A $\beta_4$ Isoform-specific Interaction Site in the Carboxyl-terminal Region of the Voltage-dependent Ca$^{2+}$ Channel $\alpha_{1A}$ Subunit

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The voltage-gated calcium channel $\beta$ subunit is a cytoplasmic protein that stimulates activity of the channel-forming subunit, $\alpha$, in several ways. Complementary binding sites on $\alpha_1$ and $\beta$ have been identified that are highly conserved among isoforms of the two subunits, but this interaction alone does not account for all of the functional effects of the $\beta$ subunit. We describe here the characterization in vitro of a second interaction, involving the carboxyl-terminal cytoplasmic domain of $\alpha_{1A}$ and showing specificity for the $\beta_4$ (and to a lesser extent $\beta_{2b}$) isoform. A deletion and chimera approach showed that the carboxyl-terminal region of $\beta_4$ poorly conserved between $\beta$ isoforms, contains the interaction site and plays a role in the regulation of channel inactivation kinetics. This is the first demonstration of a molecular basis for the specificity of functional effects seen for different combinations of these two channel components.

Voltage-dependent calcium channels have been classified into five groups, based on their electrophysiological and pharmacological properties. L-type channels are ubiquitous, present particularly in skeletal and cardiac muscle, where they play an essential role in excitation-contraction coupling. T-type channels are important for cardiac pacemaker activity and the oscillatory activity of several thalamic neurons, while N- and P/Q-type channels are important in the control of neurotransmitter release in the central and peripheral nervous systems, and the role of R-type channels remains unclear. Two of these channels have been purified to homogeneity, the skeletal muscle L-type channel and the brain N-type channel (1, 2). Although these channels differ dramatically in function, their subunit compositions are very similar, the core subunit composition of a high voltage-activated channel consisting of an $\alpha_1$ subunit, the ionic pore of the channel, and two auxiliary subunits, $\beta$ and $\alpha_2\delta$, that confer native biophysical and pharmacological properties to the channel. These subunits are encoded by at least six $\alpha_1$, four $\beta$, and one $\alpha_2\delta$ gene, for which numerous splice variants have been identified (3).

The $\beta$ subunit is a cytoplasmic protein of 52–78 kDa that, when coexpressed with the $\alpha_1$ subunit, results in an increase (of up to 100-fold) in current amplitude, alteration of both the kinetics and voltage dependence of activation and inactivation, and an apparent increase in recognition sites for channel-specific toxins (e.g. see Refs. 4–8). The regulatory effects of $\beta$ vary in importance, depending on the combination of channel subunits studied. Although $\beta$ regulation seems to be highly conserved from $\beta_1$ to $\beta_4$ and on $\alpha_{1S}$ to $\alpha_{1P}$, some important differences between these various isoforms have nevertheless been noted. The different $\beta$ subunits produce consistently different channel inactivation behaviors, $\beta_1$ producing fast inactivation, $\beta_2$ slow channel inactivation, and $\beta_1$ and $\beta_2$ more intermediate behaviors (9–11). The $\beta$ effect also appears to be $\alpha_1$ isoform-dependent; the $\beta$-induced shift in voltage dependence of inactivation has been reported for non-L-type channels, A, B, and E (4, 12), whereas it is absent for L-type channels, S, C, and D (13). Since the interaction between calcium channel subunits is promiscuous, at least for $\alpha_1$ and $\beta$ subunits (11, 14), the heterogeneity of combinations observed so far in two native channel types (N-type (15) and P/Q-type (16)) must be of functional significance in cell biology.

