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How do G proteins directly control neuronal calcium channel function?

By

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Abstract

Ca$^{2+}$ entry in neuronal cells is modulated by the activation of numerous G protein coupled receptors (GPCR). Much effort has been invested in studying direct G protein inhibition of voltage-dependent Cav2 type calcium channels. This inhibition occurs through a series of landmark convergent modifications in channel biophysical properties. An integrated view of the structural organization of the G$\beta\gamma$ dimer binding site pocket on the channel is progressively emerging. In this review, it is shown how a variable geometry of the G$\beta\gamma$ binding pocket can yield distinct sets of channel inhibition. In addition, specific mechanisms are proposed for the channel regulation by G proteins which take into account the regulatory input of each G$\beta\gamma$ binding element.
Neuronal voltage-dependent calcium channels (CaV2) which give rise to P/Q-, N- and R-type currents, are in great part localized in synaptic terminals where they control neurotransmitter release and thereby synaptic communication. Their involvement is thus essential for the normal functioning of the peripheral and central nervous system [1-3] and any mechanism that leads to their dysfunction is responsible for severe neuronal diseases. As for most key contributors to cell physiology, these molecular structures are under heavy regulatory control. One of such regulation comprises the negative control provided by G protein coupled receptors (GPCR) whereby activation of one GPCR activates a signaling mechanism that terminates calcium influx and hence inhibits neurotransmitter release [4]. This process can be derived for therapeutic purposes. For instance, pain treatment is provided through the administration of a specific agonist of opioid receptors (morphine) that inhibits N-type channel activity at peripheral synapses. The number of GPCRs (600 estimated from the Human genome) along with the diversity of G proteins identified (27 $G_\alpha$, 5 $G_\beta$ and 14 $G_\gamma$ genes) implies a great diversity in the forms of inhibition. No less than 20 known GPCRs have been shown to inhibit N-type channel activity. This inhibition can occur as the result of a feedback mechanism whereby the neurotransmitter, just released in the synaptic cleft, activates a GPCR adjacent to the channel to inhibit the process responsible for its own release. It can also be the result of the activity of an inhibitory neuromediator released from a nearby site.

Understanding how the activation of various GPCRs can lead to calcium channel inhibition is thus essential for the understanding of the physiological implications of this regulation and for the design of new therapeutic strategies. Many structure-function approaches have been undertaken to reach this goal. However the actual mechanistic picture of calcium channel regulation by G proteins remains unclear and the goal of this review is to sharpen our understanding of this important regulatory process. After a period of controversial reports (1990-1995), Herlitze et al. (1996) [5] and Ikeda (1996) [6] established that this inhibition can be produced by $G_\beta_\gamma$ dimers which with $G_\alpha$-GTP can be produced following GPCR activation. Expression experiments clearly demonstrated that the pore-forming CaV2 subunit alone is sufficient to display direct G-protein-mediated inhibition [7-9]. The identification of multiple $G_\beta_\gamma$ binding sites on CaV2 channels leads to an unclear structural picture of the direct G protein inhibition. Here, all CaV2 channels are considered to contain a single $G_\beta_\gamma$ protein binding pocket (GPBP) with multiple interactive binding sites. The aim of the present review is to dissect out the functional consequence of $G_\beta_\gamma$ binding on the GPBP of CaV2 channels.
The number of active $G_{\beta\gamma}$ binding sites on $Ca_{\gamma}2$ depends on the composition of the implicated dimer, the state of the channel and its protein environment, with here a particular emphasis put on $Ca_{\gamma}\beta$, one of the three calcium channel auxiliary subunits.

**$G_{\beta\gamma}$ binding sites on $Ca_{\gamma}2$ channels and their relative contribution to the direct GPCR-induced calcium channel inhibition**

GPCR activation produces in fact two signaling molecules, $G_{\alpha}$-GTP and the $G_{\beta\gamma}$ dimer (Fig 1). The focus in the present review is on the direct role of $G_{\beta\gamma}$ in the G protein mediated inhibition which does not preclude a functional implication of $G_{\alpha}$-GTP in channel regulation [10]. Very few of the possible $G_{\beta\gamma}$ combinations have been tested. However, the rank order of efficacies of the various $G_{\beta}$ isoforms is strongly linked to the type of $Ca_{\gamma}2$ and $Ca_{\gamma}\beta$ combination [11]. Additionally, it also seems related to the isoform type of $G_{\gamma}$ that pairs with $G_{\beta}$ [12] and most data converge to establish that $G_{\gamma}$ is mandatory for observing a G protein induced inhibition [6,13,14].

