Receptors & Clinical Investigation 2015; 2: e515. doi: 10.14800/rci.515; © 2015 by Dongil Keum, *et al.*
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RESEARCH HIGHLIGHT

Molecular basis for N-type voltage-gated Ca^{2+} channel modulation by G_q protein-coupled receptors

Dongil Keum, Byung-Chang Suh

Department of Brain Science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 711-873, Korea

Correspondence: Byung-Chang Suh
E-mail: bcsuh@dgist.ac.kr
Received: January 08, 2015
Published online: March 11, 2015

N-type voltage-gated Ca^{2+} ($Ca_V2.2$) channels, which enable synaptic transmission by triggering neurotransmitter release, are tightly modulated by G protein-coupled receptors (GPCRs) via several downstream signaling messengers, such as Gβγ, calmodulin, arachidonic acid and PIP_2. However, the molecular mechanism by which $G_{q/11}$-coupled receptors (G_qPCRs) suppress $Ca_V2.2$ currents remains unclear. In this research highlight, we review our recent finding that M_1 muscarinic receptors inhibit $Ca_V2.2$ channels through both Gβγ-mediated voltage-dependent (VD) and $G\alpha_{q/11}$/PLC-mediated voltage-independent (VI) pathways. Our photometry results also demonstrate that Gβγ-mediated VD inhibition of $Ca_V2.2$ channels initiates approximately 3 s earlier than VI inhibition, and is strongly potentiated in cells expressing plasma membrane-localized $Ca_V\beta$ subunits. Our observations demonstrate a novel mechanism for $Ca_V2.2$ channel modulation by G_qPCRs where the subcellular location of $Ca_V\beta$ subunits plays a critical role in determining the voltage-dependence of current suppression by M_1 receptors.

Keywords: $Ca_V2.2$ channels; G protein-coupled receptors; voltage-dependent; Gβγ subunits; phosphatidylinositol 4,5-bisphosphate

To cite this article: Dongil Keum, *et al*. Molecular basis for N-type voltage-gated Ca^{2+} channel modulation by G_q protein-coupled receptors. Receptor Clin Invest 2015; 2: e515. doi: 10.14800/rci.515.

N-type voltage-gated Ca^{2+} ($Ca_V2.2$) channels are central to synaptic transmission [1] in response to the propagation of electrical stimulations and to the processes it underlies, such as learning and memory [2] and gene transcription [3]. $Ca_V2.2$ channels are widely expressed throughout the brain [4] and spinal cord [5], and knockout mice who lack $Ca_V2.2$ channels show cardiovascular impairment [6], hyperactivity [7], reduced alcohol consumption [8], and hyperaggressive behavior [9]. The biophysical and pharmacological properties of $Ca_V2.2$ channels are determined by diverse combinations of channel subunits. $Ca_V\alpha1B$ and $Ca_V\alpha2\delta$ are transmembrane proteins. $Ca_V\alpha1B$ subunits are responsible for forming the voltage-sensitive pore of the channel and $Ca_V\alpha2\delta$ subunits are responsible for promoting $Ca_V\alpha1$ subunit stabilization at the plasma membrane [10]. $Ca_V\beta$ subunits are intracellular components that play an essential role in regulating the gating properties and receptor modulation of Ca_V channels. They bind to the I-II linker of the $Ca_V\alpha1$ subunit and finely tune the trafficking of α1 channel proteins to the plasma membrane, current density, channel inactivation and channel regulation by phospholipids [11-14].

