# Direct binding of G-protein $\beta\gamma$ complex to voltage-dependent calcium channels

**Michel De Waard*†, Hongyan Liu*, Denise Walker†, Victoria E. S. Scott*, Christina A. Gurnett* & Kevin P. Campbell***

* *Howard Hughes Medical Institute, Department of Physiology and Biophysics and Program in Neuroscience, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA*

† *INSERM U464, Institut Jean Roche, Faculté de Médecine Nord, Boulevard Pierre Dramard, 13916 Marseille 20, France*

**Voltage-dependent $Ca^{2+}$ channels play a central role in controlling neurotransmitter release at the synapse[1,2]. They can be inhibited by certain G-protein-coupled receptors, acting by a pathway intrinsic to the membrane[3–6]. Here we show that this inhibition results from a direct interaction between the G-protein $\beta\gamma$ complex and the pore-forming $\alpha_1$ subunits of several types of these channels[7]. The interaction is mediated by the cytoplasmic linker connecting the first and second transmembrane repeats. Within this linker, binding occurs both in the $\alpha_1$ interaction domain (AID)[8], which also mediates the interaction between the $\alpha_1$ and $\beta$ subunits of the channel, and in a second downstream sequence. Further analysis of the binding site showed that several amino-terminal residues in the AID are critical for G$\beta\gamma$ binding, defining a site distinct from the carboxy-terminal residues shown to be essential for binding the $\beta$-subunit of the $Ca^{2+}$ channel[9]. Mutation of an arginine residue within the N-terminal motif abolished $\beta\gamma$ binding and rendered the channel refractory to G-protein modulation when expressed in *Xenopus* oocytes, showing that the interaction is indeed responsible for G-protein-dependent modulation of $Ca^{2+}$ channel activity.**

*In vitro* translation of G-protein subunits ($^{35}$S-$\alpha_0$, $^{35}$S-$\beta_1$ and $^{35}$S-$\gamma_2$) yielded products of relative molecular mass ($M_r$) 40K, 32K and 8K (Fig. 1a), respectively. These G-protein probes were used to study their interaction with glutathione *S*-transferase (GST) fusion proteins containing cytoplasmic loops (Fig. 1b, left) of the neuronal $\alpha_{1A}$ voltage-dependent $Ca^{2+}$ channel[10]. The $^{35}$S-G$\beta_1\gamma_2$ complex interacts with a GST fusion protein expressing the cytoplasmic linker connecting repeats I and II of the $\alpha_{1A}$ subunit (I–II–GST, amino acid 360–486), as demonstrated by the detection of $^{35}$S-G$\beta_1$ on the autoradiogram (Fig. 1b, right). The I–II–GST fusion protein also binds native G$\beta\gamma$ complexes solubilized from rat brain in solution, as detected by immunoblot using a common anti-G$\beta$ antibody (Fig. 1c). The binding of G$\beta_1\gamma_2$ to I–II–GST is specific, as no $^{35}$S-labelled G-protein probe reacts with the control GST or a GST fusion protein expressing the II–III loop of the $\alpha_{1A}$ subunit (II–III–GST; Fig. 1d). Similarly, no binding is detected on fusion proteins expressing the II–III loop of rabbit $\alpha_{1C}$ (amino acids 772–856) and rat $\alpha_{1B}$ (amino acids 720–1139; data not shown). Finally, binding of the GTP$\gamma$S-activated $^{35}$S-G$\alpha_0$ subunit could not be detected on any of these fusion proteins (Fig. 1d). These results are thus consistent with recent findings that the G$\beta\gamma$ complex can regulate transfected P/Q-type $Ca^{2+}$ channels[11] or N-type $Ca^{2+}$ channels in sympathetic neurons[12].

Because N- and P/Q-type $Ca^{2+}$ channels can be modulated by activated G proteins[13–16], we investigated whether the $^{35}$S-G$\beta_1\gamma_2$ complex could also interact with the I–II cytoplasmic linker of several other $\alpha_1$ subunit classes, including the L-type $\alpha_{1S}$ and $\alpha_{1C}$ channels, and non-L-type $\alpha_{1B}$ and $\alpha_{1E}$ channels (Fig. 2a). The $^{35}$S-G$\beta_1\gamma_2$ complex also interacts with GST fusion proteins expressing the full-length I–II loops of the $\alpha_{1B}$ and $\alpha_{1E}$ channels, but not with those of the $\alpha_{1S}$ or $\alpha_{1C}$ channels (Fig. 2b). These results provide a molecular mechanism for earlier reports that $\alpha_{1A}$, $\alpha_{1B}$ (ref. 16) and $\alpha_{1E}$ (ref. 14) channels are modulated by G proteins.