Figure 2 summarizes the actual state of knowledge on $G_{\beta\gamma}$ / $Ca_{\gamma}2$ interaction. $G_{\beta\gamma}$ regulation requires several structural and functional $Ca_{\gamma}2$ channel determinants: one in the amino-terminus (Ns), several in the I-II loop (I-II$_{S1}$, I-II$_{S2}$, and II$_{S3}$) and two in the carboxyl-terminus of the channel (Cs). I-II$_{S2}$ contains the QXXER $G_{\beta\gamma}$ binding motif and I-II$_{S3}$ contains the GID (G protein interaction domain). In the QXXER motif, mutation of its R residue blocks G-protein-mediated slowing of activation kinetics of $Ca_{\gamma}2.1$ [15], whereas an additional mutation at the third position (I to L) decreases G protein modulation and enhances the rate of reversal of G protein effects [16]. The GID denomination was introduced because the 21-mer peptide I-II$_{S3}$ blocked paired-pulse facilitation of channels under tonic $G_{\beta\gamma}$ inhibition [17]. Both the 60 and 20 nM binding affinities of the QXXER domain and of the I-II$_{S3}$ site [15] are one order of magnitude lower than for the $Ca_{\gamma}2/Ca_{\gamma}\beta$ interaction [18]. The interaction point Cs, in the middle of the carboxyl-terminus of $Ca_{\gamma}2.3$ [19] has homologous sequences in $Ca_{\gamma}2.1$ and $Ca_{\gamma}2.2$ [20]. After truncation of Cs, together with the remaining downstream carboxyl-terminal $Ca_{\gamma}2.3$ sequence, current is no longer reduced by GPCR activation [19]. Interestingly, this Cs site for $G_{\beta\gamma}$ is close to the $C_{\alpha\omega}$ binding site in $Ca_{\gamma}2.1$ and $Ca_{\gamma}2.2$ [21] and to the $G_{\alpha\omega}$ binding site [20], which opens the possibility of a large $Ca_{\gamma}/G_{\alpha\omega\beta\gamma}/GPCR$ complex [22,23].
Importantly, the amino-terminus and the carboxyl-terminus of the channel have been shown to interact with the I-II loop [24,25]. This pattern of interactions likely brings the various Gβγ interaction points in close spatial proximity of each other. Convincingly, Zamponi and Snutch (1998) [26] demonstrated that the reassociation of Gβγ onto CaV2.2 follows a mono-exponential time course whose time constant linearly depended on Gβγ concentration. It was therefore concluded that Gβγ reassociation to the channel occurred as a bimolecular reaction implicating the binding of a single Gβγ onto each channel.

All the above observations suggest that there is a single GPBP on CaV2 and that the activation of GPCR produces variable sets of G protein-mediated regulation. Figure 3 summarizes all the effects that are considered as hallmarks of direct G protein regulation. Four “On” effects converge to achieve an efficient inhibition of CaV2 and washout of the receptor agonist leads to full recovery of the current amplitude. Recovery (“Off” effect) kinetics appear to be systematically slower that the “On” effects, lasting a few seconds [27,28]. Experimentally, “Off” effects are also observed when large prepulse depolarizations are applied (Fig 3b) [29]. However, recovery of the current amplitude is then rarely complete [30]. Prepulse application has always been assumed to reverse the direct G protein “On” inhibition by producing unbinding of the Gβγ dimer from the channel.

