

the membrane, and they provide a mechanistic framework for understanding the hallmark inhibition of these channels by ATP. Although we do not expect exact overlap of the PIP<sub>2</sub> and ATP binding sites, a negative heterotropic cooperativity is expected, so that individual residues may contribute to the binding of each ligand. Intact cell channel activity is reduced in the K<sub>ir</sub>6.2[R176A] mutant, demonstrating the physiological relevance of this finding. Membrane PIP composition may vary physiologically (16), and this may explain the wide variability in ATP sensitivity of native K<sub>ATP</sub> channels (17). It has long been recognized that activation of K<sub>ATP</sub> channels occurs under conditions where the cytoplasmic concentration of ATP is much higher than that required to inhibit channels in excised membrane patches (1, 6, 9). The profound effects of PIP<sub>2</sub> on ATP sensitivity would suggest that as membrane PIP levels increase, K<sub>ATP</sub> channels will be rendered insensitive to ATP, providing a mechanism for physiological activation.

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7. COSm6 cells were plated at  $\sim 2.5 \times 10^5$  cells per well (30-mm six-well dishes) and cultured in Dulbecco's modified Eagle's medium plus 10 mM glucose (DMEM-HG) with fetal calf serum (FCS, 10%). Cells were transfected by incubation for 4 hours at 37°C in DMEM medium plus 10% Nuserum; diethylaminoethyl-dextran (0.4 mg/ml); 100  $\mu$ M chloroquine; and 5  $\mu$ g each of pCMV6b-K<sub>ir</sub>6.2, pECE-SUR1, and pECE-GFP (green fluorescent protein) cDNA. Cells were incubated for 2 min in phosphate-buffered saline buffer containing DMSO (10%) and returned to DMEM-HG plus 10% FCS. Patch-clamp experiments were made at room temperature in a chamber (6) that allowed rapid change of the bathing solution. Micropipettes were filled with K-INT solution (below) electrode resistance  $\sim 0.5$  to 1 M $\Omega$ ). Patches were isolated from cells that fluoresced green under ultraviolet illumination and then voltage-clamped (Axopatch 1B, Axon, Foster City, CA). The standard bath (intracellular) and pipette (extracellular) solution (K-INT) contained 140 mM KCl, 10 mM K-Hepes, and 1 mM K-EGTA (pH 7.3). PIP<sub>2</sub> was sonicated in ice for 30 min before application to inside-out patches. ATP sensitivity was assessed by brief exposures to ATP-containing solutions without PIP<sub>2</sub> (Fig. 1, A and B). Inward currents (shown as upward deflections) were measured at  $-50$  mV, filtered at 0.5 to 3 kHz, digitized at 22 kHz (Neurocorder, Neurodata, NY), and stored on videotape for offline analysis with the Microsoft Excel and Solver programs. Data are presented as the mean  $\pm$  the standard error of the mean.
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12. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
13. Mutant constructs were prepared by overlap extension at the junctions of the relevant residues by sequential polymerase chain reaction (PCR). Before transfection, PCR products were subcloned into pCMV6b vector and sequenced. K<sub>ir</sub>6.2-C was constructed by fusing a DNA fragment containing K<sub>ir</sub>6.2 170-390 to pGEX2T vector. GST and K<sub>ir</sub>6.2-C, expressed in *Escherichia coli*, were purified with glutathione-agarose beads and dialysis in K-INT solution (7).
14. Cells were incubated for 24 hours in culture medium containing <sup>86</sup>Rb Cl (1  $\mu$ Ci/ml) 2 to 3 days after

transfection. Before measurement of Rb efflux, cells were incubated for 30 min at 25°C in Krebs' Ringer solution with metabolic inhibitors [oligomycin (2.5  $\mu$ g/ml) plus 1 mM 2-deoxy-D-glucose]. At selected time points, the solution was aspirated and replaced. The <sup>86</sup>Rb<sup>+</sup> in the aspirated solution was counted.

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18. We are grateful to J. Shepard for technical assistance and to S. Seino and J. Nerbonne for K<sub>ir</sub>6.2 and dissociated ventricular myocytes, respectively. This work was supported by grant HL45742 from NIH (C.G.N.), an Established Investigatorship from the American Heart Association (C.G.N.), a Career Development Award from the American Diabetes Association (S.L.S.), and by the Washington University Diabetes Research Training Center.

