Fitting K_V potassium channels into the PIP_2 puzzle: Hille group connects dots between illustrious HH groups

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In the August 2012 issue of the *JGP*, the Bertil Hille group continues its exploration of the physiological significance and cell signaling roles of PIP_2 interactions with potassium channels. As we have come to expect, the group brings surprises and deep insights. In their article (Kruse et al., 2012), they have tackled the question of which voltage-gated potassium (K_V) channels may be affected by, and therefore regulated by, changes of PIP_2 that can occur in intact cells. The work brings equally clarity and sobriety to an area of ion channel regulation that has seen false starts. Whereas most, if not all, inward rectifier potassium channels are strongly PIP_2 dependent, the situation with K_V channels is very different. PIP_2 effects on K_V channels can be large, small, variable, or absent, and PIP_2 can evidently modify primarily activation/deactivation parameters, inactivation, or channel availability per se in different cases.

Two cases can illustrate the nature of the issues at hand for K_V channels. First, many K_V channels, like other channels, are functionally labile in excised patches. Knowing that PIP_2 activates many ion channels, we tested many years ago whether the notoriously labile $K_V2.1$ channels (Zühlke et al., 1994) might be stabilized in excised patches by incorporating PIP_2. In our hands, the $K_V2.1$ channels were completely rescued from rundown and remained stable for unlimited recording periods (Hilgemann et al., 2001). Second, as just noted, K_V channel inactivation can be drastically "PIP_2 dependent" in excised patches. When recording $K_V1.1$ or $K_V3.4$ channel currents in excised patches, the application of PIP_2 micelles to the cytoplasmic side of patches can rapidly abolish inactivation (Oliver et al., 2004). One explanation for the inactivation block is that PIP_2 interacts with the positively charged inactivation domain of K_V channels. Similar to suggestions for Na/Ca exchangers (Hilgemann and Ball, 1996; He et al., 2000), positively charged inactivation domains might be able to reach and bind to PIP_2 in the plasmalemma, thereby preventing them from causing inactivation. Both of these cases, and others, suggested to many of us that PIP_2 would turn out to be a very important regulator of K_V channels.

Enter now the Hille group, which is focused on cell signaling roles of PIP_2 that can be demonstrated to occur in intact cells. Previous efforts of this group (Suh and Hille, 2002; Suh et al., 2004; Falkenburger et al., 2010) and the David Brown group (Hughes et al., 2007) established by far the best models yet of physiological regulation of ion channels by PIP_2, namely by classical pathways involving Gq-coupled receptors whose activation can lead to PIP_2 depletion and subsequent M-type ($K_V7.2/7.3$) potassium channel inhibition. These groups established beyond any reasonable doubt that this pathway controls the firing pattern of these neurons that in turn controls the subsequent release of catecholamines. In addition to modulating M-currents, PIP_2 depletion consequent to muscarinic activation also clearly inhibits N-type Ca channel currents (Gamper et al., 2004). There are in fact very few other examples in which PIP_2 has clearly been shown to play a second messenger role in intact primary cells without receptor overexpression. One very important case, still not resolved clearly, is light-induced depolarization of the *Drosophila melanogaster* rhabdomere, which may be related to PIP_2 depletion or generation of DAG during light-induced phospholipase C (PLC) activation (Huang et al., 2010).

To probe whether K_V potassium channels (and other channels) can be regulated by physiological PIP_2 changes, the Hille group used several powerful molecular biological tools to rapidly deplete PIP_2 and simultaneously monitored the PIP_2 changes that occurred by FRET between two fluorescent PIP_2-binding proteins. To deplete PIP_2, the group used overexpressed muscarinic receptors that couple to endogenous PLCs, PIP_2-selective phosphatases that are voltage dependent in the sense of being activated by depolarization, and they used PIP/PIP_2-selective phosphatases that can be brought to the cell surface rapidly "on order" by applying rapamycin. The outcomes were clear and surprising. The same K_V channels that many of us expected to be PIP_2 sensitive were unaffected by PIP_2 depletion. Thus, large PIP_2 changes in the cell surface of intact cells will not modulate the function of these K_V channels.

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There are at least three reasons in my opinion for the striking disparities between expectations from previous experiments and the results of the Hille group. First, the application of PIP_2 micelles to excised patches may generate many physiologically irrelevant results: PIP_2 micelles may interact with positively charged protein domains in ways that never occur physiologically; PIP_2 micelles may extract nonintegral PIP_2-binding proteins from membranes; and PIP_2 may incorporate to a physiologically irrelevant extent in the cytoplasmic membrane monolayer, thereby generating unphysiological protein confirmations.

Second, PIP_2 may modulate K_V channels in a constitutive fashion (i.e., in a quasi-structural sense), whereby high affinity PIP_2 interactions might never be subject to regulation by plasmalemmal PIP_2 changes that can occur physiologically. In our experience, this appeared to be the case more often than not, leading us to suggest that PIP_2 is usually acting in a constitutive manner to activate channels and transporters when they are resident in the surface membrane of cells (Hilgemann et al., 2001). Lack of PIP_2, and perhaps other membrane composition differences, would keep these same channels and transporters inactive when trafficking on internal membranes (Hilgemann et al., 2001). Consistent with this explanation, the Logothetis group has recently described that changes of the gating properties of Shaker-type $K_V1.1$ channels, which occur on excision of membrane patches, are largely explained by a loss of PIP_2 (Rodriguez-Menchaca et al., 2012). Because this group used multiple experimental approaches, besides the application of PIP_2 micelles, it would be hard to deny a constitutive function of PIP_2 at these K_V channels. That said, the Logothetis group does find substantial changes of Shaker potassium channel gating when they induce PIP_2 changes in intact cells. Potential reasons for differences between results of the two groups include both the cell types used and the methods to change PIP_2.

