The modulation of N-type calcium channels is a key factor in the control of neurotransmitter release. Whereas N-type channels are inhibited by Gβγ subunits in a G protein β-isofrom-dependent manner, channel activity is typically stimulated by activation of protein kinase C (PKC). In addition, there is cross-talk among these pathways, such that PKC-dependent phosphorylation of the Gβγ target site on the N-type channel antagonizes subsequent G protein inhibition, albeit only for Gβγ-mediated responses. The molecular mechanisms that control this G protein β subunit subtype-specific regulation have not been described. Here, we show that G protein inhibition of N-type calcium channels is critically dependent on two separate but adjacent amino acid regions of the Gβγ subunit, plus a highly conserved Asn-Tyr-Val motif. These regions are distinct from previously identified previously in Gβγ signaling to other effectors such as G protein-coupled inward rectifier potassium channels, phospholipase β2, and adenylyl cyclase, thus raising the possibility that the specificity for G protein signaling to calcium channels might rely on unique G protein structural determinants. In addition, we identify a highly specific locus on the Gβγ subunit that serves as a molecular detector of PKC-dependent phosphorylation of the G protein target site on the N-type channel α1 subunit, thus providing for a molecular basis for G protein-PKC cross-talk. Overall, our results significantly advance our understanding of the molecular details underlying the integration of G protein and PKC signaling pathways at the level of the N-type calcium channel α1 subunit.

The modulation of N-type calcium channels at presynaptic nerve terminals is a key factor in regulating synaptic efficacy (1, 2). It is now well established that the activation of G protein-coupled receptors inhibits presynaptic calcium channel activity and thus neurotransmitter release (3). G protein inhibition of both N-type and P/Q-type calcium channels appears to be exclusively mediated by the G protein βγ subunit (4, 5), with the Gβ subunit being the main determinant of calcium channel inhibition. Putative G protein βγ subunit interaction sites have been identified within the intracellular loop linking domains I and II of the calcium channel α1 subunit (6–8), as well as in the C-terminal region (9). To date, five different types of G protein β subunits have been identified and shown to mediate varying effects on native and transiently expressed N-type calcium channels (10, 11). Moreover, N-type and P/Q-type calcium channels appear to be differentially modulated by different types of G protein β subunits (12), thus providing for a mechanism by which different G protein-coupled receptors may selectively regulate individual presynaptic calcium channel subtypes.

In contrast, activation of protein kinase C (PKC) results in an up-regulation of N-type channel activity (13, 14). There is a complex interplay between PKC and G protein pathways such that activation of PKC antagonizes subsequent receptor-mediated G protein inhibition of presynaptic calcium channels (15, 16). This effect is mediated via PKC-dependent phosphorylation of a single threonine residue located in the G protein interaction site within the domain I-II linker region of the N-type calcium channel α1 subunit (17), thus allowing the channel protein to integrate multiple modulatory inputs. Interestingly, this cross-talk between G protein and PKC pathways appears to be a selective feature of the G protein βγ subunit, thus allowing PKC to selectively antagonize G protein inhibition mediated by a subset of (i.e. predominantly Gβγ-coupled) receptors (18). However, although calcium channel structural determinants of G protein regulation and PKC cross-talk have received considerable attention (3), there is relatively scant information that concerns the G protein structural determinants that underlie N-type channel regulation and PKC-G protein cross-talk. Alanine mutagenesis of Gβγ residues known to interact with Ga disrupts Gβγ coupling to a series of downstream effectors, including calcium channels, adenylyl cyclase, GIRK channels, and phospholipase β2 (19, 20). This suggests that there may be partial overlap in the G protein β subunit structural determinants that control the functional interactions both within the heterotrimeric G protein complex and with downstream signaling targets. Ford et al. (19) identified six amino acid residues (Lys-78, Met-101, Asn-119, Thr-143,
were excised from the pMT2-XS vector using previously engineered

gelases. However, these residues are completely conserved across
types of G protein β subunit subtypes, which implies that they
cannot account for the differential effects of different types of
Gβ subunits on calcium channel activity. Hence, additional
Gβ structural determinants control G protein modulation of
N-type channels.

Here, we utilized chimeric and mutant G protein β subunits to
systematically identify G protein structural determinants that
control their action on N-type channels. We identify a hot
spot of amino acid sequences in the Gβ subunit that is essen-
tial for N-type channel modulation. In addition, we localize
cross-talk behavior to a single locus on the Gβ subunit, thus
identifying a molecular switch that allows the G proteins to
detect a phosphorylated N-type calcium channel. In this con-
text, our data close a major gap in our understanding of the
complex interplay between G protein and PKC regulation of
presynaptic calcium channel activity.