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## PIP<sub>2</sub> and PIP as Determinants for ATP Inhibition of K<sub>ATP</sub> Channels

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Adenosine triphosphate (ATP)-sensitive potassium (K<sub>ATP</sub>) channels couple electrical activity to cellular metabolism through their inhibition by intracellular ATP. ATP inhibition of K<sub>ATP</sub> channels varies among tissues and is affected by the metabolic and regulatory state of individual cells, suggesting involvement of endogenous factors. It is reported here that phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) and phosphatidylinositol-4-phosphate (PIP) controlled ATP inhibition of cloned K<sub>ATP</sub> channels (K<sub>ir</sub>6.2 and SUR1). These phospholipids acted on the K<sub>ir</sub>6.2 subunit and shifted ATP sensitivity by several orders of magnitude. Receptor-mediated activation of phospholipase C resulted in inhibition of K<sub>ATP</sub>-mediated currents. These results represent a mechanism for control of excitability through phospholipids.

Modulation of K<sub>ATP</sub> channels by activation of metabotropic receptors and cell metabolism is an important pathway for regulation of cell excitability (1). A common feature of these regulatory effects is that inhibition of K<sub>ATP</sub> channels by ATP can be antagonized and activation can be mimicked by so-called K channel openers (2). These drugs are known to activate K<sub>ATP</sub> channels even in the presence of millimolar concentrations of ATP (3) and involve the sulfonylurea receptor (SUR) to exert their effect (4, 5).

The effect of the K channel opener diazoxide on the current mediated by K<sub>ATP</sub> channels in response to voltage steps in giant inside-out patches from *Xenopus* oocytes ex-

pressing K<sub>ir</sub>6.2 and SUR1 subunits is demonstrated in Fig. 1A (6). Inhibition of the K<sub>ATP</sub>-mediated current by an initial application of 100  $\mu$ M ATP was partly reversed by the addition of 100  $\mu$ M diazoxide. After wash-out of both ATP and diazoxide, the current amplitude rapidly recovered to the level present before ATP application. This protocol was repeated four times after the patch had been intermittently exposed to the phospholipid PIP<sub>2</sub>. Besides its known effect of inhibiting run-down (7, 8), exposure to 5  $\mu$ M PIP<sub>2</sub> reduced the inhibitory effect of ATP and removed activation of channel activity by diazoxide ( $n = 3$ ). Patch excision into Mg-ATP-free solution resulted in substantial run-down of K<sub>ATP</sub> channel activity (Fig. 1B). This phenomenon is known for a variety of native and cloned K<sub>ir</sub> and K<sub>ATP</sub> channels (7-10) and has recently been shown to be induced by wash-out of phospholipids such as PIP<sub>2</sub> and PIP (8). Run-down of K<sub>ATP</sub> channels was accompanied by a marked increase in ATP sensitivity ( $n = 6$ ). Immediately after patch excision, 10  $\mu$ M ATP