We have previously shown that the I–II cytoplasmic linker of all six classes of $\alpha_1$ subunits contain the AID site, a sequence of 18 amino acids, of which 9 residues are universally conserved (QQ-E--L-GY--WI---E) (1–18). The AID site is both necessary and sufficient for the binding of the $Ca^{2+}$-channel $\beta$-subunit[8,9]. The AID sequence separates two domains in the I–II linker, one upstream of conserved length (23 amino acids; domain I, D1), and a downstream sequence of variable length (between 55 and 87 amino acids; domain II, D2). To define precisely the G$\beta\gamma$ binding site on the I–II linker of the $\alpha_{1A}$ subunit, we generated several GST fusion proteins consisting of partial I–II loop sequences (Fig. 2c). The results demonstrate that $^{35}$S-G$\beta_1\gamma_2$ interacts with D1–AID and AID-D2, but not D1 alone, demonstrating the importance of AID itself in G$\beta\gamma$ binding (Fig. 2d). Consistent with this interpretation was the finding that $^{35}$S-G$\beta_1\gamma_2$ could also interact with a fusion protein (AID–GST) expressing AID and a few flanking amino acids in D1 and D2 (Fig. 3a). Surprisingly, $^{35}$S-G$\beta_1\gamma_2$ also interacts with the D2–GST fusion protein independent of AID (Fig. 2d). It is