MATERIALS AND METHODS
cDNAs—The cDNAs encoding human Gβ1 and Gγ, rat Gβ2, and
EGFP-tagged Gβ1 and Gβ2, subunits were described by us previously
(12, 21). Wild type rat calcium channel subunits were donated by Terry
Snutch (University of British Columbia), and the T422E mutant N-type
channel was described previously (17).

Chimeric Constructs—Chimeras were created in two steps. For
an initial round of chimeras, MuLu, ApaLI, and Eagl sites were inserted
into both Gβ1 and Gβ2, encoding cDNAs at exactly complimentary
positions, using the QuikChange™ site-directed mutagenesis kit (Strat-
agene, La Jolla, CA). In each case, the entire coding region was se-
quenced after mutagenesis. Together with the 5′ and 3′ cloning sites
(KpnI and XhoI, respectively) and the presence of additional ApaLI and
Eagl sites in the pMT2 vector sequence, this allowed the swapping of
four different regions (KpnI and XhoI, respectively) and the presence of additional ApaLI and
Eagl sites in the pMT2 vector sequence, this allowed the swapping of
four different regions (i.e., residues 1–47, 48–168, 168–280, and 280–
340; numbering according to Gβ2, sequence; see Fig. 1A) between Gβ1
and Gβ2, through cutting and ligating. Successful creation of the chime-
ras was confirmed via sequencing. Note that positions 47, 168, 280, and
340 in Gβ2 correspond to positions 54, 179, 294, and 353 in Gβ1.

A second round of chimeras was created via PCR, using wild type Gγ
subunits and the initial set of chimeras as templates. Briefly, sense and
antisense oligonucleotides, spanning the junctions of the new chimeras,
were generated by PCR were sequenced after cloning to ensure that no
digestion and cloned in the appropriate vector. All of the fragments
were excised from the pMT2-XX vector using previously engineered

recognition sites (XhoI and KpnI found in the 5′ and 3′ regions, respec-
tively) (12) and subcloned in-frame into the EGFP vectors. Correct
insertion within the EGFP vector was confirmed with both restriction
enzyme digestion and sequencing.

EGFP-tagged Constructs—N-terminal fluorescently tagged chimeric
Gβ proteins were created using Clontech (Palo Alto, CA) Living Col-
ors™ C-terminal EGFP vectors. Briefly, chimeric G proteins were
cloned into the pMT2-XX vector using previously engineered restriction sites (XhoI and KpnI found in the 5′ and 3′ regions, respec-

tively) (12) and subcloned in-frame into the EGFP vectors. Correct
insertion within the EGFP vector was confirmed with both restriction
enzyme digestion and sequencing.

Tissue Culture and Transient Transfection of tsA-201 Cells—Human
encephaloblastoma (tA-201) cells were grown and transfected with calcium
phosphate as described by us previously in detail (21). In each experiment
involving calcium channels, wild type or mutant rat Ca2,2 calcium chan-
nel α1 subunits were co-transfected with rat β2, rat α2,δ5, Gγ, and an
EGFP expression marker (except in the case where EGFP-tagged G pro-
terin was used), plus one of wild type or chimeric Gβ subunits. For
experiments involving GIRK channels, GIRK1 and GIRK4 subunits were
used instead of calcium channel subunits. To prevent cells from overgrow-
ing, the cells are routinely placed in a 28 °C incubator 12 h after trans-
fection. Under these conditions, tsA-201 cells change their morphology
such that they appear rounded (see Fig. 1C, inset).