The molecular determinants implicated in the various effects of G protein regulation have been dissected out by mutagenesis of G protein-sensitive channels and sequence swapping between G protein-sensitive and G protein-insensitive channels. Despite the variability among the data, the following conclusions can be extracted. First, to observe any kind of effect, the Ns site is mandatory. Evidence comes from the fact that G protein inhibition of CaV2.3 is only observed for a long isoform that comprises the Ns site, but not for a short isoform that lacks it [31]. Conversely, a CaV2.2/CaV1.2 chimera with the Ns of CaV2.2 is more sensitive to G protein regulation [32]. Second, the carboxyl-terminus of all CaV2 channels play an obligatory or facilitating role (depending on CaV2 or GPCR isoform) when G protein inhibition is mediated by GPCR activation [19,21], whereas it does not seem to contribute when a combination of Gβγ complex is over-expressed [32]. Also, peptides mimicking the binding site for Gα, present on the carboxyl-terminus of CaV2.1 and CaV2.2, impair the GPCR induced channel inhibition [21]. Deletion of the Cs site in CaV2.3 induces a complete loss of regulation by the activation of the muscarinic M2 receptor [19], whereas a similar deletion in CaV.2.2 reduces somatostatin regulation by about 50% [33]. These findings are in favor of the hypothesis along which the initial binding of the heterotrimeric G protein onto the carboxyl-
terminus of \( \text{Ca}_V \) facilitates channel regulation [22]. Third, the binding determinants of the I-II loop of \( \text{Ca}_V \) do not seem to be obligatory for observing current inhibition, one of the “On” effects of G protein inhibition [19,34]. However, the presence of the I-II loop makes G protein inhibition more prominent [32]. There is also some evidence [35] to believe that the I-II loop of some \( \text{Ca}_V \) are more efficient than others in controlling the \( G_{\gamma} \) binding affinity to the channel, thus probably imposing the well established rank order of sensitivity to G protein inhibition \( \text{Ca}_V2.2 > \text{Ca}_V2.1 >> \text{Ca}_V2.3 \). At the functional level, one convincing role of I-II\( \text{S}_2 \) is its implication in the rate of channel facilitation by prepulse application [16,36]. This index is interpreted as measuring \( G_{\gamma} \) dissociation from the channel complex (“Off” effect); the I-II\( \text{S}_2 \) would act as a voltage-sensor and its movements during membrane depolarization constitute one step in the pathway leading to channel facilitation. Actually, voltage-sensitivity of I-II\( \text{S}_2 \) has recently been evidenced [37]. Interestingly, peptides mimicking either I-II\( \text{S}_1 \), I-II\( \text{S}_2 \) or I-II\( \text{S}_3 \) are all able to prevent G protein inhibition [16,17,21]. The poor involvement of \( \text{Ca}_V \) I-II loop in the “On” effects of G protein regulation suggests that I-II loop determinants might rather contribute to “Off” effects. This hypothesis will require specific testing and, if proven true, implicates that the GPBP undergoes a real dynamic of sequential interacting events during the “On” and “Off” course of G protein regulation.

**Contribution of the \( \beta \) subunit to G protein regulation**

Any protein that forms part of a larger Ca channel protein complex, such as the machinery regulating transmitter release, is expected to interact with \( G_{\beta\gamma} \) determinants and to alter G protein inhibition as a consequence. For instance, this was shown to be the case with syntaxin 1A ([38,39]). Here, focus will be put on the “constitutive” \( \text{Ca}_V\beta \), whose modulatory effects on the activity of the pore forming \( \text{Ca}_V \) subunit have been extensively studied. Furthermore the structures of three \( \text{Ca}_V\beta s \) have been obtained in 2004 (for a review [40]).