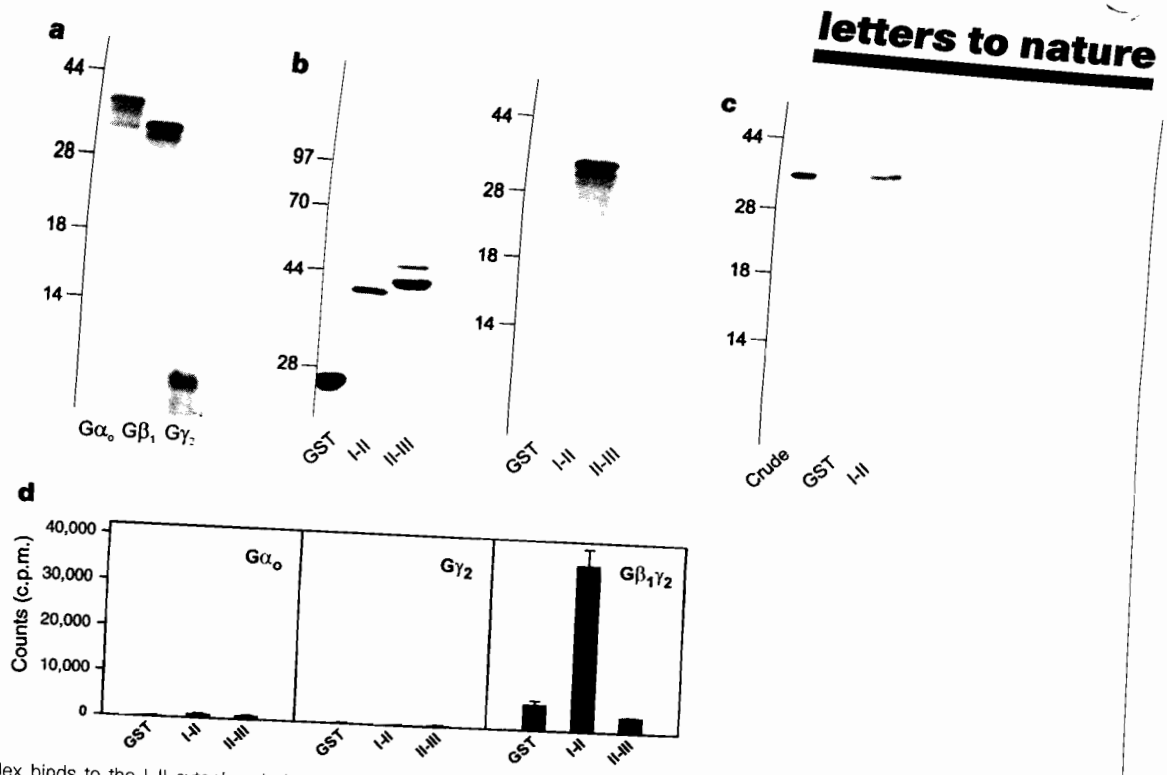


**Figure 1** G-protein βγ complex binds to the I–II cytoplasmic loop of the $\alpha_{1A}$ channel. **a**, Autoradiogram of *in vitro* translated G-protein $\alpha_o$, $\beta_1$ and $\gamma_2$ subunits separated on SDS–polyacrylamide gel. **b**, Left, Coomassie blue-stained purified control GST, $\alpha_{1A}$ I–II and II–III loop GST fusion proteins (I–II–GST and II–III–GST) separated on a 10% SDS–polyacrylamide gel. Right, autoradiogram of bound proteins from incubation of co-translated $^{35}$S-Gβ$_1$γ$_2$ with glutathione–Sepharose beads coupled with control GST, I–II–GST and II–III–GST fusion proteins. Detection of $^{35}$S-Gγ$_2$ is hampered by its comparatively weak labelling and small size. **c**, Western blot using common Gβ antibody (Dupont NEN) on crude rat brain membrane (crude) or brain extract bound to GST or I–II–GST glutathione beads. **d**, Binding of $^{35}$S-Gα$_o$, Gγ$_2$ or co-translated $^{35}$S-Gβ$_1$γ$_2$ to GST, I–II–GST and II–III–GST.

therefore concluded that the interaction of Gβγ with the I–II cytoplasmic linker can occur on two separate regions, the AID site (required for $Ca^{2+}$-channel β-subunit binding), and the D2 sequence.

We next investigated the binding properties of Gβγ to the AID and the D2 sequences. Analysis of the binding of AID–GST to $^{35}$S-Gβ$_1$γ$_2$ demonstrates that the specific binding is saturable and occurs on a single site with an apparent $K_d$ of 63 nM (Fig. 3a). The affinity of Gβγ for the AID site is thus 10- to 20-fold lower than that of the $Ca^{2+}$-channel β subunit[17], which may predict a more labile interaction at that site. Saturation binding analysis with a D2–MBP fusion protein shows that the binding of D2 is of slightly higher affinity ($K_d$ = 24 nM) than AID–GST (Fig. 3b). *In vitro* binding affinity may differ from physiological affinity owing to difference in $\alpha_1$ sequence conformation and membrane localization of G proteins. Taken together, these results suggest that the D2 region is a more stable Gβγ attachment site than AID. To test this hypothesis, we expressed full-length I–II–GST fusion proteins containing AID mutants or AID chimaeras ($D1_A$-$AID_{R387E}$-$D2_A$ mutant, $D1_A$-$AID_S$-$D2_A$ and $D1_A$-$AID_C$-$D2_A$ chimaeras) and tested their ability to bind the Gβγ complex. All these fusion proteins were able to interact with Gβγ, as well as the wild-type I–$II_A$–GST fusion protein (Fig. 3c). In contrast, $D1_A$, $D1_S$-$AID_S$-$D2_S$ (I–II loop of $\alpha_{1S}$), and $D1_C$-$AID_C$-$D2_C$ (I–II loop of $\alpha_{1C}$) were unable to bind Gβγ (Fig. 2b, d). These results demonstrate that, although $AID_A$ can bind Gβγ, it is not absolutely essential for the attachment of the Gβγ complex to the I–$II_A$ cytoplasmic linker.

Because both Gβγ and the $Ca^{2+}$-channel β-subunit bind to AID, it is important to define the critical AID residues involved in Gβγ

