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Dynamic Sensitivity of ATP-sensitive K^+ Channels to ATP*

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ATP and MgADP regulate K_{ATP} channel activity and hence potentially couple cellular metabolism to membrane electrical activity in various cell types. Using recombinant K_{ATP} channels that lack sensitivity to MgADP, expressed in COSm6 cells, we demonstrate that similar on-cell activity can be observed with widely varying apparent submembrane [ATP] ($[ATP]_{sub}$). Metabolic inhibition leads to a biphasic change in the channel activity; activity first increases, presumably in response to a fast decrease in $[ATP]_{sub}$, and then declines. The secondary decrease in channel activity reflects a marked increase in ATP sensitivity and is correlated with a fall in polyphosphoinositides (PPIs), including phosphatidylinositol 4,5-bisphosphate, probed using equilibrium labeling of cells with [^{3}H]*myo*-inositol. Both ATP sensitivity and PPIs rapidly recover following removal of metabolic inhibition, and in both cases recovery is blocked by wortmannin. These data are consistent with metabolism having a dual effect on K_{ATP} channel activity: rapid *activation* of channels because of relief of ATP inhibition and much slower *reduction* of channel activity mediated by a fall in PPIs. These two mechanisms constitute a feedback system that will tend to render K_{ATP} channel activity transiently responsive to a change in $[ATP]_{sub}$ over a wide range of steady state concentrations.

ATP-sensitive K^+ channels (K_{ATP})[1] were first identified by Noma (1) in atrial and ventricular cardiac myocytes in 1983. He hypothesized that this channel may be responsible for the outward current that is activated during hypoxic conditions and that is inhibited by intracellular injection of ATP. That K_{ATP} channels serve to couple cellular metabolic status to the membrane electrical activity has essentially been confirmed by subsequent work in a variety of tissues. In pancreatic β-cells, inhibition of K_{ATP} channels leads to a depolarization of the membrane, provoking calcium entry and insulin secretion (2). In the heart, K_{ATP} channels may be involved in ischemic protection (3), a leading hypothesis being that K_{ATP} channel activation prevents calcium overload through membrane hyperpolarization (3–5). Although a large number of pharmacological studies confirm the activation of cardiac K_{ATP} channels during ischemia in several animal models (6), the link between the metabolic status of the cell and channel activity remains unclear. The marked ATP sensitivity of the K_{ATP} channel led to the hypothesis that submembrane [ATP] ($[ATP]_{sub}$) is the link (1). However, K_{ATP} channels are also modulated by MgADP (7–11) and negatively charged polyphosphoinositides (PPIs), of which phosphatidylinositol monophosphate (PIP), PIP_2, and phosphatidylinositol triphosphate are among the most potent (12–16).

K_{ATP} channels are complexes of four regulatory subunits (SURx) and four pore-forming subunits (Kir6.x). SURx confers the sensitivity of the channel to the stimulatory effects of MgADP (17, 18), and the weight of evidence indicates that ATP inhibits the channel by direct interaction with the Kir6.x subunit (17, 19, 20). Certain mutations in SUR1 (*e.g.* SUR1(G1485D) (17)) abolish MgADP activation, and channels containing such mutant subunits are predicted to be modulated solely by submembrane ATP concentration ($[ATP]_{sub}$). In a previous paper, we showed that such mutants can be used to infer $[ATP]_{sub}$ (21). In further pursuit of this approach, we now report a large range of apparent $[ATP]_{sub}$ values in COSm6 cells utilizing the same method. Strikingly, this variability of $[ATP]_{sub}$ is reflected in the altered ATP sensitivity of excised K_{ATP} channels rather than altered on-cell activity with constant ATP sensitivity. This observation logically implies that the ATP sensitivity of the channels is actually changing in response to $[ATP]_{sub.}$ To deliberately lower $[ATP]_{sub}$, we applied metabolic inhibitors. Consistent with a feedback effect of $[ATP]_{sub}$ on ATP sensitivity, metabolic inhibition has only a short term stimulatory effect; channel activation, because of a fall in $[ATP]_{sub}$, is rapidly followed by a decrease in channel activity that is paralleled by an increase in ATP sensitivity and a decrease in membrane [PPI].

EXPERIMENTAL PROCEDURES

Patch Clamp Measurements—COSm6 cells were plated at a density of ~2.5×10^5 cells/well (30-mm six-well dishes) and cultured in Dulbecco's modified Eagle's medium plus 10 mM glucose, supplemented with fetal calf serum (10%), penicillin (100 units·ml^{-1}), and streptomycin (100 μg·ml^{-1}). The following day, cells were transfected with pCMV6b-Kir6.2 (with mutations as described (21), pECE-SUR1, and pGreenLantern (Life Technologies, Inc.) using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer's suggestions.

