PIP_2: A critical regulator of vascular ion channels hiding in plain sight

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The phosphoinositide, phosphatidylinositol 4,5-bisphosphate (PIP_2), has long been established as a major contributor to intracellular signaling, primarily by virtue of its role as a substrate for phospholipase C (PLC). Signaling by G_q-protein–coupled receptors triggers PLC-mediated hydrolysis of PIP_2 into inositol 1,4,5-trisphosphate and diacylglycerol, which are well known to modulate vascular ion channel activity. Often overlooked, however, is the role PIP_2 itself plays in this regulation. Although numerous reports have demonstrated that PIP_2 is critical for ion channel regulation, how it impacts vascular function has received scant attention. In this review, we focus on PIP_2 as a regulator of ion channels in smooth muscle cells and endothelial cells—the two major classes of vascular cells. We further address the concerted effects of such regulation on vascular function and blood flow control. We close with a consideration of current knowledge regarding disruption of PIP_2 regulation of vascular ion channels in disease.

PIP_2 | GPCR | ion channel | smooth muscle cell | endothelial cell

The purpose of the vertebrate cardiovascular system is to deliver sufficient oxygen and nutrients to, and remove CO_2 and waste products from, all cells of the body. The basic features of this system are familiar: The heart pumps blood into the vasculature, a delivery system of gradually narrowing arteries and arterioles that terminates in a vast arborizing network of capillaries—the sites of oxygen and nutrient exchange with tissue—before transitioning to a venous network of gradually increasing vessel diameter that collects the deoxygenated blood and sends it via the right ventricle to the lungs for reoxygenation and then back to the heart, where the cycle starts over again. Arteries and arterioles of the peripheral circulation are the main determinants of vascular resistance, which, together with cardiac output, determines blood pressure. These vessels have an outer layer of connective tissue, one (arterioles) or more (arteries) layers of smooth muscle cells (SMCs), and an interior lumen lined by endothelial cells (ECs). SMCs and ECs are equipped with a repertoire of voltage-gated ion channels, ligand-gated ion channels, and G-protein–coupled receptors (GPCRs). Collectively, these receptors and channels endow SMCs and ECs with the ability to sense, respond to, and balance multiple physiological inputs.

SMCs of arteries and arterioles possess the ability to contract in response to increases in intravascular pressure to produce a reduction in vessel diameter (1, 2). This feature, known as the myogenic response, is a homeostatic mechanism that establishes the tone (contractile activity) of the vessel and is an essential regulatory feature of small arteries and arterioles that contributes to the maintenance of relatively constant blood flow in the face of changes in blood pressure. As the name implies, the myogenic response is intrinsic to the smooth muscle myocytes, but SMC contractility is further controlled by ECs, changes in tissue metabolism, and importantly, humoral and neural stimuli.

Smooth muscle contractility is principally set by changes in SMC intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which reflects Ca^{2+} release from intracellular stores and influx of Ca^{2+} from the extracellular space. The primary driver of Ca^{2+} influx into the cell is a change in SMC membrane potential (V_M), and the central player governing Ca^{2+} entry in SMCs—and thus a major determinant of vascular tone—is the smooth muscle voltage-dependent Ca^{2+} channel, Cav1.2, which is activated by V_M depolarization (2, 3). The membrane potential of SMCs and ECs in arteries constricted by physiological intravascular pressures is typically around −40 mV. Under these conditions, Cav1.2 channels mediate Ca^{2+} influx, which increases $[Ca^{2+}]_i$ and leads to phosphorylation of myosin light chain, actin–myosin cross-bridge cycling, and ultimately, smooth muscle contraction and vasoconstriction. Hyperpolarization, on the other hand, deactivates Cav1.2 channels, decreasing Ca^{2+} entry into SMCs and leading to vasodilation. Therefore, signals that depolarize SMCs will tend to constrict arteries, and hyperpolarizing signals will counteract tone development and evoke vasodilation (Fig. 1). The relationship between membrane potential and arterial diameter is steep, with maximum dilation occurring at about −60 mV and maximum constriction at about −30 mV (2).

In addition to Cav1.2 channels, SMCs also express a variety of Na^+-, Ca^{2+}-, or Cl^--permeable ion channels that when activated, cause membrane depolarization, and thereby induce vasoconstriction (Fig. 1). Among these additional SMC depolarizing channels are transient receptor potential (TRP) cation channels of the canonical (TRPC3, TRPC6) and melastatin (TRPM4) subfamilies, and anion channels such as the Ca^{2+}-activated

Significance

Phosphatidylinositol 4,5-bisphosphate (PIP_2), a plasma membrane lipid, is hydrolyzed by G_q-protein–coupled receptor (G_qPCR) signaling into inositol 1,4,5-trisphosphate and diacylglycerol—extensively studied second messengers with profound regulatory effects in the vasculature. However, there is extensive evidence that PIP_2 directly regulates ion channels, a finding with significant implications for vascular function. Beyond providing a previously unexplored perspective on how vascular G_qPCR signaling influences vascular function, the concept of PIP_2-mediated ion channel regulation helps to explain how vascular cell excitability is coordinated to support cerebral blood flow control mechanisms. Importantly, the link between the metabolic state of vascular cells and PIP_2 content may provide insight into how metabolism affects vascular ion channel activity and, ultimately, vascular function in health and disease.

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Cl^- channel, TMEM16A. Among ion channels in SMCs that contribute to V_M by exerting a hyperpolarizing influence are voltage-dependent K^+ ($K_V1.2$, $K_V1.5$, $K_V2.1$) channels, strong inward-rectifier K^+ (Kir2.1) channels, large-conductance, Ca^{2+}- and voltage-sensitive K^+ (BK) channels, and ATP-sensitive K^+ (K_{ATP}) channels. Activation of any of these K^+ channels mediates K^+ efflux and causes SMC hyperpolarization and vasodilation (4).

SMC V_M is also influenced by the endothelium. Heterocellular coupling between ECs and SMCs, enabled by gap junctions localized to specialized microdomains termed myoendothelial projections (Fig. 1), allows the transfer of electrical signals from ECs to SMCs. This electrical coupling between ECs and SMCs guarantees that a change in endothelial V_M will alter the smooth muscle contractile state (tone). A prominent ion channel in the arterial/arteriolar endothelium that contributes to endothelial regulation of SMC V_M is the Ca^{2+}-permeable TRPV4 channel. When activated by epoxyeicosatrienoic acids (EETs) or acetylcholine-stimulated PKC, endothelial TRPV4 channels mediate an influx of Ca^{2+} that subsequently activates endothelial intermediate-conductance (IK) and small-conductance (SK) Ca^{2+}-activated K^+ (K_{Ca}) channels, triggering endothelial V_M hyperpolarization via K^+ efflux. This not only hyperpolarizes ECs, it also hyperpolarizes the membrane of adjacent SMCs (5). Importantly, ECs are electrically coupled to one another through EC–EC gap junctions, forming what can be viewed as an electrical syncytium that facilitates the transfer of electrical signals initiated in one cell to neighboring cells. ECs (and some types of SMCs) express Kir2.1 channels; notably, endothelial Kir2.1-mediated hyperpolarization can be transmitted directly to overlying SMCs in arteries, leading to vasodilation (6). Additionally, we have recently found that Kir2.1 channels are expressed in capillary ECs, which lack surrounding SMCs. Here, Kir2.1 activation hyperpolarizes capillary ECs, producing an electrical signal that is conducted to neighboring ECs by connexins (Fig. 1) until it reaches the upstream arteriole, whereupon it relaxes smooth muscle and evokes vasodilation (7). Another notable ion channel in ECs is the mechanosensitive Piezo1 channel, which senses mechanical forces in the vessel lumen, leading to influx of Ca^{2+} and Na^+. It has therefore been suggested that the Piezo1 channel directly depolarizes ECs or, alternatively, is functionally associated with synthesis of the potent vasodilator, nitric oxide (for review, see ref. 8).