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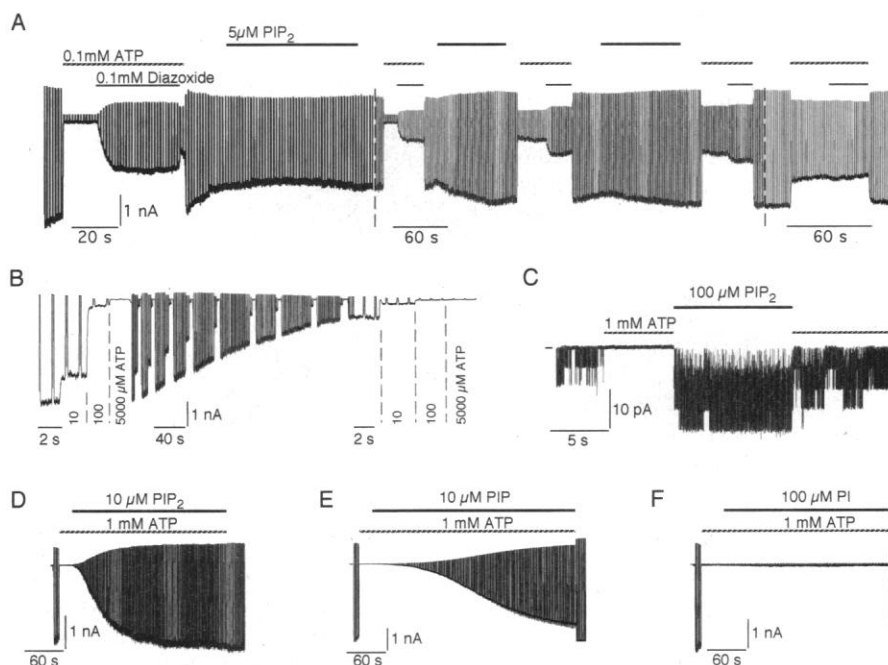
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blocked about 30% of the current. This block increased to about 70% after several minutes, suggesting that the change in ATP sensitivity results from wash-out of phospholipids (Fig. 1B). The effect of PIP<sub>2</sub> on ATP inhibition was further characterized in experiments with

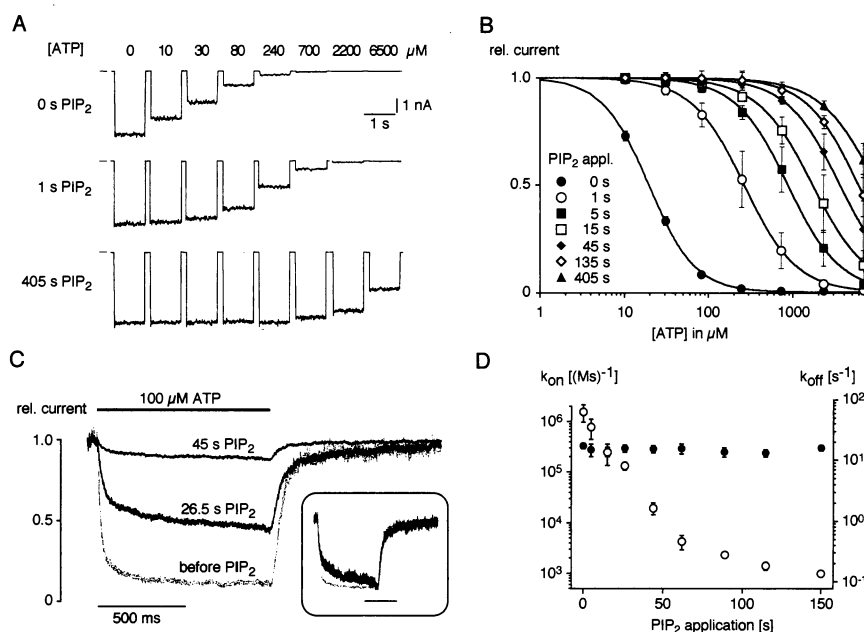
single channels (Fig. 1C) and metabolites of PIP<sub>2</sub> (Fig. 1, D to F). Prolonged application of 10  $\mu$ M PIP<sub>2</sub> completely removed inhibition of K<sub>ATP</sub> channels by 1 mM ATP (Fig. 1D;  $n = 3$ ). The current amplitude in the absence of ATP remained unchanged in this

type of experiment, indicating that the PIP<sub>2</sub>-mediated decrease in ATP sensitivity can be separated from the effect on run-down. Moreover, PIP<sub>2</sub> had no effect on the single-channel amplitude but increased the channels' open probability with respect to that observed after

