Korean J Biol Sci 1: 589-594, 1997

Coordinated Spatial and Temporal Expression of Voltage-sensitive Calcium Channel α_{1A} and β_4 Subunit mRNAs in Rat Cerebellum

Dong Sun Kim* and Hemin Chin[1]

Department of Anatomy, School of Medicine, Kyungpook National University, Taegu 702-701, Korea;
[1]Laboratory of Neurochemistry, National Institute of Neurological Disorder and Stroke, NIH, Bethesda, MD 20892, USA

Key Words:
Voltage-sensitive calcium channel
Multisubunit complex
Cerebellum
Development
RT-PCR
Northern blot analysis
RNase protection assay

The neuronal voltage-sensitive calcium channels (VSCCs) are multisubunit complexes consisting of α_1, α_2-δ and β subunits. Heterologous expression and biochemical studies have shown that the activity of VSCCs is regulated by their β subunits in a β subunit isoform-specific manner. To elucidate the β subunit identity of the P/Q-type calcium channel encoded by an α_{1A} subunit, which is exclusively expressed in the Purkinje and granule cell of the cerebellum, we have examined the spatial and temporal expression patterns of β subunits and compared them with those of α_{1A} subunit in the developing rat cerebellum. Reverse transcriptase- polymerase chain reaction (RT-PCR) and Northern blot analysis have shown that β_4 subunit mRNA was prominently expressed in the cerebellum and much more abundant than any other distinct β subunits. RNase protection assay has further demonstrated that the expression of α_{1A} and β_4 subunits increased during cerebellar development, while the amount of β_2 and β_3 mRNAs did not significantly change. In addition, a β_4 transcript was present in cultured cerebellar granule cells, but not in astrocyte cells, and the level of β_4 mRNA expression increased gradually *in vitro* seen as *in vivo*. Based on the spatial and temporal expression patterns of β_4 subunit, we conclude that β_4 may predominantly associate, but probably not exclusively, with the α_{1A} subunit in rat cerebellar granule cells.

In the nervous system, voltage-sensitive calcium channels (VSCCs) play important roles in membrane excitability, neurotransmitter release, the pattern of neuronal firing, and gene expression (Tsien and Tsien, 1990; Ghosh and Greenberg, 1995). These channels have been classified by electrophysiological and pharmacological properties as six major groups (L, N, P/Q, T, and R types) (Bertolino and Llinas, 1992; Snutch and Reiner, 1992; Reuter, 1996). VSCCs are heteroligomeric complexes containing the channel-forming α_1 subunit and the auxiliary β and α_2-δ subunits serving regulatory roles. Molecular cloning has identified six different α_1 genes (S, A, B, C, D, and E), four different β genes (1, 2, 3, and 4), and one α_2-δ gene (reviewed in Hofmann et al., 1994). Multiple splice variants for α_1, β, α_2-δ genes have also been identified (reviewed in Dunlap et al., 1995), indicating that the potential for combinatorial complexity at the structural level is great.

Coexpression studies of cloned subunits in *Xenopus* oocytes revealed that α_1 subunit can reconstitute with any one of the four β subunits to yield a functional channel. Thus, the association of various β subunits with an α_1 subunit increases the heterogeneity of Ca^{2+} channels providing the molecular bases for functional diversity of neuronal Ca^{2+} channels. For example, Ca^{2+} currents carried by α_{1E} and α_{1C} inactivate faster when coexpressed with β_3 than with β_2 (Hullin et al., 1992; Ellinor et al., 1993). β_1, β_3, and β_4, but not β_2, are permissive for voltage-dependent facilitation of Ca^{2+} channels formed by α_{1C} (Welling et al., 1995). β_3 and β_1 subunits confer slightly different pharmacological properties to L-type channels (Cens et al., 1996). Furthermore, recent biochemical studies indicate that multiple β subunit isoforms can associate to different extents with neuronal N-, P/Q-, and L-type channels (Scott et al., 1996; Liu et al., 1996; Pichler et al., 1997). The α_{1B} subunit of the N-type Ca^{2+} channel in the brain is associated with three different β subunits, β_{1b}, β_3, and β_4 (Scott et al., 1996). Similarly, the high affinity ω-conotoxin (CTX) MVIIC receptor is composed of at least an α_{1A}, α_2-δ, and any one of the four different β subunits in the rabbit brain (Liu et al., 1996). An immunoprecipitation experiment in the brain has revealed that the majority of L-type channels contain either β_3 or β_4 subunit, whereas β_{1b} and β_2 are present in a smaller fraction of complexes (Pichler et al., 1997).