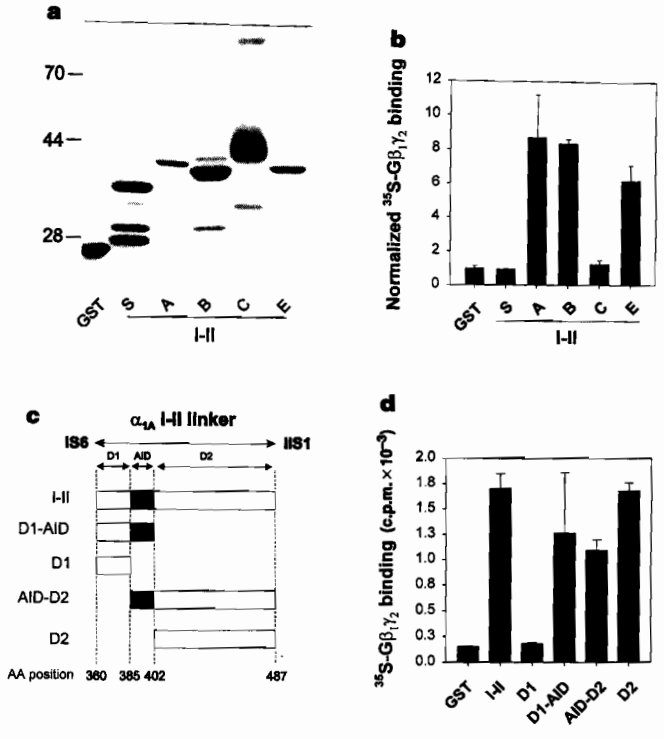


**Figure 2** Interactive channel types and localization of a G-protein βγ binding site

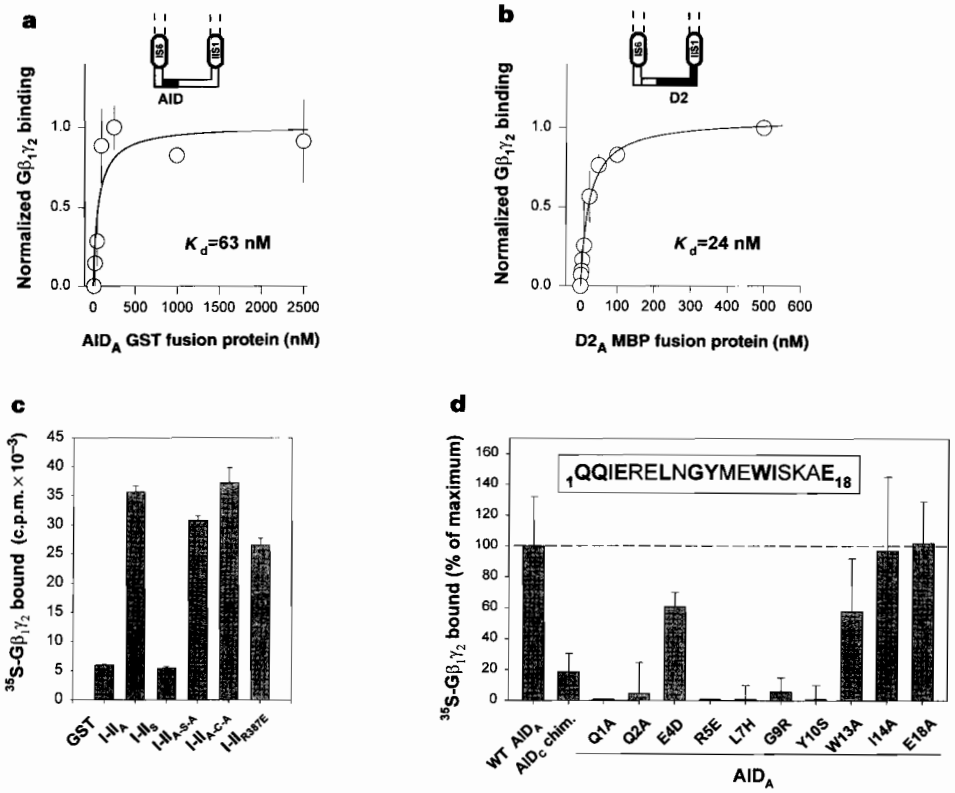


**Figure 3** Binding properties of the interacting $\alpha_{1A}$ sequences and identification of critical AID amino acids. **a**, Saturation curve showing binding of $^{35}$S-G$\beta_1\gamma_2$ to AID$_A$–GST. **b**, Saturation curve showing binding of D2$_A$-maltose binding protein (MBP) fusion protein to $^{35}$S-G$\beta_1\gamma_2$. **c**, Binding of mutant or chimaeric $\alpha_{1A}$ I–II loop to the G$\beta_1\gamma_2$ complex in the presence of the D2$_A$ sequence. I-II$_{A\text{-}S\text{-}A}$ stands for the exchange of AID$_A$ for AID$_S$ in the I-II$_A$ loop. **d**, Normalized binding of $^{35}$S-G$\beta_1\gamma_2$ to 250 nM of purified mutant and chimaeric AID–GST fusion proteins. Amino acids of AID$_A$ are numbered from 1 to 18. R5E is the same mutation as R387E in **c**.

possible involvement of other non-conserved AID residues, in addition to the conserved residues in Gβγ binding.