Recent studies have identified complementary interaction domains on the $\alpha_1$ and $\beta$ subunits (17, 18). AID$^1$ (43 subunit interaction domain), a highly conserved region in the cytoplasmic loop between transmembrane domains I and II, interacts with a stoichiometry of 1:1 (11) with BID (8 subunit interaction domain), a 30-residue region in the second conserved domain of the $\beta$ subunit (domain IV in Fig. 5A). AID and BID appear to be essential for the subunit interaction and regulation by $\beta$ subunits (17). Point mutations in AID and BID that disrupt this primary interaction also totally inhibit channel regulation by $\beta$, suggesting that it acts as an important anchoring site, due to its very high affinity (11). Several lines of evidence suggest, however, that, despite its importance in channel regulation, the AID-BID attachment site does not account for all of the regulatory potential of the $\beta$ subunit. The deletion approach used to identify the BID site revealed that it may not carry all the current stimulatory function of $\beta$, the change in inactivation kinetics (17), nor the shift in voltage dependence of inactivation.$^2$ It is also interesting that BID represents only 30 residues in a region that shows 78% identity between $\beta$ subunits over 200 residues and that, in addition, toward the amnio-terminal of $\beta$ there exists another highly conserved region (65% identity, over more than 100 residues) (17). The high level of sequence conservation is indicative of evolutionary constraint, suggesting that these regions are of functional importance. The remaining three less conserved domains (I, III, and V) undergo splicing and may also be functionally relevant to $\beta$-specific changes in inactivation as suggested by several studies (19, 20).

Viewed together, the inability of BID to account for all of the

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‡ Supported by an INSERM postdoctoral fellowship (Poste Vert).

§ An investigator of the Howard Hughes Medical Institute.

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1 The abbreviations used are: AID, $\alpha_1$ subunit interaction domain; BID, $\beta$ subunit interaction domain; PCR, polymerase chain reaction; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis.

2 M. De Waard, unpublished data.
**EXPERIMENTAL PROCEDURES**

**Preparation of Fusion Proteins—** Regions of the rabbit brain $\alpha_{1A}$ cDNA (BI-2) (21) corresponding to residues 1889–2126, 2090–2424, 2275–2424, 2070–2196, 2120–2196, and 2197–2275 were amplified by PCR, and, with the aid of BamHI and EcoRI or XbaI restriction sites included in the primers, subcloned into these sites in pGEX2TK or pGEXKG (Pharmacia Biotech Inc.). The resulting recombinant plasmids were expressed in *Escherichia coli* BL21, and GST fusion proteins were purified as described previously (11). The newly purified fusion proteins are referred to as, for example, GST-1889–2126, GST alone and a GST fusion protein of the AID region of $\alpha_{1A}$ (11) were prepared in the same way.

**In Vitro Translation of $\beta$ Subunits—** $\beta$ subunit cDNA clones used were rat $\beta_4$ (L11453), rabbit heart $\beta_4$ (X64297), rabbit heart $\beta_4$ (M88751), and rat brain $\beta_4$ (A45982). Truncated derivatives of $\beta_4$ were constructed by PCR amplification of the corresponding regions of cDNA and subcloning into pCDNA3, using HindIII and BamHI sites (added to the PCR primers), with the addition of a Kozak (22) sequence and initiation codon (ACCATGG) or termination codon (TGA) as necessary. For construction of a chimera between $\beta_4$ subunits, two primers, each with the appropriate restriction sites included in the primers, were synthesized and subcloned into pGEX2TK, using HinIII and subcloning into pCDNA3, using restriction sites included in the primers, subcloned into these sites in pGEX2TK or pGEXKG (Pharmacia Biotech Inc.). The resulting recombinant plasmids were expressed in *Escherichia coli* BL21, and GST fusion proteins were purified as described previously (11). The newly purified fusion proteins are referred to as, for example, GST-1889–2126, GST alone and a GST fusion protein of the AID region of $\alpha_{1A}$ (11) were prepared in the same way.

**Binding Assays—** Purified GST fusion proteins were coupled to glutathione-agarose beads (Sigma) by incubation for 30 min, before addition of the mixture (approximately 500 µl total concentration). Binding assays were carried out in a final volume of 200 µl, in Tris-buffered saline (0.1% Triton X-100, 25 mM Tris, 150 mM NaCl, pH 7.4), at 4 °C, for 6 h, unless otherwise stated. Beads were washed four times in binding buffer, and then analyzed either by SDS-PAGE and autoradiography or by scintillation counting.

A peptide corresponding to the AID site of $\alpha_{1A}$ (RQQIERELNGYM) was dissolved in phosphate-buffered saline (154 mM NaCl, 40 mM Na$_2$HPO$_4$, 11.5 mM NaH$_2$PO$_4$, pH 7.4) at 500 µM and added to binding reactions of 100 µM final concentration. Since addition of phosphate-buffered saline to the binding reactions introduced slight changes in binding affinity, an equal volume of phosphate-buffered saline was added to control reactions.

