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COMMENTARY

K_{ATP} Channels: Linker between Phospholipid Metabolism and Excitability

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ABSTRACT. ATP-sensitive potassium (K_{ATP}) channels couple electrical activity to cellular metabolism via their inhibition by intracellular ATP. When examined in excised patches, ATP concentrations required for half-maximal inhibition (IC_{50}) varied among tissues and were reported to be as low as 10 μM. This set up a puzzling question on how activation of K_{ATP} channels can occur under physiological conditions, where the cytoplasmic concentration of ATP is much higher than that required for channel inhibition. A new twist was added to this puzzle when two recent reports showed that phospholipids such as phosphatidylinositol-4,5-bisphosphate (PIP_2) and phosphatidyl-4-phosphate (PIP) are able to shift ATP-sensitivity of K_{ATP} channels from the micro- into the millimolar range and thus provide a mechanism for physiological activation of the channels. This commentary describes how phospholipids control ATP inhibition of K_{ATP} channels and how this mechanism is regulated effectively by receptor-mediated stimulation of phospholipase C. BIOCHEM PHARMACOL **60**;6:735–740, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. ATP-sensitive potassium channels; inward-rectifier; phosphatidylinositol-4; 5-bisphosphate (PIP_2); phosphatidylinositol-4-phosphate (PIP); phospholipase C; G-protein coupled receptors

K_{ATP}† channels are present in most, if not all, excitable tissues and share the property of being inhibited by cytoplasmic ATP. Through this ATP inhibition, K_{ATP} channels couple cell metabolism to electrical activity and thus play an important role in the physiology and pathophysiology of many tissues [1]. The prototypic example of this coupling is insulin secretion from pancreatic β-cells in response to glucose uptake. The latter results in elevation of the intracellular ATP, which, through closure of K_{ATP} channels, produces a membrane depolarization, activation of voltage-dependent Ca^{2+} channels, and influx of Ca^{2+}, which finally triggers release of insulin [1]. A further example of K_{ATP}-mediated coupling of metabolism and electrical activity is ischemia-related hyperpolarization, where a decrease in intracellular ATP releases ATP inhibition of K_{ATP} channels and thus leads to hyperpolarization of the membrane potential by an increase in potassium conductance [1, 2]. In either case, K_{ATP} channels must be able to respond to changes in ATP in the lower millimolar range, although only lower micromolar concentrations were required to inhibit these channels in excised patches [1, 3].

Recently, the molecular identity of K_{ATP} channels was elucidated: uniquely among potassium channels, they are formed from an ATP binding cassette protein, the sulphonylurea receptor (SUR1, SUR2), and a two-segment type or Kir potassium channel (Kir6.2, Kir6.1). Both subunits assemble in a 1:1 stoichiometry, with four SUR and four Kir subunits required to form functional K_{ATP} channels [4–7]. While Kir6.x acts as the pore-forming subunit of the channel complex that determines its single channel conductance, its blockade by polyamines, and its inhibition by ATP [8–10], SUR has been identified as the regulatory subunit of K_{ATP} that confers sensitivity to sulfonylureas, channel openers, and Mg-ADP [11–15]. Moreover, SUR acts as a chaperone on the Kir6.x subunits, which allows processing and transport of these proteins to the surface membrane despite an ER-retention motif present in their distal C-terminus [16].

CONTROL OF ATP INHIBITION OF K_{ATP} CHANNELS BY PHOSPHOLIPIDS

In pioneering work, Hilgemann and colleagues have shown that phospholipids such as PIP_2 and PIP are important regulators of ion transporters and channels. In excised membrane patches from cardiac myocytes, these authors showed that functional integrity of the Na^+/Ca^{2+}-exchanger as well as the K_{ATP} channel requires the presence of PIP_2. Removal of this phospholipid through phospholipase C resulted in a loss of channel or transporter activity ('run-down'), which could be reversed with PIP_2 and PIP [17].

