Sulfonylurea and K⁺-Channel Opener Sensitivity of K\textsubscript{ATP} Channels

Functional Coupling of Kir6.2 and SUR1 Subunits

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Abstract

The sensitivity of K\textsubscript{ATP} channels to high-affinity block by sulfonylureas and to stimulation by K⁺ channel openers and MgADP (PCOs) is conferred by the regulatory sulfonylurea receptor (SUR) subunit, whereas ATP inhibits the channel through interaction with the inward rectifier (Kir6.2) subunit. Phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2}) profoundly antagonized ATP inhibition of K\textsubscript{ATP} channels expressed from cloned Kir6.2+SUR1 subunits, but also abolished high affinity tolbutamide sensitivity. By stabilizing the open state of the channel, PIP\textsubscript{2} drives the channel away from closed state(s) that are preferentially affected by high affinity tolbutamide binding, thereby producing an apparent loss of high affinity tolbutamide inhibition. Mutant K\textsubscript{ATP} channels (Kir6.2[ΔN30] or Kir6.2[L164A]), coexpressed with SUR1) also displayed an “uncoupled” phenotype with no high affinity tolbutamide block and with intrinsically higher open state stability. Conversely, Kir6.2[R176A]+SUR1 channels, which have an intrinsically lower open state stability, displayed a greater high affinity fraction of tolbutamide block. In addition to antagonizing high-affinity block by tolbutamide, PIP\textsubscript{2} also altered the stimulatory action of the PCOs, diazoxide and MgADP. With time after PIP\textsubscript{2} application, PCO stimulation first increased, and then subsequently decreased, probably reflecting a common pathway for activation of the channel by stimulatory PCOs and PIP\textsubscript{2}. The net effect of increasing open state stability, either by PIP\textsubscript{2} or mutagenesis, is an apparent “uncoupling” of the Kir6.2 subunit from the regulatory input of SUR1, an action that can be partially reversed by screening negative charges on the membrane with poly-L-lysine.

Keywords: K⁺ current • sulfonylurea • MgADP • diazoxide • PIP\textsubscript{2}

Introduction

The 10 yr that followed the discovery of ATP-sensitive (K\textsubscript{ATP}) channels (Noma, 1983) led to the delineation of complex regulation by intracellular nucleotides and pharmacological agents (reviewed in Ashcroft, 1988; Nichols and Lederer, 1991; Terzic et al., 1994). The last 3 yr have seen a renewed interest in the regulation of ATP-sensitive potassium channels as a result of the cloning of the constituent subunits (Aguilar-Bryan et al., 1995; Inagaki et al., 1995, 1996). Uniquely, K\textsubscript{ATP} channels are normally formed as a complex of sulfonylurea receptor (SUR\textsubscript{x})\textsuperscript{1} and inward rectifier (Kir6.x) subunits (Inagaki et al., 1995, 1997; Clement et al., 1997; Shyng and Nichols, 1997). Recent studies demonstrate that the Kir6.x subunits form the pore, and control the hallmark inhibition by ATP (Shyng et al., 1997a; Tucker et al., 1997, 1998; Drain et al., 1998), whereas the SUR\textsubscript{x} subunit controls the sensitivity to the inhibitory sulfonylurea drugs, and to activating nucleotide diphosphates and potassium channel opening drugs (Aguilar-Bryan et al., 1995; Inagaki et al., 1996; Isomoto et al., 1996; Nichols et al., 1996; Shyng et al., 1997b; Gribble et al., 1997a,b; Schwanstecher et al., 1998).

