Identification and Differential Subcellular Localization of the Neuronal Class C and Class D L-Type Calcium Channel $\alpha 1$ Subunits

Johannes W. Hell, Ruth E. Westenbroek, Concepcion Warner, Michael K. Ahlijanian, Ward Prystay,* Mary M. Gilbert,* Terry P. Snutch,* and William A. Catterall

Department of Pharmacology, SJ-30, University of Washington, Seattle, Washington 98195; and *Biotechnology Laboratory and Departments of Zoology and Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

Abstract. To identify and localize the protein products of genes encoding distinct L-type calcium channels in central neurons, anti-peptide antibodies specific for the class C and class D $\alpha 1$ subunits were produced. Anti-CNC1 directed against class C immunoprecipitated 75% of the L-type channels solubilized from rat cerebral cortex and hippocampus. Anti-CND1 directed against class D immunoprecipitated only 20% of the L-type calcium channels. Immunoblotting revealed two size forms of the class C L-type $\alpha 1$ subunit, L_{C1} and L_{C2}, and two size forms of the class D L-type $\alpha 1$ subunit, L_{D1} and L_{D2}. The larger isoforms had apparent molecular masses of $\sim$200–210 kD while the smaller isoforms were 180–190 kD, as estimated from electrophoresis in gels polymerized from 5% acrylamide.

Immunocytochemical studies using CNC1 and CND1 antibodies revealed that the $\alpha 1$ subunits of both L-type calcium channel subtypes are localized mainly in neuronal cell bodies and proximal dendrites. Relatively dense labeling was observed at the base of major dendrites in many neurons. Staining in more distal dendritic regions was faint or undetectable with CND1, while a more significant level of staining of distal dendrites was observed with CNC1, particularly in the dentate gyrus and the CA2 and CA3 areas of the hippocampus. Class C calcium channels were concentrated in clusters, while class D calcium channels were generally distributed in the cell surface membrane of cell bodies and proximal dendrites. Our results demonstrate multiple size forms and differential localization of two subtypes of L-type calcium channels in the cell bodies and proximal dendrites of central neurons. The differential localization and multiple size forms may allow these two channel subtypes to participate in distinct aspects of electrical signal integration and intracellular calcium signaling in neuronal cell bodies. The preferential localization of these calcium channels in cell bodies and proximal dendrites implies their involvement in regulation of calcium-dependent functions occurring in those cellular compartments such as protein phosphorylation, enzyme activity, and gene expression.

VOLTAGE-dependent calcium channels play an important role in calcium-regulated neuronal functions including membrane excitability and neurotransmitter release (Miller, 1987). Four different classes of voltage-sensitive calcium channels can be distinguished by their electrophysiological and pharmacological characteristics: T, L, N, and P (Bean, 1989; Llinas et al., 1989; Hess, 1990; Tsien et al., 1991). T-type channels can be activated by small depolarizations and are designated low-threshold calcium channels, whereas stronger depolarization is required to induce the opening of the N, L, and P channel types which are designated high-threshold calcium channels. Dihydropyridines and other organic calcium channel blockers inhibit only L-type calcium channels (for review see Glossmann and Striessnig, 1990). N-type calcium channels are specifically blocked by ω-Conus geographus Toxin GVIA (ω-CgTx-GVIA)[1] (Plummer et al., 1989; Aosaki and Kasai, 1989) and P-type channels by ω-Aga-IV A (Mintz et al., 1992*a*, *b*).

The L-type calcium channels purified from skeletal muscle consist of five subunits: $\alpha 1$ (190 kD), $\alpha 2$ (143 kD), β (55 kD), γ(30 kD), and δ (24–27 kD) (Takahashi et al., 1987; Campbell et al., 1988; Catterall et al., 1988). All subunits have been cloned and sequenced from cDNA (Tanabe et al., 1987; Ellis et al., 1988; Ruth et al., 1989; Jay et al., 1990).

J. W. Hell and R. E. Westenbroek contributed equally to this work.

Address all correspondence to W. A. Catterall, Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195.

1. *Abbreviations used in this paper*: PAP, peroxidase-anti-peroxidase; SM-PBS, 10% skim milk powder in PBS; ω-CgTxGVIA, ω-Conus geographus Toxin GVIA; ω-Aga-IV A, ω-Agelenopsis toxin IV A.

The $\alpha 2$ and δ subunits are derived from a common protein precursor by posttranslational proteolytic processing (De Jongh et al., 1990; Jay et al., 1991), and the $\alpha 1$ subunit is present in two size forms of 212 and 190 kD that are likely to be derived from posttranslational proteolytic processing (De Jongh et al., 1989, 1991). The $\alpha 1$ subunit is homologous to the α subunit of the sodium channel (Tanabe et al., 1987) and forms a functional calcium channel when expressed in *Xenopus* oocytes or mammalian cells (Perez-Reyes et al., 1989; Mikami et al., 1989). However, coexpression of other subunits increases the expression of $\alpha 1$ subunits and alters their functional properties (Wei et al., 1991; Varadi et al., 1991; Singer et al., 1991). Neuronal L-type calcium channels have a subunit composition that is similar to the skeletal muscle L-type channel including $\alpha 1$, $\alpha 2$, β, and δ subunits (Takahashi and Catterall, 1987; Ahlijanian et al., 1990).

Homology screening of rat brain cDNA libraries with probes derived from the skeletal muscle $\alpha 1$ subunit cDNA has led to the identification of five different classes of calcium channels expressed in rat brain designated rbA, rbB, rbC, rbD, and rbE (Snutch et al., 1990; Soong et al., 1993), and the complete primary sequences of members of all five classes have been determined. Brain class C and Class D $\alpha 1$ subunits are most closely related to L-type calcium channels from other tissues (Hui et al., 1991; Snutch et al., 1991; Chin et al., 1991; Williams et al., 1992*a*; Seino et al., 1992) and encode calcium channels with the functional properties and high affinity for dihydropyridine calcium channel antagonists characteristic of L-type calcium channels (Williams et al., 1992*a*; Dubel et al., 1992). The neuronal class A, B, and E $\alpha 1$ subunits show a lower degree of homology with L-type calcium channels. Class B cDNAs encode an N-type calcium channel (Dubel et al., 1992; Williams et al., 1992*b*), and class A cDNAs encode high threshold channels with incompletely defined functional properties that do not correspond to either N or L-type calcium channels (Mori et al., 1991; Starr et al., 1991; Dubel et al., 1992; Niidome et al., 1992; Snutch et al., unpublished results). Class E cDNAs encode a novel type of calcium channel which has a low threshold for activation upon expression in *Xenopus* oocytes.