GPCRs, which signal through different heterotrimeric G-protein subtypes ($G_{q/11}$, G_s, $G_{i/o}$, and $G_{12/13}$) to an array of downstream signaling cascades, are key elements in the repertoire of extracellular signal-regulated receptors in vascular ECs and SMCs with particular relevance to PIP_2 regulatory dynamics. Among G-protein subtypes, G_q acts in pathways that serve to modulate arterial diameter; thus, G_q-protein–coupled receptor (G_qPCR) signaling has been a central focus in vascular physiology. In the canonical signaling pathway, stimulation of G_qPCRs activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (hereafter, PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 evokes Ca^{2+} release from the sarcoplasmic/endoplasmic reticulum (SR/ER) by sensitizing IP_3 receptors (IP_3Rs) to stimulatory Ca^{2+}, whereas DAG and Ca^{2+} activate protein kinase C (PKC). These IP_3/Ca^{2+} and DAG/Ca^{2+}/PKC cascades are major signaling pathways that have significant effects in SMCs and ECs. For instance, smooth muscle IP_3R-mediated Ca^{2+} release stimulates TRPM4 channels (9), and DAG directly activates different TRPC channel subtypes (10). Furthermore, DAG-activated PKC is an important regulator of voltage-dependent Ca^{2+} channels (11) and K_{ATP} channels (12) in SMCs. These PIP_2 metabolites are also dynamically involved in modulating endothelial ion channels: IP_3R-mediated Ca^{2+} release activates Ca^{2+}-activated IK and SK channels (13), and PKC promotes endothelial TRPV4 channel activity (14).

Although the metabolites resulting from G_qPCR signaling and PIP_2 depletion—IP_3 and DAG—are known to be important regulators of vascular ion channels, the fact that G_qPCR signaling is associated with a concomitant dramatic decrease in PIP_2 levels is often underappreciated in vascular studies. This less-studied aspect of vascular PIP_2 metabolism has profound implications for the regulation of membrane proteins, including ion channels, many of which are positively or negatively

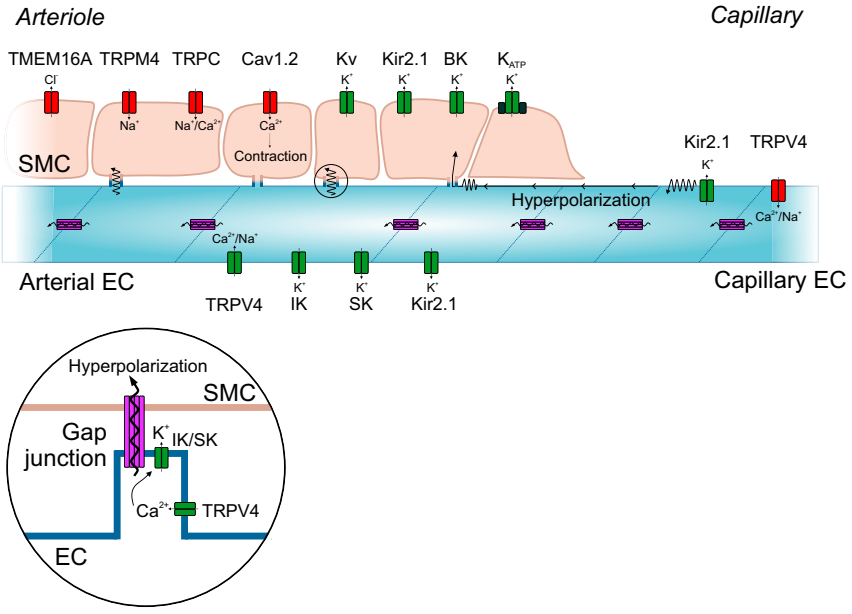

Fig. 1. Principal vascular ion channels that regulate V_M and smooth muscle contraction. A schematic depiction of prominent ion channels in SMCs and ECs. The arteriolar wall is composed of ECs facing the vessel lumen surrounded by an overlying layer of SMCs. Capillaries lack SMC coverage. Vascular cells are electrically coupled through gap junctions, which facilitate charge movement from one cell to the neighboring cell. Channels indicated in green are primary hyperpolarizing ion channels; those in red depolarize the V_M of their corresponding cell type.

regulated by association with PIP_2 in the plasma membrane (15–18). In this review, we consider the important roles of PIP_2 as a regulator of ion channels in vascular SMCs and ECs and address how this modulation affects (or could affect) vascular function. We additionally discuss how cellular PIP_2 levels are determined, as well as the basis for PIP_2–ion channel interactions. Finally, we review our current understanding of PIP_2-mediated regulation of vascular ion channels in health and disease.

Determinants of PIP_2 Levels in the Cell

Polyphosphoinositides—the phosphorylation products derived from phosphatidylinositol (PI)—exhibit different interconversions that reflect the number and sites of phosphorylated hydroxyl groups on the inositol ring (Fig. 2). The phosphoinositide PIP_2 is a minor, negatively charged phospholipid that resides primarily in the inner leaflet of the plasma membrane. An important factor to appreciate in considering PIP_2 involvement in signal transduction is that PIP_2 levels are dynamic. The cellular levels of PIP_2 reflect the net effect of lipid kinases and phosphatases, as well as G_qPCR activity-induced PIP_2 hydrolysis by phospholipases, the latter of which is the primary driver of dynamic changes in PIP_2 levels.

PIP_2 Synthesis. Distinct polyphosphoinositides can be generated from PI by phosphorylation of one to three hydroxyl groups at positions 3, 4, and 5 on the inositol ring (Fig. 2) by site-specific phosphoinositide kinases (Fig. 3). Phosphoryl transfer (to positions 4 and 5, in the case of PIP_2) by kinases requires ATP and the cofactor Mg^{2+}. Unlike protein kinases, most of which are maximally active at low-micromolar intracellular concentrations of ATP ($[ATP]_i$), lipid kinases generally require much higher concentrations of ATP (hundreds-of-micromolar range) to support their activity (19). The formation of PIP_2 reflects the sequential actions of phosphatidylinositol 4-kinase (PI4K) and phosphatidylinositol 4-phosphate 5-kinase (PIP5K). Some PIP_2 is also generated through dephosphorylation of $PI(3,4,5)P_3$ by phosphatases, such as PTEN (phosphatase and tensin homolog) (Fig. 3). Phosphorylation by PI4K is the rate-limiting step in PIP_2 synthesis, with a Michaelis–Menten constant for ATP ($K_{M,\ ATP}$) of ~0.4 to 0.9 mM (19–22). One implication of this relatively high K_M for ATP is that decreases in free cytoplasmic $[ATP]_i$, and therefore the ATP:ADP (adenosine diphosphate) ratio, could significantly slow phosphoinositide synthesis by suppressing the phosphorylation potential of lipid kinases without substantially affecting cellular reactions with a low K_M for ATP, such as those mediated by transporters or protein kinases. In other words, PIP_2 synthesis is sensitive to the physiological energy state of the cell.

PIP_2 Breakdown. The operation of kinases and phosphatases produces continuous fluctuations in polyphosphoinositides, including PIP_2 (Fig. 3). Many of these reactions, however, are not capable of milliseconds-to-seconds regulation of phosphoinositide levels (18, 23–25). Because G_q activation can rapidly activate PLC, and the rate constant for PIP_2 hydrolysis by activated PLC is high, G_q activation can decrease PIP_2 concentration within seconds (time constant, ~10 s) (18, 24) and is thus the predominant contributor to dynamic PIP_2 depletion. In fact, G_qPCR activation can rapidly deplete 90% of the cellular content of PIP_2 (23–26). Notably, the continuously changing activity of G_qPCRs, reflecting variations in the levels of receptor agonists released from perivascular cells (e.g., astrocytes, neurons) or circulating in the bloodstream, can lead to varying degrees of PIP_2 depletion. However, restoration of PIP_2 by ongoing lipid kinase-mediated synthesis is slower, spanning minutes (time constant, ~200 to 500 s) (18), resulting in long-lasting effects of G_q activation on PIP_2 cellular content and the activity of PIP_2-regulated proteins (18, 27). Thus, G_qPCR-mediated hydrolysis of PIP_2 can outstrip synthesis and could therefore represent a major influence on proteins and ion channels that are targets of PIP_2 regulation (Fig. 3).