* To whom correspondence should be addressed.
Tel: 82-53-420-6921, Fax: 82-53-427-1468

These findings raise a possibility that an α_1 subunit could associate with the different type of β subunits in each neuron at a defined cellular localization and confer an unique kinetic properties.

In the present study, we have determined the molecular identity of the β subunit associated with the P/Q-current encoded by α_{1A} subunit in the cerebellum using Northern blot analysis and RNase protection assay. Our results indicate that β_4 subunit contributes to P/Q-type channel in the cerebellum.

Materials and Methods

Primary cell culture

Cerebellar primary cultures were prepared from 8-day postnatal (P8) Sprague-Dawley rats as previously described (Levi et al., 1984). Dissociated cells were plated in 100-mm diameter plastic dishes precoated with poly-L-lysine at density of 2.5 or 1.2×10^5 cells/cm^2 (neuronal and astrocytic cultures, respectively). Cells were grown in basal Eagles medium (neuronal cultures) or D-valine-containing minimal essential medium (astrocyte cultures), containing 10% fetal calf serum, 2 mM glutamine, and 100 mg/ml gentamicine. The medium for neuronal cultures also contained 25 mM KCl (final concentration). After 16-20 h, 10 mM cytosine arabinoside was added to the neuronal cultures to prevent the replication of non-neuronal cells. Cerebellar neuronal cultures comprised more than 95% granule cells, whereas astrocyte cultures comprised more than 95% glial fibriliary acid protein (GFAP)-positive cells and a small population of galactocerebroside-positive oligodendrocytes (2-3%).

Northern blot analysis

Total RNA was isolated from adult (P21) rat brain regions and cerebella at different developmental stages, from embryonic age (E17) to 3-week old rats, as well as primary cultured cells using the RNAZol solution (Tel-Test B, Inc.). Messenger RNA was purified using an oligotex mRNA kit (Qiagen, Inc.). RNA samples (15 mg/lane for total RNA, 1 mg/lane of mRNA) were electrophoresed on an 1.2% agarose-formaldehyde denaturing gel and transferred by capillary blotting to a nytran membrane. Blots were prehybridized at 42℃ for 1 h in ExpressHyb solution (Clontech Lab.) and hybridized at 42℃ for 2 h with ^{32}P labeled 380-bp PCR fragment of the rat β_4 subunit (corresponding to the nucleotide residue number 1380-1760 as in GenBank accession no. L02315). Following hybridization the blot was rinsed in solution I (2X SSC and 0.05% SDS) at room temperature for 15 min, washed in solution II (0.1X SSC and 0.1% SDS) at 50℃ for 40 min, and finally exposed to X-ray film at -80℃ for 3 days.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

First strand cDNA was synthesized from 1 μg of total RNA of adult rat cerebellum, which had been treated with RNase-free DNase, by using SuperScript preamplification kit (Gibco BRL). Subsequently, this product was amplified with specific oligonucleotide primers of the various β subunits by Hotstart PCR for 28 cycles. Each cycle consisted of denaturation at 95℃ for 1 min, annealing at 60℃ for 1 min, and elongation at 72℃ for 1 min and final extension was carried out at 72℃ for 5 min. The amplified products were separated on an 1.2% agarose gel and visualized by ethidium bromide staining. Primer sequences used were the same as those previously described (Chick et al., 1997).

RNase protection assay (RPA)

RPA was carried out with the RPA kit II (Ambion) using the streamlined procedure. One mg mRNA isolated from different stages of rat cerebellum and 1×10^5 cpm of ^{32}P-labeled antisense probes mixed with hybridization solution. The mixture was heated at 90℃ for 5 min and incubated at 43℃ for 16 h. The hybridization mixture was diluted with RNase digestion buffer containing 5 mg/ml RNase A and 100 units/ml RNase T1 and continuously incubated for 30 min at 37℃. After digestion, the protected fragment was precipitated in the presence of RNase inactivation-precipitation mixture and the pellet was dissolved in gel-loading buffer and then heated at 90℃ for 5 min. Protected bands were separated on 6% polyacrylamide-8 M urea gels by electrophoresis and exposed to X-ray film. Probes were also hybridized to 10 mg of yeast tRNA as a negative control. The rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe was used as a RNA loading internal control. All riboprobes were generated from each template by T7 RNA polymerase; 730-bp α_{1A} (nucleotides 5907 to 6637), 309-bp β_2 (nucleotides 1494 to 1803), 251-bp β_3 (nucleotides 2015 to 2265), and 284-bp β_4 (nucleotides 1416 to 1700).