The \( \text{Ca}_V\beta \) acts by increasing the coupling efficiency between the gating charge movements and the pore opening (measured by \( \text{Ca}^{2+} \) currents) [41,42]. The voltage-dependence of gating current is however not modified. As a result, channel opening is facilitated, an effect that translates into an hyperpolarizing shift of the voltage-dependence of current activation. From this point of view, the effects of \( \text{Ca}_V\beta \) are seemingly opposite to those observed with \( G_{\beta\gamma} \). A more subtle picture emerges however when comparing \( G_{\beta\gamma} \) regulatory effects on a channel that either lacks or comprises \( \text{Ca}_V\beta \). On a “nude” channel, G-protein regulation produces 1)
marked current inhibition, 2) mild slow-down of activation kinetics, but 3) no clear-cut shifts in the voltage-dependencies of either activation or inactivation properties. Current facilitation induced by prepulse application has been observed, though not systematically. When CaVβ is added, Gβγ effect on the current amplitude is conserved, whereas the slowing of activation kinetics is enhanced. Furthermore, prepulse facilitation is clearly evidenced, the depolarizing shift of the voltage-dependence of activation is obvious, and changes in inactivation have been demonstrated. These observations rather point to a synergistic or “promoting” effect of CaVβ on Gβγ regulation. This diversity of effects might be due to distinct experimental conditions. In the actual physiological representation, one must keep in mind that the regulatory CaVβ is constitutive (with all CaV2 in a CaV2/β complex form with a generally accepted 1:1 stoechiometry) whereas Gβγ concentration varies spatio-temporally with GPCR activation. Expression of CaVβ introduces an experimental bias in the study of G protein regulation. To be optimal, the experimental conditions should be set so that Gβγ concentration rises sufficiently to also approach a 1:1 ratio between CaV2 and Gβγ. Most favorable conditions appear to be i) the acute injection of Gβγ or ii) the over-expression of a Gαβγ complex. Assuming that the stoechiometric requirements have been reached in most studies, the data indicate that the regulation by Gβγ in the presence of CaVβ can be placed into four different case scenarios.

Case 1: lack of Gβγ binding in the presence of CaVβ - There are clear-cut examples in the literature in which Gβγ regulation is ineffective on CaV2.3 in the presence of a particular CaVβ subunit isoform [43]. For instance, the co-expression of CaVβ2a with CaV2.3 blocks the M2 receptor-induced current inhibition [19]. In this study, it was found that Gβγ and CaVβ2a are both able to bind separately on the Cs interaction point. Furthermore, binding of CaVβ2a to Cs occludes the subsequent interaction of Gβγ with Cs. As suggested earlier, it is tempting to propose that Cs represents an initial anchoring point for Gβγ that controls the efficient position of Gβγ for regulation.

Cases 2 and 3: simultaneous binding of Gβγ and CaVβ. The first evidence demonstrating that Gβγ and CaVβ can bind simultaneously onto the channel comes from the fact the activation kinetics of CaV2.2 under Gβγ inhibition are much slower when CaVβ2a subunit is used over any other CaVβ [11]. The second evidence came from FRET experiments between CaV2.1 and CaVβ1b that indicated a conformational change induced by Gβ binding onto CaV2.1 carboxyl-terminus interaction site (presumably Cs) [44]. All, these observations suggest that some
isoforms of $G_\beta$ bind to the channel simultaneously with $Cav\beta$. The functional antagonism between $Cav\beta$ and $G_\beta\gamma$ might thus result from steric hindrance due to the presence of the $Cav\beta$ on the I-II loop. Consequently, binding of $G_\beta\gamma$ to the I-II loop would occur with a lower affinity (reduced interaction points and altered conformation). The induction of an inhibited channel mode induced by $G_\beta\gamma$ in the simultaneous presence of $Cav\beta$ can be envisioned along two mechanisms illustrated in Figure 4: the state-induced model (Fig 4a) or the $G_\beta\gamma$ dissociation model (Fig 4b).

