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# **How do G proteins directly control neuronal calcium channel function?**

**By**

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## **Abstract**

$\text{Ca}^{2+}$  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  $\text{Ca}_v2$  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  $\text{G}_{\beta\gamma}$  dimer binding site pocket on the channel is progressively emerging. In this review, it is shown how a variable geometry of the  $\text{G}_{\beta\gamma}$  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  $\text{G}_{\beta\gamma}$  binding element.

Neuronal voltage-dependent calcium channels ( $Ca_v2$ ) 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  $Ca_v2$  subunit alone is sufficient to display direct G-protein-mediated inhibition [7-9]. The identification of multiple  $G_{\beta\gamma}$  binding sites on  $Ca_v2$  channels leads to an unclear structural picture of the direct G protein inhibition. Here, all  $Ca_v2$  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  $Ca_v2$  channels.

The number of active  $G_{\beta\gamma}$  binding sites on  $Ca_v2$  depends on the composition of the implicated dimer, the state of the channel and its protein environment, with here a particular emphasis put on  $Ca_v\beta$ , one of the three calcium channel auxiliary subunits.

### **$G_{\beta\gamma}$ binding sites on $Ca_v2$ channels and their relative contribution to the direct GPCR-induced calcium channel inhibition**

GPCR activation produces in fact two signaling molecules,  $G_x$ -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  $G_x$ -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  $Ca_v2$  and  $Ca_v\beta$  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  $G_{\beta\gamma} / Ca_v2$  interaction.  $G_{\beta\gamma}$  regulation requires several structural and functional  $Ca_v2$  channel determinants: one in the amino-terminus (Ns), several in the HII loop (I-II<sub>S1</sub>, I-II<sub>S2</sub>, and I-II<sub>S3</sub>) and two in the carboxyl-terminus of the channel (Cs). I-II<sub>S2</sub> contains the QXXER  $G_{\beta\gamma}$  binding motif and I-II<sub>S3</sub> 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  $Ca_v2.1$  [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<sub>S3</sub> 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<sub>S3</sub> site [15] are one order of magnitude lower than for the  $Ca_v2/Ca_v\beta$  interaction [18]. The interaction point Cs, in the middle of the carboxyl-terminus of  $Ca_v2.3$  [19] has homologous sequences in  $Ca_v2.1$  and  $Ca_v2.2$  [20]. After truncation of Cs, together with the remaining downstream carboxyl-terminal  $Ca_v2.3$  sequence, current is no longer reduced by GPCR activation [19]. Interestingly, this Cs site for  $G_{\beta\gamma}$  is close to the  $G_{\alpha o}$  binding site in  $Ca_v2.1$  and  $Ca_v2.2$  [21] and to the  $G_{\alpha q}$  binding site [20], which opens the possibility of a large  $Ca_v/G_{\alpha\beta\gamma}/GPCR$  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  $G_{\beta\gamma}$  interaction points in close spatial proximity of each other. Convincingly, Zamponi and Snutch (1998) [26] demonstrated that the reassociation of  $G_{\beta\gamma}$  onto  $Ca_v2.2$  follows a mono-exponential time course whose time constant linearly depended on  $G_{\beta\gamma}$  concentration. It was therefore concluded that  $G_{\beta\gamma}$  reassociation to the channel occurred as a bimolecular reaction implicating the binding of a single  $G_{\beta\gamma}$  onto each channel.

All the above observations suggest that there is a single GPBP on  $Ca_v2$  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  $Ca_v2$  and washout of the receptor agonist leads to full recovery of the current amplitude. Recovery (“Off” effect) kinetics appear to be systematically slower than 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  $G_{\beta\gamma}$  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  $Ca_v2.3$  is only observed for a long isoform that comprises the Ns site, but not for a short isoform that lacks it [31]. Conversely, a  $Ca_v2.2/Ca_v1.2$  chimera with the Ns of  $Ca_v2.2$  is more sensitive to G protein regulation [32]. Second, the carboxyl-terminus of all  $Ca_v2$  channels play an obligatory or facilitating role (depending on  $Ca_v2$  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  $G_{\beta\gamma}$  complex is over-expressed [32]. Also, peptides mimicking the binding site for  $G_{\alpha}$ , present on the carboxyl-terminus of  $Ca_v2.1$  and  $Ca_v2.2$ , impair the GPCR induced channel inhibition [21]. Deletion of the Cs site in  $Ca_v2.3$  induces a complete loss of regulation by the activation of the muscarinic M2 receptor [19], whereas a similar deletion in  $Ca_v2.2$  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  $\text{Ca}_v2$  facilitates channel regulation [22]. Third, the binding determinants of the I-II loop of  $\text{Ca}_v2$  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  $\text{Ca}_v2$  are more efficient than others in controlling the  $\text{G}_{\beta\gamma}$  binding affinity to the channel, thus probably imposing the well established rank order of sensitivity to G protein inhibition  $\text{Ca}_v2.2 > \text{Ca}_v2.1 \gg \text{Ca}_v2.3$ . At the functional level, one convincing role of I-II<sub>S2</sub> is its implication in the rate of channel facilitation by prepulse application [16,36]. This index is interpreted as measuring  $\text{G}_{\beta\gamma}$  dissociation from the channel complex (“Off” effect); the I-II<sub>S2</sub> 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<sub>S2</sub> has recently been evidenced [37]. Interestingly, peptides mimicking either I-II<sub>S1</sub>, I-II<sub>S2</sub> or I-II<sub>S3</sub> are all able to prevent G protein inhibition [16,17,21]. The poor involvement of  $\text{Ca}_v2$  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 b 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  $\text{G}_{\beta\gamma}$  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  $\text{Ca}_v2$  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 the  $\text{Ca}_v\beta$  are seemingly opposite to those observed with  $\text{G}_{\beta\gamma}$ . A more subtle picture emerges however when comparing  $\text{G}_{\beta\gamma}$  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  $\text{Ca}_v\beta$  is added,  $\text{G}_{\beta\gamma}$  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  $\text{Ca}_v\beta$  on  $\text{G}_{\beta\gamma}$  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  $\text{Ca}_v\beta$  is constitutive (with all  $\text{Ca}_v2$  in a  $\text{Ca}_v2/\beta$  complex form with a generally accepted **1:1** stoichiometry) whereas  $\text{G}_{\beta\gamma}$  concentration varies spatio-temporally with GPCR activation. Expression of  $\text{Ca}_v\beta$  introduces an experimental bias in the study of G protein regulation. To be optimal, the experimental conditions should be set so that  $\text{G}_{\beta\gamma}$  concentration rises sufficiently to also approach a 1:1 ratio between  $\text{Ca}_v2$  and  $\text{G}_{\beta\gamma}$ . Most favorable conditions appear to be i) the acute injection of  $\text{G}_{\beta\gamma}$  or ii) the over-expression of a  $\text{G}_{\beta\gamma}$  complex. Assuming that the stoichiometric requirements have been reached in most studies, the data indicate that the regulation by  $\text{G}_{\beta\gamma}$  in the presence of  $\text{Ca}_v\beta$  can be placed into four different case scenarios.