Basis for PIP_2–Ion Channel Interactions

It has been shown that many ion channels are regulatory targets of PIP_2. As of this writing, the number of PIP_2-modulated ion channels is approaching 100 (15, 28), a number that is likely to continue to grow. In pioneering work performed over two decades ago, Hilgemann and Ball (29) reported that the K_{ATP} channel is regulated by plasmalemmal PIP_2. Many such studies have followed. In most instances, the realization that an ion channel required PIP_2 stemmed from the electrophysiological observation that ionic currents in excised patches changed over time. This phenomenon was frequently abrogated by PIP_2 supplementation or enhancement of PIP_2 synthesis. Collectively, these observations led to the recognition of PIP_2 as an important modulator of ion channel function (for review, see ref. 15).

The abundance of PIP_2 targets raises the question of whether, and if so how, PIP_2 can function to promote specific cellular functions that depend on coordinated enhancement of the activities of specific sets of channels and other proteins and suppression of the activities of others. The specificity of PIP_2 signaling could reflect cell-specific differences in expression levels of potential targets, localization of signaling and targets in

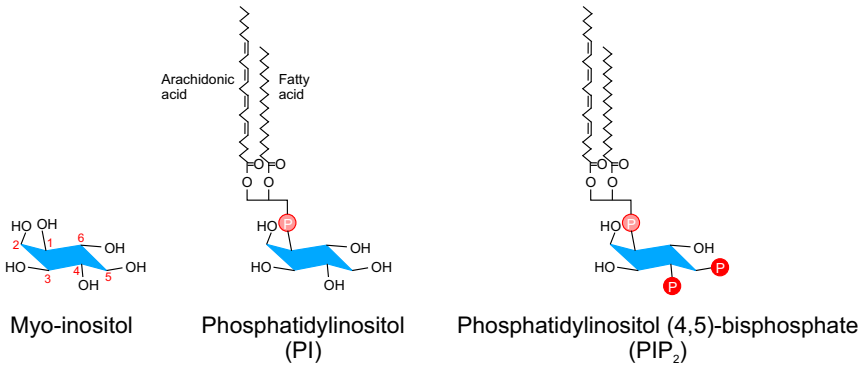

Fig. 2. Structure of PIP_2. Chemical structures of myo-inositol, phosphatidylinositol (PI), and phosphatidylinositol (4, 5)-bisphosphate (PIP_2). The numbering in the myo-inositol structure (red) refers to the different positions on the inositol ring where specific hydroxyl groups can be altered. PI is biosynthesized from phosphatidic acid via the intermediate diacylglycerol (DAG) and inositol. One to three hydroxyl groups (positions 3, 4, and 5) on the inositol ring of PI can be phosphorylated by site-specific phosphoinositide kinases to give rise to different phosphoinositides.

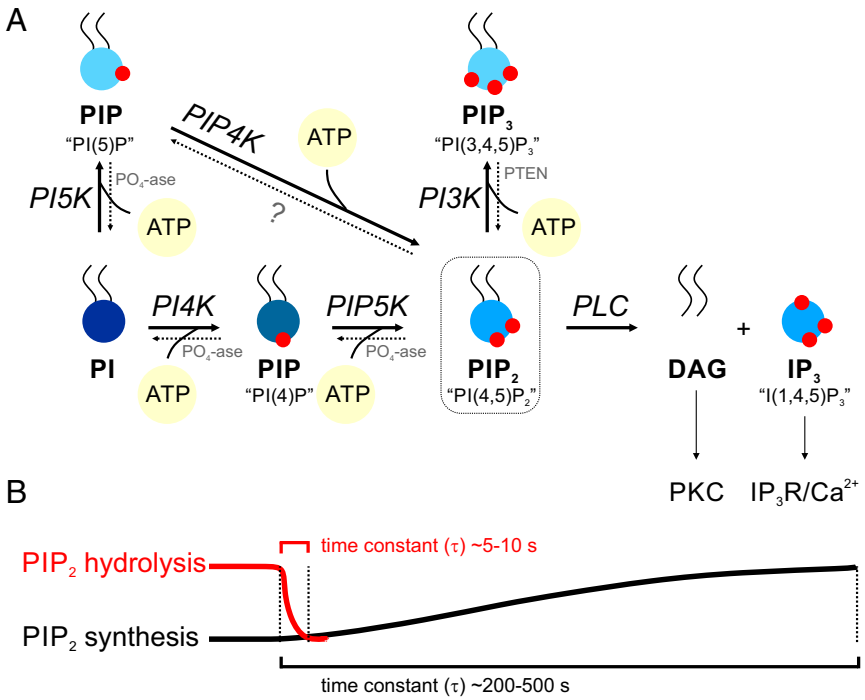

Fig. 3. PIP_2 synthesis and breakdown. (*A*) The precursor, PI, can be phosphorylated by the site-specific phosphoinositide kinase PI4K in the presence of ATP, resulting in phosphoryl transfer to position 4 on the inositol ring and formation of phosphatidylinositol-4-phosphate (PIP). The latter can be further phosphorylated at position 5 by the kinase PI(4)P5K to form PIP_2. Maximum phosphorylation can occur through the action of PI3-kinase (PI3K), which acts specifically on position 3 to form phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3). PI can alternatively be phosphorylated to PI(5)P and then to PIP_2 by the actions of PI5K and PI(5)P4K, respectively. The dotted lines represent phosphoinositide phosphatases that dephosphorylate different phosphoinositides at the 3, 4, and 5 positions of the inositol ring. PLC hydrolyzes PIP_2 to DAG, which activates PKC, and IP_3, which triggers Ca^{2+} release from intracellular stores. (*B*) An illustration highlighting the stark difference between the kinetics of PIP_2 synthesis and hydrolysis (see *Determinants of PIP_2 Levels in the Cell*).

microdomains, and/or the dependence of activities on the coincident reinforcement of multiple signals (e.g., PIP_2 and Ca^{2+} and depolarization) (24, 30–33).

PIP_2 is negatively charged (Fig. 2) and thus contributes to the negative charge of the inner leaflet of the plasma membrane (34). This negative surface charge raises local concentrations of all cations, especially multivalent inorganic (e.g., Ca^{2+}) and organic (e.g., spermine) cations, and freely diffusing, net positively charged proteins on the inner leaflet (35, 36). The high negative charge on PIP_2 can also cause neighboring intrinsic membrane proteins to orient such that net positively charged regions are closer to the PIP_2 headgroup and net negatively charged regions are farther from it (37, 38).

PIP_2 binding to an ion channel depends on the phosphoinositide specificity of the channel. Some ion channels possess specific binding pockets for PIP_2 and therefore show high PIP_2 specificity. Other channels with lower PIP_2 specificity display electrostatic binding to various negatively charged phospholipid molecules. Higher phosphoinositide specificity usually correlates with higher-affinity binding of PIP_2 to the channel (39–42). Taken together with the relative scarcity of PIP_2, the fact that PIP_2 can reversibly bind to ion channels with high affinity has given rise to the "PIP_2-gated ion channel theory," which posits that PIP_2 can be viewed as an agonist or activator of ion channels (41). Intriguingly, a lower PIP_2–ion channel binding affinity is also physiologically important because it permits binding–unbinding to occur over physiological timescales (seconds) and concentrations of receptor agonists. This could translate into more rapid modulation, relative to high-affinity binding, of ion channel activity by factors that alter PIP_2 levels.