Results

Spatial distribution of β_4 subunit expression

For identification of the β subunit associated with the P/Q-current encoded by α_{1A} subunit, which is abundantly expressed in the cerebellum, we have initially examined which one of the four β subunit mRNAs is highly expressed in the rat cerebellum by RT-PCR. Electrophoresis of RT-PCR products on agarose gel showed that all β subunits were present in cerebellum in that each set of specific β subunit primers yielded the amplified products of expected sizes. Subsequently, their identity were confirmed by DNA sequencing (not shown). The relative abundance of each isoform was $\beta_4 >> \beta_2 > \beta_1 = \beta_3$ (Fig. 1A). Our observation is consistent with a recent biochemical analysis showing that the high affinity ω-conotoxin MVIIC receptors in the rabbit brain contain diverse β subunits in the following rank

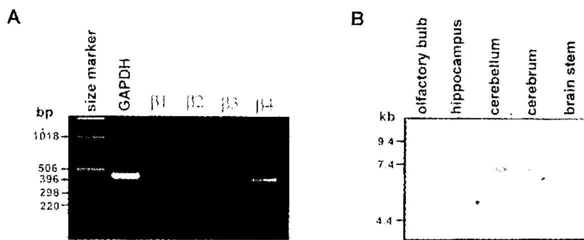

Fig. 1. The β_4 subunit mRNA is the most abundant message in the adult rat cerebellum. A, RT-PCR analysis of all β subunit mRNA expression in the adult (P21) rat cerebellum. Amplified products were analyzed on 1.2% agarose gel. Relative to the DNA size marker, the amplified DNA appears at the positions corresponding to the predicted base-pair lengths of 221, 691, 600, and 379 bp for β_1, β_2, β_3, and β_4 subunit, respectively. B, Northern blot analysis in rat brain regions. Fifteen mg of total RNAs isolated from various brain regions were hybridized with the radiolabeled PCR fragment of β_4 subunit as indicated in *Materials and Methods.*

order, $\beta_4 > \beta_3 >> \beta_1 = \beta_2$ (Liu et al., 1996). Regional expression of β_4 subunit within the brain was also examined by Northern blot analysis. A large transcript of approximately 7.0 kb, which is the same as previously reported (Castellano et al., 1993), was detected in all brain regions that were tested (Fig. 1B). The level of β_4 mRNA expression was highest in the cerebellum, followed by the cerebrum, brain stem, hippocampus, and olfactory bulb. Our observation is in good agreement with *in situ* hybridization results reported by Ludwig and colleagues (Ludwig et al., 1997). Thus, the regional expression of β_4 closely resembles that of P/Q-type α_{1A} subunit since they are both predominantly expressed in the cerebellum.