The expression of at least five distinct mRNAs encoding calcium channel $\alpha 1$ subunits in brain neurons raises questions concerning the biochemical properties, subcellular localization, and functional roles of the corresponding polypeptides. In these experiments, we have used site-directed anti-peptide antibodies against unique sequences in the class C and class D L-type calcium channel $\alpha 1$ subunits to identify the corresponding polypeptides and define their subcellular localization in central neurons.

Materials and Methods

Materials

[^{3}H]PN200-110 (3200 GBq/mmol) and [^{125}I]ω-CgTx GVIA (81.4 TBq/mmol) were obtained from New England Nuclear-DuPont (Boston, MA), digitonin from Gallard-Schlesinger (Carle Place, NY), the ECL detection kit for immunoblotting from Amersham Corp. (Arlington Heights, IL), and protein A-Sepharose, heparin-agarose, WGA, CNBr-activated Sepharose 4B, and 3,3'-diaminobenzidine tetrahydrochloride (DAB) from Sigma Chemical Co. (St. Louis, MO). WGA-Sepharose was synthesized using CNBr-activated Sepharose according to the manufacturer's instructions. Rabbit and mouse IgG (used as control antibodies), rabbit anti-mouse IgG, goat anti-rabbit IgG, and rabbit peroxidase-anti-peroxidase (PAP) were purchased from Zymed Laboratories, Inc. (South San Francisco, CA) while the biotinylated anti-rabbit IgG and Avidin D fluorescein were obtained from Vector Laboratories, Inc. (Burlingame, CA). All other reagents were from commercial sources. 2-mo-old Sprague-Dawley rats were obtained from Bantin and Kingman (Bellevue, WA).

Partial Purification and Immunoprecipitation of L-Type Calcium Channels

The procedure for the solubilization and partial purification of neuronal calcium channels was described recently (Westenbroek et al., 1992). In brief, 10 rat cerebral cortices and hippocampi were homogenized in 110 ml 320 mM sucrose with a glass-teflon homogenizer (12 strokes, ~1,000 rpm). The supernatant (S1) from a short preclearing spin (5,000 rpm, 2 min, SS 34-rotor) was incubated with 370 kBq [^{3}H]PN200-110 for 30 min. Membranes were pelleted (50,000 rpm, 35 min, 70.1 Ti-rotor) and solubilized with 170 ml 1.2% digitonin, 300 mM KCl, 150 mM NaCl, and 10 mM sodium phosphate buffer, pH 7.4, for 20 min. Unsolubilized material was removed by centrifugation as before, and the supernatant (S3) was slowly run through a 40-ml WGA-Sepharose column (50 ml/h). The column was washed with 300 ml 0.1% digitonin, 75 mM NaCl, 50 mM sodium phosphate, 10 mM Tris-Cl, pH 7.4, at a flow rate of 150 ml/h, and bound calcium channels were eluted with 100 mM N-acetyl-D-glucosamine in the same buffer at a flow rate of 50 ml/h. 2-ml fractions were collected. PN200-110 receptors were detected by scintillation counting of 30-μl samples, and ω-CgTx GVIA-receptors were measured by the filtration assay with [^{125}I]ω-CgTx GVIA (see Westenbroek et al., 1992). Up to 4 ml of fractions containing L- and N- type channels were centrifuged through 5-20% (wt/wt) sucrose gradients (36 ml containing 0.1% digitonin and 10 mM Tris-Cl, pH 7.4) at 45,000 rpm for 2 h and 15 min in a 50VTi-rotor. 2-ml fractions were collected, frozen in liquid N_2 and stored at −80°C.

Determination of [^{3}H]PN200-110 and [^{125}I]ω-CgTx GVIA Receptors

Receptor-bound [^{3}H]PN200-110 was measured by direct scintillation counting of samples virtually free of unbound ligand such as fractions eluted with *N*-acetylglucosamine from WGA-Sepharose chromatography or sucrose gradient sedimentation. To determine the amount of receptor-bound [^{3}H]PN200-110 in samples containing free ligand, aliquots (50–300 μl) were precipitated by incubation with 4 ml of ice-cold 10% polyethylene glycol (average molecular weight: 8,000) in 10 mM $MgCl_2$ and 10 mM Tris-Cl, pH 7.4, for 5 min and poured over Whatman GF/F filters, which were washed three times with 4 ml of ice-cold PEG-solution and counted by scintillation spectrometry. ω-CgTx GVIA receptors were detected by the same procedure after labeling an aliquot of the fractions by incubation with [^{125}I]ω-CgTx GVIA for 15 min at room temperature.

To determine what percentage of the labeled calcium channels would be retained on the filters, a WGA-fraction containing only bound [^{3}H]PN200-110 was assayed by both direct scintillation counting and by filtration and washing. ~78% of the total amount of [^{3}H]PN200-110 labeled receptor could be detected on the filters. All values obtained by filtration assays were corrected accordingly.

Immunoprecipitation

An aliquot of the [^{3}H]PN200-110-labeled fraction S3 (see above) was incubated with affinity-purified CNC1, CND1, or control antibodies (rabbit IgG) for 2 h on ice. A saturating concentration of protein A-Sepharose (preswollen and washed with PBS) was added, and the samples were stirred on a tilting mixer for 4 h at 4°C. The protein A-Sepharose antibody/antigen complex was collected by centrifugation, washed three times with 0.1% digitonin in PBS, and the radioactivity determined in a scintillation counter.

Immunoblotting of Calcium Channels

Since heparin-agarose binds neuronal L- and N-type calcium channels (Sakamoto and Campbell, 1991), it was used to concentrate them after sucrose gradient centrifugation; 1-ml samples were incubated with 40 μl of heparin agarose for 2–3 h at 4°C. The resin was washed three times with PBS containing 0.1% digitonin and extracted with 40 μl 3% SDS, 20 mM DTT, 10% sucrose and 125 mM Tris-Cl, pH 6.8, for 30 min at 50–60°C with protease inhibitors present as described above. The proteins were separated by SDS-PAGE in discontinuous gels with a stacking phase polymer-

ized from 3% acrylamide and a separating phase polymerized from 5% acrylamide as described by Laemmli (1970). The separated proteins were electrotransferred wet to nitrocellulose, and the blots were blocked by incubation for 1 h with 10% skim milk powder in PBS (SM-PBS). Blots were incubated with affinity-purified antibodies in SM-PBS for 1–2 h. The blots were washed five times with SM-PBS, incubated with HRP-protein A diluted 1:1,000 in SM-PBS containing 0.05% Tween 20, washed 8–10 times with 0.05% Tween 20 in PBS (5–6 h total washing time), and developed with the ECL reagent.