*Case 4: Binding of $G_\beta\gamma$ complex produces $Cav\beta$ dissociation*

One simple explanation for some of the $G$ protein and $Cav\beta$ opposite effects would be that $G_\beta\gamma$ displaces the $Cav\beta$ from its binding site on the channel, thereby reversing its regulatory properties (Fig 4b, $\beta$ dissociation” model). $Cav\beta$ association to AID has been shown to modify this site from a random coiled structure to an $\alpha$-helix with a concomitant increase in affinity [45,46]. This observation strongly suggests that the interaction of $G_\beta\gamma$ with I-II$_{S2}$ would be able to destabilize the $\alpha$-helical structure and shifts AID from a high to a low affinity binding site for $Cav\beta$ (binding antagonism). Though steric information is lacking, simultaneous binding of $Cav\beta$ and $G_\beta\gamma$ onto the I-II loop (required as the initiation step for $Cav\beta$ dissociation) is likely considering the number of high affinity sites for $G_\beta\gamma$ present downstream of AID. In agreement with these structural considerations, such a $Cav\beta$ displacement by $G_\beta\gamma$ has been observed experimentally [47]. Using a I-II loop/ $Cav\beta$ chimera to impose the 1:1 stoichiometry of interaction, it was shown that $G_\beta\gamma$ binding on the I-II loop produces a loss of internal AID / $Cav\beta$ interaction. This conclusion was also reached with a FRET approach using the full-length GFP-tagged $Cav2.1$ channel and a chemically modified Cy3-$Cav\beta$. $Cav\beta$ dissociation may require specific conditions: 1) a single anchoring point for $Cav\beta$ onto the channel (AID), 2) a peculiar $G_\beta\gamma$ combination, and 3) a specific $G_\gamma$ subunit for an interaction with the I-II loop [48].

Further investigations are required to determine which combination of subunits leads to one or the other type of $G_\beta\gamma$ interactions. Importantly, this variety of interactions is likely to occur also when any constituent of a $Cav\gamma$ complex is substituted with a related isoform.

**Ending the channel inhibition produced by activated $G$ proteins**

Ending the GPCR-induced channel inhibition occurs physiologically on recapture of the agonist, pharmacologically by application of a specific antagonist or experimentally by
application of a strong depolarization. The latter relief of inhibition is transient because as soon as the depolarizing trigger is lost, re-inhibition occurs with well-defined kinetics that are dependent on $G_{\beta\gamma}$ concentration. Although the end result is similar, at least temporary for the prepulse application, the “Off” position of $G_{\beta\gamma}$ is maybe not identical whether the “Off” signal is provided by $G_{\alpha}$ or prepulse application.

In a physiological context, hydrolysis of GTP bound on $G_{\alpha}$ converts the latter from a low to a high affinity ligand for $G_{\beta\gamma}$. Rebinding of $G_{\alpha}$ to $G_{\beta\gamma}$ terminates the inhibitory signal and may follow two possible paths. First, $G_{\alpha}$ may naturally chelate free $G_{\beta\gamma}$ that would come on and off the channel as a result of binding equilibrium. Second, $G_{\alpha}$ may bind onto $G_{\beta\gamma}$ while on the channel which implies that $G_{\beta\gamma}$ determinants essential for $G_{\alpha}$ association remain accessible in spite of the association of $G_{\beta\gamma}$ to the GPBP. The consequences of this $G_{\alpha}$ re-association to $G_{\beta\gamma}$ can leave the $G_{\beta\gamma}$ associated to Cav. In this case, the trimeric G protein is permanently associated to the channel, a view that is probably incompatible with data suggesting that $G_{\beta\gamma}$ may come on and off the channel in a concentration-dependent manner [26]. Alternatively, $G_{\alpha}$ binding produces a complete dissociation of $G_{\beta\gamma}$ from the GPBP which however does not preclude that the trimeric G protein complex may remain associated to the channel through $G_{\alpha}$. In all these potential mechanisms for signal termination, what matters the most is the loss of influence of a critical $G_{\beta\gamma}$ element onto a structural element that controls channel opening.

In that respect, termination of G protein inhibition by prepulse application has to share this critical step with the physiological termination.

The channel structural elements required for prepulse termination of $G_{\beta\gamma}$ inhibition have not yet been identified. Expectations are that these channel structural elements are voltage-sensitive and translate their conformational changes to the structural elements that bind $G_{\beta\gamma}$. The molecular schemes just proposed for the physiological termination of $G_{\beta\gamma}$ inhibition should also apply to prepulse termination. Full $G_{\beta\gamma}$ dissociation is most frequently assumed but clearly lacks a molecular demonstration. A fourth case scenario should be introduced based on the proposal that a strong depolarizing prepulse introduces a temporary conformational change in the channel [49] that would be sufficient to convert the channel from a “reluctant” to a “willing” mode (see Figure 4) with the assumption that these states are controlled respectively by $G_{\beta\gamma}$ and Cav$\beta$ association.