*Case 1: lack of  $\text{G}_{\beta\gamma}$  binding in the presence of  $\text{Ca}_v\beta$*  - There are clear-cut examples in the literature in which  $\text{G}_{\beta\gamma}$  regulation is ineffective on  $\text{Ca}_v2.3$  in the presence of a particular  $\text{Ca}_v\beta$  subunit isoform [43]. For instance, the co-expression of  $\text{Ca}_v\beta_{2a}$  with  $\text{Ca}_v2.3$  blocks the M2 receptor-induced current inhibition [19]. In this study, it was found that  $\text{G}_{\beta\gamma}$  and  $\text{Ca}_v\beta_{2a}$  are both able to bind separately on the Cs interaction point. Furthermore, binding of  $\text{Ca}_v\beta_{2a}$  to Cs occludes the subsequent interaction of  $\text{G}_{\beta\gamma}$  with Cs. As suggested earlier, it is tempting to propose that Cs represents an initial anchoring point for  $\text{G}_{\beta\gamma}$  that controls the efficient position of  $\text{G}_{\beta\gamma}$  for regulation.

*Cases 2 and 3: simultaneous binding of  $\text{G}_{\beta\gamma}$  and  $\text{Ca}_v\beta$* . The first evidence demonstrating that  $\text{G}_{\beta\gamma}$  and  $\text{Ca}_v\beta$  can bind simultaneously onto the channel comes from the fact the activation kinetics of  $\text{Ca}_v2.2$  under  $\text{G}_{\beta\gamma}$  inhibition are much slower when  $\text{Ca}_v\beta_{2a}$  subunit is used over any other  $\text{Ca}_v\beta$  [11]. The second evidence came from FRET experiments between  $\text{Ca}_v2.1$  and  $\text{Ca}_v\beta_{1b}$  that indicated a conformational change induced by  $\text{G}_{\beta}$  binding onto  $\text{Ca}_v2.1$  carboxyl-terminus interaction site (presumably Cs) [44]. All, these observations suggest that some



isoforms of  $G_{\beta}$  bind to the channel simultaneously with  $Ca_v\beta$ . The functional antagonism between  $Ca_v\beta$  and  $G_{\beta\gamma}$  might thus result from steric hindrance due to the presence of the  $Ca_v\beta$  on the III 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  $Ca_v\beta$  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 $Ca_v\beta$ dissociation*

One simple explanation for some of the G protein and  $Ca_v\beta$  opposite effects would be that  $G_{\beta\gamma}$  displaces the  $Ca_v\beta$  from its binding site on the channel, thereby reversing its regulatory properties (Fig 4b, "β dissociation" model).  $Ca_v\beta$  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  $III_{S2}$  would be able to destabilize the  $\alpha$ -helical structure and shifts AID from a high to a low affinity binding site for  $Ca_v\beta$  (binding antagonism). Though steric information is lacking, simultaneous binding of  $Ca_v\beta$  and  $G_{\beta\gamma}$  onto the I-II loop (required as the initiation step for  $Ca_v\beta$  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  $Ca_v\beta$  displacement by  $G_{\beta\gamma}$  has been observed experimentally [47]. Using a I-II loop/  $Ca_v\beta$  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 /  $Ca_v\beta$  interaction. This conclusion was also reached with a FRET approach using the full-length GFP-tagged  $Ca_v2.1$  channel and a chemically modified Cy3- $Ca_v\beta$ .  $Ca_v\beta$  dissociation may require specific conditions: 1) a single anchoring point for  $Ca_v\beta$  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  $Ca_v$  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  $Ca_v$ . 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  $Ca_v\beta$  association.

In a model in which  $Ca_v\beta$  dissociation would occur, an additional molecular event should take place upon termination of  $G_{\beta\gamma}$  inhibition. This step is linked to  $Ca_v\beta$  re-association to the

channel once its binding site has been freed from the competitive action of  $G_{\beta\gamma}$ . 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  $G_{\beta\gamma}$  interaction on  $Ca_v2$  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  $Ca_v\beta$  is not expected to vary, as opposed to the  $G_{\beta\gamma}$  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  $Ca_v\beta$  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  $G_{\beta\gamma}$ , and its essential binding determinants.** The crystal structure of  $G_{\beta\gamma}$  points to a complex of 80 x 50 x 45 Å thick (PDB accession 1TBG; [50]). CPK model of transducin  $G_{\beta\gamma}$  crystal structure [50] shown at two opposing faces (180° rotation). Considering its membrane attachment,  $G_{\beta\gamma}$  should be oriented with its longest axis perpendicular to the plasma membrane positioning the  $G_{\beta\gamma}$  at the periphery of  $Ca_v2$ .  $G_{\beta}$  is shown in blue, whereas  $G_{\gamma}$  is depicted in green. The  $G_{\beta\gamma}$  is oriented such that  $G_{\gamma}$  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  $G_{\beta}$  amino acid residues for the regulation of calcium channels (shown in red) [51-58]. Amino acid residues common to  $G_{\beta}$  subunits that bind to the I-II loop, but absent from  $G_{\beta}$  subunits that do not bind this loop include R19, S31, N35, P39, A193, R197 and A305 according to  $G_{\beta1}$  numbering. Some of these residues are within or near regions involved in the interaction with the  $G_{\gamma}$  subunit. The observation that  $G_{\beta}$  isoforms differ in their ability to interact with the III loop of  $Ca_v2.2$  is confirmed by a study demonstrating that the antagonistic effect of PKC phosphorylation of the I-II loop is only observed with  $G_{\beta1}$  [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  $G_{\beta\gamma}$  dimer shown here. Interestingly,  $G_{\alpha}$  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  $G_{\beta2}$  [65]. Figure created with RasMol v2.7.2.1 (Raswin Molecular Graphics, Berntein H. 1998-2001).

Figure 2 – **Important G protein determinants on  $Ca_v2$ -type channels.** Top diagram shows the characteristic topology of the pore-forming  $Ca_v$  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  $G_{\beta\gamma}$  determinants on  $Ca_v$  cytoplasmic domains (Ns, QXXER, GID and Cs; see text) are reported in red and detailed in the three lower panels, amino-terminal, III loop and carboxyl-terminal regions. The Ns determinant has been described on  $Ca_v2.3$  [31] and on  $Ca_v2.2$  [31,32] and identified on the basis of functional evidence only. An equivalent sequence is found on  $Ca_v3.2$  but on the I-II

loop [66]. Two binding domains, QXXER and GID, have been identified in the I-II loop using either peptides (peptides I-II<sub>S1</sub> to I-II<sub>S3</sub> [17] and peptide PL1 [21], illustrated by underlined sequences), mutagenesis [15] or binding experiment [19].

