The ATP-sensitive potassium channel (KATP) controls insulin release in pancreatic β-cells and also modulates important functions in other cell types. In this study we report that anionic phospholipids activated KATP in pancreatic β-cells, cardiac myocytes, skeletal muscle cells, and a cloned KATP composed of two subunits (SUR/Kir6.2) stably expressed in a mammalian cell line. The effectiveness was proportional to the number of negative charges on the head group of the anionic phospholipid. Screening negative charges with polyvalent cations antagonized the effect. Enzymatic treatment with phospholipases that reduced charge on the lipids also reduced or eliminated the effect. These results suggest that intact phospholipids with negative charges are the critical requirement for activation of KATP, in distinction from the usual cell signaling pathway through phospholipids that requires cleavage. Mutations of two positively charged amino acid residues at the C terminus of Kir6.2 accelerated loss of channel activity and reduced the activating effects of phospholipids, suggesting involvement of this region in the activation. Metabolism of anionic phospholipids in plasmalemmal membrane may be a novel and general mechanism for regulation of KATP and perhaps other ion channels in the family of inward rectifiers.

The ATP-sensitive potassium channel (KATP) is a highly regulated channel type important in the physiology and pathophysiology of pancreas, heart, vascular smooth muscle, and perhaps other tissues as well (1–5). KATP activity can be reconstituted by co-expression of an inward rectifying channel (Kir6.2) (6, 7), and a sulfonylurea receptor (SUR) (8, 9). Major regulatory mechanisms are postulated to reside in the nucleotide binding domains on SUR (8–10). Activation of KATP generally requires a reduced ATP concentration. This regulation, including inhibition of KATP activity by other nucleotides, does not require phosphorylation. Activation of KATP, however, also depends on another less well characterized regulatory process, because KATP gradually inactivates when the integrity of the cell is disrupted by, for example, the excised patch method of voltage clamp (11–13). This property is shared not only by most KATP channels from different tissues, but also by other channels of the inward rectifier potassium (Kir) superfamily (for reviews, see Refs. 14 and 15). Despite intense investigation, the mechanisms for maintaining activation of KATP have not been fully identified and characterized, although previous work has focused on protein phosphorylation processes on the channel or related subunit (for a review, see Ref. 16). We found that anionic phospholipids, especially phosphoinositides, are necessary and sufficient to activate and maintain KATP activity in native and cloned KATP and in two other Kir channels. We also propose and test a novel hypothesis involving the cytoplasmic tail of the pore-forming subunit to account for this regulation. Modulation of channel activity through the composition and phosphorylation of membrane phospholipids may represent an important regulation for KATP in particular, and for channels in the Kir superfamily in general.

EXPERIMENTAL PROCEDURES

Cell Isolations—Single pancreatic β-cells were enzymatically (0.05% trypsin) dispersed from isolated islets of Wistar rats collected by centrifugation through a discontinuous gradient of Ficoll (17). Single ventricular myocytes were isolated from rabbit hearts by an enzymatic digestion of (0.1% collagenase, Williston) dissociation method (17, 18). Single skeletal muscle cells were dissociated enzymatically (0.3% collagenase, Williston) from the flexor digitorum brevis muscles of the hind legs of Wistar rats (13). Isolated single skeletal muscle cells were suspended in Dulbecco’s modified Eagle’s medium until they were used. Membrane vesicles were formed by washing the isolated cells with high K+ solution.