**Electrophysiological Recordings—** Stage V and VI Xenopus oocytes were injected with BI-2-specific mRNA (400 ng/µl) in combination with $\beta_4$-specific mRNA (100 ng/µl) or truncated ΔC mRNA (100 ng/µl). Cells were incubated for 3 days in defined nutrient oocyte medium as described previously (11). Whole cell recordings were performed at room temperature (22–24 °C) using the two-microelectrode voltage clamp configuration of a GeneClamp amplifier (Axon Instruments, Foster City, CA). The extracellular recording solution was of the following composition (in mM): BaCl$_2$ 40, NaOH 50, KCl 3, HEPES 5, niflumic acid 1, pH 7.4 with methane sulfonic acid. Electrodes filled with 500 mM cesium acetate, 10 mM EGTA, 3 mM KCl, and 10 mM HEPES, pH 7.2, had resistances comprised between 0.5 and 2 megohms. The bath solution was filtered at 1 kHz, leak-subtracted on-line by a P6 protocol, and sampled at 2–4 kHz. Data were analyzed using pCLAMP version 6.02 (Axon Instruments). All values are mean ± S.D.

**RESULTS**

Purified GST fusion proteins carrying amino acids 1889–2126 (GST-1889–2126)$\alpha_4$ and 2090–2424 (GST-2090–2424)$\alpha_4$ of the $\alpha_{1A}$ subunit carboxy-terminal region were coupled to glutathione-agarose beads at a concentration of 5 µM and assayed for interaction with a 35S-labeled in vitro translated rat $\beta_4$ subunit (Fig. 1). GST-1889–2126$\alpha_4$ showed no significant binding, as seen for the control GST protein alone, while GST-2090–2424$\alpha_4$ showed a significant level of interaction, comparable to that seen for a 500 nM, saturating (11) concentration of a GST fusion protein carrying the AID region of $\alpha_{1A}$ (GSTM-AID)$\alpha_4$.

Analysis of the binding of various concentrations of GST-2090–2424$\alpha_4$ to 35S-$\beta_4$ (Fig. 2A) demonstrates that binding is saturable; specific binding appears at about 25 nM and saturates at 500 nM. Comparison of the saturation curve of GST-2090–2424$\alpha_4$ binding to $\beta_4$ to the dose-response curve of GST-AID$\alpha_4$ reveals a dissociation constant ($K_d$) of 93 nM for GST-2090–2424$\alpha_4$, which is an approximately 30-fold lower affinity compared with the GST-AID$\alpha_4$-$\beta_4$ interaction. Association kinetics (Fig. 2B) are relatively slow compared with those previously seen for the AID-BID interaction (11), with a half-time of association of approximately 120 min at 5 µM compared with 20 min at 500 nM GST-AID$\alpha_4$.

We next tested whether the AID-BID interaction had any effect on the interaction between GST-2090–2424$\alpha_4$ and $\beta_4$. In the presence of a 21-amino acid synthetic peptide containing...
the AID sequence of α1A, specific binding of β4 to GST-AID$_\alpha$ is diminished by over 90%, demonstrating the effectiveness of the peptide. The same peptide had no significant effect on the affinity of GST-2090–2424A subunit (data not shown), indicating that the BID region is not implicated in this interaction. The peptide did not modify the maximum binding of GST-2090–2424A, indicating that, at least in vitro, binding of AID to the β subunit does not induce conformational changes capable of favoring (or indeed disfavoring) this interaction. This was further investigated by analyzing the effects of AID$_\alpha$ peptide on the binding to β4 at various concentrations of GST-2090–2424A (Fig. 2D). The data show that the peptide also had no significant effect on the affinity of β4 for GST-2090–2424A.

To identify the region of the carboxyl-terminal domain that interacts with β4, we constructed a series of smaller GST fusion proteins encoding smaller fragments of this region (Fig. 3A) and compared their binding to 35S-labeled β4 (Fig. 3B). Within the region from residues 2120 to the carboxyl terminus of the molecule, a whole series of subcloned fragments maintained an ability to interact with 35S-β4. Further investigations suggested, however, that these interactions occur with a weaker affinity than GST-2090–2424A. For example, we found a $K_d$ of 225 nM for GST-2070–2196A binding to β4 (data not shown), i.e. 2-fold lower. These data indicate that a series of “microsites” are responsible for the binding activity of the α1A carboxyl terminus, perhaps together forming a binding pocket, although dependence on overall conformation of the binding domain appears to be limited. This further contrasts with the β interaction to AID, which relies on only three crucial AID residues (14).