When comparing the binding of Gβγ on the mutant AID$_A$–GST fusion proteins to the wild-type AID$_A$–GST fusion protein (Fig. 3d), Gβγ binding was lost upon mutation of Q1, Q2, L7, G9 and Y10. The binding was, however, maintained for mutants of $E_4$, $W_{13}$, $I_{14}$ and $E_{18}$. These data remain consistent with the identification of Y- -WI (10–14) as being critical for the binding of the $Ca^{2+}$-channel β-subunit[9]. Y10 could possibly be a common interactive residue for both the $Ca^{2+}$-channel β-subunit and the Gβγ complex, perhaps being responsible for some of the functional antagonism observed between activated G proteins and the $Ca^{2+}$-channel β-subunit[16,18]. Noticeably, the N-terminal part of the AID region of the three interacting $\alpha_1$ subunits contains a motif (Q- -ER) (1–5) that was previously identified in a Gβγ-interacting type of adenylyl cyclase and is necessary for its regulation by the Gβγ complex[19]. In this motif, Q1 and E4 are conserved in all $\alpha_1$ subunits, while R5 is present only in the Gβγ-interacting $\alpha_1$ subunits (A, B and E). The importance of Q1 and R5 is demonstrated in Fig. 3d, although E4 seemed not to be as essential as in the case of adenylyl cyclase (a similar result was obtained when E4 is substituted by a serine; data not shown). Overall, these results define QQ- -R-L-GY (1–10) as an essential motif for Gβγ binding to the AID site in voltage-dependent $Ca^{2+}$ channels. The involvement of additional AID residues in the interaction can not be excluded, however, as not all AID residues were tested.

A deletion approach to determine the D2 sequence responsible for the binding of Gβγ demonstrated that several stretches of the D2$_A$ sequence (401–435, 401–461, 436–487 and 450–487) were all capable of interacting with Gβγ (data not shown), suggesting that the D2 binding domain could not be localized further, and may be comprised of several microsites.

Next, we analysed the functional importance of G-protein binding to the I–II cytoplasmic linker. It is well established that activation of G proteins leads to several biophysical changes in the gating of voltage-dependent $Ca^{2+}$ channels through a membrane-delimited[20] and voltage-dependent[21] pathway that does not involve a second messenger system[22]. Injection of cRNAs encoding the $\alpha_{1A}$ and $\beta_4$ subunits[10,23] in *Xenopus laevis* oocytes results in the expression of functional voltage-dependent $Ca^{2+}$ channels (Fig. 4a). A membrane depolarization to 0 mV triggers a maximum inward current of $-1{,}831 \pm 893$ nA ($n = 5$) that peaks at $11.1 \pm 1.9$ ms ($n = 15$) after the start of the pulse (Fig. 4a, left). Irreversible activation of several endogenous[24] G proteins ($G_o$, $G_i$ and $G_s$), by injection of 200–500 μM of GTP-γS in *Xenopus* oocytes, bypassing receptor activation, results in a significant slowing of the activation process. This slowing can be measured by a delayed latency of the peak current that now occurs $26.4 \pm 2.3$ ms ($n = 4$) after the start of the depolarization (Fig. 4a, right). The effect of GTP-γS can be reversed by a strong depolarizing prepulse (data not shown). Slowing of the activation kinetics has been interpreted as the gradual and partial dissociation of activated G protein from the channel in its open state[6], although a conformational change producing a slower gating cannot be ruled out[5]. The rather slow time course of GTP-γS action (maximum effect $8 \pm 2$ min after injection) corresponds well with the slow activation of G proteins by GTP analogues in the absence of receptor agonists[25]. Activation of G proteins had a very limited effect on current amplitude itself, with an average reduction of $12.4 \pm 0.1\%$ ($n = 4$), which suggests that the effects of G proteins on the current kinetics and amplitude can be independent of each other.

Expression of the mutant $\alpha_{1A}$ R387E subunit (substitution of the R residue of the Gβγ interacting motif (QQ- -R-L-GY) (1–10)) along with $\beta_4$ results in a current that activates at a rate similar to that of the control channel, with a time to peak of $13.6 \pm 2.4$ ms