Solutions and Chemicals—The pipette solution (extracellular side, 140-K+ -bath) contained (in mM) KCl (142.0), HEPES (5.0), glucose (5.5), pH 7.4. The pipette solution for membrane vesicles from skeletal muscle cells and cloned KATP (SUR/Kir6.2) contained (in mM) NaCl (140.0), CaCl2 (1.8), MgCl2 (0.5), HEPES (5.0), and glucose (5.5). The bath solution (cytoplasmic side, 140-K+ -bath) contained in mM KCl (142.0), HEPES (5.0), glucose (5.5), and EGTA (2.0), pH 7.4. EGTA was included unless indicated otherwise. For whole cell clamp experiments, the pipette solution (140-K+ with Mg-ATP) contained (in mM) KCl (140.0), EGTA (2.0), HEPES (5.0), glucose (5.5), and Mg-ATP (0.5), pH 7.3. The bath solution (5.4-Tyrode solution) contained (in mM) KCl (115.0), CaCl2 (1.8), MgCl2 (0.5), HEPES (5.0), and the pH was adjusted to 7.4. K+ -ATP (Sigma) was dissolved into the bath solution just before use, and the pH of the solution was readjusted. DNase I and cytochalasin B were products of Sigma. Polyvalent cations were prepared in stock solutions and added to the solution prior to use. The concentration of free cations was calculated (19). A 2 ml bath was perfused with a solution of dispersed ligands at concentrations of 0.005–1 mg/ml. PPIs (a mixture of PI(4,5)P2, PI(4/P, phosphatidylinositol 4,5-bisphosphate; PI(4/PI, phosphatidylinositol 4-phosphate; PI, phosphatidylinositol; PS, phosphatidylyserine; PC, phosphatidylethanolamine; IP3, inositol 1,4,5-trisphosphate; DG, 1,2-diacylglycerol (8:0 DG); DPG, 1,2-diacylglycerol (16:0 DG); PLC, phospholipase C; PLCβ1, phospholipase Cβ1; PLα1, phospholipase Aβ1; PLD, phospholipase D; AMP-PNP, adenosine 5′-(β,y-methylene)triphosphate; AMP-PCP, adenosine 5′-(β,y-methylenetriphosphate); HEC, human embryonic kidney.)
Phospholipids Activate KATP

Phospholipids Activate KATP (small colonies surviving were isolated and grown to harvest in the continuous pressure of selection with 200–300 μg/ml active G418.)

Electrophysiological Recordings and Data Analysis—Currents were recorded using inside-out patch clamp (cytoplasmic membrane exposed to the bath) or whole cell clamp (external membrane exposed to bath) through a patch clamp amplifier (Axopatch 1-D or Axopatch 200A, Axon Instruments) and filtered through a built-in low-pass filter at 1 kHz, unless indicated otherwise. Leak current was compensated electronically on-line or subtracted off-line. Data were acquired by digitizing at 2 kHz and analyzed by a pClamp 6.0 (Axon Instruments) and graphic plotting software running on a PC-compatible computer (Gateway 2000).

RESULTS

Currents of native KATP were recorded from inside-out patches of pancreatic β-cells (Fig. 1A), cardiac ventricular myocytes (Fig. 1B), and sarcomeric membrane vesicles from skeletal muscle cells (Fig. 1C). KATP current expressed in HEK cells stably transfected by cloned cDNA of rSUR and rKir6.2 was also studied (Fig. 1D). Open channel activity was increased within 1–2 s to a maximal value after excising the patch membrane into an ATP-free solution; this was followed by decreased channel activity for pancreatic cells, cardiac cells, and transfected HEK cells. In the vesicles from skeletal muscle cells, the patch was initially devoid of KATP activity in most cases (Fig. 1A), presumably because these channels had inactivated during the vesicle formation process. An anionic phospholipid-rich tissue extraction containing PPIs (0.05–1 mg/ml) consistently activated KATP in cells from all three tissues and also the cloned KATP. Maximal effects were reached within 30 s to 2 min and did not change with time if the perfusion of the lipid-containing solution was maintained. After wash-out of PPIs from the bath solution, the activation effect remained for a time but diminished slowly with a time course that depended upon the previous concentration of PPIs. PPIs had no effects on single channel current amplitudes or ATP sensitivity. A non-KATP inwardly rectifying K+ channel, presumably the cardiac inward rectifier, or IRK1, noted coincidentally in Fig. 1B (small conductance channel observed before excision) also lost activity and was then activated by PPIs (small conductance apparent after ATP suppressed KATP). In separate experiments, PPIs activated currents expressed from clones of mouse inwardly rectifying K+ channels mIRK1 and the ATP-regulated inward rectifier hROMK1 (Fig. 2).