In a model in which Cav$\beta$ dissociation would occur, an additional molecular event should take place upon termination of $G_{\beta\gamma}$ inhibition. This step is linked to Cav$\beta$ re-association to the
channel once its binding site has been freed from the competitive action of Gβγ. This second step is required for shifting back the channel from a “reluctant” to a “willing” state. A consequence of this two-step procedure is that the kinetics of termination of G protein inhibition is also bi-modal: a rapid reversal for current amplitude and activation kinetics, and a slower one for the shift from “reluctant” to “willing”.

**Concluding remarks**

The study of Gβγ interaction on CaV2 channels in expression system has opened the way to vary independently the various molecular components. However, following the basic rules of physiology, the concentration of CaVβ is not expected to vary, as opposed to the Gβγ concentration which is strictly controlled by GPCR activation. Thus, G proteins and not calcium channel auxiliary subunits are the key regulatory elements. In the present model of G protein regulation, a G protein binding pocket of variable geometry, as defined by the direct protein environment of the channel, governs the various mechanisms of G protein inhibition. Landmark effects that at once seemed all to depend of different mechanisms can be regrouped in a single one, but still adapt the possibility of interesting variations such as CaVβ dissociation. Channel remodeling under G protein regulation is by far one of the most interesting concepts as it depicts a calcium channel with all its dynamics and opens intriguing new roles for each of its elementary components.
Figure 1 – **Structure of Gβγ, and its essential binding determinants.** The crystal structure of Gβγ points to a complex of 80 x 50 x 45 Å thick (PDB accession 1TBG; [50]). CPK model of transducin Gβγ crystal structure [50] shown at two opposing faces (180° rotation). Considering its membrane attachment, Gβγ should be oriented with its longest axis perpendicular to the plasma membrane positioning the Gβγ at the periphery of Cav2. Gβ is shown in blue, whereas Gγ is depicted in green. The Gβγ is oriented such that Gγ binds the plasma membrane by its farnesyl residue at the amino-terminus. Noteworthy a positively charged pocket is also oriented towards the plasma membrane. Various structural and functional studies have defined critical Gβ amino acid residues for the regulation of calcium channels (shown in red) [51-58]. Amino acid residues common to Gβ subunits that bind to the I-II loop, but absent from Gβ subunits that do not bind this loop include R19, S31, N35, P39, A193, R197 and A305 according to Gβ1 numbering. Some of these residues are within or near regions involved in the interaction with the Gγ subunit. The observation that Gβ isoforms differ in their ability to interact with the HI loop of Cav2.2 is confirmed by a study demonstrating that the antagonistic effect of PKC phosphorylation of the I-II loop is only observed with Gβ1 [52,53]. Additional residues required for binding peptides with the QXXER motif are shown in light purple [59,60]. The yellow arrow depicts the phosphorylation sensor of GID [53]. All important functional residues for calcium channel regulation are distributed on the two faces of the Gβγ dimer shown here. Interestingly, Gα binding site (dark purple) is almost completely masked by the “interaction” with the calcium channel with the exception of a few amino acid residues [54,56-59,61-64]. The masked residues are outlined by a dark purple dashed line. Of note, the important functional channel regulation provided by a peptide that comprises amino acid residues 270-305 of Gβ2 [65]. Figure created with RasMol v2.7.2.1 (Raswin Molecular Graphics, Berntein H. 1998-2001).