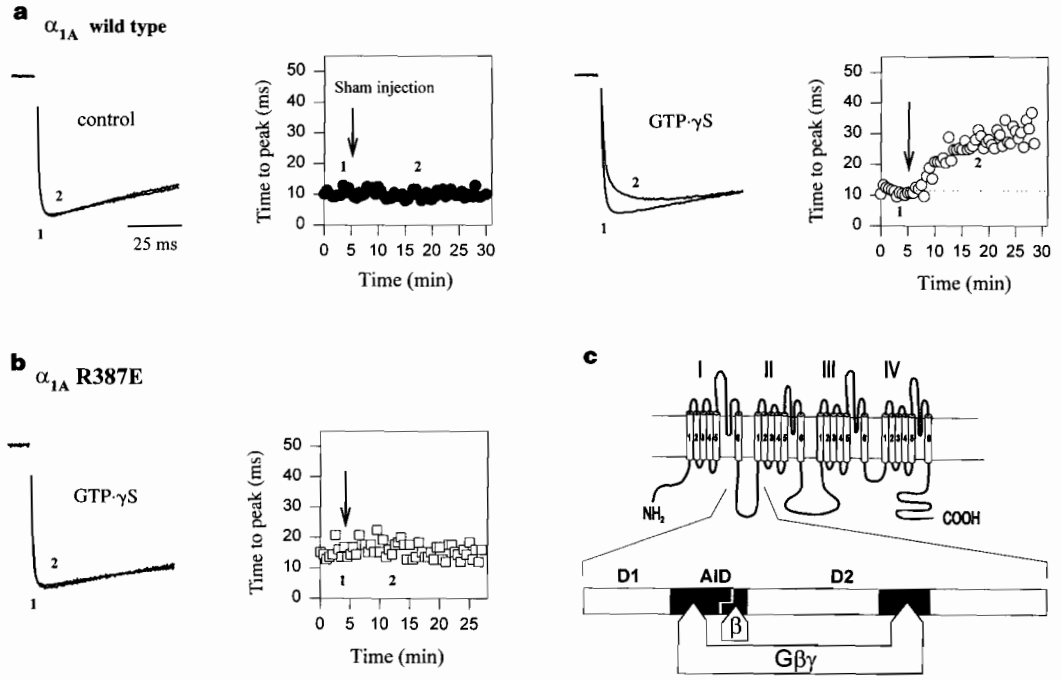


**Figure 4** Effect of activated G proteins on the activation kinetics of the wild type and the mutant $\alpha_{1A}$ R387E channel (AID R5E). **a**, Left, no effect of sham injection on activation of wild-type $\alpha_{1A}$ channel. Right, slowing of activation kinetics of wild-type $\alpha_{1A}$ channel by injection of 300 μM GTP-γS (indicated by arrow). Currents were normalized to each other to illustrate the slowing of current activation. **b**, Absence of effect of GTP-γS on the activation time course of the $\alpha_{1A}$ R387E mutant channel. Addition of 300 μM GTP-γS indicated by arrow. Peak current amplitudes are −2.8 μA (**a**, left), −2.9 μA (**a**, right) and −2 μA (**b**). **c**, Schematic representation of the Gβγ interaction site on the I–II cytoplasmic linker of the $\alpha_1$ subunit.

($n = 7$) at 0 mV. In contrast to the wild-type $\alpha_{1A}$ channel, the time to peak of the mutant $\alpha_{1A}$ R387E channel was not regulated by the injection of GTP-γS into the cell ($n = 5$; Fig. 4b), whereas two chimaeras in which the entire $AID_A$ sequence was replaced by $AID_B$ or $AID_E$ could still be regulated (data not shown). These results emphasize the importance of this arginine residue, not only in binding of Gβγ to the AID region (Fig. 3d), but also for regulation of channel activity. Mutation of this arginine residue does not affect the full-length $I–II_A$ loop binding to Gβγ (Fig. 3c) or to the $Ca^{2+}$ channel β-subunit (data not shown). The fact that most of the G-protein regulation can be blocked by this AID mutation also suggests that the D2 site attachment is not sufficient for G-protein modulation, or at least for its kinetic effect.

We have shown that the Gβγ complex interacts directly with three types of voltage-dependent $Ca^{2+}$ channels ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1E}$), which is consistent with early observations that G-protein regulation is membrane delimited and voltage dependent. These channels therefore represent a second class of ion channels, in addition to the inwardly rectifying potassium channel[26,27], that can be regulated directly by the Gβγ complex. The binding site of this complex is localized within the cytoplasmic linker that connects the hydrophobic repeats I and II of these $\alpha_1$ subunits (Fig. 4c), and mediates G-protein modulation, $Ca^{2+}$-channel β-subunit stimulation, and protein kinase C upregulation[8,28]. Similar to the GIRK1 potassium channel[27], the binding site comprises two regions, the AID region and the D2 region. The AID sequence is required for channel regulation, whereas it is possible that the D2 sequence positions the Gβγ close to the $\alpha_1$ channel. The proximity of the G-protein binding site to the $Ca^{2+}$-channel β-subunit interaction site on AID probably represents the structural basis for some of the functional antagonism (current amplitude) observed between these molecules[16,18]. The proximity of the Gβγ binding site to the AID motif means that it will be interesting to determine whether the binding of the $Ca^{2+}$-channel β-subunit may allosterically modify the regulatory effect of Gβγ complex. □