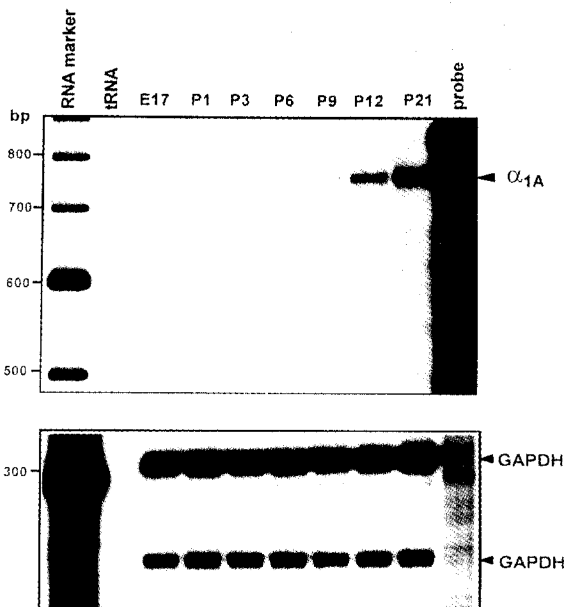

Fig. 2. The developmental expression of α_{1A} subunit in the cerebellum. RPA was carried out using 1 mg of mRNA isolated from various developmental stages of rat cerebellum and 1×10^5 cpm of ^{32}P-labeled α_{1A} and GAPDH probes as described in *Materials and Methods.* Protected fragments to the α_{1A} and GAPDH were indicated by arrowheads at 732 and 310 nucleotides, respectively. The labeled 100-bp DNA ladder was used as molecular weight marker.

Temporal expression of α_{1A}, β_2, β_3, and β_4 subunit during cerebellar development

The relative α_{1A} mRNA levels were determined during cerebellar development from E17 to P21 by RNase protection assay. The protected fragment with the expected size for the α_{1A} transcript was detected as early as E17 in embryonic rat cerebellum, although mRNA amount was very low. The α_{1A} mRNA expression level continued to increase during cerebellar development (Fig. 2). The remarkable increase occurred postnatally at three different stages, P6, P12, and P21. Similarly, the various β subunit mRNA levels were analyzed by the same method. In the case of β_4 subunit, the corresponding fragment was present in E17 stage at an approximately the same level as α_{1A} but continuously increased to its maximal level at P21 (Fig. 3). The time-course of β_4 mRNA expression closely followed that of α_{1A}. However, the level of β_2 and β_3 subunit mRNAs, which were more abundant than α_{1A} and β_4 at E17, did not significantly change, except that the expression level of β_2 subunit mRNA was slightly augmented between P12 and P21 (Fig. 3). None of the protected fragments was detected in yeast tRNA

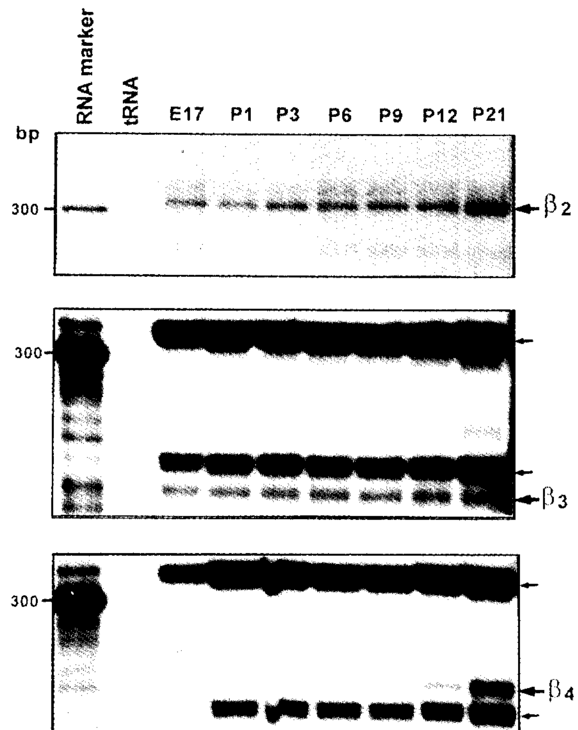

Fig. 3. The temporal expression of various β subunits during the cerebellar development. RPA was carried out using 1 mg of mRNA isolated from various developmental stages of the rat cerebellum and 1×10^5 cpm of ^{32}P-labeled β_2, β_3, β_4 and GAPDH probes as described in *Materials and Methods.* Protected fragments of 310, 251, 284, and 310 bases were shown by β_2, β_3, β_4 (thick arrow) and GAPDH (thin arrow) antisense RNA probes, respectively. The labeled 100-bp DNA ladder was used as molecular weight marker.