Patch Clamp Recordings and Data Analysis—Glass coverslips carry-
ing transfected cells were transferred to a 3-cm culture dish containing
recording solution comprised of 20 mM BaCl2, 1 mM MgCl2, 10 mM
HEPES, 40 mM tetrathylammonium hydrogenode, 10 mM glucose, 65 mM
CaCl2 (pH 7.2 with tetrathylammonium-hydroxide). Whole cell patch
clamp recordings were performed using an Axopatch 200B amplifier
(Axon Instruments, Foster City, CA) linked to a personal computer equip-
ed with pCLAMP version 5.0, 8.0, or 9.0. Borosilicate glass, 150-180 μm
in diameter (Sutter boroisilicate glass, BF150-86-15) were pulled using a Sutter P-87 micro-
electrode puller, fire-polished, and showed typical resistances of 3–4
MΩ. The internal pipette solution contained 108 mM cesium methane-
sulfonate, 4 mM MgCl2, 9 mM EGTA, 9 mM HEPES (pH 7.2). Series
resistance was compensated by 80–85%. Leak currents were negligible.
The data were filtered at 1 kHz and recorded directly onto the hard
drive of the computer. Unless stated otherwise, the currents were
evoked by stepping from −100 mV to a test potential of +20 mV. G
protein inhibition was assessed by application of a strong depolarizing
(+150 mV) prepulse (PP) for 50 ms. Typically, only cells with current
amplitudes greater than 50 pA were used for analysis. The degree of
prepulse relief of tonic G protein inhibition was determined as the ratio
of peak current amplitudes seen after (Ipp) and before (Ipp) the prepulse
and reflects the ability of a given G protein β subunit to inhibit
N-type current activity. The PP paradigms were programmed using the
“train” and “user list” functions in pCLAMP. For experiments involving
activation of protein kinase C, PMMA (Sigma) was dissolved in dimethyl
sulfoxide at a 1 mM stock concentration and diluted into the recording
solution at a final concentration of 30 mM. Control solution or solution
containing PMMA was perfused onto cells via a home-built gravity-driven
micropipette system.

For recordings involving GIRK1 and GIRK4 subunits, whole cell
recordings were conducted using an internal solution of 100 mM potassium
chloride, 440 mM KF, 5 mM EGTA 5, 1 mM MgCl2, and 5
mM NaCl (pH 7.4 with KOH). The external solution contained 25 mM KCl, 10 mM HEPES, 10 mM glucose, and 116 mM NaCl (pH 7.4 with NaOH).
Under these conditions, the predicted reversal potential for potassium is
approximately −30 mV. GIRK channel activity was assessed by holding
the cells at −35 mV, followed by an application of a voltage ramp from
−120 to +60 mV over 525 ms. Only cells displaying inward rectification
were used for analysis, and whole cell GIRK conductance was obtained by
fitting the data to a linear function, ls the inward current. Whole cell capacitance ranged from 5 to
40 pF. In this range, there was no correlation between capacitance and
whole cell conductance (r2 = 0.15; not shown), and hence the data are
plotted in Fig. 4 as whole cell conductance rather than current densities.
For chimeric constructs, we determined the GIRK activity in the absence of Gβ subunits.

All of the data were analyzed using Clampfit (Axon Instruments) and
fitting in SigmaPlot 4.0 (Jandel Scientific). Statistical analysis was
 carried out in SigmaStat via t tests, or as appropriate via ANOVA with
a post hoc Tukey test or, for GIRK experiments, ANOVA on the ranks
(Dunn method).

Confocal Microscopy—Imaging was carried out at the Seamian Fam-
ily MR Research Center Confocal Microscopy and Imaging Facility.
Briefly, tA-201 cells were transfected with DNA encoding the N-type
calcium channel (Ca2,2 α, α5, δ, and β1), Gγ, and EGFP-tagged G
protein β subunits. Initially coverslips containing cells of interest were
placed in a glass-bottomed Petri dish and visualized with an inverted
IX70 Olympus microscope. Confocal images were created using an
Olympus Fluoview confocal laser scanning microscope (confocal aper-
ture 2). The cells were stimulated with an argon 488-nm laser. Settings
were chosen for laser power, photon multiplier tube gain, and offset
so that maximum green fluorescence was maintained. Transmitted
image visualization was conducted with Noronski’s red interference
contrast microscopy (21).