Third, another perspective says that "PIP_2 is not alone." The surface membrane is more "anionic" than other membranes of cells, owing to the presence of multiple anionic lipid species that may (or may not) be subject to regulation (Yeung et al., 2008). Our experience is that transporters (Collins et al., 1992) and ion channels are usually not highly selective for PIP_2 versus other anionic lipids (Hilgemann et al., 2001). Thus, biochemically labile anionic lipids besides PIP_2 may activate K_V channels in intact cells, in concert with PIP_2 or as substitutes for PIP_2. Phosphatidylserine and phosphatidate are obvious candidates. Whereas phosphatidylserine in the cytoplasmic monolayer may change with alterations of membrane asymmetry that can occur in pathological cell circumstances after membrane patch excision (Hilgemann and Collins, 1992), phosphatidate can alter dramatically with cell signaling changes (e.g., Lopez et al., 2012). Decades have passed since these issues first arose, and yet we still need to know whether metabolism of lipids besides PIP_2 might underlie the rundown of different ion channels upon excision of membrane patches from cells.

There are many more cases where the methods used by the Hille group (Kruse et al., 2012) can tell us whether channels and transporters might be regulated by changes of surface membrane PIP_2 during cell signaling, starting with a long list of TRP channels that appear to be lipid sensitive (Rohacs, 2009). Ca-activated "BK" potassium channels also belong in this list. In our hands, BK potassium channels were completely insensitive to PIP_2 changes, using patches from tracheal smooth muscle cells, thereby verifying to us that PIP_2 sensitivity was specific to specific channel types (Hilgemann and Ball, 1996). However, it turned out that the cell-specific expression of β-1 subunits of these channels confers PIP_2 sensitivity (Vaithianathan et al., 2008). The involvement of subunits in establishing PIP_2 sensitivity is clearly consistent with the idea that PIP_2 signaling to these channels will not only be "constitutive" in nature but also occurs in a cell environment–critical fashion. Cardiac Na/Ca exchangers and K_{ATP} channels were the first two ion transport systems that my group found to be PIP_2 sensitive. Both cardiac Na/Ca exchangers and K_{ATP} channels were strongly activated by the ATP-dependent generation of PIP_2 in membrane patches, as well as by exogenous PIP_2 (Hilgemann and Ball, 1996). Nevertheless, the physiological roles that PIP_2 plays in both systems remain rather enigmatic (Hilgemann, 2007).

These examples illustrate important gaps in our knowledge about the role of PIP_2 for individual channels and transporters, gaps that can be filled by using the tools used by the Hille group. These tools can also be used to address larger questions about PIP_2 functions in cells. Besides its complex roles in cytoskeleton regulation and classical endocytic processes, PIP_2 appears to drive the formation of membrane domains, membrane budding, and membrane turnover (Fine et al., 2011; Hilgemann and Fine, 2011; Lariccia et al., 2011). In this context, the present studies of K_V channel regulation by PIP_2 reflect a convergence of the work of two great "H&H" research teams of the early 1950s. The first team was of course Hodgkin and Huxley, whose analysis of voltage-gated ion channels is known to every reader of this journal. The second team, less well known to the readers of this journal, was Hokin and Hokin, who described in the same years that phospholipids become phosphorylated in response to autonomic hormones, specifically that acetylcholine activates phosphorylation of lipids in secretory cells (Hokin and Hokin, 1953). Hokin and Hokin's results represent the activation of synthesis pathways for multiple phospholipids, including PIP_2, other phosphoinositides, and

phosphatidate, by the same general pathways that activate PIP_2 breakdown by PLCs (Hokin, 1996). As surprising as it may be, these pathways remain poorly understood because turnover of these lipids is connected to membrane turnover, so that an understanding of the underlying mechanisms becomes highly cell biological. For certain, phosphatidylinositol must be continuously provided to the surface membrane from intracellular organelles, where it is generated (Kim et al., 2011). There are also indications that PI4P is not generated at the cell surface but is transferred from other membrane sources (Yaradanakul et al., 2007; Balla et al., 2008), whereas PIP_2 is lost from the surface membrane during endocytosis (Doherty and McMahon, 2009). These issues must ultimately be understood to understand how PIP_2 metabolism controls ion channels in intact cells. To no surprise, Bertil Hille's group is now investigating these more cell biological issues (Dickson, E.J., G.R.V. Hammond, and B. Hille. 2012. Biophysical Society 56th Annual Meeting. Abstr. 1681) in addition to PIP_2–channel interactions. With new light-activated enzymatic probes moving onto their stage (Idevall-Hagren et al., 2012), we may soon expect new insights into both the biophysical and cell biological basis of K_V channel regulation by PIP_2.

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