Production and Purification of Peptides and Antibodies

The sequences of the CNC1 peptide (KYTTKINMDDLQPSENEDKS; residues 818 to 835, Snutch et al., 1991) and the CND1 peptide (KYDNKVTIDDYQEEAEDKD; residues 809 to 825, Prystay, W., and T. P. Snutch, unpublished results; Hui et al., 1991) correspond to highly variable sites within the intracellular loops between domain II and III of the class C and class D α1 subunits of rat brain calcium channels. The NH_2-terminal lysine and tyrosine were added for cross-linking and labeling purposes and are not part of the channel sequences. Peptides were synthesized by the solid phase method (Merrifield, 1963), purified by reversed-phase HPLC on a Vydac 218 TP10 column, and confirmed by amino acid analysis.

The purified peptides were coupled with glutaraldehyde to bovine serum albumin (Orth, 1979), dialyzed against PBS, and emulsified in the same volume of Freund's complete (initial injection) or incomplete adjuvant. Injections were done in multiple subcutaneous sites on New Zealand white rabbits at three week intervals. Antisera were collected, and antibodies were purified by affinity chromatography on CNC1 or CND1 peptides coupled to CNBr-activated Sepharose. 2 ml of antiserum were adsorbed to the column matrix and incubated at room temperature for 5 h with stirring on a tilting mixer. The columns were then washed with TBS, and bound IgG was eluted with 2.8 M $MgCl_2$ (CNC1) or 0.1 M glycine (pH 2.7, CND1). The affinity-purified anti-CNC1 was then dialyzed against TBS with the Centriprep 30 system from Amicon, while the affinity-purified anti-CND1 was brought to neutral pH using 0.1 M Tris. The mAb MANC1 was purified from ascites as previously described (Ahlijanian et al., 1990).

Immunocytochemistry

Adult Sprague-Dawley rats were anesthetized with sodium pentabarbitol and intracardially perfused with 4% paraformaldehyde in PB (0.1 M sodium phosphate, pH 7.4), containing 0.34% L-lysine and 0.05% sodium m-periodate) (McLean and Nakane, 1974). The brains were removed from the cranium and postfixed for 2 h (single label procedures) or 10 h (double-label procedures). The brains were then sunk in successive solutions of 10, 20, and 30% (wt/vol) sucrose in PB at 4°C over 72 h. Sagittal and coronal sections (25-35 μm) were cut on a sliding microtome.

Free-floating sections were processed for immunocytochemistry with the indirect PAP technique (Sternberger, 1979) or by immunofluorescence. Procedures for processing of tissue samples using the PAP technique have been reported previously (Westenbroek et al., 1992). Briefly, sections stained using the CNC1 antibody were incubated in affinity-purified anti-CNC1 (diluted 1:20) for 1 h at room temperature followed by 36 h at 4°C. Anti-CNC1 was diluted in PBS containing 0.1% Triton X-100 and 1% normal goat serum. The sections were then incubated in goat anti-rabbit IgG diluted 1:30 for 1 h at 37°C, incubated in rabbit PAP diluted 1:100 for 1 h at 37°C, treated with 0.04% 3,3'-diaminobenzidine and 0.003% H_2O_2 in TB for 10 min, rinsed, mounted on subbed glass slides, dehydrated, cleared in xylene, and coverslipped. Tissue sections stained with anti-CND1 were incubated in a similar manner to anti-CNC1 except the affinity-purified anti-CND1 (diluted 1:30) was incubated in Tris-buffered solutions instead of phosphate buffered solutions.

For immunofluorescence, free-floating sections were first rinsed in 0.1 M Tris for 5 min, then in 0.05 M TBS for 10 min, blocked in TBS containing 3% normal goat serum for 1 h, and rinsed in TBS for 30 min. Sections were incubated in affinity-purified CNC 1 or CND 1 antibody for 1 h at room temperature followed by 36 h at 4°C. All antibodies were diluted in 0.05 M TBS containing 0.1% Triton X-100 and 1% normal goat serum. The sections were rinsed for 1 h in TBS, incubated in fluorescein-tagged goat anti-rabbit IgG (1:100 dilution with TBS) for 3 h at 37°C, rinsed in TBS for 10 min, and in 0.1 M Tris for 20 min at room temperature, mounted, and coverslipped using Vectashield.

To determine the level of nonspecific staining, sections were incubated without primary antibody or, before incubation with the tissue sections, primary antibody was blocked by preincubation for 6–8 h with the corresponding peptide (25 μM, unless specific otherwise) or an unrelated peptide. The immunocytochemical reaction was then carried out as described above.

Results

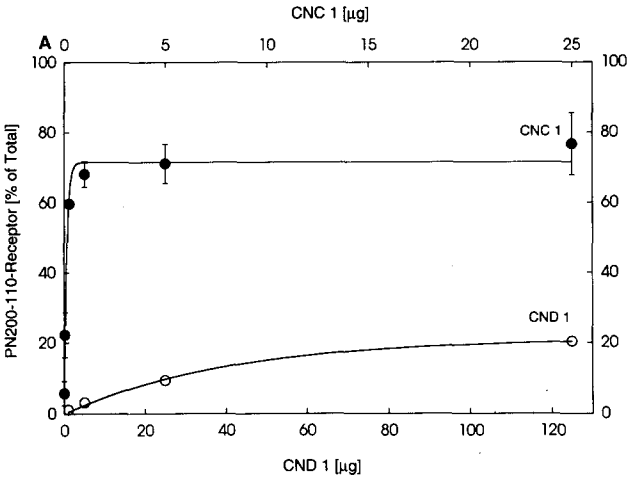
Immunoprecipitation of Neuronal L-type Calcium Channels by CNC1 and CND1

The site-directed anti-peptide antibodies anti-CNC1 and anti-CND1 were prepared against peptides corresponding to unique sequences in the class C and class D calcium channels as described in Materials and Methods. Both the anti-CNC1 and anti-CND1 antibodies immunoprecipitated high affinity [^{3}H]PN200-110 receptor sites solubilized from rat brain membranes confirming that the class C and class D calcium channels are L-type channels (Fig. 1). To determine the fraction of L-type calcium channels in rat dorsal cortex and hippocampus which are encoded by the class C or by class D genes, immunoprecipitations with increasing amounts of anti-CNC1 and anti-CND1 were performed. At the highest concentrations tested, anti-CNC1 immunoprecipitated up to 70% of the L-type calcium channels labeled by [^{3}H]PN200-110 (Fig. 1 *A*). Previous results have shown that immunoprecipitation of L-type calcium channels by this antibody is specifically blocked by the CNC1 peptide and that N-type calcium channels are not immunoprecipitated by anti-CNC1 confirming the specificity of this antibody (Dubel et al., 1992). Anti-CND1 precipitated up to 20% of the solubilized L-type channels (Fig. 1 *A*).