## Methods

**Preparation of fusion proteins.** DNA constructs for GST fusion proteins of the $\alpha_{1A}$ subunit (BI-2 clone[10]) were constructed by subcloning base pairs 1080–1459 (I–II–GST), 2633–3201 (II–III–GST[29]), 1080–1199 (D1–AID–GST), 1080–1145 (D1), 1146–1460 (AID–D2–GST) and 1200–1460 (D2–GST) into pGEX 2TK or pGEX KG vectors. GST fusion proteins of the full-length I–II linkers were expressed from base pairs 1228–1521 ($I–II_S$–GST, amino acids 334–432), 1066–1449 ($I–II_B$–GST, amino acids 356–483), 1303–1662 ($I–II_C$–GST, amino acids 435–554) and 903–1281 ($I–II_E$–GST, amino acids 302–427) of rabbit $\alpha_{1S}$, rat $\alpha_{1B}$, rabbit $\alpha_{1C\text{-}a}$ and rat $\alpha_{1E}$. The $AID_A$–GST fusion protein expresses $AID_A$ and 14 N- and 18 C-terminal sequences of $D1_A$ and $D2_A$, respectively ($AID_A$–GST)[8]. Mutations of AID residues were prepared as described previously[8]. Mutant $AID_A$ R5E was constructed by cassette mutagenesis using the mutagenic primer 5′-CAGCAGCAGATTGAA-GAGGAGCTCAACGGGTAC-3′. The $D2_A$–MBP fusion-protein construct was prepared by subcloning base pairs 1200–1460 of the $\alpha_{1A}$ into pMal-c2 vector. Fusion-protein constructs of the truncated $D2_A$ segment contain base pairs 1200–1304 (401–435 of D2), 1200–1382 (401–461 of D2), 1305–1460 (435–487 of D2) and 1347–1460 (450–487 of D2). The fusion proteins were sequenced and purified according to standard procedures[17].

**Binding assays.** Full-length $G\alpha_0$, $G\beta_1$ and $G\gamma_2$ cDNA sequences were subcloned into pcDNA3 vector. The $^{35}S$-labelled G-protein $\alpha_0$, $\gamma_2$ and $\beta_1\gamma_2$ probes were synthesized *in vitro*[30] by coupled transcription and translation (TNT system, Promega). Free $^{35}S$-methionine was removed with a PD10 column (Pharmacia) from the radiolabelled proteins for all binding experiments. Binding of the G-protein probes was performed by coupling equal amounts of the fusion proteins to glutathione–sepharose beads, and incubating with 0.5–2.0 pM of probes overnight at 4 °C in buffer consisting of (in mM) 10 HEPES, pH 7.4, 1 DTT, 1 EDTA, 150 NaCl, 0.1% CHAPS, 4 mg ml$^{-1}$ BSA. The $G\alpha_0$ subunit was activated by addition of 100 μM GTP-γS in the binding buffer. The beads were washed on ice with 4 ml 0.05% CHAPS in PBS. The beads were then loaded on SDS–PAGE or subject to scintillation counting. Rat brain membranes were solubilized for 1 h at 4 °C in buffer containing (in mM) 50 Tris, pH 7.4, 1 DTT, 1 EDTA, 1,000 NaCl, 1.5% CHAPS, with a cocktail of protease inhibitors (Complete, Boehringer Mannheim). Insoluble material was

removed by centrifugation at 100,000 r.p.m. for 12 min in a TL100.3 rotor (Beckman). The supernatant was diluted 10-fold with buffer (in mM): 20 Tris, pH 7.4, 1 DTT, 1 EDTA, 1 GDP, 10 NaF, 10 $MgCl_2$, 0.05 $AlCl_3$, with the protease inhibitor cocktail. Fusion proteins 5–20 μg were added to 25 ml of diluted brain extract, incubated at 4 °C overnight and washed as described above. Proteins analysed on SDS–PAGE (16% gel) were either dried and exposed to autoradiogram film (X-OMAT AR, Kodak) or transferred to nitrocellulose for western blot analysis. The $D2_A$–MBP protein was used for dose–response analysis because it purified better than the GST–$D2_A$ fusion protein. The saturation curve was generated as previously described[17].

