Biochemical and Biophysical Research Communications 424 (2012) 208–213

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

PIP_2 modulation of Slick and Slack K^+ channels

Maria de los Angeles Tejada, Lars Jørn Jensen, Dan A. Klaerke *

Department of Physiology and Biochemistry, IBHV, Faculty of Health and Medical Sciences, University of Copenhagen, Groennegaardsvej 7, 1870 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 3 June 2012
Available online 21 June 2012

Keywords:
Slick (Slo2.1)
Slack (Slo2.2)
$PI_{(4,5)}P_2$
Cell volume regulation
Phosphoinositides

ABSTRACT

Slick and Slack are members of the Slo family of high-conductance potassium channels. These channels are activated by Na^+ and Cl^- and are highly expressed in the CNS, where they are believed to contribute to the resting membrane potential of neurons and the control of excitability. Herein, we provide evidence that Slick and Slack channels are regulated by the phosphoinositide PIP_2. Two stereoisomers of PIP_2 were able to exogenously activate Slick and Slack channels expressed in *Xenopus* oocytes, and in addition, it is shown that Slick and Slack channels are modulated by endogenous PIP_2. The activating effect of PIP_2 appears to occur by direct interaction with lysine 306 in Slick and lysine 339 in Slack, located at the proximal C-termini of both channels. Overall, our data suggest that PIP_2 is an important regulator of Slick and Slack channels, yet it is not involved in the recently described cell volume sensitivity of Slick channels, since mutated PIP_2-insensitive Slick channels retained their sensitivity to cell volume.

1. Introduction

Na^+-activated K^+ channels (K_{Na}) are relatively newly characterized channels which were first identified in cardiac myocytes and CNS [1–3]. K_{Na} channels are activated by Cl^- as well, and they have been associated with tuning the resting membrane potential and basal excitability of neurons [4]. Two members of the K_{Na} channel family have been cloned within the last decade, namely Slick (Slo2.1) and Slack (Slo2.2) high-conductance potassium channels [4,5]. Is has been suggested that they may play an important role for neuronal slow after-hyperpolarization [6]; however neither their physiological importance nor their regulation is at present entirely understood. We have recently shown that Slick channels, but not Slack channels, are precisely regulated by changes in cell volume [7], and this is consistent with a possible role for Slick channels in cell volume regulation, e.g. during ischemia [4]. The mechanism for regulation of ion channels by changes in cell volume is unknown, but it has recently been suggested that phosphatidylinositol biphosphate (PIP_2) interaction may confer sensitivity to cell volume changes to ion channels [8].

PIP_2 is a common signalling phospholipid present in eukaryotic cells. This phosphoinositide, localized at the inner leaflet of the plasma membrane, exists under different isoforms which are characterized by phosphorylation at distinct positions of the inositol ring; namely phosphatidylinositol 3,4-biphosphate ($PI_{(3,4)}P_2$), phosphatidylinositol 4,5-biphosphate ($PI_{(4,5)}P_2$), and phosphatidylinositol 3,5-biphosphate ($PI_{(3,5)}P_2$). The most abundant isoform is $PI_{(4,5)}P_2$, which is often referred to as PIP_2. PIP_2 is a substrate for phospholipase C (PLC) for production of inositol triphosphate (IP_3) and diacylglycerol (DAG), which in turn, trigger Ca^{2+} release from intracellular stores and activate Ca^{2+}/calmodulin-dependent protein kinases II (CamKII), Protein kinase C (PKC) and other major signalling pathways. PIP_2 is now also recognized as a signalling molecule *per se* and it has been suggested to modulate the activity of a number of ion channels, either by direct interaction or indirectly through one of the above mentioned signalling pathways [9]. At present, certain members of most ion channel families, i.e. Ca^{2+} channels, Cl^- channels, Na^+ channels, TRP channels and K^+ channels have been shown to be regulated by PIP_2 [9]. Recently, also two members of the family of high conductance K^+ channels, namely Slo1 (BK channels) and Slo3 channels have been shown to be activated by PIP_2 [10,11].