Patch clamp experiments were carried out in a chamber that allowed the solution bathing the exposed surface of the isolated patch to be changed rapidly (22). The solution containing metabolic inhibitors was warmed to 37 °C before application (temperature controller TC-324B; Warner Instrument Corporation). The temperature was monitored using a thermistor at the level of the pipette. The other solutions were applied at room temperature. The standard bath (intracellular) and pipette (extracellular) solution used in these experiments (K-INT) had

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[1] The abbreviations used are: K_{ATP}, ATP-sensitive K^+ channel; $[ATP]_{sub}$, submembrane ATP concentration; PPIs, polyphosphoinositides; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP, phosphatidylinositol monophosphate; PI, phosphatidylinositol.

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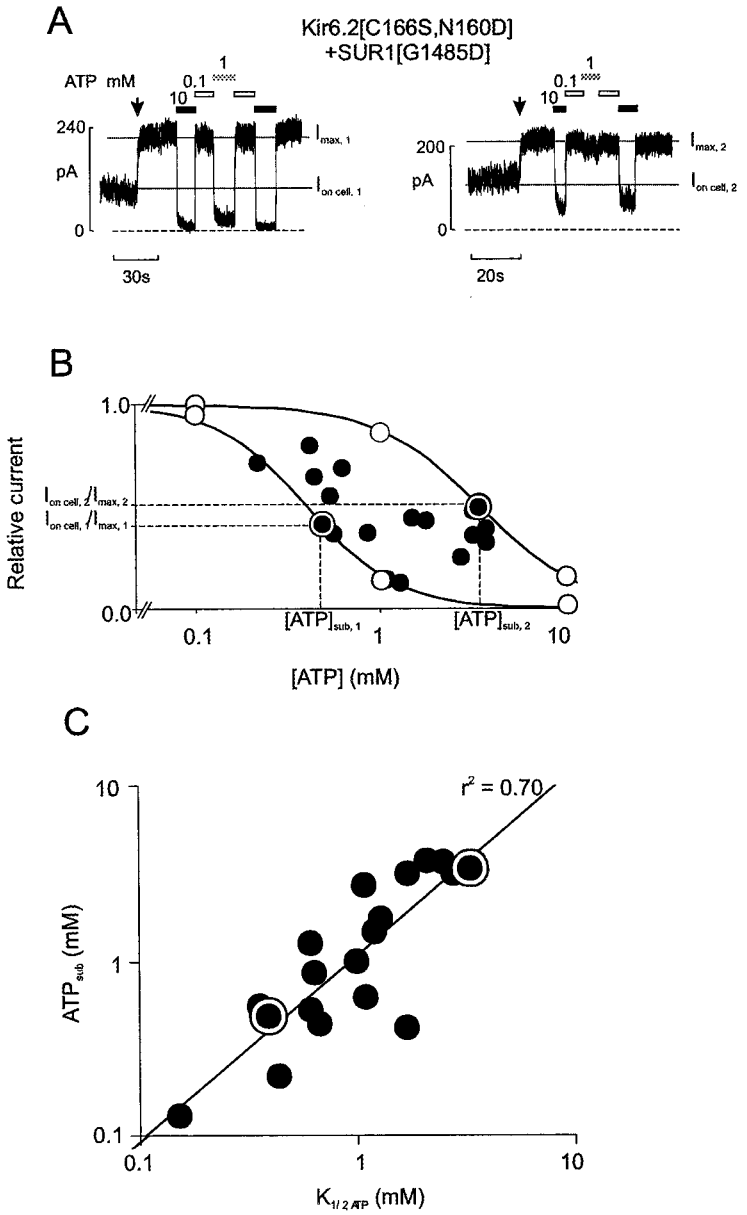
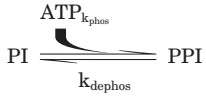


FIG. 1. *A*, macroscopic currents recorded from macropatches of two COSm6 cells expressing Kir6.2(C166S,N160D) + SUR(G1485D). The current increase at the *arrow* corresponds to patch excision in ATP free K-INT (see "Experimental Procedures"). ATP was applied as indicated. In this and subsequent figures, inward currents at −50 mV are shown as upward deflections. Zero current is indicated by a *dashed line*. *B*, calculation of $[ATP]_{sub}$ for 18 patches. Steady state dependence of membrane current (relative to current in zero ATP) on [ATP] for the two patches shown in *A*. *Open circles* represent the relative current in the presence of 0.1, 1, and 10 mM of ATP. For these two patches, *lines* correspond to the least squares fits of the Hill equation: relative current = $1/(1 + [ATP]/K_{½, ATP})^H$), where $K_{½, ATP}$ is the half-inhibitory [ATP] and H the Hill coefficient. On each curve, the *double circle* corresponds to the relative on-cell current, and the $[ATP]_{sub}$ is inferred. Additional *closed circles* correspond to on-cell current and estimated $[ATP]_{sub}$ for 16 other patches analyzed in the same way (the ATP dose-response curves are not shown). *C*, plot of the estimated $[ATP]_{sub}$ for each patch *versus* the ATP sensitivity ($K_{½, ATP}$) of the channels expressed in the patch. $K_{½, ATP}$ is deduced from the ATP dose-response curves (intersection of the curves with relative current = 0.5). The *double circles* correspond to the two patches presented in *A*. The *solid line* in this and similar figures is the correlation line, and r^2 is the coefficient of determination.