**Electrophysiological recordings.** Complementary RNAs were transcribed *in vitro* using the SP6 RNA polymerase with the rabbit brain $\alpha_{1A}$ plasmid and T7 RNA polymerase with rat brain $\beta_4$ plasmid. Wild-type, mutant or chimaeric $\alpha_{1A}$ (0.4 μg $\mu l^{-1}$) were co-injected with 0.2 μg $\mu l^{-1}$ $\beta_4$ cRNA into stage V or VI *Xenopus* oocytes. $Ba^{2+}$ currents were recorded[17] using the following bath solution (in mM): 10 $Ba(OH)_2$, 80 NaOH, 2 KCl, 1 niflumic acid, 0.05 EGTA, 5 HEPES, pH 7.4, adjusted with methanesulphonic acid. The membrane potential was held at −90 mV and transiently pulsed at 0 mV for 500 ms every 30 s. Injection of GTP-γS was performed 5–6 min after control run-up was achieved.

Received 29 August; accepted 11 December 1996.

1. Takahashi, T. & Momiyama, A. *Nature* **366,** 156–158 (1993).
2. Wheeler, D. B., Randall, A. & Tsien, R. W. *Science* **264,** 107–111 (1994).
3. Dolphin, A. C., Forda, S. R. & Scott, R. H. *J. Physiol. (Lond.)* **373,** 47–61 (1986).
4. Holz, G. G., Kream, R. M., Spiegel, A. & Dunlap, K. *J. Neurosci.* **9,** 657–666 (1989).
5. Kasai, H. *J. Physiol. (Lond.)* **448,** 189–209 (1992).
6. Boland, L. M. & Bean, B. P. *J. Neurosci.* **13,** 516–533 (1993).
7. Birnbaumer, L. *et al. Neuron* **13,** 505–506 (1994).
8. Pragnell, M. *et al. Nature* **368,** 67–70 (1994).
9. De Waard, M. *et al. FEBS Lett.* **380,** 272–276 (1996).
10. Mori, Y. *et al. Nature* **350,** 398–402 (1991).
11. Herlitze, S. *et al. Nature* **380,** 258–262 (1996).
12. Ikeda, S. R. *Nature* **380,** 255–258 (1996).
13. Plummer, M. R., Rittenhouse, A., Kanevsky, M. & Hess, P. *J. Neurosci.* **11,** 2339–2348 (1991).
14. Yassin, M., Zong, S. & Tanabe, T. *Biochem. Biophys. Res. Commun.* **220,** 453–458 (1996).
15. Mintz, I. M. & Bean, B. P. *Neuron* **10,** 889–898 (1993).
16. Bourinet, E., Soong, T. W., Stea, A. & Snutch, T. P. *Proc. Natl Acad. Sci. USA* **93,** 1486–1491 (1996).
17. De Waard, M. *et al. J. Biol. Chem.* **270,** 12056–12064 (1995).
18. Campbell, V. *et al. J. Physiol (Lond.)* **485,** 365–372 (1995).
19. Chen, J. *et al. Science* **268,** 1166–1169 (1995).
20. Delcour, A. H. & Tsien, R. W. *Science* **259,** 980–984 (1993).
21. Bean, B. P. *Nature* **340,** 153–155 (1989).
22. Hirning, L. D., Fox, A. P. & Miller, R. J. *Brain Res.* **532,** 120–130 (1990).
23. Castellano, A., Wei, X., Birnbaumer, L. & Pere-Reyes, E. *J. Biol. Chem.* **268,** 12359–12366 (1993).
24. Olate, J. *et al. FEBS Lett.* **268,** 27–31 (1990).
25. Brown, A. M. & Brinbaumer, L. *Annu. Rev. Physiol.* **52,** 197–213 (1990).
26. Wickman, K. D. *et al. Nature* **368,** 255–257 (1994).
27. Huang, C.-L. *et al. Neuron* **15,** 1133–1143 (1995).
28. Zamponi, G. W., Bourinet, E., Nelson, D., Nargeot, J. & Snutch, T. P. *Nature* **385,** 442–446 (1997).
29. Liu, H. *et al. J. Biol. Chem.* **271,** 13804–13810 (1996).
30. Neer, E. J., Denker, B. M., Thomas, T. C. & Schmidt, C. J. *Methods Enzymol.* **237,** 226–239 (1994).

**Acknowledgements.** M.D.W. and H.L. contributed equally to this study. We thank R. R. Reed, A. G. Gilman, M. I. Simon and N. Gautam for the $G\alpha_0$, $G\beta_1$ and $G\gamma_2$ clones; L. Y. Jan, C.-L. Huang and D. E. Clapham for GIRK1 constructs; and G. Krapivinsky, N. Artemyev, A. Robertson, B. A. Adams and R. Felix for comments on the manuscript. K.P.C. is an investigator of Howard Hughes Medical Institute.

Correspondence and requests for materials should be addressed to K.P.C. (e-mail: kevin-campbell@uiowa.edu).