJ, Boora P, Steckley D, de Vries G, Light PE: Kir6.2 polymorphisms sensitize beta-cell ATP-sensitive potassium channels to activation by acyl CoAs: a possible cellular mechanism for increased susceptibility to type 2 diabetes? *Diabetes* 52:2630–2635, 2003
 57. Dzeja PP, Terzic A: Phosphotransfer reactions in the regulation of ATP-sensitive K⁺ channels. *FASEB J* 12:523–529, 1998
 58. Smith PA, Proks P, Moorhouse A: Direct effects of tolbutamide on mitochondrial function, intracellular Ca²⁺ and exocytosis in pancreatic beta-cells. *Pflugers Arch* 437:577–588, 1999
 59. Crawford RM, Ranki HJ, Botting CH, Budas GR, Jovanovic A: Creatine kinase is physically associated with the cardiac ATP-sensitive K⁺ channel in vivo. *FASEB J* 16:102–104, 2002