Structural studies have demonstrated channel protein moieties complexed with PIP_2 and confirmed that PIP_2 positioning is essential for normal ion channel gating. A good example is the inward-rectifier K^+ channel, Kir2.2 (KCNJ12). Elegant work by the MacKinnon laboratory (43) has revealed crystal structures of apo- and PIP_2-bound Kir2.2 channels, clearly demonstrating that PIP_2 binding induces profound structural changes to open the channel's pore. Alternatively, PIP_2 can alter ion channel function by interfering with channel binding to other regulators, such as ATP, calmodulin, βγ complexes of heterotrimeric G proteins, or even other lipids (44–48).

The fact that all ion channels possess basic motifs means that, in theory, all channels are capable of interacting with PIP_2. An important question, however, is whether such interactions actually occur in vivo and, if so, whether they affect function in a physiologically meaningful manner. This highlights the necessity of performing experiments in native cells and tissues, where ion channels are expressed at physiological levels. It is also important to test whether physiological stimuli capable of changing PIP_2 levels can alter the corresponding ion channel activity and thereby modulate tissue function.

Concerted PIP_2 Regulation of Vascular Ion Channels

Our focus here will be on the physiological regulation of vascular ion channels in ECs and SMCs by PIP_2. For information on the broader topic of phosphoinositides and ion channel function, we refer readers to excellent recent reviews (15, 49, 50). There are many reports describing the ability of PIP_2 to regulate ion channel targets based on electrophysiological recordings of ionic currents in expression systems. In contrast to the wealth of literature on ion channel regulation by PIP_2 in cell culture and cell-free systems, there are relatively few reports of such regulation in native vascular cells (27, 51–53).

Vascular K^+ Channels. As noted above, Hilgemann and Ball (29) were the first to describe K^+ channel regulation by PIP_2. Their work opened the door for subsequent studies that have implicated PIP_2 as a modulator of most inwardly rectifying (Kir), voltage-gated (K_V), and Ca^{2+}-activated (K_{Ca}) K^+ channels (for review, see ref. 15). Members of these K^+ channel families are expressed in the vasculature and play key roles in vascular function.

Strong inward-rectifier K^+ channels. The Kir channel family is divided into seven subfamilies, Kir1–7, with variations within subfamilies giving rise to a total of 15 known isoforms (Kir1.1, Kir2.1–4, Kir3.1–4, Kir4.1–2, Kir5.1, Kir6.1–2, and Kir7.1). Among these, there is compelling evidence for the expression of strong inward-rectifier Kir2.1 (KCNJ2) and Kir2.2 (KCNJ12) channels, and weak inward-rectifier Kir6.1 (KCNJ8) channels, in the vasculature (Fig. 1).

In strong inward-rectifier Kir2 channels, outward current is blocked by voltage-dependent binding of intracellular polyamines (e.g., spermine) to the inner pore (54). Kir2.1 is highly expressed at both mRNA and protein levels in the vascular endothelium of arteries, arterioles, and capillaries (6, 7, 52, 55). It is also present and functional in most arterial SMCs, where it is necessary for K^+-mediated vasodilation (56–59). Although Kir2.2 mRNA is present in ECs and SMCs, there is little evidence for Kir2.2 involvement in vascular function (7, 52, 58–60).

Fan and Makielski (61) provided evidence that a mixture of anionic phospholipids that included PIP_2 activated strong inward-rectifier Kir2.1 channels overexpressed in cultured mammalian cells. Huang et al. (16) subsequently discovered that, when expressed in *Xenopus* oocytes, Kir2 channels were directly activated by PIP_2. Using electrophysiological approaches, they showed that Kir2.1 currents gradually declined and that providing PIP_2 to the cytoplasmic side reversed this effect. They further showed that channel activity was enhanced by ATP, which promotes PIP_2 generation by supporting lipid kinase activity, and was inhibited by antibodies against PIP_2. Inhibition of Kir2.1 current, whether due to the lack of PIP_2 or induced by an anti-PIP_2 antibody, was found to be slow compared with that of other inwardly rectifying K^+ channels. On the other hand, they found that addition of PIP_2 (or ATP) led to a recovery of Kir2.1 channel activity (16). About a decade later, MacKinnon's laboratory (43) solved the crystal structure of Kir2.2 channel subunits (encoded by *KCNJ12*), revealing that PIP_2 molecules are bound to a highly structured site in the channel transmembrane domain comprising positively charged residues. This Kir2.2–PIP_2 relationship was shown to be essential for normal channel activity. Like Kir2.2, the Kir2.1 channel (KCNJ2) requires PIP_2 to function (62–64).

Only recently has the PIP_2–Kir2 relationship in the vasculature been explored. Both arterial SMCs and ECs express Kir2 channels (Fig. 1) (6, 56, 59). Recent studies have additionally shown that brain capillaries, composed only of ECs without surrounding SMCs, express Kir2.1 channels that act as sensors of neural activity (7). Conventional whole-cell recordings from freshly isolated and dialyzed mouse capillary ECs revealed a decline in Kir2.1 currents (27) reminiscent of Kir2.2 current rundown reported in excised patches from *Xenopus* oocytes (43). When recordings were made using the perforated-patch configuration, which maintains an intact cytoplasm, capillary Kir2.1 currents did not decline, suggesting the presence of an intracellular factor that sustains Kir2.1 activity. Dialyzing capillary ECs with Mg-ATP, but not with nonhydrolyzable ATP-γ-S, prevented the decline of Kir2.1 channel currents, suggesting that dialysis of the cell caused the loss of ATP, resulting in deactivation of the lipid kinases that synthesize PIP_2. Consistent with this, dialysis with water-soluble PIP_2 in the absence of Mg-ATP was sufficient to sustain Kir2.1 activity (27). Collectively, these observations are

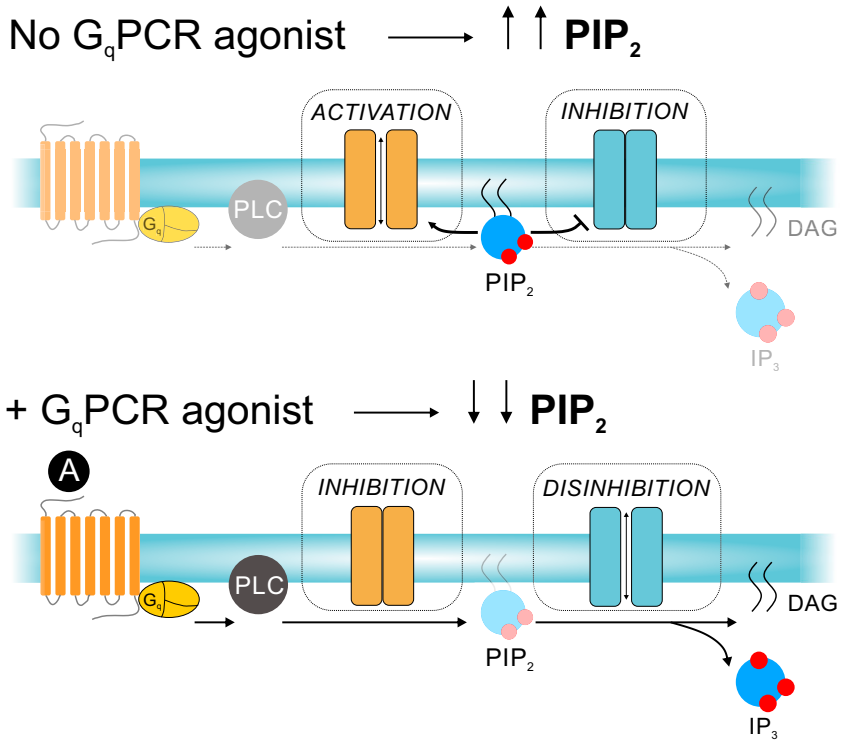

Fig. 4. G_qPCR signaling alters PIP_2 levels and ion channel activity in a coordinated manner. In the absence of G_qPCR agonists (or when G_qPCR activity is minimal), PIP_2 levels at the inner leaflet of the plasma membrane are maintained. PIP_2 can have dual effects on different ion channels, supporting (activating) one ion channel type while simultaneously inhibiting another (tonic inhibition). As G_qPCR activity increases, PIP_2 is hydrolyzed to the metabolites DAG and IP_3. The attendant dramatic depletion of PIP_2 can exert an inhibitory effect on ion channels that require PIP_2 for activation and a disinhibitory effect on ion channels that are tonically inhibited by PIP_2.

consistent with the idea that intracellular ATP helps sustain PIP_2 synthesis, and that PIP_2, in turn, is necessary for capillary EC Kir2.1 activity. The arterial EC Kir2.1 channel similarly requires PIP_2 for activity (52).