**Fig. 1.** PPIs activated KATP from diverse tissues. Activity was studied in inside-out patch membranes containing between 10 and 100 channels from a pancreatic beta cell (A), a cardiac cell (B), an isolated membrane vesicle from skeletal muscle cells (C), and KATP (rSUR/rKir6.2) channels stably expressed in HEK cells (D). The membranes were clamped at −50 mV (A and B), 0 mV (C), or −80 mV (D), and the inside-out patch was formed at the time indicated by the arrow labeled i-o. The pipette solution contained 140 mM K+ for A, B, and D and 10 mM K+ for C, with 140 mM K+ in the bath for all. Open channel currents were inward for A, B, and D and outward for C. For clarity of illustration, all currents are shown as a downward deflection from the zero current (dotted line). The bath solution contained EGTA for A, C, and D but not for B. Omitting EGTA from the bath solution potentiated loss of KATP activity by increasing the residual Ca2+ concentration in the bath solution. PPIs were effective in cardiac KATP with or without EGTA. The cytoplasmic surface of the patch membrane was perfused with PPIs (0.25 mg/ml) or ATP (2 mM) as indicated by bars. PPIs activated KATP in the examples shown and in n = 6, 5, 3, and 3 for β-cells, cardiac cells, membrane vesicles of skeletal muscle cells, and rSUR/rKir6.2, respectively.

**Fig. 2.** PPIs activated currents in cloned non-KATP Kir channels expressed in cultured cells. Methods used were the same as for Fig. 1. A, activity of a cloned mouse heart Kir (mIRK1, Kir2.1) was inhibited by Ca2+ and then activated by the addition of PPIs to the cytoplasmic surface. B, activity of an ATP-regulated Kir cloned from human kidney (hROMK1, Kir1.1) was also activated by application of PPIs.
The preparation of PPIs was a mixture of PI(4,5)P2, PI(4)P, PI, PS, and PC; to evaluate the contribution of the individual components to the activation effect, we individually tested purified PI(4,5)P2 (5 negative charges), PI(4)P (3 negative charges), PI (1 negative charge), PS (1 negative charge), PC (neutral), and another neutral phospholipid, PE, using the same protocol. All negatively charged phospholipids tested activated KATP. Phospholipids were incubated with PLC-PI (1 unit/ml), PLC (5 units/ml), PLA2 (1 unit/ml), and PLD (20 units/ml) (indicated in the figure by a plus sign) for 2 h or overnight at 37 °C. In the case of PLC, 1 mM Ca2+ was added to the incubating solution, and the reaction was stopped by adding 5 mM EGTA to the Ca2+-containing solution. To determine relative activity for D and E, the open probability (NP_open) was first calculated as the average current in a 5-s time window divided by the known single channel current amplitude under the same recording conditions. Relative activity was then obtained by normalizing NP_open to the NP_open value measured within the initial 20 s (initial) after the formation of the inside-out patch. NP_open measured after the inactivation and just before the application of lipids was taken as a control. Control mean value is indicated by a dotted line in both D and E. Most data are from cardiac cells, with some data from pancreatic β-cells included in the effects of PPIs, PI(4,5)P2, PI, PS, and PE. Data are expressed as mean ± S.D., with numbers from 3 to 5 for each point except for the control (n = 67), and for DOG, PI(4)P plus PLC-PI, and PC plus PLD (n = 1). Experimental conditions were the same as described in the Fig. 1 legend, except in some cardiac cells EGTA was included. The lipids were sonicated immediately before the experiment. The lipids were sonicated again after incubation with phospholipase and before the experiments.

The quantities of the lipids indicated in the figure are per ml volume of the bath solutions.