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† *Abbreviations*: K_{ATP}, ATP-sensitive potassium channels; PI, phosphatidylinositol; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP, phosphatidyl-4-phosphate; PIP_3, phosphatidylinositol-1,4,5-trisphosphate; SUR, sulfonylurea receptor; Kir, inward-rectifier K^+ channels; PLC, phospholipase C; ER, endoplasmic reticulum; PH, pleckstrin homology; GFP, green fluorescent protein; and PAF, platelet activation factor; PIPs, phosphahidylinositol phosphates.

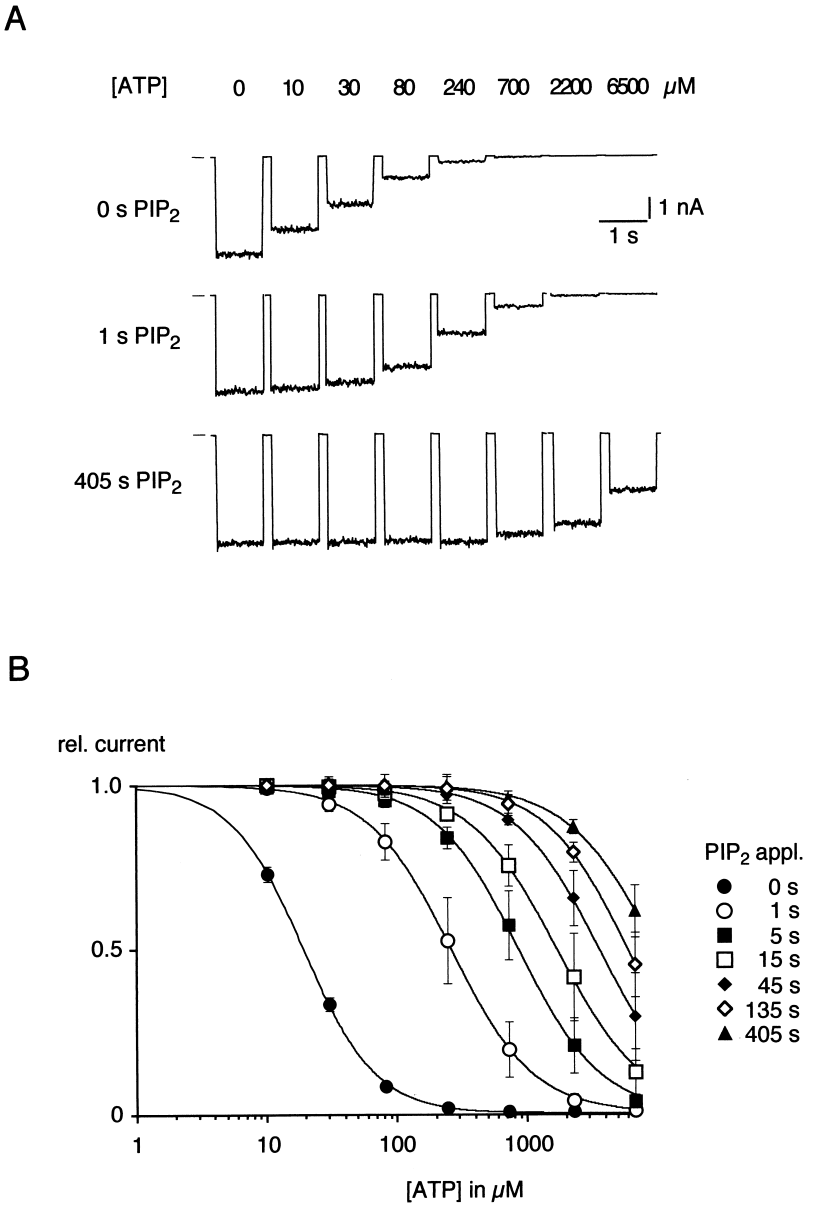

FIG. 1. Shift in ATP sensitivity of K_{ATP} channels by application of PIP_2. Panels A and B: the shift in the steady-state concentration–response relationship for ATP inhibition is shown as a function of PIP_2 application time; recordings as in panel A were performed with giant inside-out patches from *Xenopus* oocytes expressing Kir6.2/SUR1 channels.