Kir2 channels are activated by external K^+ through relief of block by intracellular cationic polyamines (54), a biophysical property that confers on SMCs and ECs the ability to sense local increases in $[K^+]_o$ and convert them into membrane hyperpolarization and vasodilation (7, 65, 66). Membrane potential hyperpolarization also activates Kir2 channels by driving polyamines out of the pore. This property enables electrically coupled ECs to transmit a regenerating hyperpolarizing signal through the endothelium to the smooth muscle layer (7, 67) (Fig. 1).

In the cerebral circulation, activation of the Kir2.1 channel by neuronal activity-driven elevation of $[K^+]_o$ provides a mechanism for translating neural activity into vascular responses, a process termed neurovascular coupling that links neural demand to increases in blood flow and thus O_2 and glucose delivery (7, 65). Modest increases in $[K^+]_o$ during neural activity—a local consequence of neuronal action potentials—leads to sustained hyperpolarization only when Kir2.1 outward current exceeds overall cellular inward current (68). Thus, this mechanism, in which retrograde electrical signaling to upstream arterioles increases cerebral blood flow, is critically dependent on the number of functional Kir2.1 channels, which, in turn, is dependent on PIP_2 occupancy (27). Although dialysis of the cytoplasmic compartment is capable of causing ATP depletion and inhibition of PIP_2 synthesis in whole-cell patch-clamp electrophysiology experiments, under physiological conditions, it is unlikely that ATP would fall to levels low enough to deactivate lipid kinases and suppress PIP_2 synthesis. Instead, hydrolysis of PIP_2 by G_qPCR activation is likely the major mechanism for producing rapid changes in PIP_2 in vivo. In fact, acute depletion of endothelial PIP_2 due to G_qPCR activation and PLC-mediated hydrolysis inhibits capillary Kir2.1 channel activity and abolishes the ability of these channels to communicate to upstream arterioles to enhance cerebral blood flow (Fig. 4) (27). Whether this regulatory dynamic operates in ECs in all vascular beds, however, is not clear. Distinct behaviors at different points in the vascular tree could, in theory, reflect differences in the availability of PIP_2 or cellular localization of receptors and proteins that determine PIP_2 levels (30, 31).

In summary, the activity of strong inwardly rectifying K^+ channels in the cerebral vasculature depends on PIP_2 (Fig. 5). Therefore, the ability of arteries/arterioles to hyperpolarize and dilate in response to neuronal activity and consequent increases in extracellular K^+ is weakened by the loss of PIP_2.

Weak inward-rectifier K^+ channels. In contrast to the strong inward rectification of Kir2.1 and Kir2.2, the Kir6.1 isoform, which is expressed primarily in SMCs and pericytes, is a weak inward-rectifier channel that associates with sulfonylurea receptor subunits (SUR2B) to form ATP-sensitive K^+ (K_{ATP}) channels (69, 70). Notably, the first demonstration that PIP_2 acts as an ion channel regulator was provided by studies on the cardiac K_{ATP} channel, Kir6.2 (encoded by *KCNJ11*), in guinea pig cardiac myocytes (29). The closely related channel, Kir6.1 (encoded by *KCNJ8*), is the pore-forming subunit of K_{ATP} channels in SMCs. Several vasoconstrictor G_qPCR agonists clearly inhibit K_{ATP} channel currents in arterial smooth muscle. However, this inhibition has been attributed to downstream activation of PKC rather than PIP_2 depletion via PLC (12, 70). Several studies support this conclusion. Although PIP_2 can bind to the C terminus of the Kir6.1 channel (71), the effects of PIP_2 depletion on SUR2B/Kir6.1, the complex found in SMCs, appear to be minimal (72). In contrast, the SUR2A/Kir6.2 channel, the predominant complex in ventricular cardiac myocytes, is inhibited by G_qPCR activation through PIP_2 depletion independent of PKC (72). In summary, the lack of PIP_2 regulation of smooth muscle K_{ATP} channels—likely reflecting structural differences in channel complex composition between cardiac myocytes (SUR2A/Kir6.2) and vascular SMCs (SUR2B/Kir6.1)—suggests that PIP_2-mediated regulation of vascular K_{ATP} channels is not of major physiological significance.

Ca^{2+}-activated K^+ channels. Ca^{2+}-activated K^+ (K_{Ca}) channels are important players in vascular physiology and are classified based on their conductances into small (SK)-, intermediate (IK)-, and large-conductance (BK) channels. Activation of K_{Ca} channels leads to membrane hyperpolarization (Fig. 1). SK3 ($K_{Ca}2.3$/KCNN3) and IK ($K_{Ca}3.1$/KCNN4) channels, which are expressed in most ECs (but see below), are voltage insensitive and are thus activated solely by elevations in cytosolic $[Ca^{2+}]_i$. BK channels, in contrast, are voltage and Ca^{2+} sensitive. Although it has been shown that other SK channels in nonvascular cells are modulated by PIP_2 levels (73, 74), this has not been investigated in ECs.

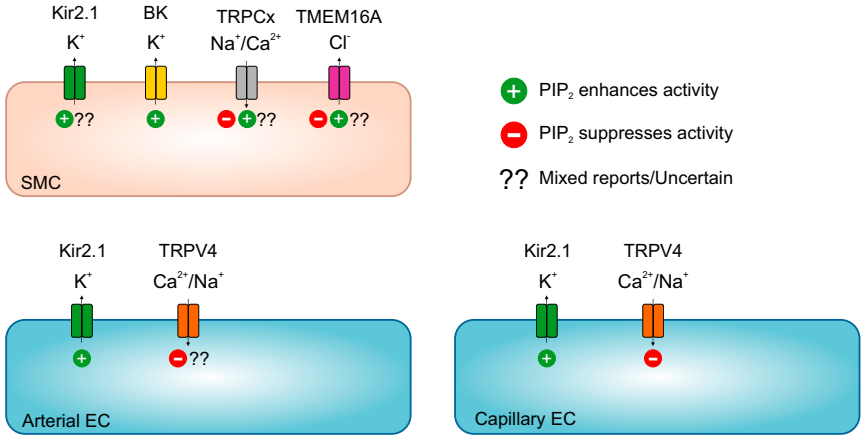

Fig. 5. PIP_2-mediated regulation of vascular ion channels. Different vascular cells express ion channels that are regulatory targets of PIP_2. Capillary ECs: PIP_2 enhances Kir2.1 activity and suppresses TRPV4 activity (27, 51). Arterial ECs: PIP_2 is essential for Kir2 channel activity (52) and presumably suppresses TRPV4 channels, similar to capillary TRPV4. Smooth muscle cells: Extensive evidence supports the conclusion that PIP_2 is required for Kir2 channel activity, although Welsh and colleagues (52) have suggested that PIP_2 is a minor physiological regulator of Kir2 channels in SMCs. PIP_2 directly activates BK channels (53). Studies have variably reported that PIP_2 facilitates or inhibits TRPC channels (see *TRPC Channels*). Greenwood and colleagues (136) have suggested that PIP_2 inhibits TMEM16A in pulmonary artery smooth muscle, but several groups studying nonvascular TMEM16A suggest otherwise (see *Vascular Chloride Channels*).