**Fig. 1.** Polyphospholipids act as K channel openers. **(A)** Responses to voltage steps from  $-80$  to  $20$  mV in a giant inside-out patch with K<sub>v</sub>6.2/SUR1 channels. Solutions were exchanged with a multibarrel application system. One hundred micromolar ATP (in K-Int<sub>0Mg</sub>) blocked almost all channel activity (left). Addition of  $100 \mu$ M diazoxide activated channels in the presence of Mg-ATP. Wash-out of diazoxide and Mg-ATP recovered current to the level present before Mg-ATP application before run-down continued. Application of  $5 \mu$ M PIP<sub>2</sub> stopped current run-down and slightly increased current. Reapplication of Mg-ATP produced less current inhibition, and diazoxide was less effective in antagonizing ATP inhibition (middle panel). Three subsequent applications of PIP<sub>2</sub> further decreased ATP sensitivity and finally abolished the diazoxide effect (right). Time scales are as indicated. **(B)** Repetitive dose-response measurements after patch excision with ATP concentrations as indicated. The first and last dose responses are shown on an expanded time scale. The first application of  $10 \mu$ M ATP produced about 30% block, whereas the last application produced about 70% block. **(C)** Inside-out patch with four active channels at  $-80$  mV. Initial application of  $1$  mM ATP (in K-Int<sub>0Mg</sub>) blocked channel activity, application of  $100 \mu$ M PIP<sub>2</sub> for  $10$  s increased channel activity, and reapplication of  $1$  mM ATP produced only partial channel inhibition. PIP<sub>2</sub> had no obvious effect on the single-channel amplitude. **(D to F)** Application of  $10 \mu$ M PIP<sub>2</sub> (D),  $10 \mu$ M PIP (E), or  $100 \mu$ M PI (F) on inside-out patches with K<sub>ATP</sub> channels in the presence of  $1$  mM ATP (in K-Int<sub>0Mg</sub>). PIP<sub>2</sub> and PIP recovered K<sub>ATP</sub>-mediated currents, whereas PI failed.



**Fig. 2.** Effect of PIP<sub>2</sub> on steady state and time course of ATP inhibition. **(A)** Dose-response measurements of ATP inhibition (ATP concentrations as indicated, voltage steps from  $0$  to  $-80$  mV) in an inside-out patch before (top) and  $1$  s (middle) or  $405$  s (bottom) after PIP<sub>2</sub> ( $100 \mu$ M) application. **(B)** Dose-response curves from experiments as in (A); data points are mean  $\pm$  SD from three experiments. Continuous lines represent fit of a Hill equation:  $I/I_{\max} = 1/[1 + ([ATP]/IC_{50(ATP)})^n]$ , where  $I$  is the current in the presence of ATP,  $I_{\max}$  is the current amplitude in the absence of ATP,  $IC_{50(ATP)}$  is the concentration for half-maximal inhibition, and  $n$  is the Hill coefficient. PIP<sub>2</sub> (cumulative application time as indicated) shifted ATP sensitivity without affecting the Hill coefficient. **(C)** On and off kinetics of ATP inhibition measured with a piezo-driven application system allowing a solution exchange at an inside-out patch in less than  $2$  ms. Time course of block and block release by  $100 \mu$ M ATP (in K-Int<sub>0Mg</sub>) as measured before and after application of  $10 \mu$ M PIP<sub>2</sub> (duration as indicated). Each trace is the average current from five subsequent ATP applications. Currents were normalized to the current preceding ATP application. Time course was slowed and steady state of ATP inhibition was decreased by PIP<sub>2</sub>, whereas block release remained unchanged. **(Inset)** Current traces normalized to their amplitude for better comparison of time courses for block and unblock. **(D)** Time dependence of PIP<sub>2</sub> effect on ATP inhibition kinetics. Off-rates were calculated from the time constants obtained by monoexponential fits to the release of ATP inhibition ( $\tau_{\text{off}}$ ) as  $k_{\text{off}} = 1/\tau_{\text{off}}$ . Apparent on-rates were calculated from  $k_{\text{off}}$ , steady-state block ( $b$ ), and ATP concentration ( $[ATP]$ ) as  $k_{\text{on}} = (bk_{\text{off}})/(1 - b[ATP])$ ; this procedure was used because fit of the on-reaction required more than a single exponential. The last four data points were obtained with  $1$  mM ATP to obtain sufficient current inhibition.  $\circ$ ,  $k_{\text{on}}$ ;  $\bullet$ ,  $k_{\text{off}}$ . Data points are mean  $\pm$  SD from two to four experiments.