Immunoprecipitation of [^{3}H]PN200-110-labeled calcium channels with CND1 was inhibited by 90% with 15 μM CND1 peptide (Fig. 1 *B*). Control peptides of similar charge did not significantly affect immunoprecipitation, and anti-CND1 did not immunoprecipitate N-type channels labeled by binding of ω-CgTx GVIA (data not shown). Although the relatively low affinity and limited supply of anti-CND1 prevented development of complete immunoprecipitation curves (Fig. 1 *A*), the large fraction of L-type calcium channels immunoprecipitated by the two antibodies suggests that near saturation with anti-CND1 was attained. Together these data indicate that class C and class D represent most, if not all, of the neuronal L-type channels from rat cerebral cortex and hippocampus.

Two Size Forms of the α1 Subunits of Class C and Class D Calcium Channels

To identify the polypeptides recognized by anti-CNC1 and anti-CND1, immunoblotting was performed with partially purified glycoprotein fractions using affinity-purified antibodies as described in Materials and Methods. With both antibodies, two bands with apparent molecular masses of ~180–190 and 210–235 kD were detected (Fig. 2 *A*). The specificity of the interaction of CNC1 and CND1 antibodies with these protein bands was tested by competition with the corresponding peptides (Fig. 2 *B*). After preincubation of the CNC1 antibody with 7 μM of the CNC1 peptide, no signal could be detected after prolonged exposure in order to reveal even a low level of reactivity (Fig. 2 *B*, *left*). Similarly, CND1 immunoreactivity was completely blocked by preincubation with 50 μM CND1 peptide (Fig. 2 *B*, *right*). Control experiments using similar concentrations of nonspecific



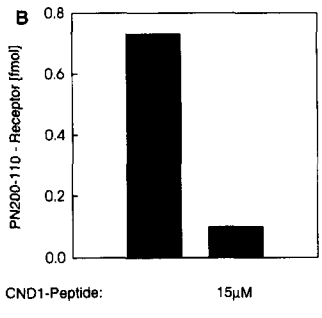


Figure 1. Immunoprecipitation of [^{3}H]PN200-110–labeled L-type calcium channels by CNC1 and CND1 antibodies. (*A*) 100-μl aliquots of the [^{3}H]PN200-110–labeled S3-fraction were incubated with the indicated amount of affinity-purified anti-CNC1 or anti-CND1 and protein A–Sepharose and treated as described in Materials and Methods. Nonspecific immunoprecipitation estimated with a high concentration of nonspecific rabbit IgG antibody was <1% of the total labeled PN200-110 receptor and was neglected. The total amount of labeled L-type channels was determined by the filter assay and corresponds to 100%. The data are mean values ± range from two independent experiments. Similar results were obtained in two other experiments. Saturation curves were fitted to the data according to the equations $y = 71.5 \times [1\text{-exp}(-x/0.123)(\text{CNC1})]$ and $y = 21.3 \times [1\text{-exp}(-x/41.0)(\text{CND1})]$. (*B*) Immunoprecipitation was performed as in *A* with 3.4 μg anti-CND1 which had been preincubated with 15 μM CND1 peptide where indicated.

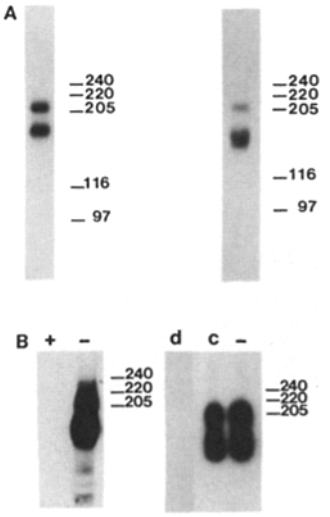


Figure 2. Immunoblotting with CNC1 and CND1 reveals two different length forms of α1 subunits for both class C and class D calcium channels. (*A*) Calcium channels were concentrated from 1-ml samples of sucrose gradient fractions with heparin-agarose, extracted with SDS-sample buffer, separated by SDS-PAGE, blotted, incubated with affinity-purified CNC1 (*left*) or CND1 (*right*) antibodies, and detected with the ECL method as described in Materials and Methods. The migration positions of α- and β-spectrin (240 and 220 kD), myosin heavy chain (205 kD), β-galactosidase (116 kD), and phosphorylase b (97 kD) are indicated at the right sides of the gels. (*B*) Specific block of immunoblotting with CNC1 and CND1 antibodies by the corresponding peptides. Immunoblots of sucrose gradient fractions enriched with L-type channels as in *A* were probed with affinity-purified CNC1 or CND1 antibodies. (*Left*) CNC1 antibodies were preincubated in the presence (+) or absence (–) of 7 μM CNC1 peptide for 1 h. (*Right*) CND1 antibodies were preincubated over night on ice with (*d*) or without (–) 50 μM CND1 peptide or with 50 μM CNC1 peptide (*c*) as a control of the specificity of the peptide block. Note that the blots were overexposed to demonstrate that no antibody had bound to the blots in the presence of the specific peptides. Molecular weight standards are indicated beside the gel as given in *A*.

peptides did not affect the recognition of α1 subunits in immunoblots with anti-CNC1 or anti-CND1. For example, the immunoreactivity with the CND1 antibody was completely blocked by the CND1 peptide, but was not significantly affected by the same concentration of the CNC1 peptide (Fig. 2 *B*, *right*). In addition, none of the four bands was immunostained when probed with nonspecific antibodies (data not shown).

To confirm that the two bands detected with CNC1 and CND1 antibodies are indeed L-type α1 subunits, their sedimentation behavior was compared with that of [^{3}H]PN200-110–labeled L-type and [^{125}I]ω-CgTx GVIA-labeled N-type calcium channels (Fig. 3). The N-type channels migrated faster during sucrose gradient centrifugation than the L-type calcium channels and had their maximum level in fraction 7, whereas the highest concentration of the L-type channels was found in fraction 9 (Fig. 3 *C*). Both bands detected by anti-CNC1 and anti-CND1 co-sedimented with the L-type channels, with the highest level of antigens and PN200-110 receptor in fraction 9 (Fig. 3). Neither the CNC1 or CND1 antibodies detectably crossreacted with the N-type α1 subunits which have significantly larger apparent molecular masses (Westenbroek et al., 1992) and were presented in highest concentration in fraction 7.