The current study presents evidence for phosphoinositide mediated activation of Slick and Slack K^+ channels as a novel regulatory mechanism for these channels. We show that two isomers of PIP_2 namely $PI_{(3,4)}P_2$ and $PI_{(4,5)}P_2$, are able to increase currents through Slick and Slack channels expressed in *Xenopus laevis* oocytes. We also identify a lysine residue at the C-terminus of both channels, which probably interacts directly with PIP_2. Finally, our results show that the strict regulation of Slick by small, fast changes in cell volume is independent of PIP_2.

2. Methods

2.1. Heterologous expression and Molecular Biology:

Xenopus laevis oocytes were prepared as previously described by Grunnet [12]. All procedures were approved by the Danish

* Corresponding author. Fax: +45 35332525.
E-mail address: dk@life.ku.dk (D.A. Klaerke).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.038

National Committee for Animal Studies. Stage V and VI oocytes were chosen for injections and cultured in Kulori medium at 19 °C (Kulori: 90 nM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.4). Site-directed mutagenesis was performed using QuikChange (Stratagene) and mutations were verified by sequencing (Eurofins MWG Operon, Ebersberg, Germany). Wild-type hSlick (Slo2.1) and rSlack (Slo2.2) in the pOX vector were kindly provided by L. Salkoff and Aquaporin-1 (AQP1) in pBluescript was kindly provided by P. Agre. Vectors were linearized with *Not1*, for Slick and Slack or *Pst1* for AQP1. mRNA was produced using the mMessage mMachine kit and purified with MEGAclear (Ambion). For expression 10 ng (50 nl) of mRNA of Slick, Slack or Slick + AQP1(3:1 ratio) was injected into oocytes.

2.2. Electrophysiology

Total currents were measured 4–5 days after injection by two-electrode voltage clamp (TEVC) as described before [12]. Currents through expressed channels were elicited using either a step protocol (500 ms depolarizations from 100 to +80 mV, holding potential −80 mV for 4 s) or a pulse protocol (500 ms depolarizations to +80 mV from −80 mV, holding the membrane potential at −80 mV for 3 s). Changes in cell volume were achieved by exposure of the oocytes to isotonic, hypotonic (−50 mOsm/l) or hypertonic (+50 mOsm/l) media as described by Grunnet [12]. To evaluate the effect of phosphoinositides, their water soluble analogs diC8 $PI_{(3,4)}P_2$ (Kemtec) and diC8 $PI_{(4,5)}P_2$ (Cayman), were directly dissolved in Kulori at a final concentration of 10 μM. Currents through expressed Slick or Slack channels were carefully measured by TEVC and oocytes were allowed to rest for 2 h in Kulori. Subsequently oocytes were incubated for 2 h with added phosphoinositides before the currents were measured a second time by TEVC. In order to chelate the effect of $PI_{(4,5)}P_2$, oocytes were incubated with neomycin or wortmannin dissolved in Kulori; pH:7.4 to final concentrations. All chemicals were from Sigma (unless otherwise stated).

2.3. Data analysis and Statistics

Data acquisition and analysis were performed using pClamp (Molecular Devises), GraphPad Prism 5® and Excel (Microsoft) packages. Sequences were analyzed with CLC Main Workbench 6.5 (Cambridge, MA, USA). Data are presented means ± S.E.M. (unless otherwise stated). Statistical comparisons were evaluated by paired Student's *t*-tests (for two means), one-way ANOVA with Tukey's post-test (for more than two means), or two-way ANOVA with Bonferroni post-test for grouped analysis. Statistical significance of *p*-values: * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0005$).

3. Results

3.1. PIP_2 effect on Slick and Slack K^+ channels

In order to analyze the sensitivity of Slick and Slack channels to phosphoinositides, DiC8 $PI_{(3,4)}P_2$ and DiC8 $PI_{(4,5)}P_2$, were applied at 10 μM to *Xenopus laevis* oocytes expressing either of the channels. Whole cell currents were measured by TEVC before and after a 2 h incubation period using a step protocol. Fig. 1 shows that both phosphoinositide isoforms were able to activate currents through Slick and Slack channels. $PI_{(4,5)}P_2$, which is the most abundant isoform naturally occurring in eukaryotic cells, activated Slick channels (225% ± 22% of control) more potently than Slack channels (168% ± 6% of control) (Fig. 1; A1, A2). $PI_{(3,4)}P_2$ stimulated currents