BK channels are activated by depolarization, with the voltage for half-maximal activation (V_{50}) depending on intracellular $[Ca^{2+}]_i$. They are expressed in virtually all SMCs, where they are arrayed in the plasma membrane in close apposition to ryanodine receptors (RyRs) in the SR. RyR-mediated Ca^{2+}-release events from the SR, termed "Ca^{2+} sparks" (75), provide the Ca^{2+} that supports activation of these channels. During a Ca^{2+} spark, $[Ca^{2+}]_i$ transiently rises well into the micromolar range. One Ca^{2+} spark has been estimated to increase nearby BK channel activity in cerebral arterial SMCs by ~10^5- to 10^6-fold (76). This high $[Ca^{2+}]_i$ causes channel opening, and rapidly hyperpolarizes the SMC membrane, thereby closing voltage-dependent Ca^{2+} channels and limiting Ca^{2+}-dependent contraction.

BK channels in vascular SMCs are activated by endogenous PIP_2 (53). In cerebral arterial SMCs, polyphosphoinositides, including PIP_2, activate BK channels independently of the PIP_2 metabolites, IP_3 and DAG. The PIP_2 headgroup has been proposed to bind to the BK channel, increasing its Ca^{2+} sensitivity and thereby increasing BK open probability (53). Ca^{2+} binding to the channel also enhances the positive effects of PIP_2 on BK channels (77). In the absence of accessory β-subunits, the open probability of the pore-forming α-subunit of the BK channel (encoded by *KCNMA1*) is enhanced approximately fivefold by PIP_2 in vascular SMCs, an effect that is attributable to a leftward shift in V_{50} and amplification of Ca^{2+}-driven gating. However, the accessory β-subunit strongly enhances PIP_2-mediated regulation of BK channels. Association of the vascular SMC BK α-subunit with the β1-subunit (KCNMB1), the predominant β-subtype in vascular SMCs (78), potentiates PIP_2 effects on BK channel open probability, increasing it by ~25-fold (vs. 5-fold in the absence of the β1-subunit) (53). In contrast to the β1-subunit, the β4-subunit (KCNMB4), which predominates in skeletal muscle, does not sensitize BKα to PIP_2 (53).

In summary, PIP_2 increases BK channel activity in vascular SMCs (Fig. 5) by as much as 25-fold. However, this pales in comparison with the ~10^5- to 10^6-fold increase in open probability induced by a single Ca^{2+} spark. Because BK channel activity in vascular smooth muscle is largely controlled by the frequency of Ca^{2+} sparks (79), it is unlikely that the functional output of these channels is significantly impacted by dynamic modulation of PIP_2.

Voltage-gated K^+ channels. Vascular SMCs express functional $K_V1.2$, $K_V1.5$, and $K_V2.1$ channels (Fig. 1). PIP_2 has been shown to modulate $K_V1.2$ (80, 81), $K_V1.5$ (82), and $K_V2.1$ (83) channels in heterologous expression systems, and may also promote changes in their biophysical properties. However, direct evidence for a regulatory role of PIP_2 on SMC K_V channels in native preparations is lacking. It has also been reported that $K_V7.x$ channels, which appear to be expressed by SMCs in some vascular beds (for review, see ref. 84), but not others (85), are activated by PIP_2, although different $K_V7.x$ subtypes display widely varying affinities for PIP_2 (86–88). The activity of several K_V7 channel subtypes is suppressed by depletion of PIP_2 (89, 90), consistent with an enhancing role for PIP_2. These studies on all K_V7 subtypes, performed in cultured cells, cell-free systems, or nonvascular systems (88, 91, 92), show that PIP_2 acts by stabilizing an open state of the channel. Whether PIP_2 plays a physiological role in regulating SMC K_V7 activity awaits experiments in native cells and vascular tissues.

Vascular TRP Channels. TRP channels are a family of cation channels whose members are ubiquitously expressed across diverse cell types. There are 28 TRP channel members in mammals that vary with respect to their expression pattern, cation permeability, activation mechanism, and cellular function. A diverse array of TRP channels has been reported in the vasculature (for review, see ref. 10). For instance, TRPC channels and TRPM4 channels are expressed in vascular SMCs and play key roles in membrane potential depolarization (Fig. 1). The vanilloid subfamily channel, TRPV4, is widely expressed in the vascular endothelium and represents a crucial Ca^{2+}-influx route in ECs. Studies performed to date suggest that PIP_2 modulates almost all TRP channels, sensitizing some (93–95) and desensitizing others (96–99).

TRPC channels. It has been shown that norepinephrine, angiotensin II, endothelin-1, uridine triphosphate, and vasopressin, acting through G_qPCRs or receptor tyrosine kinases, activate canonical TRP (TRPC) channels in vascular SMCs (10, 100, 101). Determining the contribution of each TRPC channel subtype to SMC function has been difficult and has led to disparate conclusions. Studies of this type face several obstacles. First, available pharmacological agents exhibit limited subtype selectivity; second, genetic manipulations often lead to compensatory changes in other TRPC subtypes; and third, TRPC channels heteromultimerize. Collectively, these factors complicate interpretation of experimental results, making it difficult to discern the roles of specific isoforms (for review, see refs. 10 and 101).

There is considerable evidence supporting the expression of five TRPC channel subtypes in SMCs: TRPC1, TRPC3, TRPC4, TRPC5, and TRPC6 (101). The TRPC1 subtype mediates depolarization in SMCs in response to endothelin-1 or norepinephrine exposure (102, 103). The TRPC3 channel has been implicated in vasoconstriction induced by different G_qPCR agonists via a mechanism involving DAG and possibly IP_3R, but it does not appear to contribute to constriction in response to increased intravascular pressure (myogenic response) (104–106). TRPC3 subunits can multimerize with TRPC1 and TRPC6, and also directly associate with IP_3Rs (102–104, 107). TRPC4 and TRPC5 subtypes, which are also thought to heteromultimerize with other subunits (108–111), have poorly defined roles in vascular SMCs. The TRPC6 channel likely plays a key role in receptor-mediated vasoconstriction, reflecting production of DAG, an activator of TRPC6, by PLC-mediated hydrolysis of PIP_2 downstream of activated G_qPCRs. The other product of this hydrolysis, IP_3, might also enhance TRPC6 activity (112–115). Ca^{2+} influx through TRPC6 can facilitate IP_3R-mediated Ca^{2+} release from the SR, which subsequently activates depolarizing TRPM4 channels. This latter outcome could explain the contribution of TRPC6 channels to the development of myogenic tone (116, 117).

Earlier studies explored the potential regulation of TRPC channels in vascular SMCs by PIP_2 (95, 118, 119). Such regulation is complicated by the fact that the PIP_2 metabolites, DAG and IP_3, are themselves standard activators and/or modulators of TRPC channels. In vascular SMCs, it has been shown that PIP_2 is obligatory for TRPC1 activation (95), a regulatory role that requires PKC activation and subsequent channel phosphorylation and ultimately leads to PIP_2 binding to the channel (118). These observations suggest that both PIP_2 and PKC are required for TRPC1 activation. Studies of PIP_2 regulation of TRPC6 channels have produced contradictory results, with one group reporting that PIP_2 enhances TRPC3 and TRPC6 activity in heterologous expressions systems (120), and another proposing that PIP_2 in mesenteric artery SMCs associates with TRPC6 and inhibits its angiotensin II-induced activity (119). Because TRPC6 opening requires PLC-mediated hydrolysis of PIP_2 to DAG, as noted above, it is conceivable that both PIP_2 depletion and DAG generation lead to channel activation. However, prolonged depletion of PIP_2 could ultimately reduce DAG production and decrease channel activation. Thus, the role of PIP_2 in the regulation of SMC TRPC6 activity remains unclear (Fig. 5).

TRPV4 channel. The TRPV4 channel is permeable to Ca^{2+} and Na^+ (121). It is activated by EETs (122), heat (123), and possibly mechanical stimuli (124). TRPV4 channels are also activated by G_qPCR signaling (for review, see ref. 125). Several studies have established TRPV4 expression and function in vascular SMCs

and ECs and shown that activation of the channel in either cell type leads to arteriolar dilation, albeit through different mechanisms in each cell type (5, 126).