GID forms part of the III<sub>S3</sub> sequence [67], whereas PL1 is part of both III<sub>S1</sub> and III<sub>S2</sub> [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 Ca<sub>v</sub>β. Brown refers to the EF hand, preIQ, IQ and CBD domains [73,74] which constitute the carboxyl-terminal Ca<sup>2+</sup> binding domains. G<sub>βq</sub> [20] and G<sub>βo</sub> [68] binding sites are reported in dark purple. Lower panels show sequence alignments for Ca<sub>v</sub>2.1 (SwissProt accession number: P54282, rat), Ca<sub>v</sub>2.2 (Q02294, rat), Ca<sub>v</sub>2.3 (Q15878, human) for non L-type channels, and Ca<sub>v</sub>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 **a1**: an inhibition of the current amplitude (ranging from 15 to 80% depending on the Ca<sub>v</sub>/GPCR combination) which is far more pronounced at the start of the depolarization than at the end of the pulse, **a2**: a slowing of the kinetics of current activation between 10 to 100 ms, **a3**: a depolarizing shift of the voltage-dependence of channel activation which has been described in terms of channel modes, and **a4**: 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<sub>βγ</sub> inhibition (relative facilitation). Current traces and curves were obtained from various Ca<sub>v</sub>2.2/Ca<sub>v</sub>β combinations expressed in *Xenopus* oocytes (all data obtained with the combination Ca<sub>v</sub>2.2/β<sub>3</sub> except panel 2 traces obtained from Ca<sub>v</sub>2.2/β<sub>2a</sub>).

Figure 4 – **Willing and reluctant modes of Ca<sub>v</sub>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<sub>βγ</sub> (state-induced, β dissociation and G<sub>βγ</sub> dissociation). a) State-induced model. This model assumes that willing and reluctant modes are intrinsic modes of Ca<sub>v</sub>2. Alteration of voltage-detection induced by G<sub>βγ</sub> binding preferentially shifts the equilibrium between willing and

reluctant modes towards the reluctant mode. In favor of this model, a mutation of Ca<sub>v</sub>2.2 (G177E) in the IS3 segment can shift the channel in a reluctant mode without the contribution of G<sub>βγ</sub> [76]. The state-induced model implicates that simultaneous binding of G<sub>βγ</sub> and Ca<sub>v</sub>β is possible. **b) Ca<sub>v</sub>β dissociation model.** Binding of Ca<sub>v</sub>β to the Ca<sub>v</sub>2 channel induces a shift from a reluctant state to a willing state [77]. The binding of G<sub>βγ</sub> physically displaces the Ca<sub>v</sub>β from its binding site and simultaneously imposes the reluctant mode of the channel. In this model, the converse displacement of G<sub>βγ</sub> by the Ca<sub>v</sub>β is not likely to occur because G<sub>βγ</sub> occupies multiple binding sites on Ca<sub>v</sub>2 and the Ca<sub>v</sub>β concentration is not supposed to vary. Signal termination occurs with the departure of G<sub>βγ</sub> without modal shift. Re-association of the Ca<sub>v</sub>β and induction of the willing mode will only occur after G<sub>βγ</sub> departure. The Ca<sub>v</sub>β dissociation model implicates that simultaneous binding of G<sub>βγ</sub> and Ca<sub>v</sub>β is not possible. Evidence for this model was provided for the Ca<sub>v</sub>2.1 channel [47]. **c) G<sub>βγ</sub> 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<sub>βγ</sub> from the channel complex (Ca<sub>v</sub>2/G<sub>βγ</sub>/β). 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 Ca<sub>v</sub>2/β/G<sub>βγ</sub> and Ca<sub>v</sub>2/β complexes. Small depolarization favors the undissociated state, whereas strong depolarization favors the dissociated state. G<sub>βγ</sub> departure induces an increased Ca<sup>2+</sup> entry, probably by an increase in opening probability. The inhibitory effect of G<sub>βγ</sub> becomes voltage-dependent as illustrated by the bottom current traces. Panel 3: two relationships were drawn, one in which G<sub>βγ</sub> does not dissociate from the channel (grey line) and another one in which increased dissociation of G<sub>βγ</sub> from the channel occurs with increased membrane depolarization (red line). Panel 4: I-V relationships before (control) and after G<sub>βγ</sub>-binding in two conditions (without and with a voltage-dependent G<sub>βγ</sub> dissociation during pulse application). In the case where G<sub>βγ</sub> does not dissociate a constant 50% inhibition of the current occurs at all potentials (grey line). With a voltage-dependent G<sub>βγ</sub> 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<sub>βγ</sub> 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  $Ca_v\beta$  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.

## References

- 1 Wheeler, D.B. *et al.* (1994) Roles of N-type and Q-type  $Ca^{2+}$  channels in supporting hippocampal synaptic transmission. *Science* 264, 107-111
- 2 Takahashi, T. *et al.* (1996) Presynaptic calcium current modulation by a metabotropic glutamate receptor. *Science* 274, 594-597
- 3 Dietrich, D. *et al.* (2003) Functional specialization of presynaptic  $Ca_v2.3$   $Ca^{2+}$  channels. *Neuron* 39, 483-496
- 4 Hille, B. (1994) Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci* 17, 531-536
- 5 Herlitze, S. *et al.* (1996) Modulation of  $Ca^{2+}$  channels by G-protein  $\beta\gamma$  subunits. *Nature* 380, 258-262
- 6 Ikeda, S.R. (1996) Voltage-dependent modulation of N-type calcium channels by G-protein  $\beta\gamma$  subunits. *Nature* 380, 255-258
- 7 Roche, J.P. *et al.* (1995) Abolition of G protein inhibition of  $\alpha_{1A}$  and  $\alpha_{1B}$  calcium channels by co-expression of the  $\beta_3$  subunit. *FEBS Lett* 371, 43-46
- 8 Bourinet, E. *et al.* (1996) Determinants of the G protein-dependent opioid modulation of neuronal calcium channels. *Proc Natl Acad Sci USA* 93, 1486-1491
- 9 Herlitze, S. *et al.* (2001) Allosteric modulation of  $Ca^{2+}$  channels by G proteins, voltage-dependent facilitation, protein kinase C, and  $Ca_v\beta$  subunits. *Proc Natl Acad Sci USA* 98, 4699-4704
- 10 Kammermeier, P.J. *et al.* (2000) A voltage-independent calcium current inhibitory pathway activated by muscarinic agonists in rat sympathetic neurons requires both  $G\alpha_q/11$  and  $G\beta\gamma$ . *J Neurosci* 20, 5623-5629
- 11 Feng, Z.P. *et al.* (2001) Calcium channel  $\beta$  subunits differentially regulate the inhibition of N-type channels by individual  $G\beta$  isoforms. *J Biol Chem* 276, 45051-45058
- 12 Zhou, J.Y. *et al.* (2000) Selective regulation of N-type Ca channels by different combinations of G-protein beta/gamma subunits and RGS proteins. *J Neurosci* 20, 7143-7148
- 13 Delmas, P. *et al.* (1998) On the role of endogenous G-protein  $\beta\gamma$  subunits in N-type  $Ca^{2+}$  current inhibition by neurotransmitters in rat sympathetic neurones. *J Physiol* 506, 319-329
- 14 Ruiz-Velasco, V. and Ikeda, S.R. (2000) Multiple G-protein  $\beta\gamma$  combinations produce voltage-dependent inhibition of N-type calcium channels in rat superior cervical ganglion neurons. *J Neurosci* 20, 2183-2191
- 15 De Waard, M. *et al.* (1997) Direct binding of G-protein  $\beta\gamma$  complex to voltage-dependent calcium channels. *Nature* 385, 446-450

