Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

# Lipid agonism: The $PIP_2$ paradigm of ligand-gated ion channels

Scott B. Hansen *

*Department of Molecular Therapeutics, The Scripps Research Institute, Scripps-Florida Campus, Jupiter FL 33458, USA*
*Department of Neuroscience, The Scripps Research Institute, Scripps-Florida Campus, Jupiter FL 33458, USA*

ARTICLE INFO

*Article history:*
Received 28 November 2014
Received in revised form 5 January 2015
Accepted 17 January 2015
Available online xxxx

*Keywords:*
Lipid gated
Ion channel
$PIP_2$
Signaling lipid
G-protein
Lipid raft
Lipidomics

ABSTRACT

The past decade, membrane signaling lipids emerged as major regulators of ion channel function. However, the molecular nature of lipid binding to ion channels remained poorly described due to a lack of structural information and assays to quantify and measure lipid binding in a membrane. How does a lipid–ligand bind to a membrane protein in the plasma membrane, and what does it mean for a lipid to activate or regulate an ion channel? How does lipid binding compare to activation by soluble neurotransmitter? And how does the cell control lipid agonism? This review focuses on lipids and their interactions with membrane proteins, in particular, ion channels. I discuss the intersection of membrane lipid biology and ion channel biophysics. A picture emerges of membrane lipids as bona fide agonists of ligand-gated ion channels. These freely diffusing signals reside in the plasma membrane, bind to the transmembrane domain of protein, and cause a conformational change that allosterically gates an ion channel. The system employs a catalog of diverse signaling lipids ultimately controlled by lipid enzymes and raft localization. I draw upon pharmacology, recent protein structure, and electrophysiological data to understand lipid regulation and define inward rectifying potassium channels ($K_{ir}$) as a new class of $PIP_2$ lipid-gated ion channels.



## 1. Introduction

Signaling lipids are important regulators of ion channels and exert a central role in tissue function including functional heartbeat, neuronal signaling, kidney dialysis, sight, smell, pain, and touch [1–5]. In the past, most biochemist and ion channel experts viewed lipids as unwieldy, hydrophobic molecules physically supporting ion channels in a cell membrane or liposomes but not as ligands. Recent past models of lipid signaling to ion channels suggested that the formation of anionic lipids caused a change in the plasma membrane surface charge. Little was known about how lipids engaged and disengaged the channel or how the contact of a lipid with protein might affect the conformation of ion channels in the membrane. A lack of binding constants for lipids and ion channels challenged our ability to think about lipids as ligands. Aspects of this problem remain an important hurdle.

In 1998, Hilgemann and colleagues [6] eloquently showed that a signaling lipid could directly activate an ion channel. The lipid, phosphatidylinositol 4,5-bisphosphate ($PIP_2$), a minor constituent of the plasma membrane, was required and sufficient for the activation of a potassium channel [6]. Despite more than a decade of experimentation, the nature of $PIP_2$ binding remained clouded by an inability to accurately measure its concentration in the membrane and directly detect binding to protein. Simple terminology such as lipid concentration and affinity are difficult to define for insoluble molecules in an aqueous environment [7]. Absent a well-characterized ligand protein interaction, the initial non-specific theories of surface charge and membrane curvature dominated [8,9] but struggled to account for the specificity of signaling lipids in many systems. Recently, a more accurate model emerges that includes structural and pharmacological evidence that lipids bind to and activate ion channels analogous to classic ligand-like agonist properties [10,11].

Herein, a model of lipid agonism is built on $PIP_2$ and inward rectifying potassium ($K_{ir}$) channels. Aspects of many other classes of channels and signaling lipids appear to function in a similar way; select examples are included throughout this review. The intent of this review is to

*Abbreviations:* AA, arachidonic acid; ASIC, acid-sensing ion channel; ATP, adenosine triphosphate; BK, big conductance potassium channel; $Ca_v$, voltage-dependent calcium channel or VDCC; Ci-VSP, *Ciona intestinalis* voltage-sensitive phosphatase; CoA, coenzyme A; CTD, cytoplasmic domain; $C8PIP_2$, dioctanoyl $PIP_2$; DAG, diacylglycerol; DRM, detergent-resistant membrane; ER, endoplasmic reticulum; GIRK, G-protein inward rectifying potassium channel or $K_{ir}3$; $G\beta\gamma$, G-protein beta gamma subunit; GPCR, G-protein-coupled receptor; HCN, hyperpolarization-activated cyclic nucleotide-gated; $IP_3$, inositol triphosphate; $K_{atp}$, ATP-sensitive potassium channel or $K_{ir}6$; $K_{ir}$, inward rectifying potassium channel; $K_v$, voltage-gated potassium channel; $K_{2P}$, two pore domain potassium channel; LAT, lipid acyl transferase; $L_d$, liquid-disordered phase; MARCKS, myristoylated alanine-rich C-kinase substrate; Mg, magnesium; NMDA, *N*-methyl-D-aspartate receptor; nAChR, nicotinic acetylcholine receptor; PA, phosphatidic acid; PH, pleckstrin homology; PI, phosphoinositide; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; $PIP_3$, phosphatidylinositol 3,4,5-triphosphate; PI3 kinase, phosphatidylinositol-4,5-bisphosphate 3-kinase; $PLA_2$, phospholipase $A_2$; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; PTEN, phosphatase and tensin homolog; PUFA, polyunsaturated fatty acid; P2X, purinergic receptors; Sn2, stereospecific numbering position 2 or the second hydroxyl group of glycerol; TMD, transmembrane domain; TM1, transmembrane helix 1; TREK, TWIK related potassium channel or $K_{2P}2.1$; TRP, transient receptor potential channel; VSD, voltage sensor domain