Fig. 3. Intact anionic phospholipids, but not their breakdown products, activated KATP. Effects of PI(4,5)P2 (25 μg/ml) (A), IP3 (0.1 mg/ml) (B), and phosphatidylinositol-specific PLC-PI (C) treated versus untreated PI(4,5)P2 (25 μg/ml in both cases) on KATPs are shown. KATPs in A and C were recorded from cardiac cells, while those in B were recorded from pancreatic β-cells. The same results were obtained with both tissue types, and thus summary data were pooled. D, summaries of the effects of different phospholipids and their cascade products on KATP activity. The quantities of the lipids indicated in the figure are per ml volume of the bath solutions. E, summaries of the effects of phospholipase on the phospholipid activation of KATP. Phospholipids were incubated with PLC-PI (1 unit/ml), PLC (5 units/ml), PLA2 (1 unit/ml), and PLD (20 units/ml) (indicated in the figure by a plus sign) for 2 h or overnight at 37 °C. In the case of PLC, 1 mM Ca2+ was added to the incubating solution, and the reaction was stopped by adding 5 mM EGTA to the Ca2+-containing solution. To determine relative activity for D and E, the open probability (NP_open) was first calculated as the average current in a 5-s time window divided by the known single channel current amplitude under the same recording conditions. Relative activity was then obtained by normalizing NP_open to the NP_open value measured within the initial 20 s (initial) after the formation of the inside-out patch. NP_open measured after the inactivation and just before the application of lipids was taken as a control. Control mean value is indicated by a dotted line in both D and E. Most data are from cardiac cells, with some data from pancreatic β-cells included in the effects of PPIs, PI(4,5)P2, PI, PS, and PE. Data are expressed as mean ± S.D., with numbers from 3 to 5 for each point except for the control (n = 67), and for DOG, PI(4)P plus PLC-PI, and PC plus PLD (n = 1). Experimental conditions were the same as described in the Fig. 1 legend, except in some cardiac cells EGTA was included. The lipids were sonicated immediately before the experiment. The lipids were sonicated again after incubation with phospholipase and before the experiments.
an anion to the head group of neutral PC, making this observation consistent with the effectiveness sequence depending upon net negative charge. Finally, exogenous PLA2 decreased KATP activity in inside-out patches, consistent with a recent report (23). This effect of PLA2 was reversed by subsequent application of PPIs.

We further tested the hypothesis that the negative charges in the anionic heads of the anionic phospholipids are the functional group critical to the activation of KATP by screening those charges with polyvalent cations. As an example (Fig. 4A), the polyvalent cation antibiotic neomycin decreased KATP activity, and inhibition was reversed by application of PPIs. Symbols represent the mean, and bars show the S.D., with n = 3–5 for each point. The lines are fits of the data for each polyvalent cation to an apparent binding isotherm $y = 1/(1 + C/IC_{50})$, where C is the polyvalent cation concentration. The fits are presented for descriptive purposes only, and no implication for binding versus shielding mechanism is intended.

**Fig. 4. Polyvalent cations inhibited KATP activity, and inhibition was reversed by application of PPIs.** A, neomycin in combination with PPIs on KATP from an inside-out patches from a cardiac cell. Neomycin and PPIs were perfused on the membrane cytoplasmic side during the period indicated (bars), at the concentrations noted. The bath solution contained 2 mM EGTA to slow down inactivation. Inhibitory effects of polyvalent cations on KATP of cardiac cells at different concentrations are summarized in B. Relative activity was calculated by normalizing the $NP_{\text{open}}$ (as described in the legend to Fig. 3) at the end of a 1-min application of the polyvalent cations to the membrane surface, to $NP_{\text{open}}$ just prior to application. Symbols represent the mean, and bars show the S.D., with n = 3–5 for each point. The lines are fits of the data for each polyvalent cation to an apparent binding isotherm $y = 1/(1 + C/IC_{50})$, where C is the polyvalent cation concentration. The fits are presented for descriptive purposes only, and no implication for binding versus shielding mechanism is intended.