the following composition: 140 mM KCl, 10 mM K-HEPES, 1 mM K-EGTA, pH 7.3, with additions as described. All currents were measured at a membrane potential of −50 mV. The details of the method (material, pipettes preparation, and off-line analysis) have been described previously (23–25).

Measurement of Phosphoinositide Levels—COSm6 cells were plated in 35-mm dishes. Two days prior to use, the medium was aspirated and replaced with inositol-free Dulbecco's modified Eagle's medium containing 3% fetal calf serum and 1 μCi/ml [^{3}H]*myo*-inositol. For measurement of phosphoinositide levels during metabolic inhibition, the labeling medium was removed and replaced with either Ringer's solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES, pH 7.4, with NaOH) or Ringer's plus oligomycin (2.5 μg/ml) and 2-deoxy-D-glucose (1 mM). The cells were incubated at 37 °C for the indicated length of time and washed once in cold phosphate-buffered saline. For studies of the effect of wortmannin on PPI levels during recovery from metabolic inhibition, the cells were incubated at 37 °C with metabolic inhibitors for 30 min, followed by 10 min of incubation with metabolic inhibitors plus either 10 μM wortmannin or vehicle. The solution containing the metabolic inhibitors was then removed and replaced with Ringer's solution containing 10 μM wortmannin or vehicle. The cells were incubated at room temperature for the indicated length of time and washed once in cold phosphate-buffered saline. For both assays, cells were scraped into 1 ml of methanol with concentrated HCl (10:1 v/v). One ml of water was added, and the samples were extracted with 2 ml chloroform. The upper, aqueous layer was removed, and the organic layer was re-extracted with methanol with 1 M HCl (1:1 v/v). The samples were evaporated to dryness, and the phosphoinositides were separated on thin layer plates as described previously (26). Plates were sprayed with En3Hance (PerkinElmer Life Sciences) and exposed to x-ray film. PI, PIP, and PIP_2 were identified by co-migration with standards. Bands corresponding to these lipids were scraped from the plates and counted for ^{3}H.

Simulations of Channel Response to $[ATP]_{sub}$ Changes—To simulate the biphasic response of channel activity following a step change in $[ATP]_{sub}$ (see Fig. 6), we assumed the following simplistic dependence of [PPI] on $[ATP]_{sub}$.



SCHEME 1

where PI is an inactive precursor, $k_{phos} = 1\ min^{-1} \cdot mM^{-1}$, and $k_{dephos} = 1\ min^{-1}$. An empirical function relating ATP sensitivity to active [PPI], which fitted the observed data (see Fig. 4) for the Kir6.2(C166S) + SUR1(G1485D) channels, is assumed.

$$K_{½,\,ATP} = 0.034*\exp(3.17*[PPI]) \quad \text{(Eq. 1)}$$

giving $K_{½,\,ATP}$ (the half-maximal inhibitory concentration of ATP) = 0.81 mM at steady state PPI = 1 (no units) at [ATP] = 1 mM. The channel open probability (P_{open}) is defined as an instantaneous function of [ATP] and $K_{½,\,ATP}$ as follows.

$$P_{open} = 1/(1 + ([ATP]/K_{½,\,ATP})^{H}) \quad \text{(Eq. 2)}$$

where $H = 1.3$.

RESULTS

ATP Sensitivity of K_{ATP} Channels Depends on Submembrane ATP Concentration—To investigate the correlation between the on-cell K_{ATP} channel activity and $[ATP]_{sub}$, we used a Kir6.2 mutant (Kir6.2(C166S,N160D)) that interacts with SUR1(G1485D) to generate channels that exhibit on-cell activity that is around half (0.45 ± 0.04, $n = 20$, mean ± S.E. (21)) of that observed in excised patches in the absence of ATP. Because this on-cell activity should be determined solely by the inhibitory action of ATP (because of the G1485D mutation in SUR), $[ATP]_{sub}$ can be estimated from the channel ATP sensitivity measured after patch excision (21).