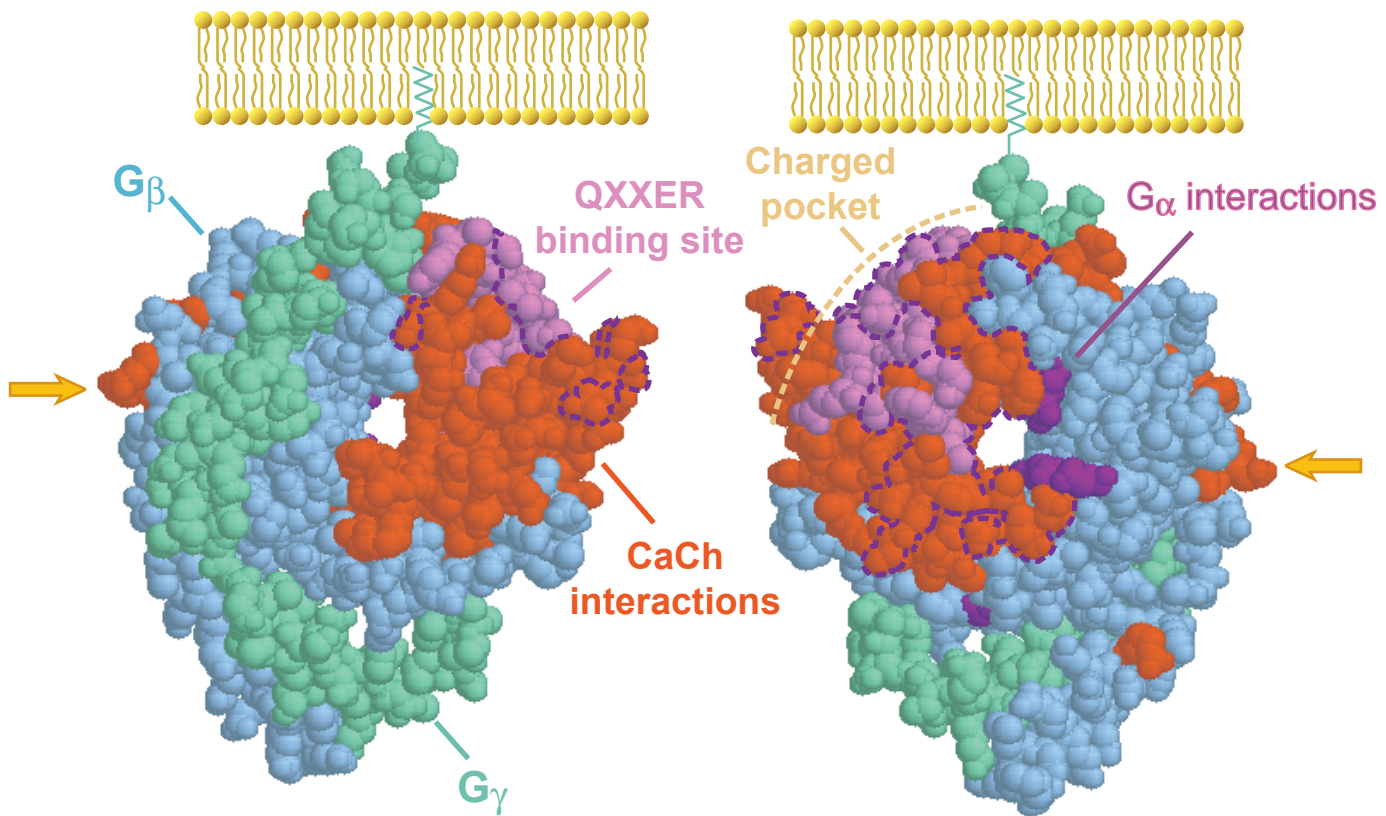
- 16 Herlitze, S. *et al.* (1997) Molecular determinants of inactivation and G protein modulation in the intracellular loop connecting domains I and II of the calcium channel  $\alpha_{1A}$  subunit. *Proc Natl Acad Sci USA* 94, 1512-1516
- 17 Zamponi, G.W. *et al.* (1997) Crosstalk between G proteins and protein kinase C mediated by the calcium channel  $\alpha_1$  subunit. *Nature* 385, 442-446
- 18 De Waard, M. and Campbell, K.P. (1995) Subunit regulation of the neuronal  $\alpha_{1A}$   $Ca^{2+}$  channel expressed in *Xenopus* oocytes. *J Physiol* 485, 619-634
- 19 Qin, N. *et al.* (1997) Direct interaction of  $G\beta\gamma$  with a C-terminal  $G\beta\gamma$ -binding domain of the  $Ca^{2+}$  channel  $\alpha_1$  subunit is responsible for channel inhibition by G protein-coupled receptors. *Proc Natl Acad Sci USA* 94, 8866-8871
- 20 Simen, A.A. *et al.* (2001) The C terminus of the Ca channel  $\alpha_{1B}$  subunit mediates selective inhibition by G-protein-coupled receptors. *J Neurosci* 21, 7587-7597
- 21 Furukawa, T. *et al.* (1998) Differential interactions of the C terminus and the cytoplasmic I-II loop of neuronal  $Ca^{2+}$  channels with G-protein  $\alpha$  and  $\beta\gamma$  subunits. I. Molecular determination. *J Biol Chem* 273, 17585-17594
- 22 Kitano, J. *et al.* (2003) Direct interaction and functional coupling between metabotropic glutamate receptor subtype 1 and voltage-sensitive  $Ca_v2.1$   $Ca^{2+}$  channel. *J Biol Chem* 278, 25101-25108
- 23 Beedle, A.M. *et al.* (2004) Agonist-independent modulation of N-type calcium channels by ORL1 receptors. *Nat Neurosci* 7, 118-125
- 24 Restituto, S. *et al.* (2000) The  $\beta_{2a}$  subunit is a molecular groom for the  $Ca^{2+}$  channel inactivation gate. *J Neurosci* 20, 9046-9052
- 25 Geib, S. *et al.* (2002) The interaction between the FII loop and the III-IV loop of  $Ca_v2.1$  contributes to voltage-dependent inactivation in a  $\beta$ -dependent manner. *J Biol Chem* 277, 10003-10013
- 26 Zamponi, G.W. and Snutch, T.P. (1998) Decay of prepulse facilitation of N type calcium channels during G protein inhibition is consistent with binding of a single  $G\beta$  subunit. *Proc Natl Acad Sci USA* 95, 4035-4039
- 27 Zhou, J. *et al.* (1997) Speed of  $Ca^{2+}$  channel modulation by neurotransmitters in rat sympathetic neurons. *J Neurophysiol* 77, 2040-2048
- 28 Jones, L.P. *et al.* (1997) G-protein modulation of N-type calcium channel gating current in human embryonic kidney cells (HEK 293). *J Physiol* 498, 601-610
- 29 Ikeda, S.R. (1991) Double-pulse calcium channel current facilitation in adult rat sympathetic neurones. *J Physiol* 439, 181-214
- 30 Ikeda, S.R. and Dunlap, K. (1999) Voltage-dependent modulation of N-type calcium channels: role of G protein subunits. *Adv Second Messenger Phosphoprotein Res* 33, 131-151
- 31 Page, K.M. *et al.* (1998) Identification of the amino terminus of neuronal  $Ca^{2+}$  channel alpha1 subunits  $\alpha_{1B}$  and  $\alpha_{1E}$  as an essential determinant of G-protein modulation. *J Neurosci* 18, 4815-4824
- 32 Canti, C. *et al.* (1999) Identification of residues in the N terminus of alpha1B critical for inhibition of the voltage-dependent calcium channel by  $G\beta\gamma$ . *J Neurosci* 19, 6855-6864
- 33 Hamid, J. *et al.* (1999) Identification of an integration center for cross-talk between protein kinase C and G protein modulation of N-type calcium channels. *J Biol Chem* 274, 6195-6202
- 34 Zhang, J.F. *et al.* (1996) Multiple structural elements in voltage-dependent  $Ca^{2+}$  channels support their inhibition by G proteins. *Neuron* 17, 991-1003