Deletion of up to ~36 amino acids from the COOH terminus of Kir6.2 results in the generation of ATP-sensitive channels in the absence of SUR\textsubscript{x} subunits (Tucker et al., 1997; Zerangue et al., 1999), but these channels are not activated by MgADP or potassium channel openers (PCOs), nor are they inhibited at high affinity by sulfonylurea drugs, consistent with these agents acting through the SUR\textsubscript{x} subunit (Gribble et al., 1997a). MgATP clearly binds to the nucleotide binding folds of SUR1 (Ueda et al., 1997), and ATP hydrolysis seems to be required for binding PCOs (Schwanstecher et al., 1998) and transduction (Nichols et al., 1996; Gribble et al., 1997b; Shyng et al., 1997a) of the stimulatory PCO signal to the channel. The physical nature of the coupling of SUR\textsubscript{x} to Kir6.x subunits is essentially unknown at the present time, although intriguingly, Clement et al. (1997) demonstrated that Kir6.2 could be labeled with azido-glibenclamide only in the presence of SUR1 subunits, consistent with a tight physical association of the two subunits (Lorenz et al., 1998). In the present study, we have explored the functional coupling of SUR1 to Kir6.2. The results dem-

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\textsuperscript{1}Abbreviations used in this paper: Kir, inward rectifier; PCO, potassium channel opener; PIP\textsubscript{2}, phosphatidylinositol 4,5-bisphosphate; SUR, sulfonylurea receptor.
onstrate that the pharmacological control of \( K_{\text{ATP}} \) channel function through SUR1 subunits can be “uncoupled” when the channel open-state stability is increased, either by mutation of the Kir6.2 subunit, or by manipulation of the phospholipid composition of the membrane. The results also suggest that nucleotide hydrolysis and PCO binding stimulate the channel activity by a convergent pathway with phosphatidylinositol 4,5-bisphosphate (PIP2). A preliminary report of these results was made to the Biophysical Society (annual meeting, Kansas City, MO, February, 1998).

methods

Molecular Biology

Kir6.2 mutations were prepared using PCR methods. Resulting PCR products were subcloned into the EcoRI-ClaI sites of the mammalian expression vector pCMV6b. SUR1 was cloned into the pECE expression vector. The nucleotide sequences of the mutant Kir6.2 constructs were verified by fluorescence-based cycle sequencing using AmpliTaq DNA polymerase, FS (Perkin-Elmer Cetus Corp.), and an ABI PRISM DNA sequencer (Perkin-Elmer Cetus Corp.).

Expression of \( K_{\text{ATP}} \) Channels in COSm6 Cells

COSm6 cells were plated at a density of \( \sim 2.5 \times 10^4 \) cells per well (30-mm six-well dishes) and cultured in Dulbecco’s modified Eagle medium plus 10 mM glucose (DMEM-HG), supplemented with fetal calf serum (10%). The next day, cells were transfected by incubation for 4 h at 37°C in DMEM containing 10% nuserum, 0.4 mg/ml diethylaminoethyl-dextran, 100 μM chloroquine, and 5 μg each of pCMV6bKir6.2, pECE-SUR1, and pECE-green fluorescent protein cDNA. Cells were subsequently incubated for 2 min in HEPES-buffered salt solution containing DMSO (10%), and returned to DMEM-HG plus 10% FCS. Cells were assayed for \( K_{\text{ATP}} \) currents by patch-clamp measurements, 2-4 d after transfection.

Patch-Clamp Measurements

Patch-clamp experiments were made at room temperature, in an oil-gate chamber that allowed the solution bathing the exposed surface of the isolated patch to be changed rapidly. Micro pipettes were pulled from thin-walled glass (WPI Inc.) on a horizontal puller (Sutter Instrument Co.). Electrode resistance was typically 0.5-1 MΩ when filled with K-INT solution (see below). Microelectrodes were “sealed” onto cells that fluoresced green under UV illumination by applying light suction to the rear of the pipette. Inside-out patches were obtained by lifting the electrode tip through the oil-gate. Inside-out patches were obtained by lifting the electrode and then passing the electrode tip through the oil-gate. Microelectrodes were “sealed” onto cells that fluoresced green under UV illumination by applying light suction to the rear of the oil-gate. Microelectrodes were “sealed” onto cells that fluoresced green under UV illumination by applying light suction to the rear of the oil-gate. Microelectrodes were “sealed” onto cells that fluoresced green under UV illumination by applying light suction to the rear of the oil-gate.