Figure 2 – **Important G protein determinants on Ca2-type channels.** Top diagram shows the characteristic topology of the pore-forming Ca⁺ channel. Each hydrophobic domain (I to IV) is made of six transmembrane spanning segments (1 to 6), S4 representing the voltage-sensor, rich in positively-charged amino acid residues. The major Gβγ determinants on Ca⁺ cytoplasmic domains (Ns, QXXER, GID and Cs; see text) are reported in red and detailed in the three lower panels, amino-terminal, HI loop and carboxyl-terminal regions. The Ns determinant has been described on Cav2.3 [31] and on Cav2.2 [31,32] and identified on the basis of functional evidence only. An equivalent sequence is found on Cav3.2 but on the I-II
Two binding domains, QXXER and GID, have been identified in the I-II loop using either peptides (peptides I-II$_S$ to I-II$_S$ [17] and peptide PL1 [21], illustrated by underlined sequences), mutagenesis [15] or binding experiment [19]. GID forms part of the I-II$_S$ sequence [67], whereas PL1 is part of both I-II$_S$ and I-II$_S$ [21,68]. The inhibitory phosphorylation site in GID is shown in blue [17]. Green refers to the primary site (the 18 amino-acid residues of the AID on I-II loop [69]) and the secondary sites (SS1 on the amino-terminus [70,71] and SS2 on the carboxyl-terminus [70,72]) that interact with Cavβ. Brown refers to the EF hand, preIQ, IQ and CBD domains [73,74] which constitute the carboxyl-terminal Ca$^{2+}$ binding domains. $G_{oq}$ [20] and $G_{zo}$ [68] binding sites are reported in dark purple. Lower panels show sequence alignments for Ca$_{2.1}$ (SwissProt accession number: P54282, rat), Ca$_{2.2}$ (Q02294, rat), Ca$_{2.3}$ (Q15878, human) for non L-type channels, and Ca$_{1.2}$ (P22002, rat) for L-type channel. Boxes refer to sites defined by point mutations. Sequence gaps are shown by dashes.

Figure 3 - Hallmarks of G protein modulation. a) “On” effects observed after GPCR activation are a$_1$: an inhibition of the current amplitude (ranging from 15 to 80% depending on the Cav/GPCR combination) which is far more pronounced at the start of the depolarization than at the end of the pulse, a$_2$: a slowing of the kinetics of current activation between 10 to 100 ms, a$_3$: a depolarizing shift of the voltage-dependence of channel activation which has been described in terms of channel modes, and a$_4$: a shift to hyperpolarized potentials of the steady-state inactivation curve.

b) Experimental “Off” effect. Prepulse application at 100 mV before test pulse at 10 mV reduces $G_{βγ}$ inhibition (relative facilitation). Current traces and curves were obtained from various Ca$_{2.2}$/Cavβ combinations expressed in Xenopus oocytes (all data obtained with the combination Ca$_{2.2}$/β$_3$ except panel 2 traces obtained from Ca$_{2.2}$/β$_{2a}$).

Figure 4 – Willing and reluctant modes of Ca$_{2}$-type channels. The terms willing and reluctant [75] refer to the easiness of channel activation. The willing mode corresponds to channel activation at more hyperpolarized potentials than the reluctant mode. We have identified three different possible mechanisms for the induction of the reluctant mode by $G_{βγ}$ (state-induced, β dissociation and $G_{βγ}$ dissociation). a) State-induced model. This model assumes that willing and reluctant modes are intrinsic modes of Ca$_{2}$. Alteration of voltage-detection induced by $G_{βγ}$ binding preferentially shifts the equilibrium between willing and
reluctant modes towards the reluctant mode. In favor of this model, a mutation of Cav2.2 (G177E) in the IS3 segment can shift the channel in a reluctant mode without the contribution of G\(_{\beta\gamma}\) [76]. The state-induced model implicates that simultaneous binding of G\(_{\beta\gamma}\) and Cav\(\beta\) is possible. b) Cav\(\beta\) dissociation model. Binding of Cav\(\beta\) to the Cav2 channel induces a shift from a reluctant state to a willing state [77]. The binding of G\(_{\beta\gamma}\) physically displaces the Cav\(\beta\) from its binding site and simultaneously imposes the reluctant mode of the channel. In this model, the converse displacement of G\(_{\beta\gamma}\) by the Cav\(\beta\) is not likely to occur because G\(_{\beta\gamma}\) occupies multiple binding sites on Cav2 and the Cav\(\beta\) concentration is not supposed to vary. Signal termination occurs with the departure of G\(_{\beta\gamma}\) without modal shift. Re-association of the Cav\(\beta\) and induction of the willing mode will only occur after G\(_{\beta\gamma}\) departure. The Cav\(\beta\) dissociation model implicates that simultaneous binding of G\(_{\beta\gamma}\) and Cav\(\beta\) is not possible. Evidence for this model was provided for the Cav2.1 channel [47]. c) G\(_{\beta\gamma}\) dissociation model.