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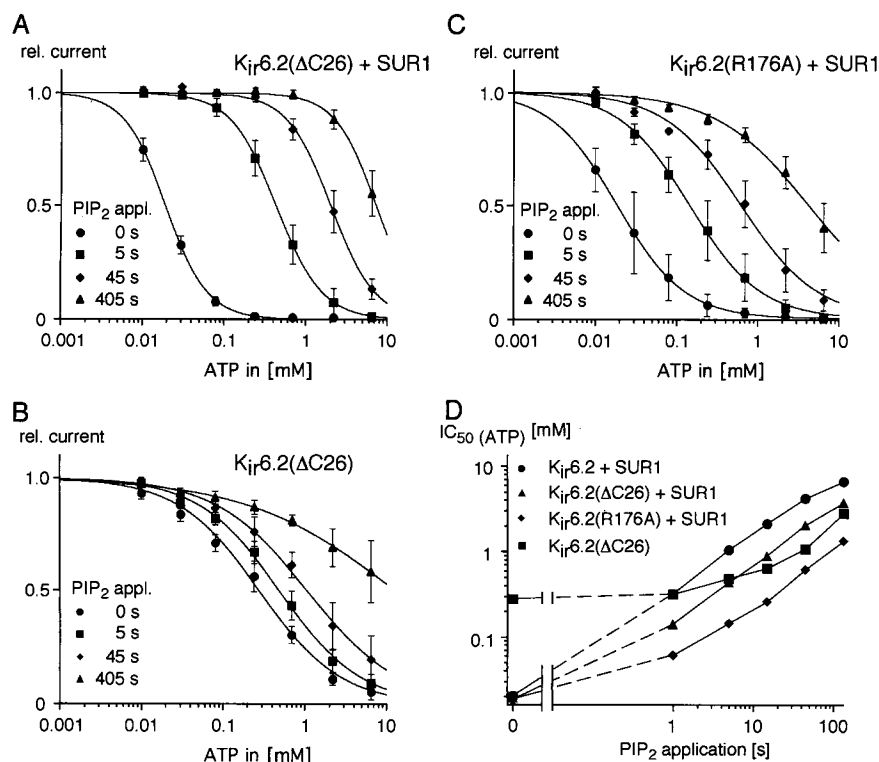
run-down of channel activity (Fig. 1C). Similar to  $\text{PIP}_2$ , PIP was also able to reduce ATP inhibition, although on a slower time scale (Fig. 1E;  $n = 6$ ), whereas phosphati-

dylinositol (PI) had no obvious effect on  $K_{\text{ATP}}$  channels even at a 10 times higher concentration (Fig. 1F;  $n = 3$ ). Furthermore, phospholipids were only active when

applied to the cytoplasmic side of the membrane; addition of 50  $\mu\text{M}$   $\text{PIP}_2$  into the pipette had no effect on ATP sensitivity in inside-out patches (11).