In line with these results, the pronounced 'run-down' of channel activity observed with cloned K_{ATP} channels (Kir6.2/SURx) in excised patches could be reversed by application of PIPs [18]. Surprisingly, however, PIPs did not only restore activity of the channels but they also changed their inhibition by ATP quite dramatically [19, 20]. Following patch excision, channels were inhibited by low micromolar ATP, whereas millimolar concentrations of the nucleotide were required for channel inhibition after PIP_2 was applied to inside-out patches for a few seconds (Fig. 1A). More prolonged exposure to PIP_2 even rendered channels completely insensitive to 1 mM ATP [19, 20].

Interestingly, when the loss of steady-state ATP inhibition was monitored with increasing intervals of PIP_2 application, the respective concentration–response curves were shown to shift to higher concentrations without any change in their shape and steepness (Fig. 1B). Thus, the phospholipid seemed not to simply switch the channel from an ATP-sensitive to an ATP-insensitive state in an 'all-or-nothing' manner (which would have resulted in an increasing fraction of unblocked current), but rather rendered the individual channel ATP-insensitive in a gradual 'step-by-step' mode. Mechanistically, this gradual shift in ATP sensitivity was shown to result from a stepwise reduction in binding of ATP to the channel [19]. Unbinding of ATP was not affected, as if the PIPs did not alter the nucleotide-binding site *per se*, but reduced its availability for ATP instead [19].

MOLECULAR DETERMINANTS OF PHOSPHOLIPID ACTION

More light on the interaction between PIPs and the K_{ATP} channel complex came from experiments that aimed to

dissect the determinants required for the effect on ATP inhibition on either of the two partner molecules.

For the phospholipid, basically two properties were found to be important. The first is the negative charge of the head group of the phospholipid. While PIP_3 and PIP exerted an effect similar to PIP_2, no shift in ATP sensitivity was observed with the dephosphorylated PI that lacks negative charges on the inositol ring [19, 20]. Furthermore, PIP_2-mediated shifts in ATP sensitivity could be abolished by application of positively charged compounds such as poly-l-lysine [18, 20], which is well known to screen negative surface charges [21]. The second requirement for the phospholipid is its insertion into the plasma membrane mediated by the lipid tail of the PIPs. Accordingly, inositol-1,4,5-trisphosphate, the 3-fold negatively charged head group hydrolyzed from PIP_2 by PLC, did not exert any effect on ATP inhibition [20]. It should be added that PIPs are only effective when inserted into the inner leaflet of the membrane bilayer; application of PIPs to the extracellular side of the membrane failed to affect channel properties [19].

The latter observation showed that the PIPs interacted with the cytoplasmic portion of the heteromeric K_{ATP} channel complex. As the actual target of the PIPs, the Kir6.x subunit was identified in experiments with a mutant of Kir6.2 that formed ATP-sensitive channels in the absence of SUR due to deletion of the ER-retention motif [19, 20]. Similar to wild type, ATP inhibition of this mutant was largely shifted by PIP_2 [19, 20]. Although the binding site(s) for PIPs has not been identified yet, a few positively charged residues in the cytoplasmic C-terminus were found to influence the effect of PIPs on ATP inhibition [18–20].

From all these results, our understanding of the phospholipid effect on ATP-inhibition emerges as follows. Membrane-inserted PIPs interact directly with the α-subunit of the K_{ATP} channel complex on the basis of electrostatics. As a result of this interaction, the Kir6.x protein gradually undergoes structural rearrangements that lead to decreased accessibility of the nucleotide-binding site for ATP and consecutively to a reduction in ATP inhibition of the channels by the nucleotide. The ATP-binding site itself seems not to be affected by the PIPs.

CONTROL OF K_{ATP} ACTIVITY BY G-PROTEIN COUPLED RECEPTORS

Although PIP_2 and PIP are effective in controlling ATP inhibition of K_{ATP} channels in excised membrane patches, the question remained of whether or not they are of importance under physiological conditions. At least some relevance might be expected, given that the IC_{50} for ATP inhibition observed in excised patches from cardiomyocytes varies by as much as two orders of magnitude [22] and that ATP sensitivity of these cells changes considerably upon metabolic stress [23]. However, it remained obscure over what range the level of PIPs might vary in plasma membranes.