Results

High Affinity Sulfonylurea Sensitivity Is Lost After Kir6.2 NH2-Terminal Deletion

Gribble et al. (1997a) reported that tobutamide inhibition of Kir6.2+SUR1 coexpressed channels in Xenopus oocytes is biphasic, consisting of low and high affinity components. The mechanistic basis of the biphasic response to tobutamide is presently unknown (see discussion), but it is clear that high affinity sulfonylurea interaction is with the SUR1 subunit (Aguilar-Bryan et al., 1995), whereas a low affinity action may occur through direct interaction with the Kir6.2 subunit (Gribble et al., 1997a). As shown in Fig. 1, similar biphasic dose-response curves are seen for both wild-type Kir6.2+SUR1 (WT+SUR1) channels and for Kir6.2[K185Q]+SUR1 channels expressed in COSm6 cells. The K185Q mutation in Kir6.2 reduces ATP sensitivity, possibly by altering ATP binding affinity, but does not affect the ATP-independent open probability (Tucker et al., 1997; Koster et al., 1999). In contrast, Kir6.2[ΔN2-30]+SUR1 channels also have a reduced ATP sensitivity, which in this case results from open-state stabilization that is reflected by near continuous bursting at the single channel level (Koster et al., 1999), and these channels show only low affinity inhibition by tobutamide (Fig. 1A). This raises alternate possibilities that high affinity tobutamide block is lacking from Kir6.2[ΔN2-30] channels because the NH2 terminus is physically involved in “coupling” to the regulatory effects of SUR1, or because the high affinity inhibitory effect of tobutamide depends on channel open state stability.

High Affinity Sulfonylurea Sensitivity Is Lost After PIP2 Treatment of Wild-Type Channels

We can explore the correlation between tobutamide sensitivity and open-state stability of the channel by applying PIP2. PIP2 increases the channel open probability by increasing bursting behavior of the single channel and decreases the sensitivity to ATP (Baukrowitz et al., 1998; Shyng and Nichols, 1998). Although direct experimental proof is not available, both actions can be...
explained by models in which the action of PIP$_2$ is to stabilize the channel open or bursting state, with ATP binding to, and stabilizing, the channel closed state (Shyng et al., 1997a). As shown in Fig. 2, treatment of wild-type Kir6.2+SUR1 channels with PIP$_2$ leads to increased overall channel activity and loss of ATP sensitivity (Baukrowitz et al., 1998; Shyng and Nichols, 1998). Concomitant with this increase in open-state stability, there is a gradual and complete loss of high affinity tolbutamide block (Fig. 2, A and C). The rate of loss of both ATP sensitivity and high tolbutamide sensitivity (Fig. 2 B) are quite variable from patch to patch. However, there is a reasonable correlation between the tolbutamide inhibition and ATP sensitivity (Fig. 2 D). The loss of high affinity tolbutamide inhibition could occur because the high affinity component actually changes affinity (i.e., the real, or apparent, binding affinity of tolbutamide is reduced), or because high affinity binding fails to cause inhibition of channel activity.
As shown in Fig. 3, the latter explanation is correct; with time after addition of PIP$_2$, the dose–response relationship can be fit by assuming that the high affinity inhibition becomes a progressively smaller fraction of the [tolbutamide]-inhibition relationship. Data points at intermediate times cannot be fit by assuming a constant high affinity fraction, with reduced affinity. This is consistent with an effect of PIP$_2$ on the coupling of high affinity binding to channel inhibition, not on modifying tolbutamide binding itself.