On-cell channel activity ($I_{on\text{-}cell}$) was measured following seal formation (Fig. 1*A*, *before arrows*). Patches were then excised, and different [ATP] levels were applied to the cytoplasmic surface to generate [ATP] inhibition curves (Fig. 1*A*, *following arrows*, and see Fig. 1*B*). $[ATP]_{sub}$ was inferred from the on-cell K_{ATP} channel activity using this calibration curve. As shown in Fig. 1*B*, this analysis indicated a wide variability in inferred $[ATP]_{sub}$ (0.2–3.7 mM). However, this variability did not correlate with alterations in on-cell K_{ATP} channel activity but instead tended to reflect patch-to-patch variability in ATP sensitivity (Fig. 1*C*). These data not only indicate that ATP sensitivity of a single molecular species of channel varies considerably from cell to cell but, moreover, that ATP sensitivity actually depends on the $[ATP]_{sub}$. It is as though the ATP sensitivity of the channel adjusts to the $[ATP]_{sub}$, and on-cell channel activity tends to remain constant.

We repeated the above experiment with other mutant channels that have different ATP sensitivity (Kir6.2(I154C) and Kir6.2(T171C)). Each gave a similar correlation between the measured $K_{½,\,ATP}$ and the estimated $[ATP]_{sub}$. As shown in Fig. 2 (*bottom panel*), essentially the same behavior is also observed for mutant Kir6.2(I154C) channels expressed without SUR1. The SUR1 subunit is therefore not directly implicated in the regulatory mechanism that maintains channel activity independent of the variation in $[ATP]_{sub}$.

Biphasic Effect of Metabolic Inhibition on Kir6.2: Stimulation and Inhibition—The above findings suggest that the channel ATP sensitivity actually adjusts to the ambient $[ATP]_{sub}$. If this is the case, then one might predict that a fall in $[ATP]_{sub}$ would be accompanied by a compensatory increase in ATP sensitivity and consequent reduction of channel activity. Metabolic inhibition can drastically decrease [ATP] in cells. Using an ATP-luciferase assay in COS cells, a 100-fold decrease in cellular [ATP] has been observed after 20 min of metabolic inhibition (27). To examine the effect of metabolic inhibition on channel activity, we applied oligomycin (2.5 μg/ml) plus 2-deoxy-D-glucose (1 mM) at 37 °C. Metabolic inhibition led to a biphasic change in the on-cell current (Fig. 3*A*): (i) a rapid increase (485 ± 144%; $n = 6$) occurred within the first minute with a time to 90% activation of 11.9 ± 3.5 s ($n = 6$) and (ii) a subsequent slower decrease to 43 ± 22% of initial on-cell current occurred with a time to 90% inactivation of 21.8 ± 2.3 min ($n = 6$).

Patches were excised at various times during application of metabolic inhibitors, and $K_{½,\,ATP}$ was immediately estimated.

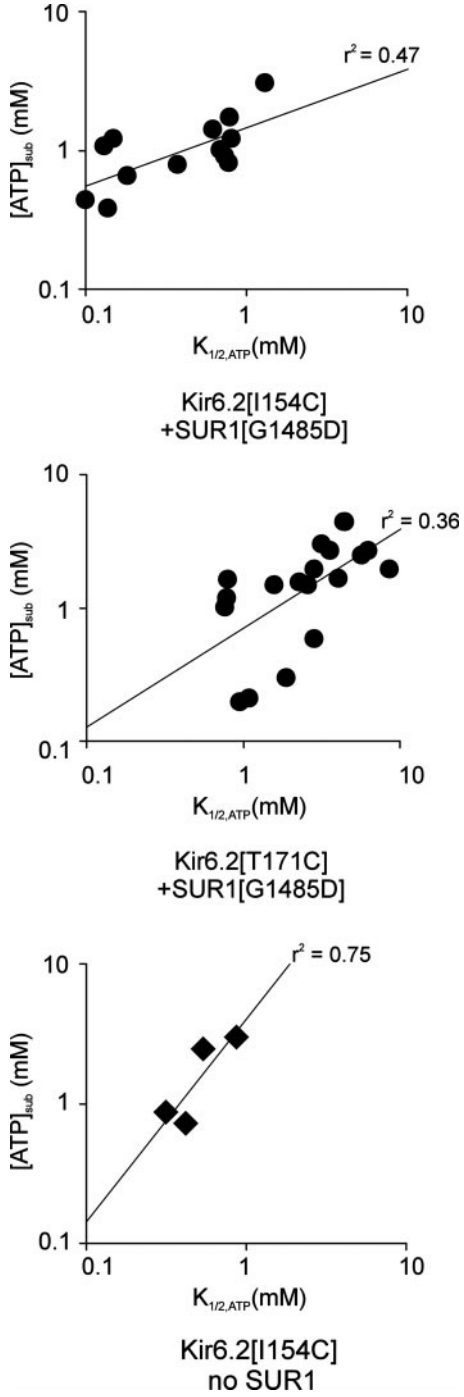


FIG. 2. **Analysis of similar experiments to those in Fig. 1 on two other mutant K_{ATP} channels as indicated, including Kir6.2(I154C)expressed without SUR1 (*bottom panel*).** Each *panel* shows a plot of the estimated $[ATP]_{sub}$ for each patch *versus* the ATP sensitivity ($K_{½,\,ATP}$) of the channels expressed in the patch.