The peptides recognized by the CND1 antibody consistently migrated slightly more rapidly compared to size markers than those recognized by the CNC1 antibody (e.g., Fig. 2 *A*). For a direct comparison of the migration positions of the immunostained bands, the immunoblot shown in Fig. 3, *A* and *B* was probed with anti-CNC1 and then stripped with 2% SDS and 20 mM DTT. The stripped blot was incubated with the ECL-reagent and exposed for 30 min. No band was detectable at that point demonstrating that the first antibody was completely washed off. The blot was reprobed with anti-CND1. A careful alignment of the two exposures revealed that both the larger and the smaller bands recognized by anti-CNC1 migrated more slowly than the corresponding anti-CND1 bands in comparison to marker proteins. These findings exclude the possibility of crossreactivity of the CNC1 and CND1 antibodies and confirm the specificity of both antibodies.

Localization of Class D L-type Calcium Channels

Throughout the rostral–caudal extent of the rat brain, there is dense labeling of the majority of neurons by the CND1 antibody. Both projection neurons and interneurons are immunoreactive for the α1 subunit of L-type calcium channels recognized by the CND1 antibody. The densest labeling is in the cell soma and proximal dendrites with diminishing density of label in more distal dendritic regions. To illustrate these points, the dorsal cerebral cortex, hippocampus, and cerebellum are described in greater detail below.

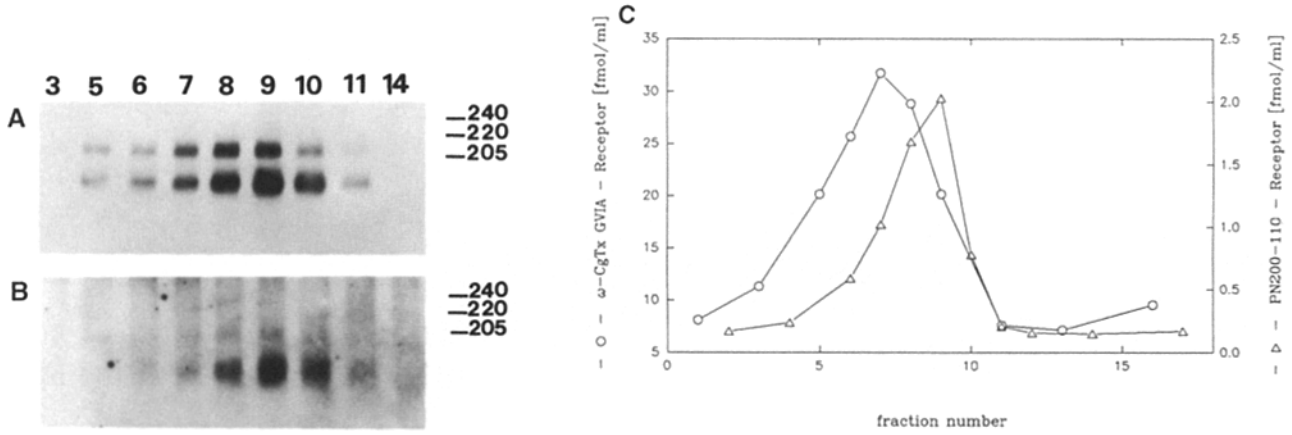


Figure 3. CNC1 and CND1 immunoreactivity comigrates with L-type, but not N-type, calcium channels during sucrose gradient sedimentation. (*A*) WGA-Sepharose fractions from [^{3}H]PN200-110–labeled rat brain membranes were loaded onto sucrose gradients and, after centrifugation, different 2-ml fractions were immunoblotted with CNC1 antibody as described in Fig. 2. The fractions are numbered from the bottom of the gradient. Molecular mass standards are indicated at the right side of the gel. (*B*) The same blot was stripped with 2% SDS and 20 mM DTT in 62.5 mM Tris-Cl, pH 6.8, for 30 min at 50°C. The stripped blot was developed with the ECL reagent and exposed for 30 min with no signal detectable any longer, demonstrating the complete absence of any antibody at that point. The blot was reprobed with CND1 antibody and exposed for 30 min. A careful alignment of exposures after incubation with CNC1 and CND1 reveals that L_{C2} (210 kD) is slightly higher than L_{D2} (200 kD). For example, compare the positions of these bands with that of the 205-kD marker. The same is true for the two lower bands, L_{C1} and L_{D1} (190 and 180 kD, respectively). (*C*) Concentrations of PN200-110– and ω-CgTx GVIA–labeled L- and N-type calcium channels in different sucrose gradient fractions. Bound [^{3}H]PN200-110 was measured by scintillation counting. To determine the amount of N-type calcium channels, aliquots of each fraction were incubated with 2 nM [^{125}I]ω-CgTx GVIA, precipitated with PEG, and filtered as described in Materials and Methods. Note that the two bands recognized by CNC1 as well as those detected with CND1 comigrate with each other and with the PN200-110 receptor.

In all regions of the dorsal cerebral cortex, neurons throughout layers 1–6 are immunoreactive for L-type calcium channels recognized by the CND1 antibody (Fig. 4 *a*). Block of the CND1 antibody using the CND1 peptide results in the complete absence of staining (Fig. 4 *b*), but preincubation of the CND1 antibody with the CNC1 peptide did not change the pattern of staining as compared to incubations with the CND1 antibody alone (Fig. 4 *c*). These two controls demonstrate that the CND1 antibody is specific for the class D α1 subunit and does not recognize the corresponding sequence of the class C α1 subunit. The densest concentration of class D L-type calcium channels is in cell bodies, especially at locations where dendrites originate (Fig. 4 *d*). Class D calcium channels are also located in the proximal portions of dendrites. The intensity of labeling with the CND1 antibody in dendrites drops off dramatically as the distance from the cell body increases. Confocal microscopy after labeling with anti-CND1 and a fluorescein-tagged secondary antibody revealed weak, but clearly detectable immunoreactivity along the dendrites farther out than the proximal portion (Fig. 4 *f*). These results indicate that many types of neurons in the cerebral cortex express class D L-type calcium channels with relatively high density in the cell bodies and proximal dendrites and diminishing density in the distal dendrites.