RESULTS

Determinants of N-Type Channel Inhibition by Gβ1 Sub-
units—We have shown previously that rat N-type calcium chan-
nels are potently inhibited by Gβ1 subunits, whereas Gβ2 sub-
units have no significant effect on current activity (Ref. 12; see
also Fig. 1C). To determine key G protein β subunit structural
determinants that control calcium channel inhibition, the differ-
Fig. 1. Effect of chimeric G protein subunits on N-type calcium channel activity. A, schematic representation of a chimeric G protein β subunit adapted from the crystal structure reported by Sondek et al. (31). The Gβ subunit is comprised of an N-terminal helix linked to a rigid seven-bladed propeller structure. An initial set of chimeras was constructed by swapping fragments between Gβ5 (indicated in blue) and Gβ1 (indicated in orange). For this purpose, the Gβ sequence was divided into four segments (i.e. N terminus-residue 47, residues 47–168, residues 168–280, and residue 280–C terminus; the numbering corresponds to Gβ1 sequence, see arrows for approximate positions within the Gβ subunit structure). The nomenclature of the chimeric constructs is based on the origin of the four segments, with the depicted 5151 construct indicating that segments 1 and 3 were derived from Gβ1, segments 2 and 4 from Gβ5. In contrast, as illustrated in Fig. 1B, replacement of either residues 47–168 or residues 168–280 with corresponding Gβ5 sequence, alone or in combination with other substitutions, reduced the degree of prepulse relief to that seen in the presence of wild type Gβ5 (note that prepulse relief observed with Gβ5 does not differ significantly from conditions where no G proteins are coexpressed). To minimize the possibility that the lack of modulation might be due to the lack of expression or due to inappropriate targeting of the Gβ subunit chimeras, we generated N-terminally EGFP-tagged versions of the wild type and key chimeric G proteins and analyzed their subcellular distribution using fluorescence confocal microscopy. As shown in the inset of Fig. 1C, the wild type and chimeric G protein subunits were detected in the plasma membrane, suggesting that they are indeed properly expressed and targeted in tsA-201 cells. Moreover, the observation that all of the Gβ constructs display robust plasma localization implies that the observed effects are not secondary due to an inability of certain chimeras to assemble into Gββ dimers. In addition, we have shown previously via Western blot and kinetic analyses that transient transfection of wild type G protein results in saturating levels of Gβ which, in turn, leads to a homogenous population of G protein bound N-
type calcium channels (21). Taken together, these data suggest that the failure to observe G protein inhibition with certain chimeras was not simply due to a lack of expression/targeting.

Overall, our data obtained with the initial set of chimeras suggest that although the N- and C-terminal regions of G8 are not critical determinants of G protein inhibition of N-type channels, one or more regions between residues 47 and 280 are essential for G protein inhibition. To further elucidate the G protein β subunit structural determinants of N-type channel modulation, we created additional chimeras with sequence substitutions in regions 47–168 and 168–280. As shown in Fig. 2, replacing G8β residues 47–116 or 116–168 with corresponding G8βn sequence resulted in the loss of G protein inhibition. Substitutions of smaller fragments within these two regions revealed that replacement of regions 47–75, 75–100, and 116–140 did not block the ability of G8β1 to inhibit N-type channel activity. On the contrary, the 47–75 construct displayed a dramatically enhanced ability to inhibit N-type channel activity. In contrast, substitution of residues 140–168 or replacement of an Asn-110, Tyr-111, and Val-112 motif (that is highly conserved in all G protein β subunits with the exception of G8β3 with G8βn i.e. Cys-Ala-Ile) sequence eliminated the ability of G8β1 to inhibit N-type calcium channels. A similar analysis for residues 168–280 is shown in Fig. 3. As shown in Fig. 3 (A and B), substitution of residues 204–248, 248–280, and 168–186 maintained the ability of G8β1 to inhibit N-type calcium channels, whereas substitution of residues 186–204 abolished G8β1 modulation. Taken together, there appear to be at least three separate regions that are responsible for the differential effect of G8β1 and G8βn on N-type calcium channel activity. It is unlikely that these effects would have arisen from a global disruption of G8β1 subunit folding because of the presence of G8βn sequence, because G8β1 activity was retained for a majority of the G8βn substitutions that were created (i.e. 1–47, 47–75, 75–100, 116–140, 168–186, 204–248, and 248–280). To ensure that the effects of the chimeras were specific rather than caused by inappropriate folding, we first attempted to create a gain of function chimera in which regions in G8βn were concomitantly replaced with corresponding G8β1 residues 110–112, 140–168, and 186–204. However, the G8β(110–112, 140–168, 186–204) chimera did not result in significant G protein inhibition ($I_{PP}/I_{PP}$ decrease was not observed). This suggests that gain of function may require additional residues outside of the three identified regions, which will be subject to further investigation. As an additional approach, we created two point mutations in which residues 111 and 153 of G8β1 were replaced with corresponding G8βn residues. These residues are located within the 110–112 and 140–168 stretches were chosen based on the surface exposure on the G8β1 crystal structure (see Fig. 5B below). Substitution of Tyr-111 with alanine resulted in a dramatic reduction of the degree of prepulse relief ($I_{PP}/I_{PP}$) to 1.48 ± 0.19 (n = 15), and replacement of Asp-153 to asparagine resulted in an even stronger reduction of prepulse relief to 1.37 ± 0.08 (n = 15). These data suggest that even single amino