The effects of phospholipids on KATP were rapid, sustained, and general to all anionic phospholipids and all types of KATPs tested. The mechanism for the effect of these lipids appeared less likely to involve a very specific or complex cellular process requiring many steps. We hypothesized that PPIs directly interact with KATPs or one of its subunits, mediated by electrostatic force, to maintain the channel in a functional state. We noted a segment of Kir6.2 composed of a high concentration of positively charged residues at the beginning of the cytoplasmic C terminus (Fig. 5A). This positioning of a cluster of positive charges has been shown to be an important determinant of protein topology in other membrane proteins (26, 27). To test our hypothesis, two mutants were made in this region: 1)
adjacent positively arginine residues at positions 176 and 177 were mutated to neutral alanines (R176A,R177A), and 2) the arginine at 176 was mutated to the negatively charged glutamic acid (R176E). Wild type Kir6.2 co-transfected with SUR expressed a peak KATP current of $1.59 \pm 0.3$ nA ($n = 8$) recorded by whole cell clamp at 0 mV with no ATP in the pipette. The current was blocked by glibenclamide and also exhibited the usual loss of activity with time. The R176A,R177Amutant co-transfected with rSUR also expressed a glibenclamide-sensitive K$^+$ current, but the current was much smaller, with a mean value of $0.32 \pm 0.07$ nA ($n = 4$, $p = 0.008$, compared with wild type) at 0 mV at maximum. Loss of channel activity was much faster than wild type, and the current showed more fluctuation. Examples of whole cell wild type and R176A,R177A currents are shown in Fig. 6, panels A and B, respectively. In inside-out patches, exogenous application of PPIs also activated the channels expressed from the R176E,R177A mutant with rSUR also expressed a glibenclamide-sensitive K$^+$ current, but the current was much smaller, with a mean value of $0.32 \pm 0.07$ nA ($n = 4$, $p = 0.008$, compared with wild type) at 0 mV at maximum. Loss of channel activity was much faster than wild type, and the current showed more fluctuation. Examples of whole cell wild type and R176A,R177A currents are shown in Fig. 6, panels A and B, respectively. In inside-out patches, exogenous application of PPIs also activated the channels expressed from the R176E, R177A mutant, but activation was less effective than for wild type, as demonstrated by a lower open probability and by the transient nature of the activation. Single channel recordings (Fig. 7A) from excised patches showed that the lower open probability in the mutant was caused by a decrease in open times (Fig. 7B) with a possible increase in the closed times. No glibenclamide-sensitive current could be recorded from the cells co-transfected with the R176E mutant and rSUR, suggesting that either this mutant did not express or that the mutation rendered it incapable of opening.

**FIG. 5.** Schematic diagrams for structure and amino acid sequence of Kir channels to account for electrostatic channel-membrane lipid interactions. A, block diagram of the linear amino acid sequence of Kir channels indicating the two transmembrane (M1 and M2) and pore-forming (H5) domains. The amino acid sequence for the initial C terminus (shaded area) for six representatives of the Kir family are shown. B, a diagram of two Kir subunits indicating how positively charged residues might interact with anionic membrane phospholipids to “tether” a “gate” away from the pore, allowing the channel to be active.

**DISCUSSION**

**Activation/Inactivation of KATP**—Gradual inactivation of KATP with time was recognized with the first description of KATP (1) using the inside-out patch clamp technique, a method that severely disrupts the cytoplasmic environment. This inactivation, sometimes called run-down, has been reported or addressed in nearly all research on KATP. Loss of activity is a consistent feature of all native and cloned KATP (Fig. 1, and Refs. 11–13 and 16). Despite much study, the mechanism required to maintain KATP activity is still unclear. Mg-ATP (28, 29) was found to transiently and partially activate and maintain KATP in cardiac, pancreatic, and skeletal muscle cells. Some anions (F$^-$, VO$_4^{2-}$) (30, 31) were also shown to have similar effects. Trypsin treatment was found to dramatically maintain KATP activation (18, 32). Mg$^{2+}$ was found to be necessary for ATP activation of KATP; therefore, many researchers speculated that phosphorylation/dephosphorylation was the key to maintenance of KATP channel activity (16). Mg-ATP, however, was often ineffective in activating KATP in rat skeletal muscles (13). In addition, intensive searches for protein kinases and the protein phosphorylation targets have not been successful (32). These results suggest that although phosphorylation may be a part of the regulatory process, additional key elements are involved. In rat skeletal muscle cells, KATP was found to be activated by high concentrations (>50 mM) of gluconate, with the effect proportional to the length of the carbon chain (13). This finding suggested to us the hypothesis that native anionic
phospholipids might be an endogenous mediator of this effect, because like gluconate they have an anionic head with a hydrophobic tail.