Within the hippocampal formation, we also observe a wide distribution of neurons which are immunoreactive for L-type calcium channels recognized by the CND1 antibody. Cell bodies of pyramidal neurons in the CA1–CA3 areas (Fig. 5, *a–d*) and granule cells in the dentate gyrus (Fig. 5 *e*) are stained with the CND1 antibody. Interneurons in the strata oriens, radiatum, and lacunosum moleculare of the hippocampus and interneurons in the molecular layer of the dentate gyrus are also immunoreactive for the α1 subunit of class D L-type calcium channels (Fig. 5, *a–e*). In each case, there is dense immunoreactivity in neuronal cell bodies and often an accumulation of immunoreactivity in the region where the basal and apical dendrites emerge from the cell body (Fig. 5, *c* and *e*). In contrast, there is relatively weak immunoreactivity for the CND1 antibody in the dendrites of neurons which diminishes progressively in more distal portions (Fig. 5, *b–d*). This is most evident in the confocal image in Fig. 5 *f* in which faint immunofluorescence above background can be detected as far as 400 μm from the cell body. Weak dendritic staining for the class D L-type calcium channels was also present in the dendrites of interneurons within the strata oriens, radiatum, and moleculare of the hippocampus.

In the cerebellum, the most intense staining with the CND1 antibody is observed in the cell bodies of neurons in the granular layer. The small cells in the granular layer exhibiting strong immunoreactivity are the cerebellar granule cells, which have a diameter of 6–9 μm (Fig. 6, *a* and *b*, layer *G*). Because of the dense staining of granule cells, it is difficult to determine whether the smaller number of Golgi cells in the granular layer are also labeled. The principal projection neurons of the cerebellum, the Purkinje cells, are also stained by anti-CND1 (Fig. 6, *a* and *b*, layer *P*). Unlike the weak, but clearly detectable staining of the dendrites of projection neurons in the dorsal cortex and the hippocampus,

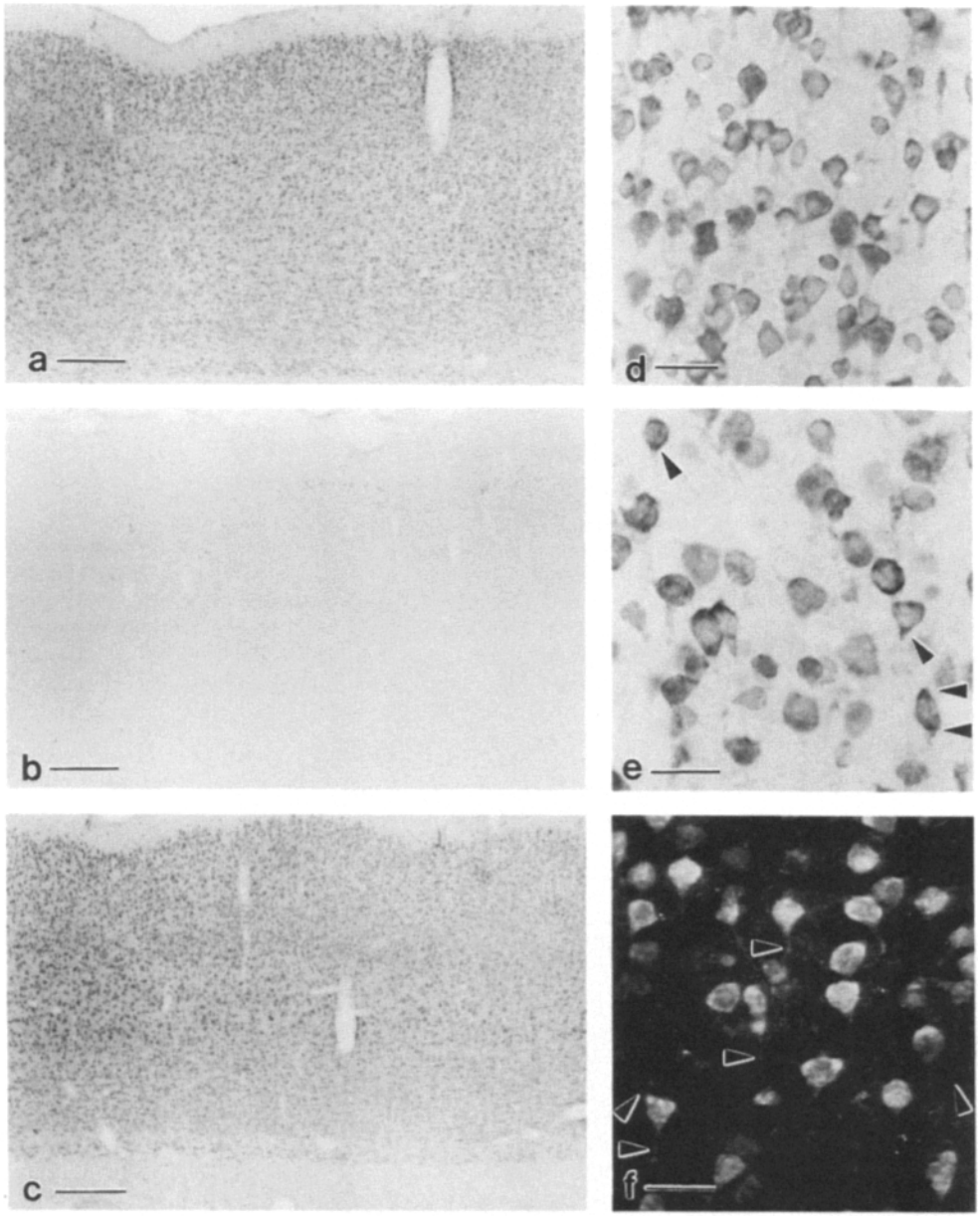


Figure 4. Localization of class D L-type calcium channels in the dorsal cortex. Sagittal sections of adult rat brain were prepared as described in Materials and Methods. (*a*) Low magnification of dorsal cortex demonstrating CND1 immunoreactive neurons throughout layers 1–6. (*b*) Control section stained with CND1 antibody preabsorbed with the CND1 peptide. (*c*) Control section stained with CND1 antibody preabsorbed with CNC1 peptide showing lack of cross-reactivity. (*d* and *e*) Higher magnifications of the dorsal cerebral cortex illustrating CND1 staining in the cell body and proximal dendrites. Note the dense immunoreactivity at the base of the dendrites (*arrowheads*). (*f*) Immunofluorescent staining viewed with the confocal microscope to demonstrate the relatively weak, but detectable anti-CND1 staining along the proximal and middle portions of the dendrites (*arrowheads*). Bars: (*a–c*) 500 μm; (*d–f*) 50 μm.

the extensively branched proximal and distal dendrites of the cerebellar Purkinje cells are not detectably stained with the CND1 antibody. In the molecular layer where the dendrites of the Purkinje cells are the predominant structure, the cell bodies of basket and stellate cells are immunoreactive, but the Purkinje cell dendrites are not observed (Fig. 6, *a* and *b*, layer *M*). As in other brain regions, the immunocytochemical staining suggests that most cell types within the cerebellum express class D L-type channels and have a significant density of these channels in their cell bodies.