**High Affinity Sulfonylurea Sensitivity Depends on the Channel Open-State Stability**

Since PIP$_2$ and NH$_2$-terminal deletion both increase the channel open state stability (Fan and Makielski, 1997; Shyng and Nichols, 1998; Koster et al., 1999), the loss of high affinity tolbutamide sensitivity in NH$_2$-terminal truncated channels, and on wild-type channels treated with PIP$_2$, suggests that the coupling of high affinity tolbutamide binding (at SUR1; Aguilar-Bryan et al., 1995) to channel inhibition may also depend on the open-state stability. To examine this possibility further, we have measured the tolbutamide sensitivity of channels expressed from Kir6.2[R176A]+SUR1, and Kir6.2[L164A]+SUR1 channels, which have intrinsically very low, and high, open-state stabilities, respectively (Shyng and Nichols, 1998). Kir6.2[R176A]+SUR1 channels have a much lower intrinsic open probability ($P_{\text{zero}}$) than wild-type channels ($P_{\text{zero}} < 0.1$), due to reduced PIP$_2$ affinity (Fan and Makielski, 1997; Huang et al., 1998; Shyng and Nichols, 1998). As shown in Fig. 4 A, it is clear that these channels have a larger high affinity component of tolbutamide inhibition than wild-type channels, but which is again lost as $P_{\text{zero}}$ increases after treatment with PIP$_2$ (Fig. 4 B). In contrast, Kir6.2[L164A]+SUR1 channels have a very high $P_{\text{zero}}$ (>0.85), corresponding to an intrinsic ATP sensitivity of $K_{1/2,\text{ATP}}$ ~ 1mM (data not shown), due to the open-state stabilizing effect of this mutation. As shown in Fig. 4 A, there is essentially no high affinity component of tolbutamide inhibition of Kir6.2[L164A]+SUR1 channels. On average, 100 µM tolbutamide inhibited wild-type Kir6.2+SUR1,
Kir6.2[L164A] + SUR1, and Kir6.2[R176A] + SUR1 channels by 33%, 36%, and 77%, respectively (n = 3 in each case).

MgADP Stimulation and Diazoxide Stimulation of Channel Activity Disappears with PIP2 Stimulation

Activation of wild-type Kir6.2+SUR1 channels by MgADP and diazoxide, at a fixed [ATP], is quite variable from patch to patch (Figs. 5 B and 6 B). As shown in Figs. 5 A and 6 A, the ability of these agents to stimulate channel activity changes after PIP2 stimulation, and in a qualitatively similar way for both Kir6.2[ΔN2-30] + SUR1 and wild-type (Kir6.2 + SUR1) channels. In each case, the stimulation tends to increase, but then gradually falls to zero with time after PIP2 application. The time course of this effect is also quite variable from patch to patch (Figs. 5 B and 6 B), but is reasonably well correlated with the accompanying change of ATP sensitivity (Figs. 5 D and 6 D). This result indicates that the stimulatory action of the PCOs, like ATP sensitivity itself, is not a fixed parameter of channel function, but is probably dependent on the open-state stability of the channel (Shyng and Nichols, 1998). As PIP2 increasingly stabilizes the open state of the channel, sojourns in an ATP-accessible closed state become less and less frequent (Baukrowitz et al., 1998; Shyng and Nichols, 1998). The present results are also consistent with PCOs acting by shifting the equilibrium between the open and closed states (see Shyng et al., 1997b), such that as the channel open-state stability approaches maximal, the stimulatory effect of the PCOs saturates.