* 130 Scripps Way #2C1, Jupiter, FL 33458, USA. Tel.: +1 561 228 2415.
*E-mail address:* shansen@scripps.edu.

http://dx.doi.org/10.1016/j.bbalip.2015.01.011
1388-1981/

facilitate an understanding at the interface of ion channel activation and membrane lipid biology, although neither field is reviewed in a comprehensive way.

## 2. The signaling lipid $PIP_2$ is an agonist that gates ion channels

$PIP_2$, arguably the best-studied signaling lipid, is comprised of an inositol head group (the named feature), a phosphoglycerol backbone, and two acyl chains (Fig. 1A). $PIP_2$ bears four negative charges and is a permanent and minor component (<1%) of the Eukaryotic plasma membrane inner leaflet [12,13].

### 2.1. $PIP_2$ ion channel physiology

$PIP_2$ signaling dictates the activatable state of a plethora of ion channels [2,14,15] (Fig. 1) with broad reaching cellular function. The first indication that a channel is $PIP_2$ dependent usually arises when a channel, excised from the plasma membrane (e.g., inside out patch), steadily decreases in conductance until the channel inactivates. This is known as "rundown" [2,16]. The excised patch lacks the cytosolic factors to maintain sufficient $PIP_2$ levels in the membrane to support ion channel function; hence, the channels in the patch close. Adding ATP and Mg was shown to delay rundown [16]. Presumably, $PIP_2$ synthesizing enzymes are excised in the patch with the channels and that these enzyme utilize the ATP to replenish $PIP_2$ [2,16]. Adding back a soluble $PIP_2$ analog dioctanoyl $PIP_2$ ($C8PIP_2$) rescues activity [2,15] of many ion channel types [17–20]. In a second method, $PIP_2$ scavengers (e.g., polyamines or $PIP_2$ antibodies) are used to deplete or mask $PIP_2$ availability [21–23]. Polyamines are positively charged polymers that bind via avidity to the multiple negative charges of $PIP_2$. More complete descriptions of $PIP_2$-dependent ion channels and $PIP_2$ cellular function are reviewed by Suh and Hille [2,11], Xie [5], and McLaughlin [9]. Recently, a voltage-sensitive phosphatase (Ci-VSP) was shown to provide direct control over $PIP_2$ signaling in the membrane [24–26]. When Ci-VSP is co transfected with $K_{ir}$ [24–26], $K_v7.1$ [27], $Ca_v2$ [28,29], and TRP [30,31], channels are voltage-dependent consistent with Ci-VSP regulation of $PIP_2$. This method provides better control of $PIP_2$; however, indirect effects of $PIP_2$ remain a possibility.

In order to directly show $PIP_2$ modulation, an ion channel can be purified and reconstituted (reinserted) into lipid vesicles with a known lipid composition. A lack of purified ion channels limited this technique, but recent advancements in membrane protein expression and purification [32,33] has overcome this problem for select channel types [34–38]. The nAChR was among the first channels to show direct dependence on a lipid for activation, phosphatidic acid (PA) [39]. Recently, $PIP_2$-dependent channels were reconstituted into lipid vesicles and shown to respond directly to $PIP_2$ modulation. This includes GIRK [40,41], TRPV1 [42], TRPM8 [43], and $K_{ir}2.1$-2 [44] channels.