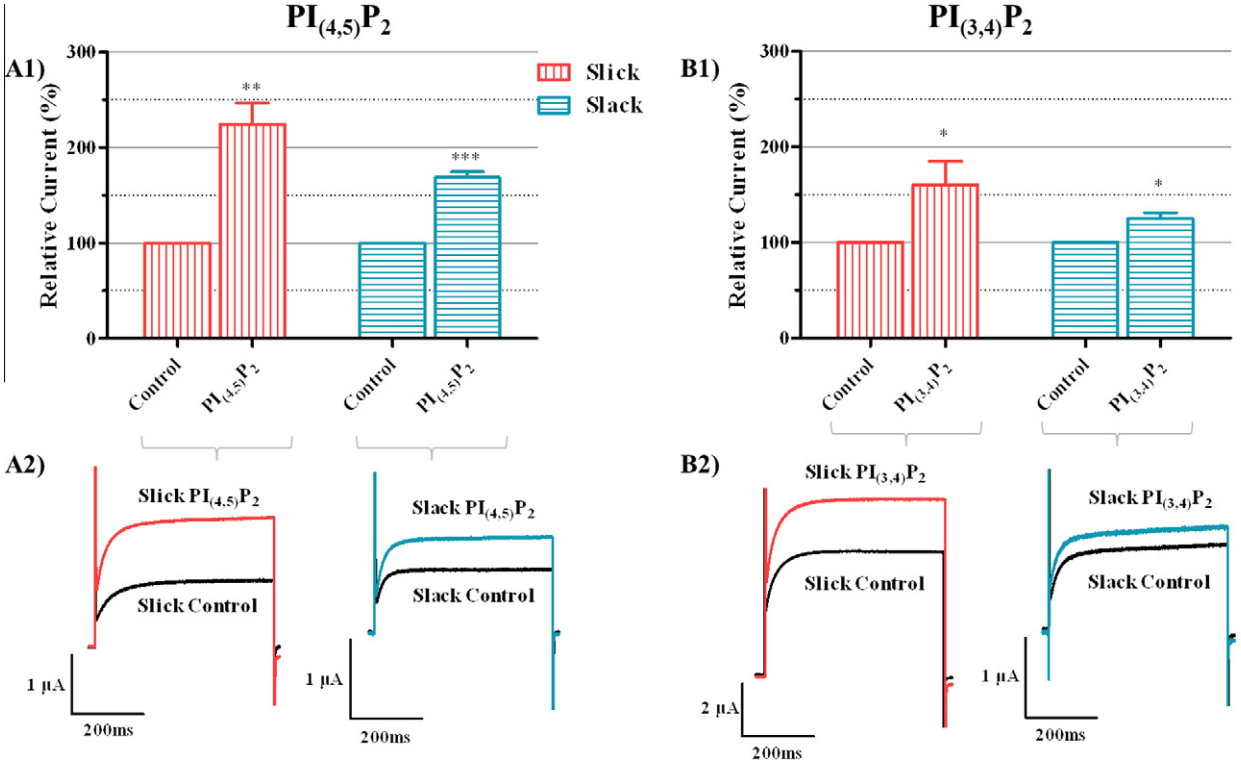

Fig. 1. Phosphoinositide-mediated activation of Slick and Slack K^+ channels. *Xenopus laevis* oocytes expressing Slick or Slack channels were stimulated by a step protocol (see Methods) and currents were measured at the end of the +80 mV step for Slick (red) and Slack (blue) channels before (control) and after a 2 h incubation with 10 μM $PI_{(4,5)}P_2$ (A1) or $PI_{(3,4)}P_2$ (B1). Currents for control oocytes were normalized to 100% and the columns show the relative effects of incubation with the phosphoinositides (means ± SEM for 5–7 independent experiments). Panels A2 and B2 represent current traces (+80 mV) for single, representative oocytes expressing Slick (red) or Slack (blue) channels before (black) or after PIP_2 treatment (Slick, red and Slack, blue). Non-injected oocytes showed currents less than 100 nA and did not respond to treatment with PIP_2 (*data not shown*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)