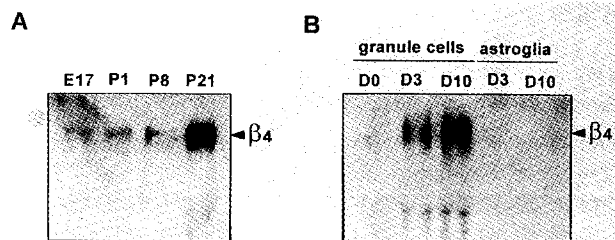

Fig. 4. The β_4 subunit was expressed in granule cells and its level increased during culture period. A, Northern blot analysis of 1 mg of mRNA isolated from different ages of the rat cerebellum. B, Northern blot analysis of 15 mg of total RNA isolated from granule cells and astrocytes which were grown for various periods *in vitro* as starting at postnatal 8-day rat cerebellum. Both blots were probed with ^{32}P-labeled PCR fragment of β_4 subunit as described in *Materials and Methods*. Blots were hybridized with the rat GAPDH mRNA to verify equal loading of RNA per lane.

and two protected fragments of GAPDH mRNA, the completely protected fragment of 310 bp and the smaller partially protected band of approximately 270 bp, were observed. Taken together, these results demonstrated that the developmental expression patterns of α_{1A} and β_4 subunit are apparently in a good agreement.

Expression of β_4 subunit in cultured granule cells

To further confirm the differential expression of β_4 subunit transcript during cerebellar development, the relative level of β_4 mRNA at various times was analyzed using Northern hybridization. The blot showed that an approximately 7.0-kb band was detected and its relative intensity increased during developmental period (Fig. 4A). This finding showed a favorable coincidence to the result obtained from our RNase protection assay. Additionally, we analyzed the time-course of β_4 subunit expression in primary cultures of cerebellar granule cells and astrocytes. The same size transcript was seen in RNA derived from cultured cerebellar granule cells, but not in an astrocytes (Fig. 4B), indicating that the expression of β_4 subunit is limited to neurons. Consistent with the *in vivo* results, its expression in primary culture continued to increase during granule cells growth (Fig. 4B).

Discussion

To determine the β subunit identity of the P/Q-type calcium channel encoded by an α_{1A} subunit, we have investigated the regional distribution of β_4 subunit of VSCCs and temporal expression of α_{1A} and various β subunits in the cerebellum at the mRNA levels. This study should also help to discriminate the subunit composition between P-type and Q-type calcium channel in the cerebellum.

An α_{1A} transcript, although present in all brain regions, is most abundantly expressed in Purkinje cells and granule cells of the cerebellum and its immunoreactivity was also highly concentrated in the cerebellar glomeruli (Starr et al., 1991; Ousley et al., 1994), suggesting that this subunit might encode the P-type calcium channel. However, the properties of channels expressed in oocytes are distinct from those of Purkinje cells with respect to inactivation kinetics and sensitivity to peptide toxins ω-agatoxin IVA (ω-Aga-IVA) and ω-conotoxin MVIIC (ω-CTX-MVIIC) (Sather et al., 1993). They were apparently more closely resemble the Q-type calcium currents detected in cerebellar granule cells (Zhang et al., 1993). These differential properties might be due to distinct splice variants of α_{1A} subunit or an intricate series of post-translational folding and modification. Alternatively, the character of calcium channels encoded by α_{1A} subunit itself was remarkably modified depending on specific β subunits associated with the α_{1A} subunit. Interestingly, coexpression of the β_2 subunit dramatically slowed the inactivation of the α_{1A} current to that typically seen for P-type Ca^{2+} currents detected in cerebellar Purkinje cells, whereas β_1 and β_3 subunit significantly accelerated the inactivation kinetics. However, none of the β subunits did have pronounced effect on the activation of the α_{1A} current and the sensitivity to block by ω-Aga-IVA (Sather et al., 1993; Stea et al., 1994). Also, a recent biochemical study using specific labeling of the high affinity ω-conotoxin MVIIC receptor and a panel of subunit specific antibodies showed that this receptor is composed of at least of an α_{1A}, α_2-δ, and any one of the four different β subunits in the rabbit brain (Liu et al., 1996). These evidences indicate that a given α_1 subunit may associate with different β subunits in a cell-type-dependent manner, thus contributing to the distinct functional properties, such as P- and Q-type. Assembly of α_1 subunits with different β subunits during biosynthesis of calcium channels or exchange of β subunits on pre-existing calcium channels is an important area of future research.