Therefore, we took a more direct approach to show that ATP inhibition of K_{ATP} channels is controlled by PIPs under cellular conditions. A purinoreceptor (P2y2) known to activate PLC via coupling to the $G_{\alpha q}$/11 subtype of G-proteins [24] was heterologously expressed in *Xenopus* oocytes together with the K_{ATP} channel. Stimulation of the P2y2 receptor (by extracellular ATP) would be expected to reduce PIP_2 in the membrane (hydrolysis into IP_3 and diacylglycerol) and thus to result in reduction of the K_{ATP}-mediated current due to the consecutive increase in ATP sensitivity. This was observed in current recordings from whole oocytes (Fig. 2A, upper panel), and the reduction in current did indeed result from an increase in ATP sensitivity of the underlying K_{ATP} channels, as seen in excised patches from such an oocyte (Fig. 2A, lower panel). Moreover, the decrease in K_{ATP}-mediated current was observed only for as long as the P2y-receptor was stimulated. After removal of the ligand, the current started to recover and reached steady-state a few minutes thereafter (Fig. 2A, upper panel).

This type of experiments implied close correlation between the membrane content of PIP_2 and activity of K_{ATP} channels; however, PIP_2 levels were not monitored directly. This 'gap' was filled in by recent work of Meyer and coworkers. With a smart approach these authors showed that receptor-induced stimulation of PLC resulted in reversible degradation of the PIP_2 pool in the plasma membrane [25]. They fused the PH domain of PLC-δ1, which is known to bind PIP_2, to GFP. When this GFP–PH fusion-construct was heterologously expressed in RBL cells, fluorescence was very much restricted to the plasma membrane, as would be expected for GFP–PH binding to membrane-inserted PIP_2 (Fig. 2B, lower panel, 0 sec). Upon stimulation of the coexpressed receptor for the PAF, which effectively couples to PLC, GFP–PH dissociated from the plasma membrane and became uniformly distributed in the cytoplasm (Fig. 2B, lower panel, 30 sec). As viewed in the last image of this series, this dissociation was only transient, and the GFP–PH relocalized to the plasma membrane after receptor stimulation was stopped (Fig. 2B, lower panel, 360 sec). According to the authors, this relocalization reflected binding of the reporter construct to PIP_2 restored in the surface membrane [25]. Interestingly, the time course of GFP–PH relocalization nicely paralleled the time course observed for the recovery of K_{ATP}-mediated currents after activation of the P2y-receptor was stopped (Fig. 2A, upper panel).

These experiments on receptor-mediated stimulation of PLC present evidence that PIPs are basically able to control ATP inhibition of K_{ATP} channels under cellular conditions and thus represent a powerful tool to control excitability. The complete picture on lipid control of excitability is certainly more complex and comprises a number of further enzymes of the PIPs metabolism such as the phosphoinositide-kinases (PI-kinase), PIPs-phosphatases, and phospho-