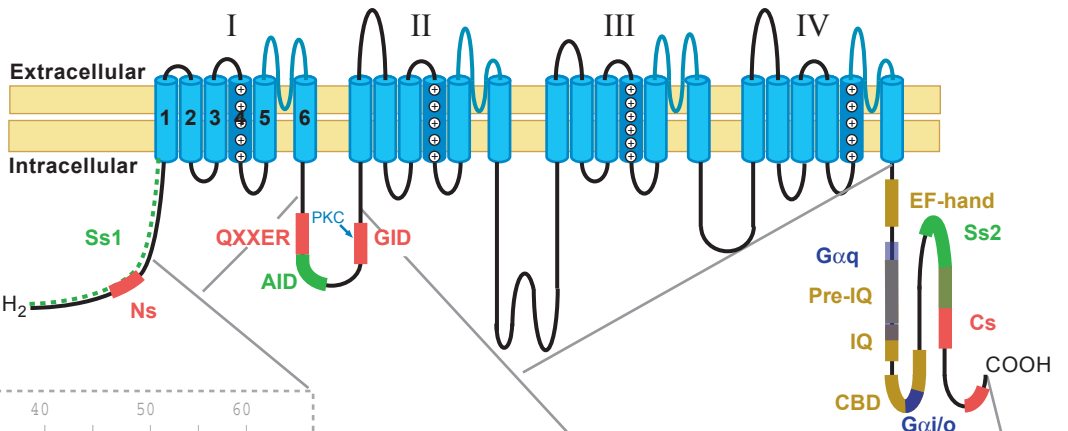


- 35 Simen, A.A. and Miller, R.J. (1998) Structural features determining differential receptor regulation of neuronal Ca channels. *J Neurosci* 18, 3689-3698
- 36 Simen, A.A. and Miller, R.J. (2000) Involvement of regions in domain I in the opioid receptor sensitivity of  $\alpha_{1B}$  Ca<sup>2+</sup> channels. *Mol Pharmacol* 57, 1064-1074
- 37 Sandoz, G. *et al.* (2004) Repositioning of charged I-II loop amino acid residues within the electric field by  $\beta$  subunit as a novel working hypothesis for the control of fast P/Q calcium channel inactivation. *Eur J Neurosci* 19, 1759-1772
- 38 Sheng, Z.H. *et al.* (1994) Identification of a syntaxin-binding site on N-type calcium channels. *Neuron* 13, 1303-1313
- 39 Jarvis, S.E. *et al.* (2000) G protein modulation of N-type calcium channels is facilitated by physical interactions between syntaxin 1A and  $G\beta\gamma$ . *J Biol Chem* 275, 6388-6394
- 40 Richards, M.W. *et al.* (2004) Ca<sup>2+</sup> channel beta-subunits: structural insights AID our understanding. *Trends Pharmacol Sci* 25, 626-632
- 41 Neely, A. *et al.* (1993) Potentiation by the beta subunit of the ratio of the ionic current to the charge movement in the cardiac calcium channel. *Science* 262, 575-578
- 42 Olcese, R. *et al.* (1996) Coupling between charge movement and pore opening in vertebrate neuronal  $\alpha_{1E}$  calcium channels. *J Physiol* 497, 675-686
- 43 Toth, P.T. *et al.* (1996) Selective G-protein regulation of neuronal calcium channels. *J Neurosci* 16, 4617-4624
- 44 Hummer, A. *et al.* (2003) Competitive and synergistic interactions of G protein  $\beta_2$  and Ca<sup>2+</sup> channel  $\beta_{1b}$  subunits with Ca<sub>v</sub>2.1 channels, revealed by mammalian two-hybrid and fluorescence resonance energy transfer measurements. *J Biol Chem* 278, 49386-49400
- 45 Opatowsky, Y. *et al.* (2004) Expression, purification and crystallization of a functional core of the voltage-dependent calcium channel  $\beta$  subunit. *Acta Crystallogr D Biol Crystallogr* 60, 1301-1303
- 46 Van Petegem, F. *et al.* (2004) Structure of a complex between a voltage-gated calcium channel beta-subunit and an alpha-subunit domain. *Nature* 429, 671-675
- 47 Sandoz, G. *et al.* (2004) Cav $\beta$ -subunit displacement is a key step to induce the reluctant state of P/Q calcium channels by direct G protein regulation. *Proc Natl Acad Sci USA* 101, 6267-6272
- 48 Fathallah, M. *et al.* (2002) Modelling of the III-IV loop, a domain involved in calcium channel Cav2.1 inactivation, highlights a structural homology with the gamma subunit of G proteins. *Eur J Neurosci* 16, 219-228
- 49 Kasai, H. and Aosaki, T. (1989) Modulation of Ca-channel current by an adenosine analog mediated by a GTP-binding protein in chick sensory neurons. *Pflugers Arch* 414, 145-149
- 50 Sondek, J. *et al.* (1996) Crystal structure of a G-protein beta gamma dimer at 2.1Å resolution. *Nature* 379, 369-374
- 51 Agler, H.L. *et al.* (2003) Custom distinctions in the interaction of G-protein beta subunits with N-type (CaV2.2) versus P/Q-type (CaV2.1) calcium channels. *J Gen Physiol* 121, 495-510
- 52 Cooper, C.B. *et al.* (2000) Cross-talk between G-protein and protein kinase C modulation of N-type calcium channels is dependent on the G-protein beta subunit isoform. *J Biol Chem* 275, 40777-40781
- 53 Doering, C.J. *et al.* (2004) A single G $\beta$  subunit locus controls cross-talk between protein kinase C and G protein regulation of N-type calcium channels. *J Biol Chem* 279, 29709-29717