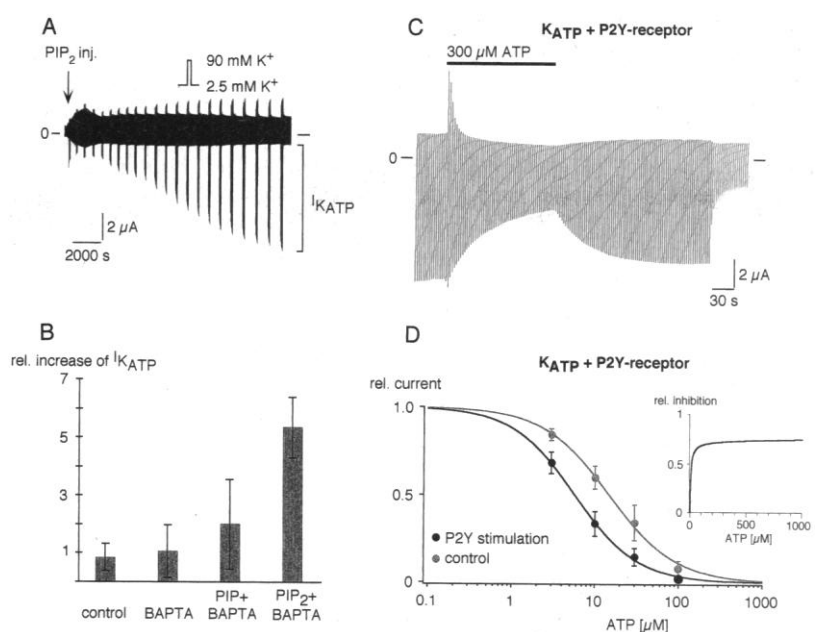
For a more quantitative investigation of the phospholipid effect,  $\text{PIP}_2$  was applied to giant inside-out patches for increasing intervals, and the ATP sensitivity of channels was determined. The dose-response curves for ATP inhibition were gradually shifted toward higher concentrations, without changes in their steepness (Fig. 2, A and B). This result suggests that increasing concentrations of  $\text{PIP}_2$  gradually change ATP sensitivity of individual channels rather than render channels ATP insensitive in an all-or-none manner. To analyze the underlying mechanism, we determined the kinetics of ATP inhibition before and after exposure to 10  $\mu\text{M}$   $\text{PIP}_2$ , using a piezo-controlled application system (12). The release of ATP inhibition exhibited a mono-exponential time course (time constant:  $69.1 \pm 10.4$  ms;  $n = 5$ ) that did not change with  $\text{PIP}_2$  application (Fig. 2C). The steady-state ATP inhibition, however, was markedly reduced by the phospholipid (Fig. 2, B and C). As a consequence, the apparent blocking rate constants ( $k_{\text{on}}$ ) calculated from steady-state inhibition and release rates ( $k_{\text{off}}$ ) changed by as much as three orders of magnitude in response to the  $\text{PIP}_2$  exposure, whereas  $K_{\text{off}}$  remained unchanged (Fig. 2D). This change suggests that  $\text{PIP}_2$  decreases the probability of ATP binding to its receptor site without affecting the stability of the ATP-receptor interaction.

This raised the question of which subunit of the  $K_{\text{ATP}}$  channel complex is involved in action



**Fig. 3.** Contribution of  $K_{\text{ATP}}$  subunits to ATP inhibition and  $\text{PIP}_2$  effect. (A to C) Dose-response curves from experiments as in Fig. 2A with  $K_{\text{ir}}6.2(\Delta\text{C}26)$  channels in the presence (A) and absence (B) of SUR1 or with  $K_{\text{ir}}6.2(\text{R}176\text{A})/\text{SUR}1$  channels (C). Data points are mean  $\pm$  SD from four experiments; cumulative application time of  $\text{PIP}_2$  is as indicated. (D) Dependence of  $\text{IC}_{50}$  for ATP inhibition as a function of  $\text{PIP}_2$  application time for the channels indicated. Data points are taken from the results shown in Fig. 2B and (A) to (C).

**Fig. 4.** Modulation of  $K_{\text{ATP}}$  currents by  $\text{PIP}_2$  injection or stimulation of a coexpressed P2Y receptor. (A) Two electrode voltage-clamp measurements from oocytes expressing cloned  $K_{\text{ATP}}$  channels in response to voltage ramps from  $-120$  to  $50$  mV. The external solution was intermittently changed from  $\text{Ext}_{90\text{K}}$  to  $\text{Ext}_{2.5\text{K}}$  to monitor both leakage and  $K_{\text{ATP}}$  current. Injection of 50 nl of 10 mM  $\text{PIP}_2$  plus 10 mM BAPTA produced an initial transient increase of the leakage current (visible at 2.5 mM  $\text{K}^+$ ) and a delayed but continuous increase in  $K_{\text{ATP}}$ -mediated ( $I_{\text{KATP}}$ ) current from about 1 to about 8  $\mu\text{A}$  over 4.5 hours. (B) Relative increase of  $K_{\text{ATP}}$ -mediated current measured 10 hours after injection of either 10 mM BAPTA, 1 mM PIP and 10 mM BAPTA, or 1 mM  $\text{PIP}_2$  and 10 mM BAPTA. Bars represent mean  $\pm$  SD of three to five experiments. (C and D) Stimulation of a coexpressed P2Y receptor decreased  $K_{\text{ATP}}$ -mediated currents by increasing ATP sensitivity. (C) Responses to voltage ramps from  $-120$  mV to  $50$  mV in 2.5 s in  $\text{Ext}_{90\text{K}}$  (traces in black) or  $\text{Ext}_{2.5\text{K}}$  (traces in grey) to which 100 nM staurosporine was added. Application of ATP evoked a transient increase in outward current followed by a reversible decrease in  $K_{\text{ATP}}$ -mediated current. Measurements of  $K_{\text{ATP}}$ -mediated currents in whole oocytes were enabled by high expression levels of the channel protein. (D) Dose response for ATP inhibition of  $K_{\text{ATP}}$  channels measured in patches from oocytes before (control) and after stimulation of P2Y receptors (P2Y stimulation). Data points are mean  $\pm$  SD from five experiments. (Inset) Relative inhibition (calculated from the ratio of the two dose responses) of  $K_{\text{ATP}}$  currents by P2Y receptor stimulation was independent of intracellular ATP for concentrations  $> 100$   $\mu\text{M}$ .