### 2.2. $PIP_2$ ion channel structure

Despite robust channel modulation by indirect methods, absent a crystal structure, an understanding of the molecular action of $PIP_2$ and the precise binding site remained speculative. In 2011, an X-ray crystal structure complex of $K_{ir}2.2$ with $PIP_2$ revealed a $PIP_2$ binding site in the channel's transmembrane domain [10] (Fig. 2). The glycerol backbone and 1′ phosphate of $PIP_2$ capped the first transmembrane spanning helix (TM1) of $K_{ir}$. An intimate coordination of the 5′ inositol phosphate in the distal end of the second transmembrane spanning helix (TM2) accounted for $PIP_2$ specificity. Moreover, a conformational change appeared to initiate or open the ion conduction pathway. Basic residues on a linker between the transmembrane domain and cytoplasmic domain directly contacted $PIP_2$, but distal basic residues proposed in the CTD [45] did not; rather, they were buried and stabilized proper folding of the cytoplasmic domain structure [10]. Prior to the $K_{ir}2.2/PIP_2$ complex, structures of $PIP_2$/protein complexes were limited to soluble membrane localization domains, which lack a transmembrane domain and share few if any functional similarities with ion channels. A lack of appropriate structural examples and an understanding of how lipids and proteins interact in the plasma membrane hindered a complete mechanistic interpretation of $PIP_2$ data. Furthermore, early studies on the C-terminus of $K_{ir}$ included residues that turned out to be in the TMD of $K_{ir}$ and key to binding the 5′ inositol phosphate [6] (Fig. 2). Only with recent structural

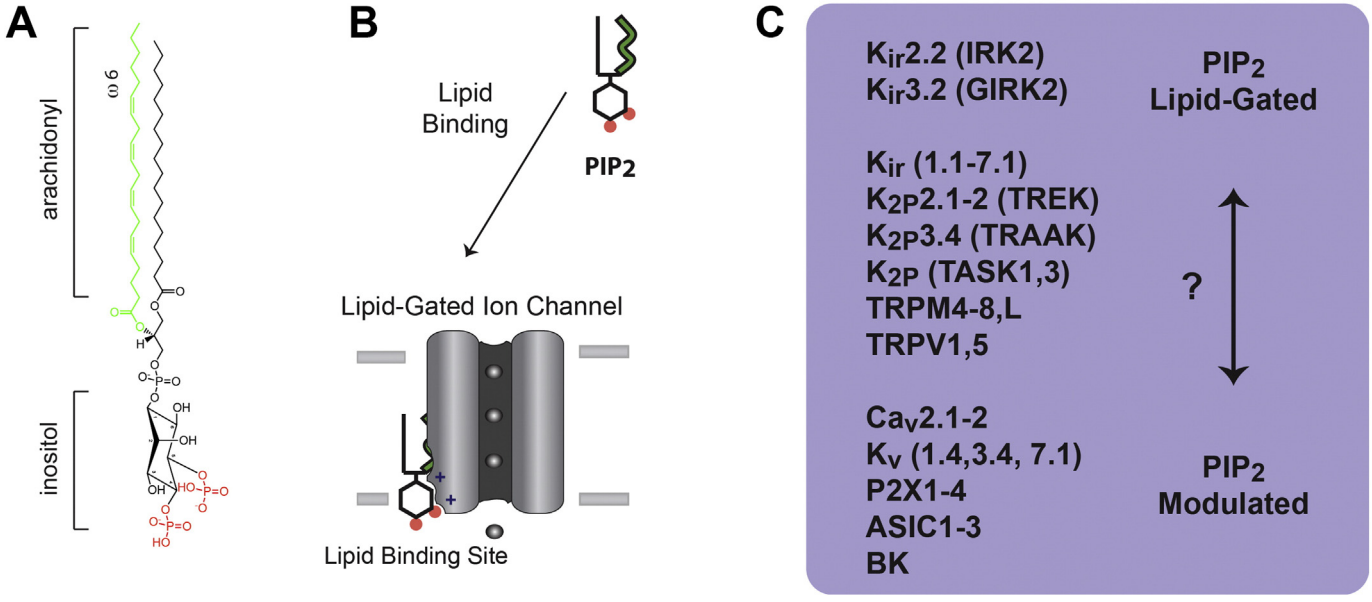


**Fig. 1.** $PIP_2$ lipid regulation of ion channels. (A) The chemical structure of plasma membrane $PIP_2$ is shown with an arachidonyl acyl chain (green) and inositol phosphates at the 4′ and 5′ position (red). (B) A cartoon representation of a $PIP_2$ lipid-gated ion channel. $PIP_2$ is shown bound to a lipid-binding site in the transmembrane domain of an ion channel. (C) List of ion channels with lipid gating properties. $K_{ir}2.2$ and 3.2 are the most clearly "lipid gated." A second group appears to be dual regulated, or "$PIP_2$ modulated." $PIP_2$ modulates channel gating, but gating also requires either voltage or a second ligand. A third group of channels behave similar to $K_{ir}$ but await definitive proof of lipid gating vs. $PIP_2$ modulation (?). The list of channels is exemplary and not comprehensive.

Please cite this article as: S.B. Hansen, Lipid agonism: The $PIP_2$ paradigm of ligand-gated ion channels, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbalip.2015.01.011