**Intact Anionic Phospholipids Maintain KATP Activity—**Activation of KATP in cardiac cells by PI(4,5)P2 and Mg-ATP/PI has been recently reported (33, 34). All negatively charged intact phospholipids we tested possess the ability to activate KATP, including PS, PI, PI(4)P, and PI(4,5)P2, directly, and in the absence of magnesium and ATP. Mg-ATP and PI, therefore, are not critical requirements to activate the channel. Although Mg-ATP is not required to mediate activation by anionic phospholipids, the anionic lipid hypothesis can account for the well known Mg-ATP activation effect on KATPs. It has previously been reported that nonphosphorylating analogs of ATP, AMP-PNP and AMP-PCP, are incapable of increasing KATP activity, suggesting that Mg-ATP acts as a phosphoryl group donor in kinase-mediated phosphorylation reaction, although such kinases have not yet been identified (29). Our effectiveness se-

**FIG. 6.** A double mutation in the proximal C terminus of Kir6.2 accelerated loss of channel activity. Shown is the inactivation of KATP current recorded from a tsA201 cell transiently co-expressed with wild type rKir6.2 and rSUR (A) and from a tsA201 cell transiently co-expressed with R176A,R177A (RR176AA) mutant and rSUR (B). The time label indicates the elapsed time after the rupture of the cell membrane. No ATP was included in the pipette solution. Currents were elicited by the voltage steps from a holding potential of −20 mV to −100 mV and +60 mV with a 20-mV increment.

**FIG. 7.** PPIs were less effective in maintaining channel activity in the mutant and resulted in shorter channel open times. A, effects of PPIs on the single channel currents of KATP expressed from the co-transfection of rKir6.2 and rSUR and KATP expressed from the co-transfection of R176A,R177A (RR176AA) and rSUR. The outward current is downward. The K+ concentrations were as follows: [K+]o = 10 mM; [K+]i = 144 mM. The holding potential was 0 mV. The currents were low-pass filtered at 100 Hz. In the patch with wild type rKir6.2/rSUR (left), a single channel was present. In the patch with R176A,R177A/rSUR (RR176AA/rSUR) (right), two channels were present. ▼ indicates the time when the excised patch formed, and ▼▼ indicates the time when PPIs (1 mg/ml) were applied to the bath. B, histogram analysis for the open times of the single channel events in the presence of PPI shown in A. The open times were fitted to two components of the exponential. The time constants were 4.3 and 121 ms for Kir6.2/SUR, and were 3.8 and 32 ms for R176A,R177A/rSUR, respectively.
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quence of PI(4,5)P$_2$ > PI(4)P > PI suggests the possibility that in vivo, Mg-ATP potentiates the activity of KATPs by serving as a substrate for the phosphoinositide kinases (35) and amphiphospholipid translocases that maintain sufficient membrane concentrations of PPIs to maintain KATP by increasing net negative membrane charge. PI(4,5)P$_2$ and phosphorylation of PI may perhaps be the important physiological regulators of KATP activity in this mechanism, a subject that requires further study.