![Chimeric analysis of residues 47–168 in the G protein β1 subunit. Top panel, chimeric G protein subunits are depicted using the same color schemes as described in the legend to Fig. 1. The numbering on the chimeric structures reflect amino acids in the G8β sequence that were replaced by corresponding G8β residues, the plus and minus signs reflect the ability or inability to inhibit N-type channels. Bottom panel, bar graph illustrating the degree of prepulse relief obtained upon coexpression of the N-type calcium channels with the chimeras depicted in the upper panel of the figure. Note the axis break on the ordinate (i.e. the 47–75 construct resulted in dramatically greater prepulse relief compared with wild type G8β). The asterisks indicate the statistical significance (p < 0.05, ANOVA) relative to wild type G8β. The dotted line indicates the level of modulation seen with the 1511 construct. All of the error bars denote the standard errors.](image-url)
acoid substitutions in at least two of the identified regions are sufficient to drastically attenuate the ability of Gβ1 to inhibit N-type calcium channels, consistent with the idea that the inability of the chimeras to regulate channel activity did not arise secondarily from global structural changes.

To confirm the functionality of those Gβ chimeras that were unable to regulate N-type channel activity, we carried out a series of experiments with GIRK channels. GIRK1 and GIRK4 subunits were coexpressed in tsA-201 cells with either wild type or chimeric/mutant Gβ subunits, and the whole cell GIRK conductance was determined via whole cell patch clamp recordings using a voltage ramp protocol. Rather than examining the entire set of chimeras, we focused on the key chimeras and mutants that most narrowly defined the regions involved in N-type channel modulation, i.e. Gβ1(100–112), Gβ1(140–168), Gβ1(186–204), Gβ1(Y111A), Gβ1(D153N), and the Gβ1(110–112, 140–168, 186–204) chimera. As shown in Fig. 4, expression of GIRK1/4 in the absence of exogenous Gβ subunits resulted in some background GIRK activity, consistent with previous work in Xenopus oocytes (22). Cotransfection of Gβ1 subunits resulted in a significant increase in whole cell conductance by ~300%. Interestingly and in contrast with a previous study (22), wild type Gβ1 subunits also effectively activated GIRK1/4 channels, thus confirming that Gβ1 subunits are indeed functionally expressed in our system. But more importantly, every single one of the chimeric and mutant Gβ subunits examined mediated a significant increase in GIRK1/4 activity to a level comparable with that seen with wild type Gβ1. Hence, we conclude that the lack of effects of these chimeras/mutants on N-type channel activity did not arise from inadequate protein expression or protein misfolding.

G Protein PKC Cross-talk—We have shown previously that PKC-dependent phosphorylation of Thr-422 in the Ca2.2 calcium channel I-II linker selectively antagonizes the Gβ1-mediated inhibition of N-type channel activity (18). We also showed that all aspects of this effect could be mimicked by replacing Thr-422 with glutamic acid (17, 18), thus eliminating the need for diffuse activation of PKC and consequently the potential for secondary effects/incomplete specificity of PKC activators. Because a chimera containing the first 47 residues of Gβ1 (Fig. 5A) appeared to behave like wild type Gβ1 with regard to N-type channel regulation (recall Fig. 1), we examined whether the PKC cross-talk could still be observed with this construct. Although the T422E mutation reduced the ability of Gβ1 to inhibit N-type channel activity, the 5111 chimera failed to recognize the presence of the T422E substitution (Fig. 5B), thus indicating that a key Gβ1 structural determinant is located in the N-terminal region. Because the PKC cross-talk is only observed with Gβ1, we examined the N-terminal 47-amino acid stretch for residues unique to this subunit (Fig. 5C, bold letters). As shown in Fig. 5D, replacement of Asp-5 with glutamic acid had no effect on cross-talk. In contrast, replacement of Asn-35 and Asn-36 with corresponding sequences in Gβ1 abolished the effects of the T422E I-II linker mutation, suggesting that one or both of these asparagine residues sense the presence of a negative charge within the I-II linker G protein-binding domain (7). If so, then one should be able to confer cross-talk onto other G protein subunits by substituting asparagi-
agine residues in complimentary positions. To test this hypothesis, we carried out site-directed mutagenesis in the G\(_{\beta3}\) subunit (which modulates N-type channels effectively but whose action is not antagonized by PKC) (18) and compared the abilities of the mutant G\(_{\beta3}(S35N,G36N)\) construct to inhibit wild type and T422E mutant N-type channels. Consistent with our hypothesis, the mutant G\(_{\beta3}\) subunit inhibited the T422E channel significantly (\(p < 0.05\)) less effectively than the wild type Cav2.2 channel (Fig. 5E).