The initial increase in the on-cell current is presumably due to a decrease in $[ATP]_{sub}$, and there was no obvious change of ATP sensitivity during this phase (Fig. 3*B*). The subsequent slow decline in current correlated with significant increase of ATP sensitivity. For the mutant Kir6.2(C166S) coexpressed with SUR1(G1485D), $K_{½,\,ATP}$ decreased from 1.11 ± 0.21 mM ($n = 16$) to 0.15 ± 0.02 mM ($n = 6$) in 20 min (Fig. 3*B*).

Concomitant PIP_2 Levels and ATP Sensitivity Changes during Metabolic Inhibition—Negatively charged PPIs modulate the ATP sensitivity of the K_{ATP} channel (14, 15). Because PPI synthesis is ATP-dependent, a decrease in $[ATP]_{sub}$ during metabolic inhibition can also lead to a decreased level of PPIs

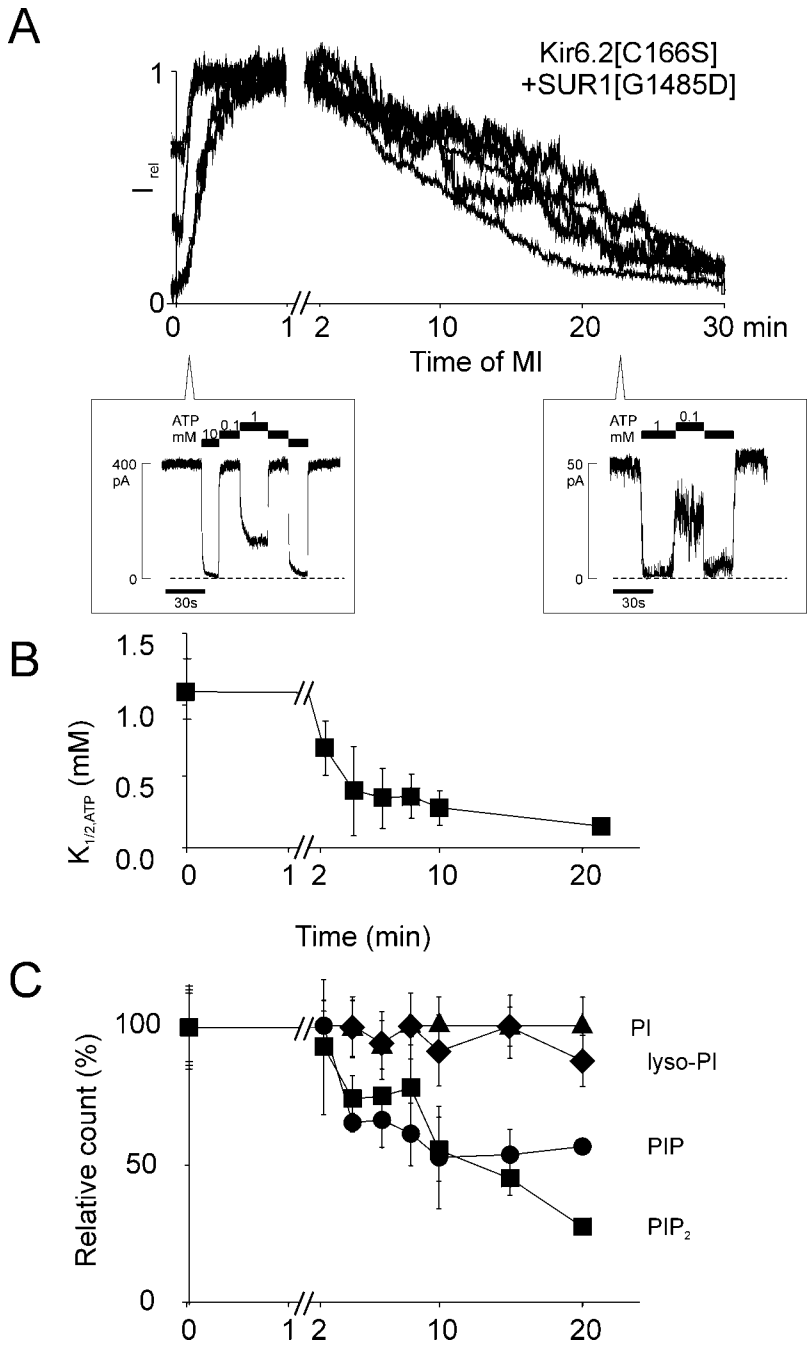


FIG. 3. *A*, on-cell current recorded from 5 cells expressing Kir6.2(C166S) + SUR1(G1485D) at 37 °C. Metabolic inhibitors (2.5 μg/ml oligomycin and 1 mM 2-deoxy-D-glucose) were applied at time 0. The currents are normalized to the peak current during metabolic inhibition. *Insets*, macroscopic currents recorded from excised macropatches of a COSm6 cell expressing Kir6.2(C166S) + SUR(G1485D) before (*left inset*) and after (*right inset*) 20 min of metabolic inhibition. ATP was applied as indicated. *B*, $K_{½,\,ATP}$ of mutant Kir6.2(C166S) + SUR1(G1485D) as a function of metabolic inhibition duration. Metabolic inhibitors (2.5 μg/ml oligomycin and 1 mM 2-deoxy-D-glucose) were applied at 37 °C. $K_{½,\,ATP}$ is estimated as above (see Figs. 1 and 2) from the relative current in presence of 1 and 0.1 mM ATP (mean + S.E., n = 3–16 patches). *C*, membrane phosphoinositide levels as a function of duration of metabolic inhibition, relative to the level at t = 0 (means + S.E., n = 3 independent measurements).

(28). COSm6 cells in monolayers were exposed to metabolic inhibition as above and rapidly harvested and lysed at various times after onset of metabolic inhibition for measurement of [PPI].