Figure 3. Mechanism of the PIP2-induced loss of tolbutamide block. (A) Representative current recorded from inside-out membrane patches containing wild-type KATP channels exposed to differing [tolbutamide] or 30 μM ATP, as shown. The gaps in the record are 4.5, 2, and 1 min. The dashed line indicates zero current (determined in 5 mM ATP). (B) Plot of relative current (Irel) versus [tolbutamide] for trace segments shown in A, both before (●) and with time after (○) PIP2 application. Data at 10 mM tolbutamide are from control and 10-min time points. Fitted lines correspond to a least squares fit of the sum of two Hill equations as described in Fig. 1, with the fraction of high affinity inhibition allowed to vary (37, 23, 13, 4, % at 0, 4.5, 7, 8 and 10 min) as indicated, after PIP2 application. The insert shows data only at 7 min. The dashed lines correspond to (A, dashed) the same curve as above, and (B, dotted) a least squares fit of the sum of two Hill equations, with the fraction of high affinity inhibition held constant at 37%, but with the affinity (i.e., K1/2) allowed to vary. It is clear that high affinity block disappears because the high affinity fraction disappears, not because there is a change in the sensitivity of this component.

Treatment with polycations can reverse the stimulatory actions of PIP2 on open probability and ATP sensitivity (Deutsch et al., 1994; Shyng and Nichols, 1998), probably by shielding the negative charges introduced by PIP2. As shown in Fig. 7, some reversal of both tolbutamide insensitivity and loss of PCO action is observed
when patches are subsequently treated with polylysine. However, some irreversible loss of high affinity tolbutamide sensitivity, as well as of diazoxide and MgADP stimulation, also occurs after prolonged PIP2 treatment, such that polylysine may only partially restore the SUR1 coupling (e.g., Fig. 7 A), even though ATP sensitivity can be restored to, or beyond, control levels (see discussion).

discussion
Loss of High Affinity Tolbutamide Sensitivity with Increased Open Probability
A biphasic dose–response relationship for tolbutamide inhibition of $K_{ATP}$ channels was demonstrated by Gribble et al. (1997a), but the mechanistic basis was not made clear. When the high affinity component is saturated, there is an ~40% reduction of wild-type currents. The high affinity binding of sulfonylureas is to the SUR1 subunit (Aguilar-Bryan et al., 1995; Gribble et al., 1997a), and channel inhibition results from an allosteric effect on the channel. By contrast, the low affinity inhibitory effect results from a direct interaction with the Kir6.2 subunit itself (Gribble et al., 1997a), and might be a pore-blocking action. The present results demonstrate that the high affinity, physiologically relevant, action can be abolished by increasing the open state stability (and hence $P_{o,zero}$) with PIP2, or by deleting the channel NH2 terminus. Kir6.2[ΔN2-30] channels, which have an intrinsically higher open state stability (Koster et al., 1999), show essentially no high affinity tolbutamide sensitivity (Fig. 1). Hence, although the high affinity sulfonylurea binding site is clearly on the SUR1 subunit (Aguilar-Bryan et al., 1995), the inhibitory effect on $K_{ATP}$ channel activity will depend critically on the functional state and molecular nature of the Kir subunit. This prediction is dramati-
cally borne out by the results (Fig. 4), which show that a mutant with even higher intrinsic open-state stability (Kir6.2[L164A]), is almost completely insensitive to tolbutamide, with no high-affinity inhibition. By contrast, in a mutant with intrinsically low open probability (Kir6.2[R176A]), putatively due to reduced PIP₂ affinity, tolbutamide sensitivity is almost all high affinity, under ambient conditions after patch isolation. However, subsequent treatment with PIP₂ still abolishes high affinity tolbutamide inhibition, as the open-state stability increases to, and beyond, that of the wild-type channel (i.e., the open probability increases and $K_{1/2,ATP}$ decreases; Fig. 4 B).