All four β subunit isoforms show a fairly similar affinity for AID. However, the functional effects of coexpression of these isoforms vary considerably and also depend on the α1 subunit tested. Functional differences among β subunits may be a reflection of the differing capacities of β isoforms to form secondary interactions with the α1 subunit concerned. We therefore tested whether the interaction observed between β4 and GST-2090–2424A also existed for other β subunits translated in vitro. Fig. 4 shows a comparison of binding of 35S-β subunits to three different concentrations of GST-2090–2424A to GST alone, and to a GST fusion protein expressing AID$_\alpha$, at a concentration expected to yield maximal binding. β4 interacts with GST-2090–2424A with a high affinity, showing maximal binding at 1 µM fusion protein concentration, β4 binds with a much lower affinity, showing only limited binding at 10 µM fusion protein concentration, while binding of β4 and β1b is insignificant even at this concentration of fusion protein.

The differences in interaction affinity observed for different β subunits and the fact that the main regions of sequence divergence among β subunits are the carboxyl- and amino-
terminal regions (Fig. 5A) suggested that one of these regions was responsible for the interaction. We investigated this possibility (Fig. 5B) by deleting either or both regions from the \( \beta_4 \) cDNA. We assayed the capacity of the resulting in vitro translated proteins to bind to two fusion proteins, GST-2070–2275 \( \alpha \) and GST-2275–2424 \( \alpha \), which represent approximately the two halves of the region of \( \alpha_{1A} \) under investigation (Fig. 2B). Deletion of the amino-terminal 48 amino acids of \( \beta_4 \) had no effect on the binding of GST-2070–2275 \( \alpha \) and GST-2275–2424 \( \alpha \). In contrast, deletion of the carboxyl-terminal 109 amino acids of \( \beta_4 \) drastically interferes with its capacity to interact with either fusion proteins. Residual weak binding of both fusion proteins seemed to be present. To check whether this residual binding was due to the amino terminus, we tested the binding of these fusion proteins to the double mutant \( \beta_4^{\Delta N} \). The results show that there was no difference between \( \beta_4^{\Delta C} \) and \( \beta_4^{\Delta N,C} \), confirming the absence of a binding function for the amino terminus. The importance of the carboxyl-terminal region was confirmed by constructing a chimera in which the carboxyl-terminal region of \( \beta_3 \) was replaced by the corresponding region of \( \beta_4 \) (Fig. 5B). The inability of \( \beta_3 \) to interact with either \( \alpha_{1A} \) fusion protein was successfully “rescued” by replacement of this region, resulting in a binding capacity approaching that of the full-length \( \beta_4 \) subunit.

Interestingly, the results obtained with the full-length, truncated, and chimera \( \beta_4 \) were very similar for the two fusion proteins assayed. This further suggests that the carboxyl ter-
**α_{1A}β_4 Interaction Site**

**DISCUSSION**

We describe here the identification of a new interaction site between the α_{1A} calcium channel subunit and the β_4 subunit. The interaction is of low affinity compared with that between the AID site on the I-II cytoplasmic loop of α subunits and the BID site of β subunits. This and the fact that mutations in AID or BID disrupt the interaction between the two subunits in expression experiments (17, 18) suggest that such an interaction would be of a secondary nature, dependent on formation of the initial AID-BID interaction to bring the interaction sites into close proximity, or to introduce conformational constraints that favor the interaction. The alternative possibility, that two
β subunits would be associated to a single α1 (e.g. as proposed by Tareilus et al. (20)), seems unlikely for several reasons. First, the molar ratio between α1 and β subunits is 1:1 in purified channels (2, 23). Second, studies of the AID-BID interaction revealed that β subunits with affinities lower than 100 nM for AID do not associate with α1 upon coexpression in oocytes (14), although, of course, it cannot be ruled out that in native conformation the affinity between the two carboxyl-terminal sequences is higher than that predicted from *in vitro* experience. Third, and most important, expression of β subunits containing disruptive BID mutations fail to modify channel properties, ruling out additional interactions in these conditions.