Both PIP and PIP_2 levels dropped significantly during 20 min of metabolic inhibition (Fig. 3*C*), and the kinetics of the decrease in PIP_2 levels approximate the kinetics of the decrease in $K_{½,\,ATP}$ (Fig. 3, *B* and *C*). Following removal of metabolic inhibitors, there was a rapid recovery of both ATP sensitivity (see Fig. 5*A*) and PPIs (see Fig. 5*B*, *filled symbols*). Interestingly, and consistent with a causal relationship, the same relationship exists between $K_{½,\,ATP}$ and PPI levels during metabolic inhibition and during recovery (Figs. 4 and 5*C*). Wortmannin is a blocker of PI 3-kinase, and, at higher concentrations, also of PI 4-kinase (29–31). At 10 μM, wortmannin had no effect on the ATP sensitivity of K_{ATP} channels in cells exposed to control solution or metabolic inhibitors. However, it significantly inhibited the subsequent recovery of both $K_{½,\,ATP}$ and PPIs following removal of metabolic inhibitors (Fig. 5, *A* and *B*, *open symbols*).

DISCUSSION

Cell-to-Cell Variability of K_{ATP} Channel ATP Sensitivity—Previous studies have demonstrated variability in the ATP sensitivity of native K_{ATP} channels (32), but the underlying mechanism has remained elusive. The present study demonstrates significant cell-to-cell variability in the ATP sensitivity of single molecular species of K_{ATP} channel. This striking result indicates that there must be cell-to-cell variability of some regulator(s) of K_{ATP} channel ATP sensitivity. We propose that this may be the cellular PPI level. The synthesis of highly phosphorylated PPIs requires ATP, and variation in $[ATP]_{sub}$ could therefore affect [PPI] (28) and hence the ATP sensitivity of the K_{ATP} channel (14, 15, 33). Consistent with this idea, we observed a correlation between the apparent $[ATP]_{sub}$ and the ATP sensitivity of recombinant K_{ATP} channels expressed in

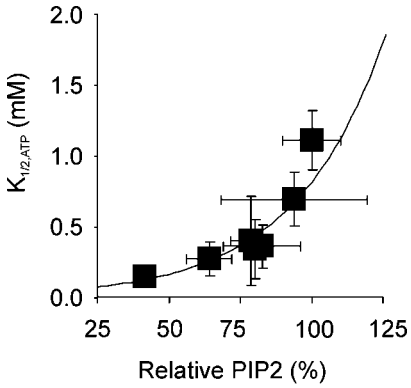


FIG. 4. **Correlation between $K_{½,\,ATP}$ (in mM) and relative PIP_2 levels during metabolic inhibition.** The *solid line* corresponds to a empirical fit: $K_{½,\,ATP} = 0.034*\exp(3.17*PIP_2)$.

COSm6 cells (Figs. 1 and 2).