of PIP<sub>2</sub> and PIP. Experiments as in Fig. 2B were performed with a mutant of K<sub>ir</sub>6.2 that has been deleted by the COOH-terminal 26 amino acids [K<sub>ir</sub>6.2(ΔC26)] and that forms ATP-sensitive channels in the absence of the SUR subunit (13). K<sub>ir</sub>6.2(ΔC26) channels displayed PIP<sub>2</sub>-dependent decrease of ATP inhibition in the absence and presence of SUR1 (Fig. 3, A and B). As described before (13), ATP sensitivity determined before PIP<sub>2</sub> application was substantially lower in homomeric K<sub>ir</sub>6.2(ΔC26) channels than in K<sub>ir</sub>6.2(ΔC26)/SUR1 channels. However, longer applications of PIP<sub>2</sub> were required to shift ATP inhibition (Fig. 3, A, B, and D). In addition, the PIP<sub>2</sub> effect was less stable in K<sub>ir</sub>6.2(ΔC26) channels in the absence of SUR1 as judged from the faster wash-out of the phospholipid effect (11). These results indicate that PIP<sub>2</sub> basically exerts its effect on ATP inhibition through interaction with the K<sub>ir</sub>6.2 subunit. Moreover, SUR1 increases sensitivity of K<sub>ATP</sub> channels for ATP and stabilizes binding of PIP<sub>2</sub>. Correlation between binding affinity for PIP<sub>2</sub> and channel inhibition by ATP was further investigated in a K<sub>ir</sub>6.2 mutant channel, in which arginine (R) 176 was changed to alanine (A). This and the adjacent residue have recently been shown to reduce interaction between K<sub>ir</sub>6.2 and K<sub>ir</sub>1.1 with PIP<sub>2</sub> (8, 10). Longer PIP<sub>2</sub> applications were indeed necessary to induce a given shift in ATP sensitivity compared with K<sub>ir</sub>6.2 wild-type channels, although the ATP inhibition measured before PIP<sub>2</sub> exposure was similar in both channels (Fig. 3, C and D).

To ensure that PIP<sub>2</sub> plays a role in the activity of K<sub>ATP</sub> channels in the cellular environment, we injected PIP<sub>2</sub> and PIP into oocytes expressing K<sub>ATP</sub> channels (14). Injection of about 50 nl of phospholipids at a concentration of 10 or 1 mM resulted in an increase of K<sub>ATP</sub>-mediated currents (Fig. 4, A and B). PIP<sub>2</sub> was more effective than PIP, and intracellular calcium had no effect on the K<sub>ATP</sub>-mediated current (Fig. 4B). We further tested the effect of transient stimulation of a coexpressed metabotropic purino-receptor of the P2Y<sub>2</sub> subtype, which is known to activate phospholipase C (PLC) (15, 16) and thus decrease the concentration of PIP<sub>2</sub>. Such receptor-activated stimulation of PLC reduces PIP<sub>2</sub> concentrations in cultured cells within minutes by about a factor of 2 (17). Application of 300 μM ATP to an oocyte coexpressing K<sub>ATP</sub> channels and P2Y<sub>2</sub> receptors resulted in a reversible reduction of the potassium current by 59 ± 3% (Fig. 4C; n = 2). To exclude effects of protein kinase C, we performed these experiments in the presence of staurosporine (100 nM). The initial increase in outward current observed in the first few seconds after ATP application is due to transient activation of calcium-dependent chloride channels endogenous to *Xenopus* oocytes through the PLC-IP<sub>3</sub> pathway (15, 18). The P2Y<sub>2</sub>-mediated increase in ATP inhibition was confirmed by dose-response experiments per-