A significant problem that has hindered studies of calcium channel assembly is that the exact subunit composition for channels is unknown. Many Ca^{2+} channels are extremely difficult to purify biochemically, largely because of their low levels of expression. Another factor is the difficulty of obtaining a homogeneous source of Ca^{2+} channel, since most tissues express various subtypes that are composed of different homologous subunit isoforms. This heterogeneity can lead to considerable difficulties in determining subunit composition and stoichiometry. In this regard, the expression of cloned channel subunits in heterologous-expression systems has provided a powerful means for investigation of Ca^{2+} channel assembly. However, careful comparison and correlation of the electrophysiological and pharmacological properties between expressed and native channels showed that the cloned channel subunits gave rise channels with similar, but not identical, properties to those of the native channels, underscoring limitations of the heterologous expression studies. In this sense, our strategy to study and compare the spatial and temporal expression patterns of each subunit at mRNA levels seems to be a good approach

for analysis of the molecular composition of P/Q-type calcium channels *in vivo*.

In the present study, we have demonstrated that β_4 mRNA is the major isoform present in the cerebellum and that α_{1A} and β_4 mRNAs levels increase concomitantly during cerebellar development. Thus, we suggest that β_4 may associate predominantly, but probably not exclusively, with the α_{1A} subunit. In this regard, it is interesting to note that mutation of the β_4 subunit gene (*Cchb4*) is associated with ataxia and seizures in the Lethargic (*lh*) mouse (Burgess et al., 1997) while an absence epilepsy in tottering (*tg*) mice, which is similar to the lethargic phenotypes, is due to calcium channel gene α_{1A} defects (Fletcher et al., 1996). These evidences indicate that *Cchb4* was an excellent candidate gene for the association of α_{1A}. On one hand, the cellular distribution and temporal expression of α_{1A} and β_4 subunit transcripts were analyzed in developing cerebellar sagittal sections by *in situ* hybridization histochemistry. α_{1A} and β_4 mRNAs were detected at E17 and their expression gradually increased during cerebellar development and especially β_4 transcript was intensively present in migrating granule cells in external granular cell layer. In contrast, they were already expressed at E17 and did not significantly change in Purkinje cells of the cerebellum (data not shown). Taken together, we speculate that β_4 subunit may associate with α_{1A} subunit in granule cells to give rise to the Q-type calcium current, which play an important role in regulating synaptic communication for the establishment of internal granule cell layer. However, we can not rule out the possibility that the association between α_{1A} and β_4 subunit might contribute a P-type calcium current, because the pharmacological dissection of multiple types of Ca^{2+} channel currents in the rat cerebellar granule neurons demonstrated that P-type current comprised 11% of the total current (Randall and Tsien, 1995). To test this hypothesis, further studies using electrophysiological and biochemical methods should be undertaken during cerebellar development; (1) whether the current density through Q-type calcium is substantially accelerated?; (2) whether the amplitude of peak current of Q-type channel is increased in granule cells?; (3) whether the number of w-conotoxin MVIIC receptor in cell surface membrane is increased?

Acknowledgements

We thank Hee Jung Cho for his encouragement and valuable advice. This study was supported by a grant from the Medical Research Institute of the Kyungpook National University Hospital (97015).

References

Bertolino M and Llinas RR (1992) The central role of voltage-activated and receptor-operated calcium channels in neuronal cells. *Annu Rev Pharmacol Toxicol* 32: 399-421.

Burgess DL, Jones JM, Meisler MH, and Noebels JL (1997) Mutation of the Ca^{2+} channel β subunit gene *Cchb4* is associated with ataxia and seizures in the lethargic (*lh*) mouse. *Cell* 88: 385-392.

Castellano A, Wei X, Birnbaumer L, and Perez-Reyes E (1993) Cloning and expression of a neuronal calcium channel β subunit. *J Biol Chem* 268: 12359-12366.

Cens T, Mangoni ME, Richard S, Nargeot J, and Charnet P (1996) Coexpression of the beta 2 subunit does not induce voltage-dependent facilitation of the class L-type Ca^{2+} channels. *Pfluegers Arch* 431: 771-774.

Chick CL, Liu Q, Li B, Klein DC, Zylka M, Kim DS, Chin H, Karpinski E, and Ho AK (1997) α_{1D} L-type Ca^{2+}-channel currents: inhibition by a β-adrenergic agonist and pituitary adenylate cyclase-activating polypeptide (PACAP) in rat pinealocytes. *J Neurochem* 68: 1078-1087.

Dunlap K, Luebke JI, and Turner TJ (1995) Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends Neurosci* 18:89-98.