Panel 1: prepulse depolarization at strong voltage is known to induce a facilitation which can be interpreted as a departure of a blocking G\(_{\beta\gamma}\) from the channel complex (Cav2/G\(_{\beta\gamma}\)/\(\beta\)). This departure is prepulse duration dependent and has been proposed to be completed in less than 100 ms [78]. Panel 2: this process is therefore likely to occur during the first depolarizing pulse itself resulting in a voltage-dependent variable proportion of mixed Cav2/\(\beta\)/G\(_{\beta\gamma}\) and Cav2/\(\beta\) complexes. Small depolarization favors the undissociated state, whereas strong depolarization favors the dissociated state. G\(_{\beta\gamma}\) departure induces an increased Ca\({}^{2+}\) entry, probably by an increase in opening probability. The inhibitory effect of G\(_{\beta\gamma}\) becomes voltage-dependent as illustrated by the bottom current traces. Panel 3: two relationships were drawn, one in which G\(_{\beta\gamma}\) does not dissociate from the channel (grey line) and another one in which increased dissociation of G\(_{\beta\gamma}\) from the channel occurs with increased membrane depolarization (red line). Panel 4: I-V relationships before (control) and after G\(_{\beta\gamma}\)-binding in two conditions (without and with a voltage-dependent G\(_{\beta\gamma}\) dissociation during pulse application). In the case where G\(_{\beta\gamma}\) does not dissociate a constant 50% inhibition of the current occurs at all potentials (grey line). With a voltage-dependent G\(_{\beta\gamma}\) dissociation, the resulting I-V curve is close to the grey line at low potentials, whereas it gets closer to the control black line at depolarized potentials. Panel 5: resulting voltage-dependence of the conductance for control and liganded G\(_{\beta\gamma}\) channels with or without dissociation. The red curve illustrates the appearance of an apparent reluctant mode. As a result, the reluctant mode and the slowing of activation kinetics are not “On” effects but become “Off” effects of G protein regulation.
Activation curves have been described by Boltzmann-derived equations. The G\(_{\beta\gamma}\) dissociation model is not yet experimentally demonstrated. Like the state-induced model, it implies that G\(_{\beta\gamma}\) and the Cav\(\beta\) subunit can bind together on the channel. Noteworthy, further complexity in the mechanism of G protein regulation can be introduced by combining the three different mechanisms that are not exclusive of each other.

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a  “On”-effects

1. Holding potential (mV) → Test pulse (mV) → Normalized $I_{Ca^{2+}}$

2. Holding potential (mV) → Normalized $I_{Ca^{2+}}$

3. Test pulse (mV) → Normalized $I_{Ca^{2+}}$

4. Holding potential (mV) → Normalized $I_{Ca^{2+}}$

b  “Off”-effects

10 mV

-90

100 mV

-90
a  State-induced model

Open probability vs. Test potential (mV)

R  W

Ca^{2+}  β

Ca^{2+}  β

b  β subunit dissociation model

Open probability vs. Test potential (mV)

R  W

Ca_{2.x}  +β

Ca_{2.x}  +G_{βγ}

G_{βγ}  dissociation model

1. Normalized P2/P1 vs. Prepulse duration (ms)

P1  P2

0  1  200 ms

2. G_{βγ} dissociation

Ca_{2.x}  Ca_{2.x}  Ca_{2.x}

-90  -90  -90

0 mV  20 mV  40 mV

3. Ca_{2.x}  β vs. Test potential (mV)

G_{βγ}  No dissociation

0  1  200 ms

4. I_{Ca} (µA) vs. Test potential (mV)

Control  +G_{βγ} (with dissociation)  +G_{βγ} (without dissociation)

-20  20  60

5. G/G_{max} vs. Test potential (mV)

Control  +G_{βγ} (with strong dissociation)  +G_{βγ} (with low dissociation)