- 54 Ford, C.E. *et al.* (1998) Molecular basis for interactions of G protein betagamma subunits with effectors. *Science* 280, 1271-1274
- 55 Garcia, D.E. *et al.* (1998) Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. *J Neurosci* 18, 2834-2841
- 56 Mirshahi, T. *et al.* (2002) Distinct sites on G protein beta gamma subunits regulate different effector functions. *J Biol Chem* 277, 36345-36350
- 57 Mirshahi, T. *et al.* (2002) G $\beta$  residues that do not interact with G $\alpha$  underlie agonist-independent activity of K<sup>+</sup> channels. *J Biol Chem* 277, 7348-7355
- 58 Wall, M.A. *et al.* (1998) Structural basis of activity and subunit recognition in G protein heterotrimers. *Structure* 6, 1169-1183
- 59 Weng, G. *et al.* (1996) G $\beta$  subunit interacts with a peptide encoding region 956-982 of adenylyl cyclase 2. Cross-linking of the peptide to free G $\beta\gamma$  but not the heterotrimer. *J Biol Chem* 271, 26445-26448
- 60 Yan, K. and Gautam, N. (1996) A domain on the G protein beta subunit interacts with both adenylyl cyclase 2 and the muscarinic atrial potassium channel. *J Biol Chem* 271, 17597-17600
- 61 Albsoul-Younes, A.M. *et al.* (2001) Interaction sites of the G protein beta subunit with brain G protein-coupled inward rectifier K<sup>+</sup> channel. *J Biol Chem* 276, 12712-12717
- 62 Lambright, D.G. *et al.* (1996) The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature* 379, 311-319
- 63 Li, Y. *et al.* (1998) Sites for G $\alpha$  binding on the G protein beta subunit overlap with sites for regulation of phospholipase C $\beta$  and adenylyl cyclase. *J Biol Chem* 273, 16265-16272
- 64 Zhao, Q. *et al.* (2003) Interaction of G protein beta subunit with inward rectifier K<sup>+</sup> channel Kir3. *Mol Pharmacol* 64, 1085-1091
- 65 Li, X. *et al.* (2005) G protein beta (sub2) subunit derived peptides for inhibition and induction of G protein pathways: Examination on voltage gated Ca<sup>2+</sup> and G protein inward rectifying K(sup+) channels. *J Biol Chem*
- 66 Wolfe, J.T. *et al.* (2003) T-type calcium channel regulation by specific Gprotein betagamma subunits. *Nature* 424, 209-213
- 67 Jarvis, S.E. and Zamponi, G.W. (2001) Interactions between presynaptic Ca<sup>2+</sup> channels, cytoplasmic messengers and proteins of the synaptic vesicle release complex. *Trends Pharmacol Sci* 22, 519-525
- 68 Furukawa, T. *et al.* (1998) Differential interactions of the C terminus and the cytoplasmic I-II loop of neuronal Ca<sup>2+</sup> channels with G-protein alpha and beta gamma subunits. II. Evidence for direct binding. *J Biol Chem* 273, 17595-17603
- 69 Pragnell, M. *et al.* (1994) Calcium channel beta-subunit binds to a conserved motif in the I-II cytoplasmic linker of the alpha 1-subunit. *Nature* 368, 67-70
- 70 Walker, D. *et al.* (1999) A new beta subtype-specific interaction in  $\alpha_{1A}$  subunit controls P/Q-type Ca<sup>2+</sup> channel activation. *J Biol Chem* 274, 12383-12390
- 71 Stephens, G.J. *et al.* (2000) The  $\alpha_{1B}$  Ca<sup>2+</sup> channel amino terminus contributes determinants for beta subunit-mediated voltage-dependent inactivation properties. *J Physiol* 525, 377-390
- 72 Walker, D. *et al.* (1998) A  $\beta_4$  isoform-specific interaction site in the carboxyl-terminal region of the voltage-dependent Ca<sup>2+</sup> channel  $\alpha_{1A}$  subunit. *J Biol Chem* 273, 2361-2367
- 73 Kim, J. *et al.* (2004) Identification of the components controlling inactivation of voltage-gated Ca<sup>2+</sup> channels. *Neuron* 41, 745-754
- 74 Liang, H. *et al.* (2003) Unified mechanisms of Ca<sup>2+</sup> regulation across the Ca<sup>2+</sup> channel family. *Neuron* 39, 951-960

- 75** Bean, B.P. (1989) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* 340, 153-156
- 76** Zhong, H. *et al.* (2001) Control of gating mode by a single amino acid residue in transmembrane segment IS3 of the N-type Ca<sup>2+</sup> channel. *Proc Natl Acad Sci USA* 98, 4705-4709
- 77** Walker, D. and De Waard, M. (1998) Subunit interaction sites in voltage-dependent Ca<sup>2+</sup> channels: role in channel function. *Trends Neurosci* 21, 148-154
- 78** Canti, C. *et al.* (2000) Interaction between G proteins and accessory subunits in the regulation of  $\alpha_{1B}$  calcium channels in *Xenopus* oocytes. *J Physiol* 527, 419-432