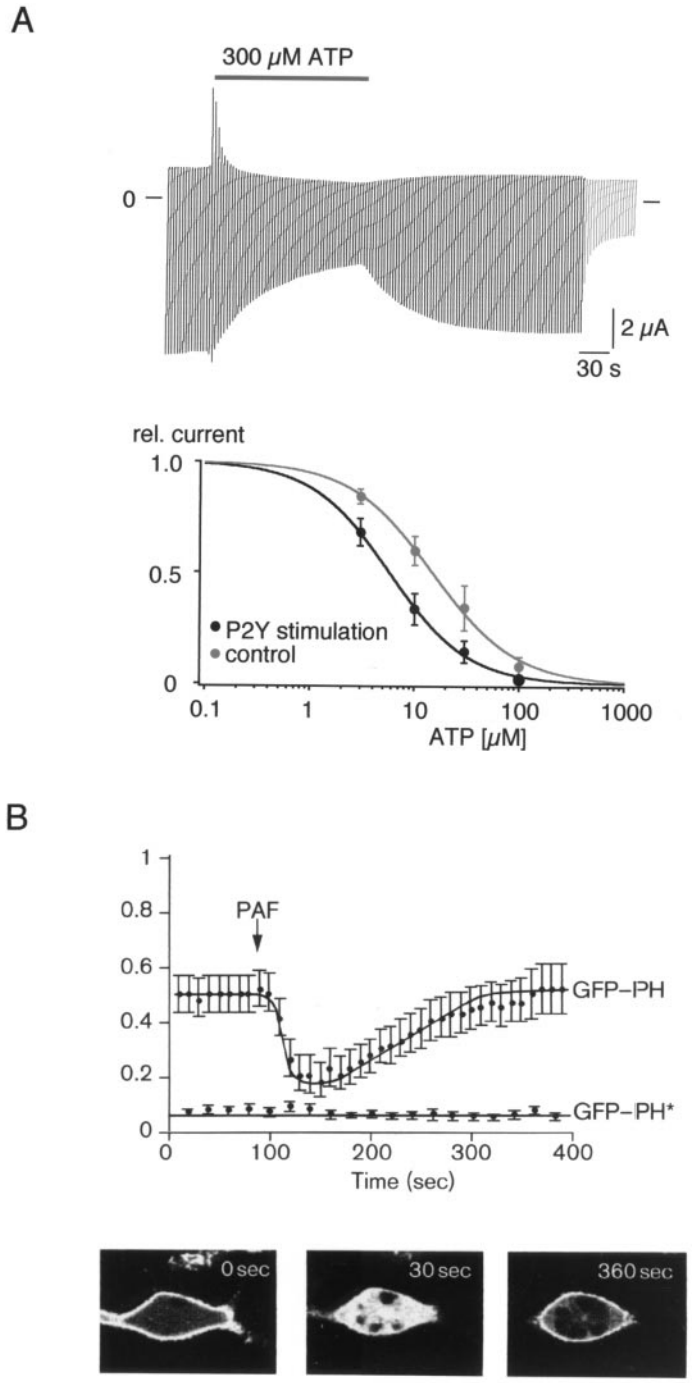

FIG. 2. Regulation of ATP inhibition of K_{ATP} channels by stimulation of G-protein coupled receptors (GPCRs) coupling to PLC. Panel A: currents mediated by K_{ATP} (Kir.6.2/SUR1) channels in a whole *Xenopus* oocyte are decreased (upper panel) by stimulation of a coexpressed P2y2-receptor that couples to PLC; this decrease in current results from an increase in ATP sensitivity of the channels as determined in inside-out patches from stimulated and unstimulated oocytes (lower panel). Panel B: transient dissociation of GFP–PH bound to PIP_2 from the plasma membrane observed after stimulation of the PLC-coupling PAF-receptor. The fraction of membrane fluorescence relative to that of the cytoplasm was calculated by measuring the fluorescence intensity at the membrane (I_{mb}) and dividing it by the average intracellular fluorescence intensity (I_{cy}); data points represent means ± SEM of 12 experiments. The images show GFP fluorescence of an RBL cell before and 30 and 360 sec after stimulation with PAF (for experimental details see text; recordings were reprinted from Stauffer *et al.*, *Curr Biol* 8: 343–346, 1998 (Ref. 25) with kind permission from Elsevier Science).

lipases. Activity of these enzymes is known to change in response to a multitude of biological responses such as activation of heteromeric G-proteins and protein tyrosine kinase receptors [26, 27]. That PI-kinases may indeed be relevant for regulation of K_{ATP} channels is supported by recent work, in which Noma and coworkers show that recovery of run-down K_{ATP} channels could be prevented by wortmannin, an inhibitor of PI-kinases [28]. Furthermore, activation of K_{ATP} channels by leptin in hypothalamus neurons and pancreatic β-cells is thought to involve PI-kinases, pointing to a possible role for the modulation by PIPs of K_{ATP} channels in body weight control [29, 30]. Figure 3 summarizes enzymes and pathways potentially involved in phospholipid-mediated control of K_{ATP} channels, although some of them await experimental verification and clearly require further investigation.