The carboxyl-terminal tails of α1 subunits play various roles in channel function. In the α1c subunit, deletion of the distal regions of the carboxyl terminus results in increased channel opening probability (24), and the more proximal EF-hand domain plays a role in Ca2+-induced inactivation (25). The carboxyl-terminal tail is also known to undergo post-translational modifications in the form of phosphorylation and proteolysis, in some cases essential to channel function (26). Interestingly, there are many phosphorylation sites present in both the α1A carboxyl-terminal and β4 carboxyl-terminal sequence that may be of functional relevance. In β4, several of these phosphorylation sites are unique to this subunit. These data point to the functional importance of this region in channel regulation and may also provide the key to the main function of the subunit interaction site we describe here.

We show that, at least *in vitro*, β4 shows a much greater affinity for the carboxyl-terminal region of α1A than does β2a, while no interaction is detected for β1b or β3. These differences in affinity suggest a functional significance that may help to explain the differences in functional effects seen for different combinations of α1 and β subunits. In light of this, it is interesting that β4 is coexpressed in the same brain regions as is α1A, particularly in the cerebellum (21, 27), and is the major β subunit associated with the α1A subunit in the P/Q channel-type (16). A similar interaction has recently been reported between an α1B splice variant and β1a (20). It will be interesting to see whether this interaction also displays a specificity for a particular subset of β subunits.

The advantage of a form of subunit specificity in the α1-β association remains largely to be investigated. Our data suggest that a secondary interaction site that favors β4 association to BI-2 rather than β3, the other predominant β in brain (28), could be determinant in underlying subtle kinetic differences induced by the various β subunits. Besides obvious functional differences, specific α1-β associations may be determinant in various aspects of channel biosynthesis such as channel targeting. Brice et al. (29) and Chien et al. (30) have indeed demonstrated that β subunits are crucial to cell surface localization of α1.

Alternatively, it is possible that the carboxyl-terminal sequences of both subunits contribute to the process of channel clustering known to occur in voltage-dependent calcium channels, with the carboxyl-terminal sequence of the β subunit interacting with the carboxyl terminus of an α1 subunit other than the one that it is attached to via its BID site. Channel clustering is known to occur in various ion channels and has best been characterized for the shaker K+ channel for which clustering is produced by the PSD-95 proteins (31). Calcium channel clustering is probably induced by a third party protein because the carboxyl-terminal interaction described herein may be of insufficient affinity to be the primary cause of such a clustering behavior.

Besides the existence of separate genes encoding Ca2+ channel subunits, alternative splicing is another process by which diversity can be introduced. The functional significance of alternative splicing in Ca2+ channel subunits is still largely unknown. Splicing can occur in several regions of the α1 protein, including the amino terminus (32), the IS6 transmembrane sequence, the cytoplasmic linkers between domain I and II and between domain II and III (33), transmembrane segment IVS3, and the carboxyl-terminal sequences (21, 33). Particularly pertinent to the data presented here is the existence of two splice alternatives in α1A described by Mori et al. (21) that result in an almost total divergence of sequence from residue 2230 onward, i.e. concerning the majority of the sequence responsible for the interaction studied here. It is therefore likely that the carboxyl terminus of the other α1 splice variant, BI-1, may not interact with β4. Generally, it remains to be seen whether the splicing occurring in the carboxyl-terminal tail of the α1 subunit plays a role in the specificity of the secondary α1-β interaction. Two case scenarios can be discussed. The first is that any deletion or insertion may modify the regulatory input of the associated β subunit at that location without modifying the type of β subunit associated. The second possibility is that splicing modifies the α1-β interaction specificity and that it favors the association of another type of β subunit, presumably to specify a different membrane targeting of the channel. In the case of α1A, it would be interesting to see whether the carboxyl terminus of BI-1 interacts with β4, the other predominant β subunit known to interact with α1A in the brain (16). Our data also shed new light on data obtained by other groups that report a lack of impact of α1A carboxyl-terminal alternative splicing on β subunit regulation (for instance in α1s, (34), but see Soldatov et al. (35)) and suggest that negative data may well be due to the use of an inappropriate combination of α1 and β subunits.