Phospholipid Control of K_{ATP} Channel Activity during Metabolic Inhibition—Many agents (*e.g.* pH, MgADP, and potassium channel openers) have been shown to affect the ATP sensitivity of K_{ATP} channels (11). However, the most dramatic modulators of ATP sensitivity in inside-out membrane patches are PPIs and other negatively charged lipids, with PIP_2 and phosphatidylinositol triphosphate being the most potent (13–15). The endogenous levels of PPIs are likely to determine the intrinsic sensitivity of the channel to ATP, and there are now several reports that pharmacological or genetic modulation of PPI levels alter intrinsic K_{ATP} channel activity (16, 34). However, the primary evidence for a direct modulatory role of membrane phospholipids still comes from inside-out patch experiments, in which PPIs are applied to the intracellular membrane surface. Our results demonstrate significant parallel changes in both [PPI] and K_{ATP} channel ATP sensitivity following inhibition of metabolism in intact cells. This strengthens the argument that PPIs can dynamically modulate K_{ATP} channel activity *in vivo* and, as discussed below, implicate PPIs in a feedback mechanism that counters the inhibitory effect of ATP.

Secondary Modulation of Channel Activity through PPIs: a Dynamic Feedback Control of Channel Activity—In various tissues, K_{ATP} channels serve as sensors of cellular metabolism, modulating the membrane potential in response to changes in the metabolic status of the cell (2). However, the mechanism by which this channel regulation is accomplished is not fully understood. The present study shows that acute inhibition of metabolism not only has a direct stimulatory effect on channel activity (presumably because of a fall in $[ATP]_{sub}$) but also causes a slower secondary inhibitory effect that is paralleled by changes in PPI levels. Following removal of metabolic inhibition, both [PPI] and ATP sensitivity recover, and the recovery of both is inhibited by the PI kinase inhibitor wortmannin. Although wortmannin may have various nonspecific effects at the concentrations used in the present experiments (35), this parallel inhibition is consistent with a causal relationship between [PPI] and channel ATP sensitivity.

What is the functional consequence of such a relationship? We suggest that by adjusting the ATP sensitivity of the channel to match the ambient $[ATP]_{sub}$, slow changes in [PPI] will exert a feedback control on channel activity that will tend to render cells responsive to a sudden change in $[ATP]_{sub}$ from any steady state level. Fig. 6 illustrates the proposed feedback mechanism and the response of a simple model of K_{ATP} channel activity. The time-dependent behavior of this model is simulated in Fig. 6*B*, together with the dependence of P_o on $[ATP]_{sub}$ (Fig. 6*C*). The model predicts that the instantaneous fall of

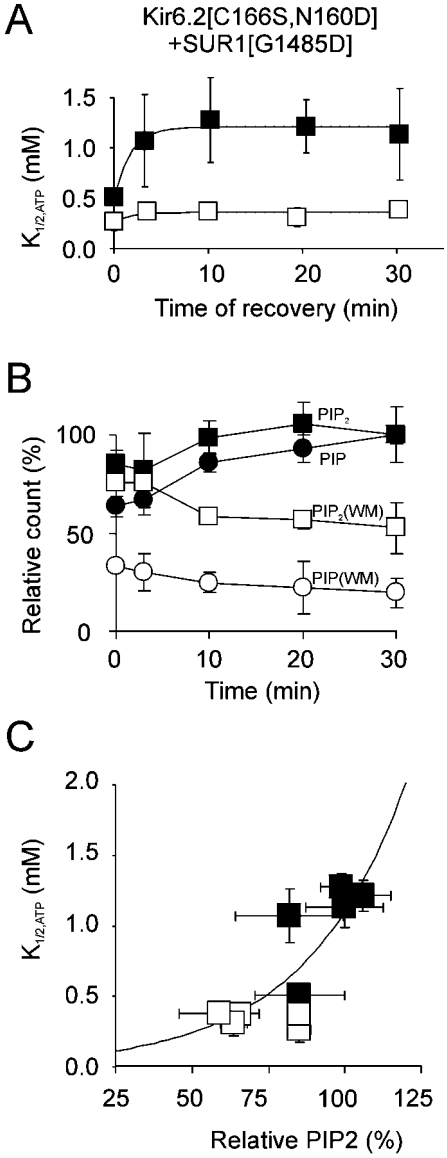


FIG. 5. *A*, $K_{½,\,ATP}$ of mutant Kir6.2(C166S) + SUR1(G1485D) as a function of time of recovery from metabolic inhibition. Recovery in the absence (*solid symbols*) and the presence (*open symbols*) of 10 μM wortmannin. (mean + S.E., n = 3–10 patches). *B*, membrane phosphoinositide levels as a function of duration of recovery from metabolic inhibition, in the absence (solid symbols) and the presence (*open symbols*) of wortmannin. The levels are normalized to the peak recovery in the absence of wortmannin (mean + S.E., n = 3). *C*, correlation between $K_{½,\,ATP}$ and relative PIP_2 levels during recovery from metabolic inhibition in the absence (*solid symbols*) and the presence (*open symbols*) of wortmannin.