To confirm that the observations were not an artifact of the T422E calcium channel mutant, we repeated the experiments shown in Fig. 5 (D and E) using proper activation of PKC via 30 nM of the phorbol ester PMA. For each cell, the degree of prepulse relief was measured before application of PMA and 3 min after PMA application. As seen in Fig. 5F, activation of PKC did not affect the degree of prepulse relief observed with the G\(_{\beta1}(N35S,N36G)\) mutant. In contrast, mutagenesis of G\(_{\beta3}\) residues 35 and 36 to corresponding G\(_{\beta1}\) sequence (i.e. G\(_{\beta3}(S35N,G36N)\)) conferred the cross-talk behavior, such that the degree of prepulse was significantly reduced (\(p < 0.01\), paired t test) following the application of PMA.

Taken together, these data suggest that the G\(_{\beta3}\) subunit contains a precise locus of two amino acids that allows this subunit to sense the presence of a negative charge (such as a phosphate group) on the N-type calcium channel \(\alpha_1\) subunit.

**DISCUSSION**

**Comparison with Previous Work**—Our work constitutes the first systematic approach toward delineating key G\(_{\beta}\) subunit regions that are essential for inhibiting N-type calcium channel activity. Unlike previous studies that were based on alanine mutagenesis of G\(_{\beta}\) subunit residues known to be involved in G\(_{\beta}\) interaction domain (Refs. 23 and 24 and Fig. 6) and has not been previously identified as an important functional domain on the G\(_{\beta3}\) subunit. G protein inhibition was also lost upon substitution of G\(_{\beta3}\) residues 140–168 and 186–204. Hence, the G\(_{\beta}\) subunit structural determinants that control signaling to N-type calcium channels appear to be different from those implicated in coupling to other effector systems such as adenyl cyclase, GIRK channels, or phospholipase \(\beta_2\) (i.e. residues 72–105, 115–135, and 143, 186, 228, and 332) (19, 22, 25–31). However, in lieu of specific examination of the action of our chimeras on effectors other than GIRK channels, it is difficult to gauge whether the
regions identified in our study are exclusively involved in signaling to N-type channels.

Within regions 140–168 and 186–204, respectively, 12 and 9 amino acid residues are identical in Gβ1 and Gβ3 and can therefore not account for the observed effects (the conserved residues include Thr-143 and Asp-186, which were implicated previously as being important for N-type channel modulation) (19). Examination of the localization of the nonconserved resi-
G Protein-PKC Cross-talk

G Protein-PKC Cross-talk—The N-type calcium channel contains at least three separate physical binding sites for G protein effectors (see above). We therefore envision a model in which Gβγ is held in place through interactions of two distinct sites on the Gβγ protein with two spatially separate regions within the calcium channel I-II linker. This model, the functional interaction between the phosphorylated Thr-422 I-II linker residue and residues 35/36 on the Gβγ subunit would destabilize the overall binding interaction, thus reducing the extent of G protein inhibition of the channel. The C terminus of the N-type channel might contribute toward stabilizing overall G protein binding (perhaps by interacting with previously identified residues in the Gα interaction region).

Taken together, our data close a major gap in our understanding of the molecular basis underlying cross-talk between G protein and PKC regulation of N-type calcium channels. The presence of a specific site on the Gβ subunit that serves as a molecular detector of PKC-dependent phosphorylation of the N-type calcium channel provides a unique means of integrating multiple signaling pathways at the level of a protein-protein interaction. This may allow for precise regulation of N-type calcium channel activity and, consequently, synaptic transmission.

Acknowledgments—We thank Dr. Terry Soutch for providing wild type calcium channel cDNAs, and Dr. Hubert van Tol for providing GIRK subunits. We also thank Dr. Scott Jarvis for help with artwork.

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J. Biol. Chem. 2004, 279:29709-29717.
doi: 10.1074/jbc.M308693200 originally published online April 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M308693200

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