G-protein coupled receptors (GPCRs) precisely regulate Ca^{2+} ion influx through $Ca_V2.2$ channels [15, 16]. The activation of GPCRs coupled to $G\alpha_{i/o}$ ($G_{i/o}$PCRs) or $G\alpha_{q/11}$ (G_qPCRs) is known to suppress $Ca_V2.2$ current through two distinct pathways. The first operates via Gβγ heterodimer dissociation from $G_{i/o}$PCR. The Gβγ heterodimer then directly binds to the I-II linker of the $Ca_V\alpha1B$ subunit, which partially overlaps with the binding site of the $Ca_V\beta$ subunit,

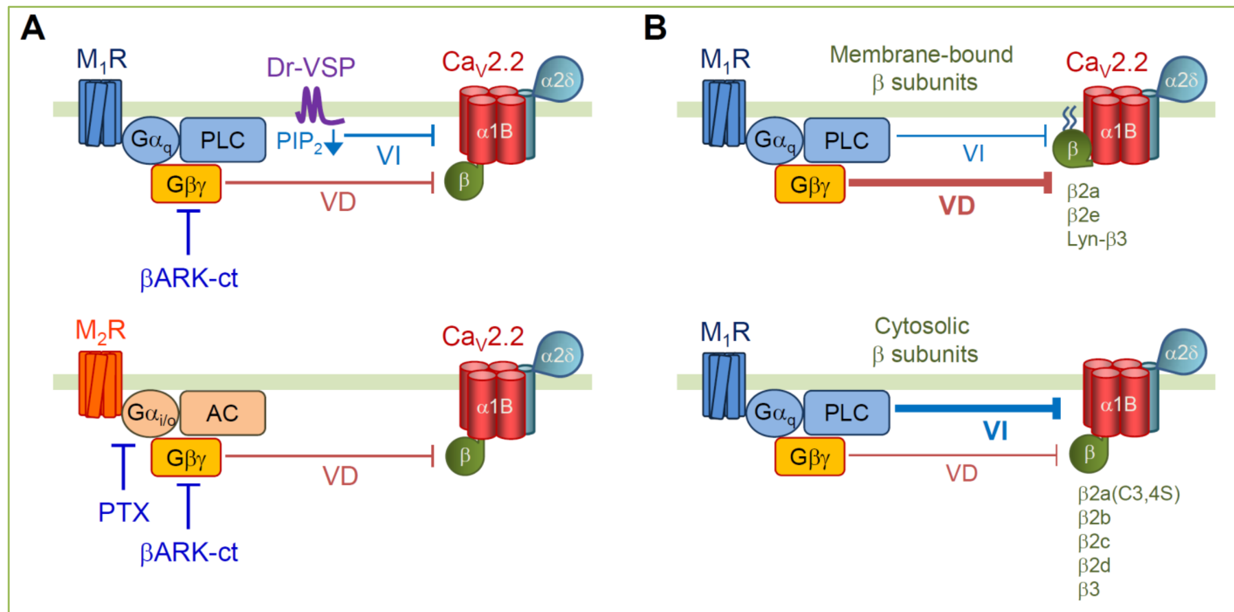

Figure 1. Diagram of inhibitory signaling to $Ca_V2.2$ channels by acetylcholine muscarinic receptors. (A) M_1 and M_2 muscarinic receptors suppress $Ca_V2.2$ current via different pathways. M_1R suppresses $Ca_V2.2$ currents through both PIP_2-dependent VI and Gβγ-mediated VD pathways, while M_2R suppresses currents only through the Gβγ-mediated VD pathway. The Gβγ scavenger βARK-ct inhibits the Gβγ-mediated pathway and PTX inhibits M_2 receptor signaling by blocking the activation of $G_{i/o}$ proteins. (B) M_1R modulates $Ca_V2.2$ channels through two separate pathways independently. The predominance of each type of modulation is determined by the $Ca_V\beta$ subunit. Membrane-associated β subunits decrease PIP_2-dependent VI regulation and enhance Gβγ-mediated VD regulation. The cytosolic β subunit increases VI regulation and decreases the effects of VD regulation. Thick line: major inhibitory pathway. Thin line: minor or weak inhibitory pathway. AC, adenylyl cyclase; PLC, phospholipase C; PTX, pertussis toxin; VD, voltage-dependent inhibition; VI, voltage-independent inhibition. Images are modified from the original work [31].