Earley et al. (126) reported that, in vascular SMCs, TRPV4 channel activation by EETs induces Ca^{2+} influx, which stimulates RyR activity through a Ca^{2+}-induced Ca^{2+}-release mechanism to generate Ca^{2+} sparks, which, in turn, activate BK channels. The resulting BK channel-mediated K^+ efflux causes SMC hyperpolarization and feedback deactivation of voltage-dependent Ca^{2+} channels, leading to arterial dilation. Santana and colleagues (127) also reported that activation of G_q-coupled angiotensin II receptors stimulates SMC TRPV4 channels in a PKC-dependent manner.

Strong evidence for TRPV4 channel expression and function in the endothelium has accumulated over the past decade. Activation of TRPV4 channels in mesenteric artery ECs, either by a potent synthetic agonist (GSK1016790A) or in response to muscarinic G_qPCR activation, results in an influx of Ca^{2+} that can be detected optically. These events, termed "TRPV4 sparklets," are elementary Ca^{2+} signals that reflect Ca^{2+} influx through individual TRPV4 channels, which appear to exist predominantly in the form of four-channel clusters in mesenteric ECs (5). This Ca^{2+} influx subsequently activates K_{Ca} channels (IK and SK), increasing K^+ efflux. This not only hyperpolarizes EC V_M, it also hyperpolarizes adjacent SMCs (5) (Fig. 1). Notably, muscarinic receptor activation of mesenteric endothelial TRPV4 channels stimulates TRPV4 sparklets only at myoendothelial projections linking ECs to SMCs (Fig. 1) (5, 14). This microdomain signaling enables activation of as few as three TRPV4 channels per EC to cause hyperpolarization and near-maximal vasodilation in response to acetylcholine (5). In addition to regulation of membrane potential and diameter, vascular TRPV4 channels have been implicated in angiogenesis and control of vascular permeability (125).

Recent work has shown that, similar to arterial ECs, brain capillary ECs express functional TRPV4 channels (51). Because capillary ECs are apparently unique compared with arterial/arteriolar ECs in that they lack functional Ca^{2+}-activated IK/SK channels (7), activation of TRPV4 channels should depolarize capillary ECs (Fig. 1), as is the case in the lymphatic system (128). One intriguing observation is that the open probability of TRPV4 channels in brain ECs is exceedingly low under basal conditions, an observation that might indicate tonic TRPV4 inhibition in the cerebral vascular bed. Electrophysiological and pharmacological experiments have revealed that PIP_2 suppresses TRPV4 channel activity in capillary ECs (51) (Fig. 5). These findings in native vascular ECs are in line with previous results in heterologous expression systems showing that PIP_2 interacts directly with N-terminal residues of the TRPV4 channel (99, 129) and suppresses TRPV4 channel activity (99). Consistent with an inhibitory role for PIP_2, we found that suppressing PIP_2 synthesis by decreasing intracellular ATP or inhibiting lipid kinases significantly enhanced TRPV4 activity. Enhancing PIP_2 hydrolysis through prostanoid or muscarinic G_qPCR signaling has also been shown to activate TRPV4 channels in brain capillary ECs (51). Therefore, receptor agonists that are postulated to mediate neurovascular coupling in the brain, such as prostaglandin E2 (PGE_2) (130, 131), could be physiological activators of brain capillary EC TRPV4 channels. Because G_qPCR signaling causes PIP_2 depletion, it not only activates a depolarizing channel (TRPV4), but simultaneously deactivates a hyperpolarizing channel (Kir2.1) (27, 51). This dual mechanism ensures more efficient control of the membrane potential of brain capillaries, such that PIP_2 facilitates K^+/Kir2.1-mediated hyperpolarization and G_qPCR-mediated PIP_2 depletion depolarizes V_M.

In contrast to cerebral ECs, where G_qPCR agonists increase TRPV4 activity through depletion of PIP_2 (51), muscarinic G_qPCR activation in mesenteric arteries promotes TRPV4-mediated hyperpolarization in a manner that depends on A-kinase anchoring protein (AKAP150)-bound PKC (14). These apparent vascular bed-specific modes of physiological TRPV4 activation are reminiscent of the different mechanisms of K_{ATP} channel inhibition by G_qPCRs in vascular SMCs (PKC-mediated) (12) and cardiac myocytes (PIP_2-dependent) (29). Another important consideration is the possibility that normal PIP_2 levels are different at different points in vascular networks. A higher PIP_2 set point could dramatically alter vascular ion channel regulation and could reflect diminished PIP_2 breakdown owing to reduced levels of G_qPCR agonists, lower constitutive G_qPCR/PLC activity, and/or decreased G_qPCR expression (24). The proximity of G_qPCRs to ion channels under PIP_2 control—and thus the ability of an agonist-activated G_qPCR to deplete PIP_2 in the vicinity of the channel—could also dictate ion channel regulation (24, 30, 31). As an alternative (or in addition) to reduced PIP_2 hydrolysis, more robust PIP_2 synthesis could translate into higher levels of PIP_2 and stronger tonic inhibition of TRPV4 channels in brain capillary ECs. Physiologically, a higher PIP_2 set point in the brain endothelium could have favorable implications for sustaining electrical signaling in the brain circulation. Specifically, the PIP_2 set point in brain capillaries is apparently sufficient to maximally activate Kir2.1 channels; this facilitates V_M hyperpolarization and suppresses TRPV4 channels, thereby limiting V_M depolarization (Fig. 5) (27, 51, 67). Conversely, one could envision a scenario in which a lower PIP_2 set point, and therefore higher activity of cerebral TRPV4 channels, would limit hyperpolarization in response to Kir2.1 activation (67). The resulting failure to evoke V_M hyperpolarization would be detrimental to Kir2.1-mediated capillary electrical signaling, with deleterious consequences for regulation of cerebral blood flow.

Vascular Chloride Channels. The chloride equilibrium potential in SMCs is between −30 and −20 mV (132, 133), and the cell's membrane potential is about −50 to −40 mV (2). Therefore, activation of chloride channels in SMCs leads to membrane depolarization. TMEM16A (transmembrane member 16A, encoded by *ANO1*) is a Ca^{2+}-activated Cl^- channel expressed in vascular SMCs that is a determinant of reactivity in some vascular beds (134, 135). Pritchard et al. (136), working on pulmonary arterial SMCs, were the first to report the regulation of TMEM16A by PIP_2. On the basis of biochemical and electrophysiological analyses, these authors suggested that PIP_2 physically associates with TMEM16A in SMCs and that this association suppresses TMEM16A currents. However, the effects of PIP_2 on TMEM16A appear more complex (Fig. 5), given that several studies from different groups have demonstrated a requirement for PIP_2 to sustain TMEM16A activity, albeit in nonvascular cells (137, 138). Along these lines, Carlson and colleagues (139) recently reported that, in addition to Ca^{2+}, PIP_2 is required for TMEM16A activity. G_qPCR signaling-induced PLC activation and subsequent Ca^{2+} signals have also been suggested to activate TMEM16A (140), despite the simultaneous PIP_2 hydrolysis that this signaling produces, highlighting the complexity of PIP_2 modulation. Recent structural findings provide some insights into the molecular basis of PIP_2-dependent regulation of the TMEM16A channel (141). The ion-conducting pore of TMEM16A has two modules: a Ca^{2+}-binding module that mediates channel activation, and a PIP_2-binding module that mediates desensitization. G_qPCR activation triggers Ca^{2+} signals that activate the TMEM16A channel. PIP_2 dissociation from the channel during prolonged stimulation and Ca^{2+} saturation, as occurs during extended G_qPCR stimulation, leads to channel desensitization due to a conformational change that causes the ionic pore to collapse (141). Clearly, further investigations are warranted to understand the consequences of

PIP_2–TMEM16A interactions in the vasculature and how such a regulatory axis could alter vascular function.