$[ATP]_{sub}$ initially produces a rapid increase of K_{ATP} channel activity, but that the secondary decrease in [PPI] subsequently increases channel ATP sensitivity, returning channel activity toward its initial value. In the model presented, a secondary rise, or fall, in [PPI] influences channel activity by decreasing or increasing the ATP sensitivity of the channel. In the case of wild type K_{ATP} channels, however, increase in [PPI] leads to increased channel activity by promoting an increase in the channel open probability, with relatively constant ATP sensitivity (33). In this case, qualitatively similar biphasic changes in channel activity would be expected to result from a step change in [ATP], but the effect of a change in the controlling equilibrium K_C would now be primarily to shift the peak P_{open} rather than the $K_{½,\,ATP}$. It is also likely that in native tissues, the compensatory effect of changes in [PPI] on K_{ATP} channel

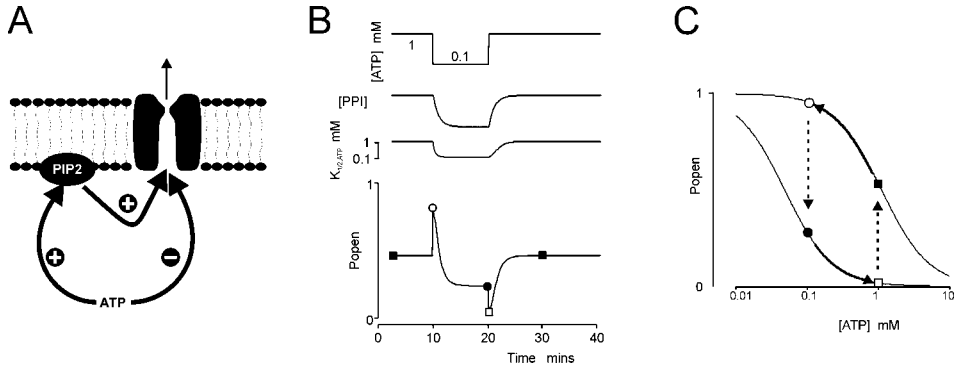


FIG. 6. *A*, cartoon summarizing two antagonistic effects of ATP on the K_{ATP} channel via interactions with the pore forming Kir6.2 subunit: a direct inhibitory effect and an indirect activatory effect through changes in PPIs. *B*, a simple quantitative model (see "Experimental Procedures") predicts biphasic current responses. Step changes of [ATP] (*top*) causes exponential change of PPIs (*middle upper*) and hence $K_{½,ATP}$ (*middle lower*) to a new level that is directly proportional to [ATP]. P_{open}, an instantaneous function of [ATP] and $K_{½,ATP}$ (*bottom*) changes biphasically. *C*, trajectory of P_{open} following step changes of [ATP]. Immediately following a step from 1 to 0.1 mM, P_{open} follows the dose-response curve from solid square to open circle (*solid arrow*). The time-dependent fall of PPIs then causes P_{open} to fall to the *solid circle* (*dashed arrow*). The recovery of ATP to 1 mM cause instantaneous and time-dependent changes of P_{open} back to the initial value.

activity will be modulated, or obscured, by the stimulatory action of MgADP, mediated via the sulfonylurea receptor.

In ventricular myocytes, anoxia has been shown to lead to a biphasic change in K_{ATP} channel activity (36). After a latency of a few minutes, on-cell current rises to a peak and then decays with time constants in the range of 30 s (36). The decay is much faster than in our experimental model. However, given the unknown metabolic differences (in overall metabolic pathways, phosphatase activity, etc.) resulting from different genetic or environmental factors, between the model and native tissues, the qualitative similarity suggests that the secondary decrease in the native cardiac K_{ATP} current may result from increased ATP sensitivity because of decreased [PPI]. Contrary to this suggestion and opposite to the present findings, it has been reported that the ATP sensitivity of cardiac K_{ATP} channels can actually decrease within the first few minutes of metabolic inhibition (37). It is possible that differential experimental conditions are responsible. Most notably, in the latter experiments, the effect depended on extracellular Ca^{2+}, suggesting that in that case, elevated intracellular Ca^{2+} may activate additional processes that ultimately lead to reduced ATP sensitivity. In addition, glycolysis was not inhibited in the earlier study, and experiments were performed at room temperature. Preliminary experiments at such temperatures revealed a much smaller decline of channel current in the present experiments (data not shown).

Conclusions—Utilizing heterologously expressed K_{ATP} channels as indicators of $[ATP]_{sub}$ in COSm6 cells, the striking observation of this study is that apparent $[ATP]_{sub}$ is quite variable from cell to cell and moreover correlates with the ATP sensitivity of K_{ATP} channels rather than with on-cell activity. That the ATP sensitivity of single molecular species is variable and controlled by $[ATP]_{sub}$ suggests a mechanistic link, which we hypothesize to be PPI levels. Parallel changes in PPI and ATP sensitivity in metabolically inhibited cells and block of these changes by a PI kinase inhibitor (wortmannin) further strengthen this hypothesis.

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