10 20 30 40 50 60

Ca<sub>v</sub>2.1 MARFGDEMPGRYGAGGGSSGPAAGVVVGAAGGRRGAGGSRQGGPGAQRM-YKQSMQARRTMAYL

Ca<sub>v</sub>2.2 MVRFGDELGGRYG-GTGG-G~-ERARG-GGAGGAGGPGQGLPPGQRVLYKQSIQAQRRTMAYL

Ca<sub>v</sub>2.3 MARFGBAVVAR~-PGSGD-GD-SDQSR~-NR~-QGTVPVPSGQAAAAYKQTKAQRRTMAYL

Ss1 Ns

370 380 390 400 410 420 430 440

Ca<sub>v</sub>2.1 GEFAKERERVENRRAPFLKLRQQQTERELNGYMEWISKAEVILAEDETDVEQRHPFDGALRRATLTKSKTDLLNPEEA

Ca<sub>v</sub>2.2 GEFAKERERVENRRAPFLKLRQQQTERELNGYLEWIFKAEVMLAEEDKNAEIKSPLDAVLKRAATKKSRLDIIHAEEG

Ca<sub>v</sub>2.3 GEFAKERERVENRRAPFLKLRQQQTERELNGYRAWIDKAEVMLAEENKNAGT-SALE-VLRRATIKRSRTEAMTRDSS

Ca<sub>v</sub>1.2 GEFKSKEREKAKARGDFQKLRKQQLLEDLKGYLEWITQAEIDIDPENEDGMDKPRNMSMPTSETESVNTENVAGGDI

peptide I-II<sub>s1</sub> I-II<sub>s2</sub> I-II<sub>s3</sub>

PL1

QXXER AID GID PKC

1770 1780 1790 1800 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910

Ca<sub>v</sub>2.1 DNFEYLTRDSSILGPHHLDEYVRVWAAYDPAACGRIHXYDMYSLLRVISPGLGKKCPHRVACKRLLRMDLPVA-DDNTVHFNSTLMALIRTALDIKIAKGGADQOMDAELRKMMAIWPNLQKTLDDLVTPEKSTDLTVGKIYAAM

Ca<sub>v</sub>2.2 DNFEYLTRDSSILGPHHLDEFIRVWAAYDPAACGRISYNDMFEMLKHMSPPLGLGKKCHARVAYKRLVRMNPISNEDMTVHFTSTLMALIRTALEIKLAPAGTKQHQCDABLRKEISSVWANLPQKTLDDLVPPEHKPDDEMTVGKIVYAAL

Ca<sub>v</sub>2.3 DNFEYLTRDSSILGPHHLDEFVRVWAAYDRAACGRIHYTEMYEMLTLMSPPLGLGKRCPSKVAYKRLVLMNMPVA-EDMTVHFTSTLMALIRTALDIKIAKGGADRQQLDSELQKETLAIWPHLSQKMLDILLVPMFKASDLTVGKIYAAM

EF-hand Gαq Pre-IQ

1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040 IQ

Ca<sub>v</sub>2.1 MIMFYRQSKAKKLOAMREE~-QNRTPLMFQRMPEPSPQEGGSPQNALP~-S-TQLDPGGGLMAQESSMKESPSVVTQRAQEMFQKTGTWSPERGPIDMPNSQPNQSQSVEMREMGTGYSDSSEHYLPMEGQTRAASMPRLP

Ca<sub>v</sub>2.2 MIFDFYKQNKTTTRDOTHQAPGGLSQMGVPSLFHPLKATLEQTPAVLRGARVFLRQKSATSLNNGGAIQTQESGIKESLSWGTQRTQDVLVEARA-PLERGHSAELPVQPGALAVDVQMQNMTLRGP-DGEPQPGLESQGRAASMPRLA

Ca<sub>v</sub>2.3 MIMFYRQSKAKKQKORQCLEE~-QKNAP-MFQRMPEPSSLPQEI IANAKALPYLQDDPVVSGLS~-GRSGYPSMSPSPQDIFQLACMDPTDDGQFQER-QSLE-P~-EVSELKSVQPSNHGIYLPDSD~-TQEHAGSGRASSMPRLT

Gai/o CBD

2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160 2170 2180

Ca<sub>v</sub>2.1 AENQRRRGRPRGNLSTISDTPMKRSASVLPKARLDDY-S-L-ERVPEENQ-RYH~-Q-RRDRGHRTSERSLGRVTDVDTGLGTDLSMTIQSGD-LPS~-KDRDQDRGPKDRKHR-PHHHHHHHHHPAP~-DRERYAQ

Ca<sub>v</sub>2.2 AETQ~-PAPNASPMKRSISTLAPRPHGTQLCNTVL-DRPPPSOVS-HHHHHRCHRRRDKORSLEK~-GPSLSVDTEGAPSTAAGSLPHGEGSTGCR~-RERKQERGRSQERRQPSSSSEKQRFYSCDRFGSREPPQP

Ca<sub>v</sub>2.3 VDPQ~-VVTDPSMRRSFSTIRDKRSNSWLEEFMSMERSS~-ENTYKS~-RRR~-SYHSSLRLSAHRLNSDGHKSDT~-HRSGGREGRSKERKH~-LLS~-PDSVRCNSEERGTQADWES

Ss2 Cs

2190 2200 2210

Ca<sub>v</sub>2.1 ~ERPDTGRARAREQRWSRSPSEGREHAT~-HRQ

Ca<sub>v</sub>2.2 KPSLSSHPI SPTAALEPGPHQGGSGSVNGSLPMTSTGASTPGRGRRQLPQTPLTPRPSITYKTANSSVPHFAEQSGLPAPFSPG~-RLSRG-LSEHNALLQKEPLSQPL~-ASGSRIGSDPYLQRLDSESAHNLPEDTLTFEEA