Possible Molecular Mechanisms for the Anionic Lipid Effect—Do anionic phospholipids activate KATPs through an intermediate structure or process or through a direct structural interaction with the channel? Some components of PPIs, especially PI(4,5)P$_2$, are known to directly regulate various cellular proteins. One of the most important proteins interacting with the anionic phospholipids is protein kinase C, which uses Ca$^{2+}$ as part of its signaling pathway. In our cell-free patch clamp experiments, however, ATP was not included in the perfusion solution to the cytoplasmic side of the membrane, and Ca$^{2+}$ was chelated by EGTA to less than $5 \times 10^{-10}$ M. Indeed, regardless of the presence of ATP, raising the cytoplasmic Ca$^{2+}$ concentration decreased, rather than increased, the effect of PPIs. Thus, ATP and Ca$^{2+}$ are not required for the phospholipid effect on KATP, making it unlikely that the protein kinase C signaling pathway is involved in these effects of anionic lipids. Our observation that the protein kinase C activator diacylglycerol was ineffective in activating KATPs is consistent with noninvolvement of protein kinase C. Likewise, the immediate requirement of other phosphorylation processes in maintaining KATP activity is unlikely because of the ATP-free conditions of our study. Another important protein group known to interact with PI(4,5)P$_2$ is the cytoskeletal regulating proteins such as gelsolin. The possibility of involvement of cytoskeletal structure in the KATP activity has been suggested (33). The effects of PPIs remained even after application of high concentrations of the cytoskeleton disrupters DNase I and cytochalasin B (data not shown). Also, PS, which does not interact with most cytoskeletal proteins (36), was effective in activating KATPs in our experiments. Participation of the cytoskeleton network is evidently not a requirement for the effect, although a modulatory role has not been excluded.

The apparent importance of electrostatic interactions suggested by our data lead us to propose a molecular/physical model involving the channel protein (Fig. 5B). Kir channels that are not thought to associate with SUR (mIRK1, but hROMK1) were also activated by anionic phospholipids. This caused us to focus on the channel structure (Kir6.2) rather than SUR for the mechanisms of the effect. In our model, the positively charged residuals at the beginning of the C terminus of Kir6.2 are anchored or tethered by the electrostatic force of the anionic phospholipid head groups at the cytoplasmic face of the membrane. A decrease in the concentration of anionic phospholipids causes a release or conformation change of the tethered portion of the C terminus, triggering the formation of a gate that closes off the inner vestibule of the channel pore. This model is highly speculative, but protein-membrane electrostatic interactions at this portion of membrane proteins have been proposed previously (26, 27). The model also has value because it can account for the known experimental data such as the inhibitory effects of pH and di- and trivalent ions (by charge screening or neutralization), the anionic charge sequence, and suggests possible explanations for such effects as trypsin (cleaving the gate) and Mg-ATP (increasing net negative membrane charge by phosphorylation of membrane phospholipids). This model was tested by the Kir6.2 mutant R176A,R177A, which reduced positive charge on the cytoplasmic tail. As the model predicted, the mutant channel activity was reduced and less sensitive to PPIs.

How widely might this protein-lipid interaction mechanism apply to ion channel activity in general? To date, KATP has been cloned from pancreatic cells (6, 7) and cardiac cells (9, 37); both require a sulfonylurea receptor and Kir6.2 to function. Kir6.2 is one member of the Kir family with functional and structural similarities, including a concentration of positive charges at the beginning of the C terminus (Fig. 5A). Loss of channel activity has been generally found in most members of the Kir family, in both native channels (Refs. 38–40, for example) and expressed channels (41, 42). Our demonstration that PPIs activated native non-KATP inward rectifier (Fig. 1B) and also two cloned non-KATP inward rectifiers (Fig. 2) indicates that this mechanism may be general to all channels of this family. The higher effectiveness for more highly charged phospholipids suggests a role for lipid phosphorylation in this regulation. Thus, the PPIs, especially PI(4,5)P$_2$ and phosphatidylinositol 3,4,5-triphosphate, along with enzymes maintaining them such as phosphoinositide kinases (35), may be important cellular regulators by maintaining a channel protein-anionic lipid interaction required to activate channels.

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