formed with excised patches from oocytes that had been incubated with ATP (1 mM) before the measurements. As shown in Fig. 4D, P2Y<sub>2</sub> stimulation resulted in an increase in ATP inhibition by about 70%, which was independent of intracellular ATP at concentrations > 100 μM.

In conclusion, inhibition of K<sub>ATP</sub> channels by intracellular ATP depends on the concentration of phospholipids such as PIP and PIP<sub>2</sub> in the cell membrane. At low concentrations of phospholipids, K<sub>ATP</sub> channels are blocked by micromolar concentrations of ATP, whereas prolonged application of PIP<sub>2</sub> renders channels ATP insensitive. This observation might underlie the variability observed for ATP sensitivity of K<sub>ATP</sub> channels in various tissues and studies (19, 20) and may contribute to the mechanism or mechanisms by which K<sub>ATP</sub> channels can overcome the high physiological concentrations of intracellular ATP.

Mechanistically, PIP<sub>2</sub> most likely binds to the K<sub>ir</sub>6.2 subunit, although SUR increases binding affinity of the phospholipids. It exerts its effect either by stabilizing a state in which the channel cannot interact with ATP (21) or by rendering the ATP-binding site inaccessible for ATP, for example, by competitive binding. However, the finding that PIP<sub>2</sub> changes the apparent on-rate for ATP inhibition by several orders of magnitude without having any effect on the off-rate argues against a change in conformation of the ATP-binding site, which would also affect the off-rate.

The observation that a metabotropic receptor coupling to PLC can control activity of K<sub>ATP</sub> channels might point toward the physiological role of the phospholipid effect presented here. This role is supported by the finding that ATP sensitivity of cardiac K<sub>ATP</sub> channels is regulated through a G-protein-related pathway (22). This was observed in a membrane patch and did not involve a diffusible second messenger. Thus, phospholipid-mediated opening of otherwise ATP-blocked K<sub>ATP</sub> channels might represent a new mechanism to control excitability in a wide variety of cells.

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6. *Xenopus* oocytes were surgically removed from adult females and manually dissected. About 50 nl of a solution containing complementary RNA was injected into Dumont stage VI oocytes. Oocytes were treated with

- collagenase type II (0.5 mg/ml; Sigma) and incubated at 19°C for 1 to 3 days before use. Experiments were done at room temperature (about 22°C) 2 to 7 days after injection. Giant patch pipettes were made from thick-walled borosilicate glass, had resistances of 0.3 to 0.6 Mohms (tip diameter of 20 to 30 μm), and were filled with 120 mM KCl, 10 mM Hepes, and 1.8 mM CaCl<sub>2</sub>. Currents were recorded and corrected for capacitive transients with an EPC9 amplifier (HEKA Electronics, Lamprecht, Germany), whose analog filter was set to 3 kHz (−3 dB). Leakage correction was not performed. Solutions were applied to the cytoplasmic side of the excised patches through a motor-driven multibarrel pipette; solutions had the following compositions (pH adjusted to 7.2 with KOH) with the concentration of free Mg<sup>2+</sup> ions calculated according to A. Fabbio [*Methods Enzymol.* **157**, 378 (1986)]: K-Int<sub>1,0Mg</sub> (120 mM KCl, 10 mM Hepes, 10 mM EGTA, and 1.44 mM MgCl<sub>2</sub>), which was also used as bathing solution, and K-Int<sub>0,0Mg</sub> (120 mM KCl, 10 mM Hepes, and 10 mM EGTA), to which K<sub>2</sub>-ATP was added as indicated. PIP<sub>2</sub>, PIP, and PI were dissolved by 30 min of sonification. Staurosporine, ATP, diazoxide, and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) were from Sigma, and PIP<sub>2</sub>, PIP, and PI were from Boehringer-Mannheim.
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