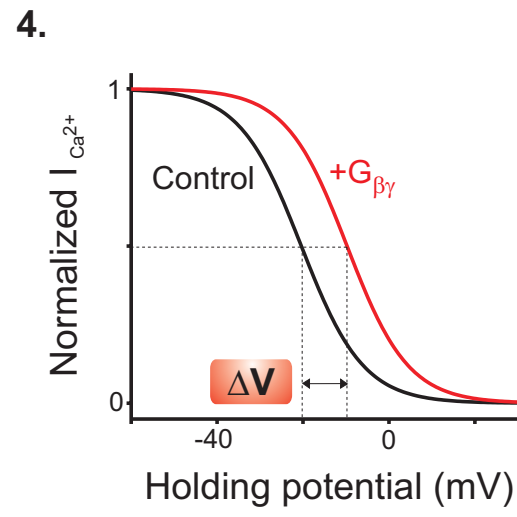
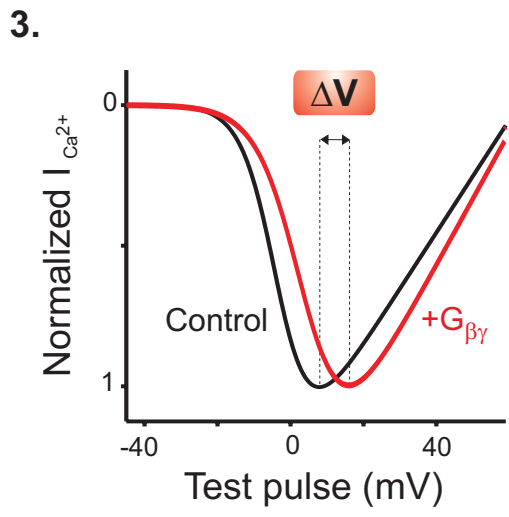
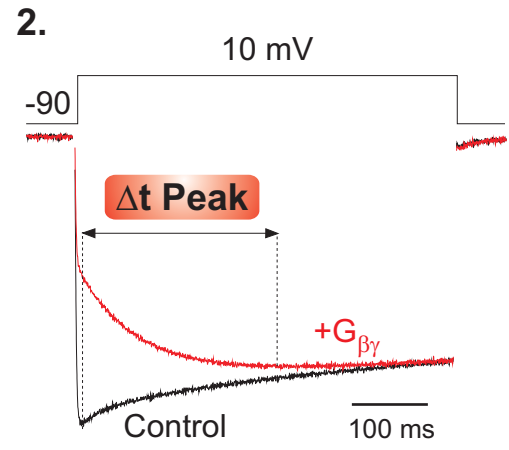
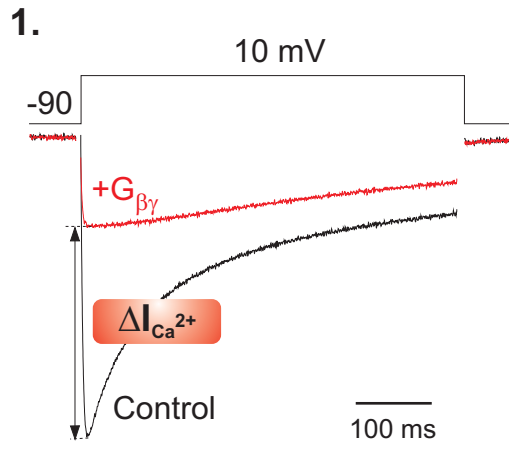
Ca<sub>v</sub>2.3 ~PERRQ-SRSPSEGRSQTENRQCTGSLSESIIPVSDTSTP-RRSRQPLPVPPKPRPLLSY~-SSLIRHAGSISPPADGSEEGSLPTSQALENNACTIETSSNSPHQSQSHASQORYISEPYLALHEDSHASDCG-EETLTFEAA

VATNSGRSSRTSYVSSL-TSQSHPI RRVPNGYHCTLGLSTGVRARHSHYHPDQ-DHWC

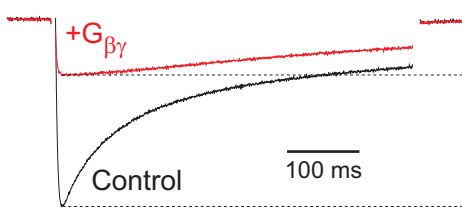
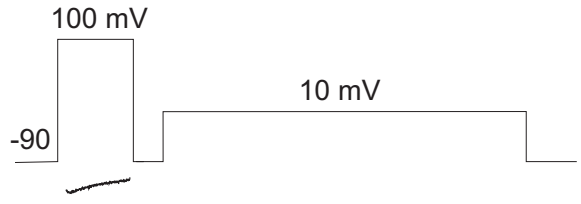
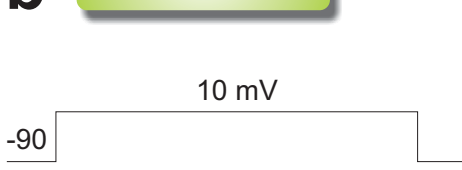
VATSLGRSNTIGSAPPLRHSWQMPNGHYRRRRRGGPGGMCGAVNNLLSDTEEDDKC

Cs

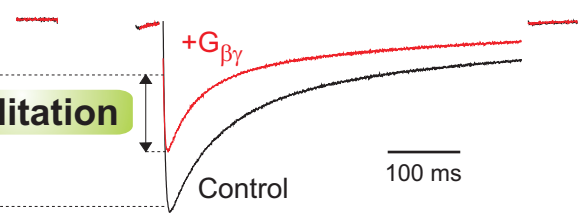
**a** "On"-effects



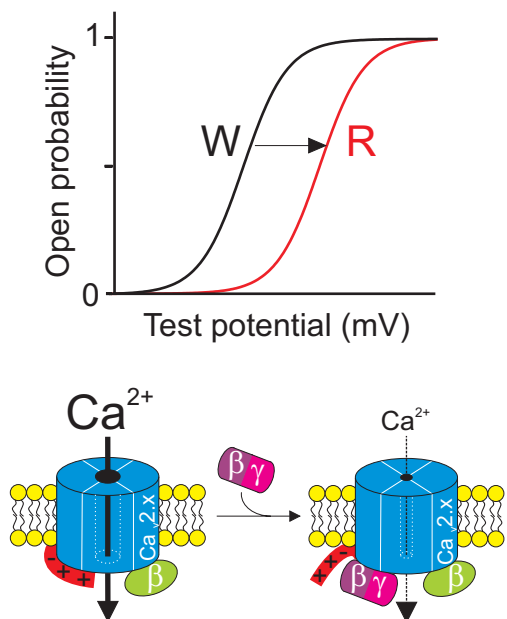
**b** "Off"-effects



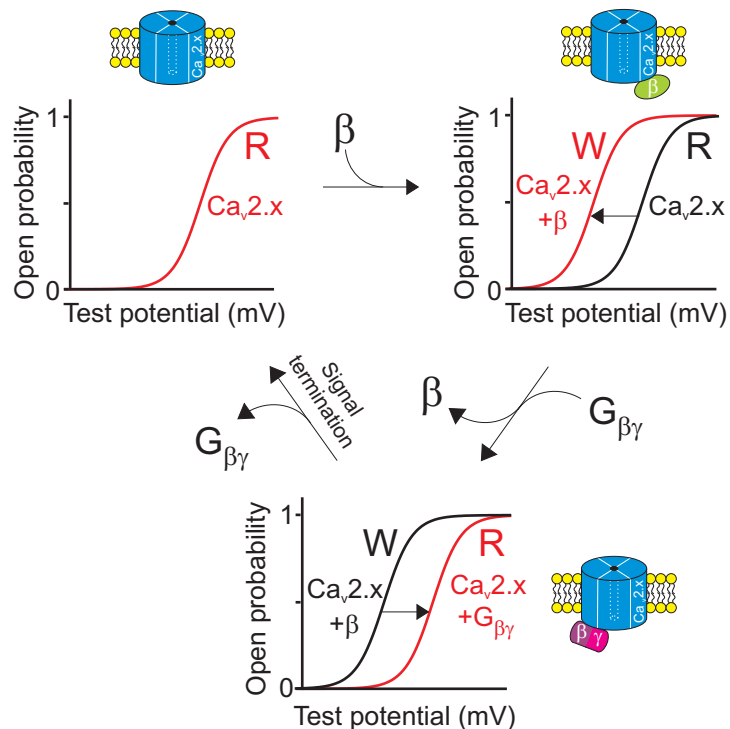
**Facilitation**



**a** State-induced model



**b** β subunit dissociation model



**c**

G<sub>βγ</sub> dissociation model