Ellinor PT, Zhang J-F, Randall AD, Zhou M, Schwarz TL, Tsien RW, and Horne WA (1993) Functional expression of a rapidly inactivating neuronal calcium channel. *Nature* 363: 455-458.

Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD, Hawkes R, Frankel WN, Copeland NG, and Jenkins NA (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* 87: 607-617.

Ghosh A and Greenberg ME (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* 268: 239-247.

Hofmann F, Biel M, and Flockerzi V (1994) Molecular basis for Ca^{2+} channel diversity. *Annu Rev Neurosci* 17: 399-418.

Hullin R, Singer-Lahat D, Freichel M, Biel M, Dascal N, Hofmann F, and Flockerzi V (1992) Calcium channel β subunit heterogeneity: functional expression of cloned cDNA from heart, aorta and brain. *EMBO J* 11: 885-890.

Levi G, Aloisi F, Ciootti MT, and Gallo V (1984) Autoradiographic localization and depolarization-induced release of acidic amino acids in differentiating cerebellar granule cell cultures. *Brain Res* 290: 77-86.

Liu H, de Waard M, Scott VES, Gurnett CA, Lennon VA, and Campbell KP (1996) Identification of three subunits of the high affinity ω-conotoxin MVIIC-sensitive Ca^{2+} channel. *J Biol Chem* 271: 13804-13810.

Ludwig A, Flockerzi V, and Hofmann F (1997) Regional expression and cellular localization of the a1 and β subunit of high voltage-activated calcium channels in rat brain. *J Neurosci* 17: 1339-1349.

Ousley AH and Froehner SC (1994) An anti-peptide antibody specific for the class A calcium channel α_1 subunit labels mammalian neuromuscular junction. *Proc Natl Acad Sci USA* 91: 12263-12267.

Pichler M, Cassidt TN, Reimer D, Hasse H, Kraus R, Ostler D, and Striessnig J (1997) β subunit heterogeneity in neuronal L-type Ca^{2+} channels. *J Biol Chem* 272: 13871-13882.

Randall A and Tsien RW (1995) Pharmacological dissection of multiple types of Ca^{2+} channel currents in rat cerebellar granule neurons. *J Neurosci* 15: 2995-3012.

Reuter H (1996) Diversity and function of presynaptic calcium channels in the brain. *Curr Opin Neurobiol* 6: 331-337.

Sather WA, Tanabe T, Zhang JF, Mori Y, Adams ME, and Tsien RW (1993) Distinctive biophysical and pharmacological properties of class A (BI) calcium channel α_1 subunits. *Neuron* 11: 291- 303.

Scott VES, de Waard M, Liu H, Gurneett CA, Venzke DP, Lennon VA, and Campbell KP (1996) β subunit heterogeneity in N-type Ca^{2+} channels. *J Biol Chem* 271: 3207-3212.

Snutch TP and Reiner PB (1992) Ca^{2+} channels: diversity of form and function. *Curr Opin Neurobiol* 2: 247-253.

Starr TVB, Prystay W, and Snutch TP (1991) Primary structure of a calcium channel that is highly expressed in the rat cerebellum. *Proc Natl Acad Sci USA* 88: 5621-5625.

Stea A, Tomlinson WJ, Soong TW, Bourinet E, Dubel SJ, Vincent SR, and Snutch TP (1994) Localization and functional

properties of a rat brain alpha 1A calcium channel reflect similarities to neuronal Q- and P-type channels. *Proc Natl Acad Sci USA* 91: 10576-10580.

Tsien RW and Tsien RY (1990) Calcium channels, stores, and oscillations. *Annu Rev Cell Biol* 6: 715-760.

Welling A, Lacinova L, Donatin K, Ludwig A, Bosse E, Flockerzi V, and Hofmann F (1995) Expression of the L-type calcium channel with two different beta subunits and its modulation by RO40-5967. *Pfluegers Arch* 429: 400-411.

Zhang JF, Randall AD, Ellinor PT, Horne WA, Sather WA, Tanabe T, Schwarz TL, and Tsien RW (1993) Distinctive pharmacology and kinetics of cloned neuronal Ca^{2+} channels and their possible counterparts in mammalian CNS neurons. *Neuropharmacology* 32: 1075-1088.

[Received October 6, 1997; accepted November 15, 1997]