Regulation of N-type calcium channels by G-proteins

Multiple pathways to control calcium entry into neurons

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Voltage-gated calcium channels represent one of the main pathways for calcium (Ca\(^{2+}\)) entry into neurons, where they control many calcium-dependent processes such as hormone secretion, gene expression or neurite outgrowth. They are expressed both in the central and peripheral nervous systems, and are comprised of a Ca\(_{\text{p}}\) pore forming subunit, plus \(\beta\), \(\gamma\) and \(\alpha_{\delta}\) auxiliary subunits, which control channel trafficking, and activity. At the presynaptic level, Ca\(^{2+}\) entry into nerve terminals is under the control of N-type voltage-gated Ca\(^{2+}\) channels, which allow functional coupling between action potentials and evoked neurotransmitter release. In turn, these channels are strongly regulated by a negative feedback following activation of G-protein coupled receptors (GPCRs) allowing a precise control of synaptic activity. This regulation is characterized by a strong membrane-delimited inhibition of Ca\(^{2+}\) influx, mediated by the direct binding of G\(_{\beta\gamma}\) dimers to specific cytoplasmic regions of the Ca\(_{2.2}\) subunit (the pore forming subunit of N-type Ca\(^{2+}\) channels) (review ref. 1). On the basis of its reversibility in response to a strong membrane depolarization, this inhibition is called “voltage-dependent inhibition”,\(^2\) which contrasts with “voltage-independent inhibition” that occurs over a slow time course and involves diffusible second messengers.\(^3\) Up to date, at least 19 GPCRs have been characterized as regulators of N-type Ca\(^{2+}\) channel activity. Recently, it was reported that some GPCRs (orphanin ORL1\(^4\) and dopamine D1\(^5\)) receptors physically interact with N-type Ca\(^{2+}\) channels within macromolecular complexes, providing for additional channel regulation such as channel trafficking to the plasma membrane or channel internalization.\(^6\) In a recent issue of Channels, Kisilevsky and Zamponi extended this study to the D2 dopamine receptor (D2R).\(^7\) They demonstrate that D2R physically associates with N-type Ca\(^{2+}\) channel allowing Ca\(^{2+}\) current inhibition in voltage-dependent and voltage-independent manners, but also by controlling channel surface expression level.

Using the patch-clamp technique on HEK 293T cells transiently expressing Ca\(_{2.2}\) channels (in combination with \(\beta\)\(_{1a}\) and \(\alpha_{\delta}\) auxiliary subunits), the authors show that activation of D2Rs by dopamine (DA) produces (within a short time frame) a strong (74%) inhibition of the Ca\(^{2+}\) influx mediated by N-type channels (Fig. 1A from ref. 7). According to the extent of current recovery assessed by strong membrane depolarizing prepulse, the authors propose that activation of D2Rs produces both voltage-dependent (i.e. reversed by prepulse application and mediated by G\(_{\gamma\delta}\) dimers) and voltage-independent (i.e. non-reversed by prepulse application) inhibition (Fig. 1B from ref. 7).

To investigate if a physical interaction could exist between N-type Ca\(^{2+}\) channels and D2Rs, as it was previously shown for ORL1 and D1 receptors, the authors performed co-immunoprecipitation experiments from rat striatal homogenate and effectively demonstrate such an interaction (Fig. 2A from ref. 7). In order to determine if this interaction occurs through a direct binding of the N-type Ca\(^{2+}\) channel with the D2R, the authors performed a set
of in vitro binding experiments using different domains of the intracellular regions of both CaV2.2 subunit and D2R and show that both the II-III linker and the carboxy-terminus domain of the CaV2.2 subunit are able to interact with the loop-3 and the carboxy-terminus domains of the D2R (Fig. 2C from ref. 7). These data were confirmed by confocal microscopy based on the capability of CaV2.2 domains to translocate to the plasma membrane in the presence of the D2R, and vice versa (Fig. 3A from ref. 7).