PIP_2–Ion Channel Vascular Pathologies

Pathological changes in PIP_2 interactions with an ion channel can increase or decrease channel function. These pathologies are categorized under one of two mechanisms, depending on the nature of the defect. In the first, an ion channel mutation involving residues critical for PIP_2 binding can cripple channel regulation, even if cellular PIP_2 levels are unchanged. In the second, compromised PIP_2 levels could lead to altered ion channel regulation in the absence of mutations in the target channel (Fig. 6). Despite the critical roles of ion channels in vascular physiology, only a few vascular pathologies are currently recognized to involve PIP_2. Given the emerging interest in PIP_2 and its crucial role in vascular ion channel regulation, it is likely that additional PIP_2-dependent channelopathies will be identified in the future. In this section, we discuss the limited available information on pathological conditions in which altered PIP_2–ion channel interactions have been implicated and speculate on others.

Ion Channel Mutation. Andersen–Tawil syndrome (ATS) is characterized by cardiac arrhythmias, periodic paralysis, and developmental abnormalities. About 60% of ATS patients harbor loss-of-function mutations in the *KCNJ2* gene encoding the Kir2.1 channel. Many of these mutations map to Kir2.1 residues involved in PIP_2 binding; these mutations lead to defective PIP_2 binding to the channel and thus decrease channel activity (142, 143). In addition to the hallmark triad of ATS symptoms—arrhythmias, periodic paralysis, and dysmorphic features—impaired flow-mediated vasodilation, indicative of impaired endothelial function and presumably reflecting dysfunctional Kir2.1 channels (144), has been reported in ATS patients (145). ATS patients also often display multiple white matter lesions characteristic of small-vessel diseases of the brain (146). Vascular abnormalities attributable to Kir2.1 mutations in ATS have yet to be systematically investigated.

Mutations in the TRPV4 gene cause a wide range of human disorders (for review, see ref. 147). Although a direct link between TRPV4 channelopathies and PIP_2 regulation has not been clearly established, many human TRPV4 mutations are located in the ankyrin repeat domain, which regulates binding of PIP_2, ATP, and calmodulin to the channel (99, 129, 147, 148). Despite the remarkably high number of disease-causing TRPV4 mutations (compared with other TRP channels), it is unclear why vascular manifestations of these mutations are mild in affected patients, who are more frequently affected by skeletal disease and neuropathies.

PIP_2 Availability. A change in PIP_2 levels in the inner leaflet of the plasma membrane, in most pathophysiological settings, a decrease, could disrupt the function of PIP_2-regulated proteins. As noted above, cellular PIP_2 levels are dynamic, such that PIP_2 availability is determined largely by PIP_2 synthesis and depletion. Other cellular mechanisms, such as PIP_2 sequestration, might also play a role in making the phosphoinositide less available to PIP_2-interacting proteins (149, 150).

Altered PIP_2 synthesis. PIP_2 synthesis requires an appropriate ATP:ADP ratio and functional lipid kinases. Interruption of either or both could decrease PIP_2 and compromise ion channel regulation (Fig. 6) (151, 152). There are pathological situations in which ATP levels decline dramatically, such as during ischemic events (e.g., cerebral or coronary ischemia) (153, 154). These events could therefore be associated with compromised PIP_2 synthesis. Mitochondrial dysfunction in the vasculature, a key feature associated with aging (155) and different diseases such as diabetes (156, 157) and CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) (158), can lead to compromised oxidative phosphorylation and ATP synthesis. Several studies have additionally reported disrupted phosphoinositide levels in the brains of Alzheimer's disease patients (159–161), changes that, in theory, could contribute to altered blood flow control. In addition to ATP, phosphoinositide kinases are required for PIP_2 synthesis, and their dysregulation causes human diseases, such as cancer and developmental disorders (162).

Altered PIP_2 hydrolysis. G_qPCR/PLC activation affects Kir2 and TRPV4 channels in a PIP_2-dependent manner (27, 51). There are several instances in which G_qPCR activity would be abnormally high, which could disrupt maintenance of PIP_2 levels (Fig. 6). For example, most capillary malformation patients (nonsyndromic and Sturge–Weber syndrome) harbor an R183Q gain-of-function or activating mutation in the $G\alpha_q$ protein, encoded by *GNAQ*, which is expressed at high levels in ECs (163–167). These patients exhibit altered cerebral blood flow [e.g., generalized hypoperfusion, severe ischemia, and impaired cerebral hemodynamic responses to seizure activity (168–170)], but how mutant *GNAQ* affects endothelial signaling and blood flow is not fully understood. It is conceivable that the constitutively active $G\alpha_q$ R183Q mutant could deplete PIP_2 in ECs, leading to Kir2 channel deactivation and TRPV4 channel activation (27, 51). If

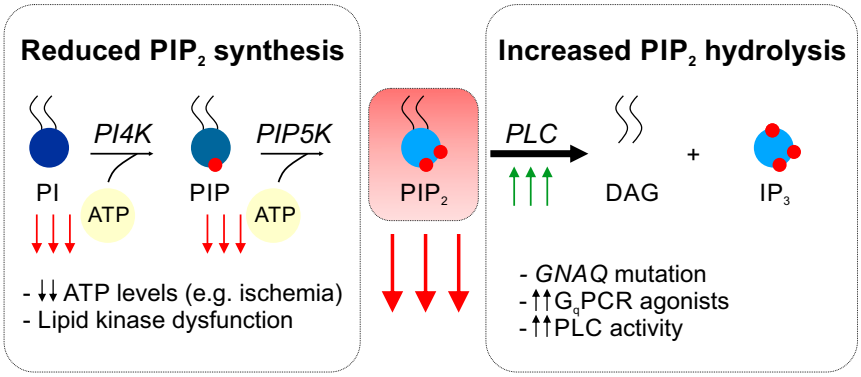

Fig. 6. Altered PIP_2 levels in pathology. A profound decline in cellular levels of PIP_2 is likely associated with different vascular pathologies. Impaired biosynthesis of PIP_2 occurs when ATP:ADP ratio necessary for phosphoryl transfer is not maintained or when phosphoinositide kinases are dysfunctional. A reduction in ATP levels is linked to certain pathological conditions (e.g., ischemia) or mitochondrial dysfunction, the latter of which is a hallmark of many vascular diseases. On the other hand, enhanced breakdown of PIP_2 by G_qPCR/PLC signaling can reduce PIP_2 levels at rates exceeding those of PIP_2 repletion. Pathological increases in G_qPCR activity (e.g., due to a gain-of-function mutation in *GNAQ* or increased receptor agonist levels) or PLC activity can result in reduced availability of PIP_2, thereby affecting vascular ion channel activity.

so, it could explain the reported cerebral ischemia and poor cerebral blood flow in infants with capillary malformation (163). Another example in the brain is cortical spreading depression, during which a slow depolarizing wave propagates across the cerebral cortex. This depolarization is associated with global release of neurotransmitters, many of which are receptor agonists and could therefore enhance G_qPCR activation and PIP_2 breakdown (171). Whether this affects vascular signaling remains to be tested. Downstream PLC activity can also be altered in disease. For example, PLC is increased and accumulates in the brains of Alzheimer's disease patients (172, 173), a finding that aligns with a reported reduction in phosphoinositides in these patients (160).

Concluding Remarks

We have summarized our current perspective on known and potential physiological regulation of ion channels in the vasculature by the phosphoinositide PIP_2. There are significant implications of such regulation for vascular function in health and disease. First, the ability of PIP_2 to tune ion channel function facilitates the coordination of vascular cell excitability through dual actions of PIP_2 on hyperpolarizing and depolarizing channels so as to favor a membrane potential shift in one direction or the other. Second, the concept of PIP_2-mediated ion channel regulation provides an important and underexamined mechanism by which vascular G_qPCR signaling is coupled to vascular function. Third, the strong link between the metabolic state of SMCs or ECs and PIP_2 content suggests a potential mechanism to explain how metabolism affects vascular ion channel activity and, ultimately, vascular function. These insights establish a strong foundation for further investigations that will advance our understanding of how disruption of PIP_2-mediated ion channel regulation—whether due to channel mutation or altered PIP_2 availability—can be detrimental to vascular function and blood flow control.

Data Availability. This article includes no unpublished data.

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