CONCLUSIONS

The membrane-bound phospholipids PIP_2 and PIP determine ATP inhibition of K_{ATP} channels by direct interaction between their negatively charged head groups and the channel α-subunit. This interaction shifts ATP sensitivity by several orders of magnitude into the millimolar range

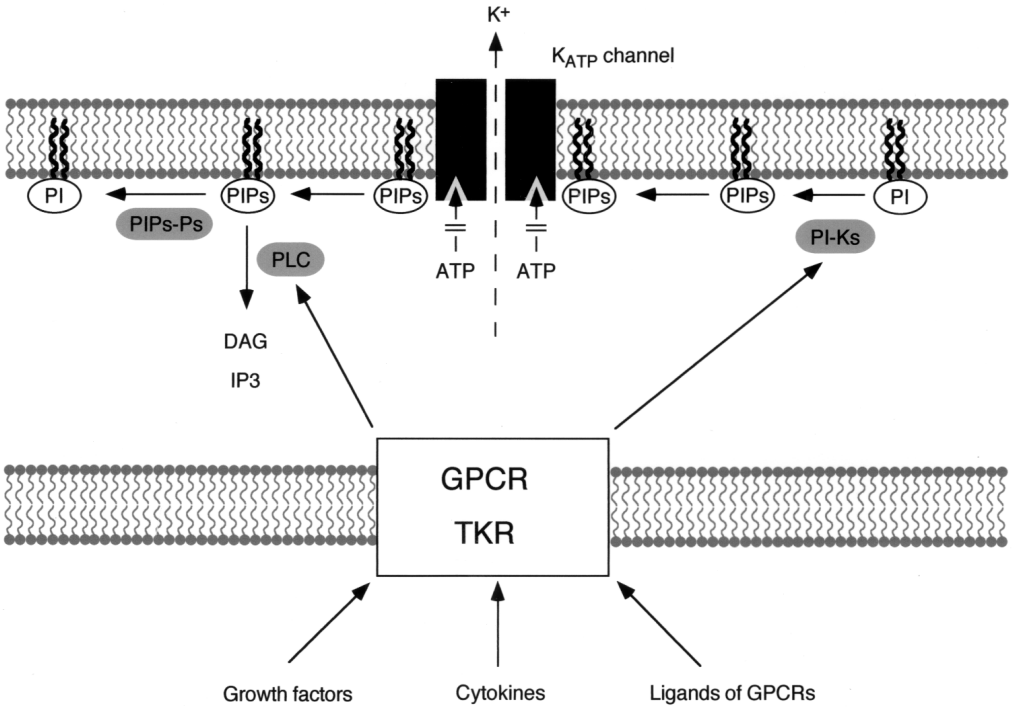

FIG. 3. Illustration summarizing interactions between K_{ATP} channels and phospholipid metabolism. K_{ATP} channels are activated upon binding of PIPs, which abolish ATP inhibition. PIPs are generated from phosphorylation of phosphatidylinositol (PI) by PI-kinases and degraded by phospholipase C (PLC) and PIPs-phosphatases (PIPs-P). PLC and PI-kinases are under the control of G-proteins and tyrosine-kinases that are activated by a multitude of biological stimuli via binding to G-protein coupled receptors (GPCR) and tyrosine-kinase receptors (TKR).

and provides further means to understand why K_{ATP} channels can be activated under physiological conditions. Moreover, the phospholipids are most likely the factor responsible for the considerable variability in ATP sensitivity observed with K_{ATP} channels in various types of cells and under various metabolic conditions.

The levels of PIPs are controlled by key enzymes such as PLC and PI-kinases, whose activity, in turn, is regulated by G-proteins and tyrosine-kinases. These cascades show how K_{ATP} channels link electrical activity to lipid metabolism and suggest interaction of PIPs and K_{ATP} as a very powerful tool to control excitability in many types of cells.

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