Localization of Class C L-type Calcium Channels

The CNC1 antibody shows immunoreactivity which is similar in its general distribution to that of the CND1 antibody: strongest in neuronal cell bodies and proximal dendrites and faint in the medial and distal portions of dendrites. However, the class C L-type calcium channels are strikingly clustered rather than smoothly distributed along the cell surface. For comparison of the distribution of the class C and class D L-type calcium channels, the distribution of immunoreactivity of the CNC1 antibody is illustrated for the dorsal cerebral cortex, hippocampus, and cerebellum as well.

In the dorsal cerebral cortex, the cell bodies of neurons of all sizes and morphologies in layers 1–6 are labeled with the CNC1 antibody (Fig. 7 *a*). Like CND1 immunoreactivity, CNC1 staining is localized predominantly in the cell body and proximal dendrites of most cortical neurons (Fig. 7, *b–f*). However, the CNC1 antibody shows a more punctate pattern of immunoreactivity compared to the CND1 antibody. There is an overall light pattern of staining of the cell body and proximal dendrites with small, roughly circular clusters of intense labeling 1.5–2 μm in diameter scattered throughout these areas (Fig. 7, *e* and *f*). In addition, there is comparatively light CNC1 staining of dendrites farther out along the more distal portions of the dendrites (Fig. 7, *b* and *c*, *arrowheads*).

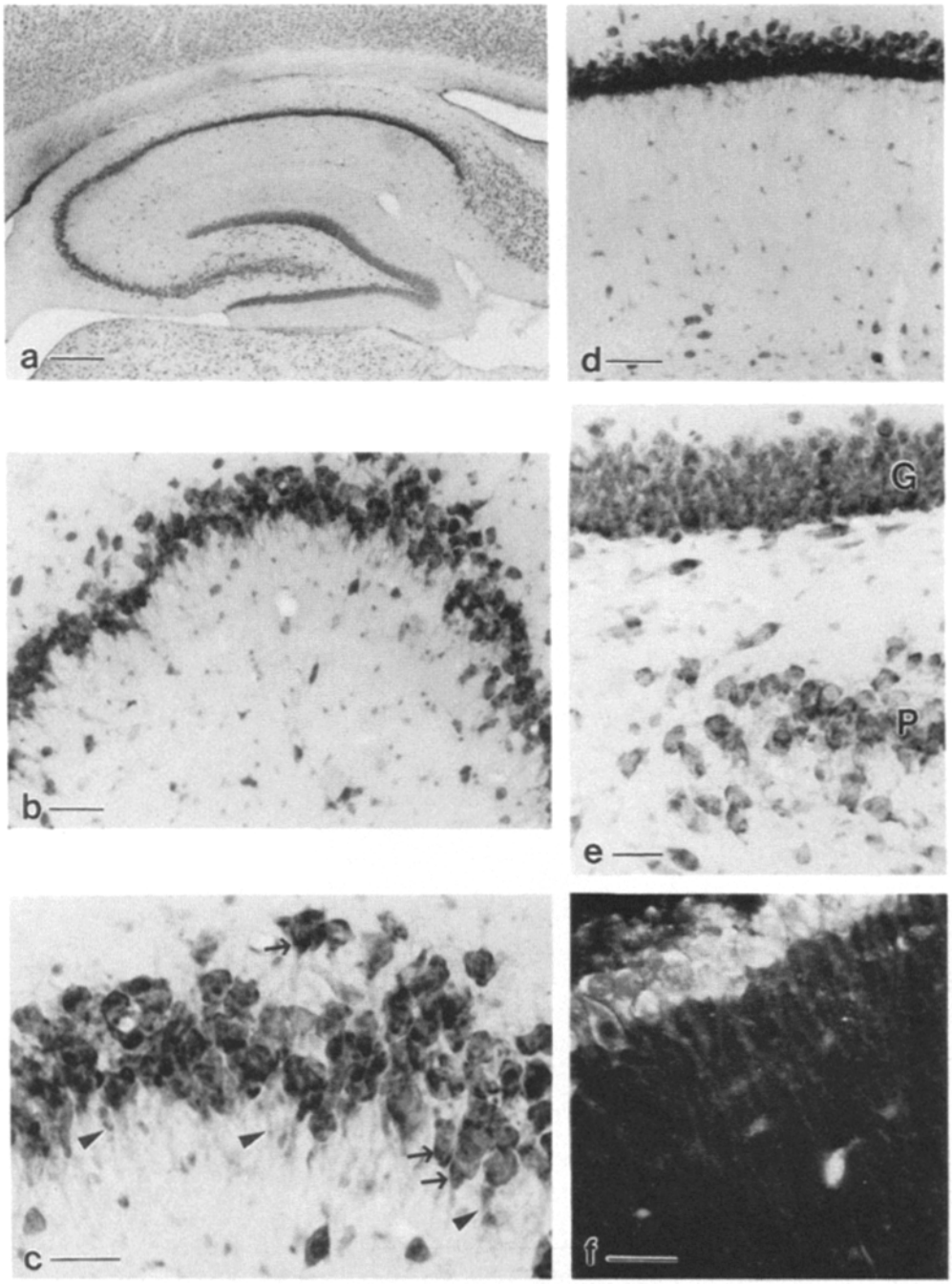


Figure 5. Localization of class D L-type calcium channels in the hippocampus. Sagittal sections of adult rat brain were stained using the methods described in Materials and Methods. (*a*) Low magnification of the hippocampal formation illustrating a wide distribution of CND1 immunopositive cells in all layers of the hippocampus and the dentate gyrus. (*b*) Section of the CA2/CA3 region illustrating CND1 labeling in the cell body and proximal dendrites of pyramidal neurons and interneurons. (*c*) Higher magnification of picture shown in (*b*) to illustrate the density of anti-CND1 staining at base of dendrites (*arrows*) and in proximal portions of pyramidal cell dendrites (*arrowheads*). (*d*) Higher magnification of the CA1 region to illustrate faint dendritic staining for some distance into the stratum radiatum. (*e*) Higher magnification of the granule cells of the dentate gyrus (*G*), CA3 pyramidal cells (*P*), and interneurons illustrating dense cell body staining in these regions. (*f*) Immunofluorescent staining of the CA1 region viewed using the confocal microscopy to illustrate the faint anti-CND1 staining in the apical dendrites in the stratum radiatum. Bars: (*a*) 500 μm; (*b* and *d*) 100 μm; (*c*, *e*, and *f*) 50 μm.