It is becoming increasingly obvious that a wide range of neurological and motor diseases result from mutations in the α1 or β subunits, and a number of these are particularly relevant to the data we have presented here. In mice, the leaner phenotype, similar to absence epilepsy, has been attributed to a mutation in a splice donor consensus sequence of α1A, resulting in aberrant splicing and therefore degeneration of the coding sequence corresponding to either residue 2026 or 2072 onward of the protein we have used (36), i.e. corresponding well to the region identified as interacting with β4. In humans, a severe form of ataxia has been shown to be associated with a 5-base pair insertion close to the stop codon that extends the translated sequence to include a glutamine repeat of variable size (37), which would presumably entail conformational changes to this region. Concerning the β4 subunit, its overall functional importance has been shown by the assignment of a lethargic phenotype in mice to a deletion of about 60% of the protein (38), although such a drastic alteration is likely to completely inactivate the β4 subunit and at least leads to the loss of the BID in addition to the carboxyl-terminal site.

Finally, the data obtained here contribute to our understanding of the general organization of high voltage-activated calcium channels. The existence of several sites of interaction between the two channel components highlights the utility of an *in vitro* approach using fusion proteins, since it enables us to study such interactions individually and therefore to assess their functional impact and to gradually dissect the conformational basis of the relationship between the two subunits. It is not always possible, however, to extrapolate directly between the situation *in vitro* and that *in vivo*. For example, our inability to demonstrate an effect of AID-β association on the affinity of the interaction between the carboxyl termini of α1A and β4 probably reflects the absence of the remainder of the α1A mol-
ecule and therefore a loss of integrity of the conformational constraints existing in native channels which may determine the overall manner in which the two subunits interact.

Acknowledgments—We thank Dr. H. Liu for the GST-2090–2424 A construct and for helpful comments on the manuscript, Dr. V. Scott for the GST-1889–2126 A construct, and Dr. R. Felix for reading the manuscript.