The present findings are significant for understanding sulfonylurea sensitivity of $K_{ATP}$ channels. They demonstrate that sulfonylurea sensitivity will depend critically on the open-state stability of the channels (manifested by open probability in the absence of ATP¹). This can change dramatically in inside-out membrane patches as a consequence of “run down” and “run up.” Run down is a gradual, variable, and probably multifactorial, reduction of channel activity, often associated with decreased open probability and increased $K_{1/2,ATP}$ (Thuringer and Escande, 1989; Deutsch and Weiss, 1993). A significant mechanism of run down is probably decreasing levels of phosphorylated phosphatidylinositol in the cell membrane. Such run down can be reversed, and the channels run up, by application of exogenous PIP₂ (Baukrowitz et al., 1998; Shyng and Nichols, 1998), MgATP (Takano et al., 1990), and by application of MgUDP (Tung and Kurachi, 1993; Terzic et al., 1994). Interestingly, Brady et al. (1998) reported an “operative condition-dependent response” of $K_{ATP}$ channels to sulfonylureas, in which stimulation of channel activity by MgUDP led to a loss of glibenclamide sensitivity, but only if the channels had not previously run down. It is likely that in vivo variability of ATP sensitivity (Findlay and Fairve, 1991) reflects cell-to-cell
variability of the open state stability, resulting in turn from variability of membrane phospholipid levels. Similarly, in vivo variability of sulfonylurea sensitivity under different conditions (Findlay, 1993; Venkatesh et al., 1991; Mukai et al., 1998) is also likely to reflect changes in open-state stability and accessibility of the closed channel.

**Figure 6.** PIP$_2$ effect on diazoxide stimulation from cells expressing Kir6.2+SUR1 and Kir6.2[ΔN2-30]+SUR1 channels. (A) Representative currents from inside-out patches containing wild-type (Kir6.2+SUR1) or Kir6.2[ΔN2-30]+SUR1 channels. The patches were exposed to diazoxide and MgATP, or PIP$_2$, as indicated. The dashed line represents zero current determined in 5 mM ATP. (Top) Open probability, estimated from noise analysis of 10 s of current in zero ATP, is indicated. (B) Plot of the percent diazoxide stimulation versus time in PIP$_2$ for five individual patches containing wild-type K$_{ATP}$ channels. Percent diazoxide stimulation was determined by calculating the increase in current in the presence of diazoxide and MgATP relative to current in MgATP alone, and then expressing this value as a fraction of the maximal current observed in the absence of ATP. (C) Percent stimulation by diazoxide and inhibition by ATP before (Precontrol) or after (Post PIP$_2$) application of PIP$_2$ (3-20 min) from patches in B. (D) Percent diazoxide stimulation versus K$_{1/2,ATP}$ after application of PIP$_2$ to patches from B.

**PIP$_2$ Activation Masks PCO Actions**

It is clear that PIP$_2$ activation of K$_{ATP}$ channels and other inward rectifiers does not require the presence of a SUR1 subunit, and probably results from a direct interaction of PIP$_2$ with the cytoplasmic portion of the channel protein itself (Hilgemann and Ball, 1996; Fan and Makielski, 1997; Baukrowitz et al., 1998; Huang et al., 1998; Shyng and Nichols, 1998). The present results indicate that PCO sensitivity, like ATP sensitivity (Baukrowitz et al., 1998; Shyng and Nichols, 1998) is a variable, dynamically dependent on membrane phospholipid levels rather than a fixed parameter. After PIP$_2$ application to inside-out patches, there is generally first an increase in the stimulatory action of PCOs, and then a gradual disappearance of their action as the PIP$_2$ stimulation saturates, such that, even though ATP inhibition is still observable at high concentrations, there is no relief of this inhibition by PCOs (Figs. 5 and 6). As discussed in Shyng and Nichols (1998) and Baukrowitz et al. (1998), it is likely that membrane phospholipid levels are variable from cell to cell, and that such variability accounts for the cell-to-cell variability of ATP sensitivity that is observed physiologically (Findlay and Faivre, 1991). By the same reasoning, the variable stimulatory action of PCOs (see, e.g., Figs. 5 B and 6 B) might be a result of cell-to-cell variability of membrane...
phospholipid levels. The results raise the question: How does the membrane phospholipid level determine the PCO sensitivity? One possibility is that PIP\(_2\) affects ATP hydrolysis, or PCO binding to the SUR1 subunit. However, as we have previously suggested (Shyng et al., 1997b), it seems likely that PCOs act ultimately to stabilize the open state of the channel itself, just as the phospholipids do. Therefore, the lack of PCO effects after elevation of phospholipids, is likely to be a consequence of the convergent action of these two agents on the energetic stability of the open state relative to the ATP-accessible closed state.