To examine the functional importance of the CaV2.2-D2R interaction in the regulation of N-type Ca2+ currents by DA, “minigenes” encoding for the different domains of the CaV2.2 subunit (II-III linker and carboxy-terminus) and D2R (loop-3 and carboxy-terminus) were expressed in HEK 293T cells in order to disrupt the interaction between CaV2.2 subunit and D2R. Patch-clamp experiments revealed that expression of these “minigenes” specifically (except for the D2 loop-3) slows down the time course of D2 loop-3) slows down the time course of DA-induced N-type Ca2+ current inhibition and reduces both its voltage-dependent and voltage-independent component (Fig. 4A and B from ref. 7).

To further investigate the functional importance of the CaV2.2-D2R interaction, the authors determined whether D2R could regulate plasma membrane expression of N-type Ca2+ channels. Using confocal microscopy on HEK 293T cells, they demonstrate that i) co-expression of the D2R with CaV2.2 subunit favors N-type Ca2+ channels surface expression and ii) application of DA results in a loss of surface expression of both D2R and CaV2.2 subunit suggesting co-trafficking of these proteins in complexes (Fig. 5 from ref. 7).

In summary, Kisilevsky and Zamponi provide evidence that CaV2.2-D2R interaction plays an important role in DA-induced regulation of N-type Ca2+ currents. They demonstrate that such a physical interaction allows a faster and more complete inhibition of N-type Ca2+ currents by DA, both in a voltage-dependent and voltage-independent manner. Furthermore, this interaction allows additional regulation levels for CaV2.2-D2R complexes trafficking to the plasma membrane or channel internalization.

These interesting results leave some questions to be discussed. First, co-immunoprecipitations from rat striatal homogenate seem to indicate that only the glycosylated form of the D2R is immunoprecipitated with the CaV2.2 subunit. Although not discussed by the authors, this could suggest a possible functional importance of this glycosylation in the D2R-induced regulation of N-type Ca2+ currents. Along these lines, although never clearly stipulated, many results in the literature indicate that co-expression of the CaV2.2-D2R interaction, which is subject to glycosylations, favors functional coupling between neuronal voltage-gated Ca2+ channels and direct G-protein regulation. Hence, it could be interesting to test by co-immunoprecipitation if GPCRs which do not physically interact with CaV2.2 channels, such as µ-opioid receptors, can indirectly interact in the presence of the D2R subunit.

Another observation concerns the functional importance of the CaV2.2-D2R interaction in DA-induced voltage-dependent inhibition of N-type Ca2+ currents. Based on the observation that over-expression of peptides corresponding to regions involved in the CaV2.2-D2R interaction, which are presumed to prevent CaV2.2-D2R interaction, alters the extent of current inhibition, the authors proposed that CaV2.2-D2R interaction is necessary for a faster and complete inhibition of N-type Ca2+ currents by DA. However, no direct experimental evidence is provided confirming the capability of these peptides to disrupt the CaV2.2-D2R interaction. Co-immunoprecipitation experiments from HEK 293T cells over-expressing CaV2.2 subunit and D2R in combination with these peptides could resolve this question. Furthermore it seems important to evaluate the biophysical characteristics of N-type Ca2+ channels in the presence of these molecular determinants (independently of the presence of the D2R), in particular to check if they do may affect channel inactivation properties. This is particularly important since it was shown that channel inactivation is a critical factor in the modulation of direct voltage-dependent inhibition of N-type Ca2+ channels by G-proteins.

Finally, this study opens a very interesting and frequently discussed question in the field G protein regulation of neuronal voltage-gated calcium channels: what are the molecular bases of the voltage-independent inhibition? Based on the time course of channels internalization observed in response to GABA-B receptors activation (within the minute), it seems that the voltage-dependent inhibition observed in this study (i.e., non-reversed by prepulse application) could be entirely the consequence of CaV2.2-D2R complexes internalization under agonist stimulation. In order to further examine this question, it could be interesting to develop a chimeric CaV2.2-µ-opioid receptor construct (which do not naturally physically interact) to examine if voltage-independent inhibition of N-type Ca2+ currents is increased under agonist stimulation.

In conclusion, the study by Kisilevsky and Zamponi provides interesting insights in the understanding of molecular mechanisms at the basis of the regulation of neuronal voltage-gated Ca2+ channels by G-proteins, and reveals many pathways by which GPCRs can finely control Ca2+ entry into neurons for precise cellular functions.

References

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