and triggers fast current inhibition [17]. Since the Gβγ binding to the α1B subunit slows channel activation and shifts the voltage dependence of the channel opening towards a positive charge, a stronger depolarization of the plasma membrane is needed for the $Ca_V2.2$ channels to open. This inhibition can be relieved by supplying a large depolarizing pulse [18, 19] and is thus referred to as "voltage-dependent" (VD) [15]. The second pathway, for G_qPCRs, depends on phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis by phospholipase C (PLC) [20-22] and/or arachidonic acid (AA) generation by phospholipase A_2 (PLA_2) activation [23] following $G\alpha_{q/11}$ subunit activation. This pathway is responsible for relatively slow and voltage-independent (VI) Ca^{2+}channel inhibition. The role of the Gβγ subunit in G_qPCR modulation of $Ca_V2.2$ channels remains unclear. It is possible that the Gβγ subunit released from the $G_{q/11}$ protein can produce fast and voltage-dependent inhibition of the $Ca_V2.2$ current [21, 22, 24-26].

Previous studies have revealed that the slow component of $Ca_V2.2$ channel modulation by the $G_{q/11}$-coupled M_1 muscarinic acetylcholine receptor (M_1R) relies on PIP_2 depletion through $G\alpha_q$/PLC activation and that the $Ca_V\beta$ subunit is an important regulator of the muscarinic modulation of the channel [13, 22]. Indeed, since G_qPCRs activation accompanies many downstream signals, such as Ca^{2+} release from the endoplasmic reticulum and PKC activation via diacylglycerol generation, discriminating between PIP_2 effects and those of other signals is almost impossible. However, with the aid of several genetically encoded tools, such as voltage-sensing phosphatase from zebra fish (Dr-VSP) [22, 27, 28] and the chemically-inducible dimerization system (Lyn-FRB/FKBP-Inp54p) [29] which cleaves the 5-phosphate of PIP_2 to generate phosphatidylinositol 4-monophosphate (PIP) on demand, the contribution of $PI(4,5)P_2$ depletion alone to Ca_V channel inhibition was uncovered in single cells. Interestingly, M_1R activation resulted in stronger Ca_V current inhibition than direct $PI(4,5)P_2$ depletion through Dr-VSP activation or through the rapamycin inducible dimerization system, which implies that pathways besides $G\alpha_q$/PLC are involved. To verify this hypothesis, N-type $Ca_V2.2$ channels, those are regulated by both VI and VD pathways, were studied [30]. We also adopted C-terminus of β-adrenergic receptor kinase (βARK-ct) as a Gβγ subunit scavenger [25, 31, 32]. When Ba^{2+} currents were measured in tsA201 cells expressing M_1R, Ca_V α1B, α2δ1 and β2a subunits upon muscarinic stimulation, the co-expression of βARK-ct resulted in the disappearance of the VD component of M_1R-induced inhibition; channel inhibition was consequently attenuated to the level due to

Dr-VSP activation alone (approximately10% of total). Similarly, substituting Gβγ-insensitive chimeric $Ca_V2.2$ (α1C-1B) for the wild type Ca_V α1B subunit also decreased M_1R-induced Ca_V current depression. In contrast, pertussis-toxin (PTX) sensitive $G\alpha_{i/o}$-coupled M_2R-induced $Ca_V2.2$ current inhibition was completely abolished by the co-expression of βARK-ct. Therefore, we concluded that unlike M_2R that mainly inhibits $Ca_V2.2$ channels through Gβγ-mediated VD pathway, M_1R inhibits the channels through both VI and VD components, such that the VI component is subject to $G\alpha_q$/PLC activation followed by PIP_2 depletion, while the VD component is mainly affected by the Gβγ subunit (**Fig. 1 A**).