The Role of SUR Subunits in Controlling \(K_{\text{ATP}}\) Channel Function

It is now clear that the pore-forming (Kir6.2) subunits can generate ATP-sensitive K channels in the complete absence of expressed SUR subunits, even without truncation of the COOH terminus (Tucker et al., 1997; John et al., 1998; Mikhailov et al., 1998). So, what is the role of the SUR1 subunit? Clearly, there is evidence for a chaperoning action to bring the channel to the surface, and with which SUR1 remains in physical proximity (Clement et al., 1997; John et al., 1998; Zerangue et al., 1999). Moreover, the physiologically, and pharma-
cologically, important regulators of the channel seem to act through an interaction with the SUR1 subunit (Aguilar-Bryan et al., 1995; Schwanstecher et al., 1998). The balance of evidence suggests that ATP hydrolysis at the nucleotide binding folds activates the channel, and that this activation is stabilized by binding of MgADP and other PCOs to the SUR1 subunit (Nichols et al., 1996; Gribble et al., 1997b; Shyng et al., 1997a,b; Schwanstecher et al., 1998). High-affinity sulfonylurea binding is to the SUR subunit (Aguilar-Bryan et al., 1995), and this effect is then transduced to inhibition of channel activity. The physical nature of the coupling between SURx and the Kir6.x subunits and the interacting regions of each subunit remain unknown. The present results show that deletion of the NH$_2$-terminus of Kir6.2 can functionally uncouple the high affinity tolbutamide sensitivity from the channel. However, it is clear that PCO actions on the channel remain for the NH$_2$-terminus truncated channel so that a physical coupling is still intact.

High affinity sulfonylurea sensitivity and PCO sensitivity is conferred by the SUR1 subunit, and is absent for Kir6.2 channels expressed in the absence of SUR1 (Gribble et al., 1997a; Tucker et al., 1997), which begs the question whether the effect of PIP$_2$ is to cause a physical, or functional, uncoupling of Kir6.2 from SUR1. A physical uncoupling seems unlikely based on the observation that treatment with polylysine leads to (a) some reversal of the PIP$_2$ abolition of pharmacological regulation, and (b) full restoration of the SUR1-dependent $K_{V_{1.2}ATP}$ of $\sim10$ μM. Nevertheless, we cannot exclude the possibility that the PIP$_2$ action physically interrupts the transduction of the inhibitory signal from SUR1 to Kir6.2.  

Conclusions

High affinity tolbutamide inhibition seems, like ATP inhibition, to be the result of a closed state stabilization, but, unlike ATP inhibition, is not likely to be a direct binding to the closed channel. Stabilizing the open state and raising the channel open probability, either by mutation or by application of PIP$_2$, reduces high affinity tolbutamide sensitivity. Similarly, PCOs act on SUR1 to stabilize the channel in the open state, convergent with PIP$_2$ action, such that PIP$_2$ treatment leads to channel activation without further activation in the presence of PCOs. Treatment with polylysine causes at least partial reversal of the uncoupling actions of PIP$_2$ effect, restoring some high affinity tolbutamide sensitivity and PCO stimulation. These results indicate that, in native cells, the pharmacological and physiological control of channel activity by the SUR1 subunit will be critically dependent on the open-state stability, itself determined by the phospholipid content of the membrane.

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References


Gribble, F.M., S.J. Tucker, and F.M. Ashcroft. 1997a. The interaction of nucleotides with tolbutamide block of cloned ATP-sensitive K channel currents expressed in Xenopus oocytes—a reinter-