REFERENCES
1. Flockerzi, V., Oeken, H.-J., Hofmann, F., Pelzer, D., Cavalie, A., and Trautwein, W. (1986) Nature 323, 66–68
2. Witcher, D. R., De Waard, M., Sakamoto, J., Franzini-Armstrong, C., Pragnell, M., Kahl, S. D., and Campbell, K. P. (1995) Science 261, 486–489
3. Snutch, T. P., and Reiner, P. B. (1992) Curr. Opin. Neurobiol. 2, 247–253
4. Stea, A., Dubel, S. J., Pragnell, M., Leonard, J. P., Campbell, K. P., and Snutch, T. P. (1993) Neuropharmacology 32, 1103–1116
5. Castellano, A., Wei, X., Birnbaumer, L., and Perez-Reyes, E. (1993) J. Biol. Chem. 268, 12359–12366
6. Castellano, A., Wei, X., Birnbaumer, L., and Perez-Reyes, E. (1993) J. Biol. Chem. 268, 12359–12366
7. Lacerda, A. E., Kim, H. S., Ruth, P., Perez-Reyes, E., Flockerzi, V., Hofmann, F., Birnbaumer, L., and Brown, A. M. (1991) Nature 352, 527–530
8. Wei, X. Y., Perez-Reyes, E., Lacerda, A. E., Schuster, G., Brown, A. M., and Birnbaumer, L. (1991) J. Biol. Chem. 266, 21943–21947
9. Perez-Reyes, E., Castellano, A., Kim, H. S., Bertrand, P., Baggett, K., Lacerda, A. E., Wei, X. Y., and Birnbaumer, L. (1992) J. Biol. Chem. 267, 1792–1797
10. Ellinor, P. T., Zhang, J.-F., Randall, A. D., Zhou, M., Schwartz, T. L., Tsien, R. W., and Horne, W. A. (1993) Nature 363, 455–458
11. De Waard, M., and Campbell, K. P. (1995) J. Physiol. 485, 619–634
12. Soong, T. W., Stea, A., Hodson, C. D., Dubel, S. J., Vincent, S. R., and Snutch, T. P. (1993) Science 260, 1136–1139
13. Tomlinson, W. J., Stea, A., Bourinet, E., Charnet, P., Nargeot, J., and Snutch, T. P. (1993) Neuropharmacology 32, 1117–1126
14. De Waard, M., Scott, V. E. S., Pragnell, M., and Campbell, K. P. (1996) FEBS Lett. 380, 272–276
15. Scott, V. E. S., De Waard, M., Liu, H., Gurnett, C. A., Venzke, D. P., Lennon, V. A., and Campbell, K. P. (1996) J. Biol. Chem. 271, 3297–3322
16. Liu, H. Y., De Waard, M., Scott, V. E. S., Gurnett, C. A., Lennon, V. A., and Campbell, K. P. (1996) J. Biol. Chem. 271, 13804–13810
17. De Waard, M., Pragnell, M., and Campbell, K. P. (1994) Neuron 13, 495–503
18. Pragnell, M., De Waard, M., Mori, Y., Tanabe, T., Snutch, T. P., and Campbell, K. P. (1994) Nature 368, 67–70
19. Olcese, R., Qin, N., Schneider, T., Neely, A., Wei, X., Stefani, E., and Birnbaumer, L. (1994) Neuron 13, 1433–1438
20. Tareilus, R., Roux, M., Qin, N., Olcese, R., Zhou, J., Stefani, E., and Birnbaumer, L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1703–1708
21. Mori, Y., Friedrich, T., Kim, M.-S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furutachi, T., Mikoshi, K., Imoto, K., Tanabe, T., and Numa, S. (1991) Nature 356, 398–402
22. Karak, M. (1986) Cell 44, 263–292
23. Jay, S. D., Sharp, A. H., Kahl, S. D., Vedvick, T. S., Harpold, M. M., and Campbell, K. P. (1991) J. Biol. Chem. 266, 3287–3295
24. Wei, X., Neely, A., Lacerda, A. E., Olcese, R., Stefani, E., Perez-Reyes, E., and Birnbaumer, L. (1994) J. Biol. Chem. 269, 1635–1640
25. De Leon, M., Wang, Y., Jones, L., Perez-Reyes, E., Wei, X., Soong, T. W., Snutch, T. P., and Yue, D. T. (1995) Science 270, 1502–1506
26. Hell, J. W., Westenbroek, R. E., Breeze, L. J., Wang, K. K., Chavkin, C., and Catterall, W. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3362–3367
27. Ludwig, A., Flockerzi, V., and Hofmann, F. (1997) J. Neurosci. 17, 1339–1349
28. Witcher, D. R., De Waard, M., Liu, H., Pragnell, M., and Campbell, K. P. (1995) J. Biol. Chem. 270, 18098–18093
29. Brice, N. L., Berrow, N. S., Campbell, V., Page, K. M., Brickley, K., Tedder, I., and Dolphin, A. C. (1997) Eur. J. Neurosci. 9, 749–759
30. Chien, A. J., Zhao, X., Shirokov, R. E., Puri, T. S., Chang, C. F., Sun, D., Rios, E., and Hosey, M. M. (1995) J. Biol. Chem. 270, 30036–30044
31. Hseuh, Y., Kim, E., and Sheng, M. (1997) Neuron 18, 803–814
32. Marubio, J. M., Roonfeld, M., Dasgupta, S., Miller, R. J., and Philipson, L. H. (1996) Recept. Channels 4, 243–251
33. Hofmann, F. (1994) Annu. Rev. Neurosci. 17, 399–418
34. Klockner, U., Mikala, G., Eisfeld, J., Iles, D. E., Strobeck, M., Mershon, J. L., Schwartz, A., and Varadi, G. (1997) Am. J. Physiol. 273, H1372–H1381
35. Soldatov, N. M., Zulke, R. D., Bouar, A., and Reuter, H. (1997) J. Biol. Chem. 272, 3565–3566
36. Fletcher, C. F., Latz, C. M., O'Sullivan, T. N., Shaughnessy, J. D. Jr., Hawkes, R., Frankel, W. N., Copeland, N. G., and Jenkins, N. A. (1996) Cell 87, 607–617
37. Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D. W., Amos, C., Dobyns, W. B., Subramony, S. H., Zoghbi, H. Y., and& Lee, C. C. (1997) Nat. Genet. 15, 62–69
38. Burgess, D. L., Jones, J. M., Meisler, M. H., and Noebels, J. L. (1997) Cell 88, 385–392