To further examine the two M_1R-mediated regulatory pathways, we simultaneously measured FRET (Förster resonance energy transfer) between the eCFP- and eYFP-tagged pleckstrin homology (PH) domain from phospholipase C-δ (PH-PLCδ), which sufficiently reflects the extent of plasma membrane PIP_2 [22, 33-36], and $Ca_V2.2$ current inhibition in single control and βARK-ct-expressing cells. In this single cell assay, $Ca_V2.2$ current depression due to M_1R activation was attenuated in cells co-expressing βARK-ct by approximately 50%. In addition, we found that current inhibition started 3-4 s before PIP_2 hydrolysis in control cells, but not in βARK-ct expressing cells, where current inhibition and PIP_2 hydrolysis started at almost the same time. Although we did not find any differences in the kinetics of current inhibition and PIP_2 hydrolysis between control and βARK-ct expressing cells, we did discriminate between the fast (τ = 1.6 s) and slow (τ = 4.1 s) components of M_1R induced $Ca_V2.2$ inhibition by subtracting the scaled Ca_V current of βARK-ct co-expressing cells from that of control cells, considering 'lag time'. The findings clearly indicated that M_1R activation mediates both slow and fast N-type $Ca_V2.2$ current suppression through the $G\alpha_{q/11}$ and Gβγ subunits, respectively. Furthermore, our findings suggest that under physiological conditions, the downstream effects of G_qPCRs may be affected by the duration, frequency or intensity of stimulation.

As mentioned earlier, the $Ca_V\beta$ subunit determines the $Ca_V2.2$ channel modulation by membrane PIP_2 turnover[13]. $Ca_V2.2$ channels expressed with membrane-localized β subunits were only very slightly inhibited by PIP_2 depletion while channels expressed with cytosolic β subunits were dramatically inhibited by PIP_2 depletion. Additionally, we also realized that the extent of M_1R-induced VI or VD inhibition of N-type Ca_V current varied with the β subunit isotype expressed. This was confirmed by measuring the $Ca_V2.2$ current suppression by M_1R in cells transfected with α2δ1, β2a, palmitoylation resistant mutant β2a(C3,4S), β2b, β3 or membrane targeted Lyn-β3. Firstly, VD inhibition by M_1R was measured by applying a strong depolarizing pulse between the pre- and post-test pulses with and without the presence of a muscarinic receptor agonist. Current inhibition in cells co-expressing membrane-localized β2a or chimeric Lyn-β3 was approximately 30%, while the inhibition in cells with cytosolic β2b, β2a(C3,4S) or β3 was 11-15%. However, the PIP_2 dependence of the current inhibition was 10-25% and 41-59% for membrane-localized and cytosolic β subunits, respectively. As depicted in **Fig. 1 B**, although $Ca_V2.2$ channels are inhibited by both VI and VD pathways, the channels with membrane-localized β subunits are more sensitive to the Gβγ-mediated VD pathway, whereas cytosolic β subunits are largely affected by the PIP_2-dependent VI pathway.

As shown above, our study demonstrates that i) G_qPCR M_1R inhibits N-type $Ca_V2.2$ channels via both Gβγ-mediated VD and $G\alpha_{q/11}$/PLC-mediated VI pathways, ii) these two pathways can be temporally separated, and iii) the cellular location of $Ca_V\beta$ subunits is critical to the determination of the voltage-dependency of $Ca_V2.2$ channel modulation by G_qPCRs. Because the expression of β subunits varies spatially and temporally in brain tissues [21, 22, 24, 26, 37-42], $Ca_V2.2$ modulation by G_qPCRs can also be gradated. Hereby, our research proposes a novel G_qPCR-mediated signaling pathway for $Ca_V2.2$ channel modulation that extends previous observations that Ca_V channels can be regulated by multiple signals [43-46].

Conflict of Interests

We declare there is no potential conflict of interests.

Acknowledgements

This work was supported by the Ministry of Education, Science & Technology (No. 2012R1A1A2044699) and the DGIST R&D Program of the Ministry of Science, ICT&Future Planning (No. 14-BD-06).

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