The hippocampal formation possesses a regionally specific pattern of CNC1 antibody immunoreactivity with strong staining of the cell bodies but not of the dendrites of pyramidal cells in area CA1, strong staining of both the cell bodies and the dendritic fields of pyramidal cells in areas CA2 and CA3, and strong staining of the dendritic field but not of the cell bodies of the granule cells of the dentate gyrus (Fig. 8 *a*). The immunoreactivity is abolished when the CNC1 antibody is blocked by the CNC1 peptide (Fig. 8 *b*), but is unaltered when the CNC1 antibody is preincubated with the CND1 peptide (Fig. 8 *c*), indicating that the CNC1 antibody is specific for the class C α1 subunit and does not cross react with the corresponding class D sequence. Higher resolution analysis of area CA1 in the confocal microscope reveals a general distribution of calcium channels in cell bodies with superimposed clusters (Fig. 8 *g*). Lighter staining of the dendrites is also resolved with confocal microscopy indicating a low, but significant amount of class C calcium channels smoothly distributed along the dendrites up to 500 μm from the cell body (Fig. 8 *g*). In contrast, higher resolution views of areas CA2 and CA3 show relatively dense labeling of both pyramidal cell bodies and their dendrites, especially in the proximal regions (Fig. 8, *d–f*). The cell bodies and the proximal dendrites of the pyramidal cells in areas CA2 and CA3 exhibit an overall distribution of class C calcium channels with numerous clusters superimposed (Fig. 8, *e* and *f*). Thus, in the hippocampus as well as the cerebral cortex, class C calcium channels are both generally

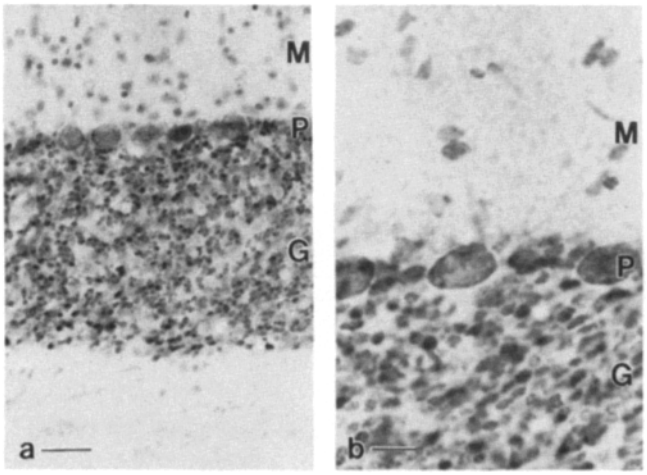


Figure 6. Localization of class D L-type calcium channels in the cerebellum. Sections were prepared as described in Materials and Methods. (*a*) Low magnification of the cerebellum illustrating the pattern of staining in the granular (*G*), Purkinje cell (*P*), and molecular (*M*) layers. (*b*) Higher magnification to illustrate the pattern of cell body staining in all three layers. Bars: (*a*) 50 μm; (*b*) 25 μm.

distributed and concentrated in clusters on neuronal cell bodies and proximal dendrites.

Class C calcium channels are found throughout the granular, Purkinje cell, and molecular layers of the cerebellum (Fig. 9 *a*). Most prominent is the staining of the cell bodies of Purkinje cells which have class C calcium channels distributed over their surface and concentrated in punctate clusters (Fig. 9, layer *P*). The main dendritic shafts of the Purkinje cell dendrites in the molecular layer are labeled, but the densest staining is usually at dendritic branch points in the proximal two-thirds of the length of the dendrites (Fig. 9, *a* and *b*). Interneurons in the molecular layer are only faintly labeled and difficult to detect (Fig. 9 *b*, *arrowhead*). In the granular layer, both Golgi cells (Fig. 9 *b*, *arrows*) and granule cells (Fig. 9 *c*) are labeled with CNC1 antibody. Fine punctate staining can be observed throughout the granular layer and the Purkinje cell layer, suggesting small clusters of class C L-type calcium channels at synaptic sites or other local specializations that do not contain class D calcium channels.

Discussion

Most Rat Brain L-type Calcium Channels Contain Class C or Class D α1 Subunits

L-type calcium channels are thought to be uniformly labeled by high affinity dihydropyridine antagonists like PN200-110 (Glossmann and Striessnig, 1990). Seventy percent of the [^{3}H]PN200-110-labeled calcium channels could be precipitated with a saturating amount of anti-CNC1 and 20% by the highest available concentration of anti-CND1. Since there is no cross-reactivity between these antibodies, it is, therefore, likely that class C or class D α1 subunits are present in most, if not all, brain calcium channels in our solubilized preparations. However, because only 50–60% of the [^{3}H]PN200-110–labeled calcium channels could be solubilized from membranes from rat cortex and hippocampus using digitonin, a relatively weak nonionic detergent that allows retention of calcium channel function, we cannot exclude that another L-type channel is enriched in the insoluble material and was not detected in our experiments. Analysis of the insoluble fraction remaining after digitonin extraction by SDS-PAGE and immunoblotting revealed a similar ratio of immunostaining by the CNC1 and CND1 antibodies, confirming that the ratio of these two calcium channel subtypes in the solubilized preparations is representative of the ratio in the tissue as a whole.

Two Different Size Forms of α1 Subunits Are Derived from both the Class C Gene and the Class D Gene

Both the CNC1 and CND1 antibodies detected two different size forms of calcium channel α1 subunits on immunoblots. Anti-CNC1 recognized proteins with apparent molecular masses of ~190 kD (L_{C1}) and 210 kD (L_{C2}), and anti-CND1 stained proteins with molecular masses of ~180 kD (L_{D1}) and 200 kD (L_{D2}) in the 5% polyacrylamide gels used for SDS-PAGE in these experiments. However, both size forms of the class C and class D α1 subunits have larger apparent molecular masses in 6% polyacrylamide gels than in 5% gels relative to the molecular mass markers. For example, the class C polypeptides appear to be 210 and 235 kD in 6% polyacrylamide gels (Hell et al., unpublished results). Molecular cloning and sequencing of mammalian brain cDNAs predicted molecular masses of 240–243 kD for the full length class C α1 subunits (Snutch et al., 1991) and 247 and 187 kD for long and short forms of the class D α1 subunit (Hui et al., 1991; Williams et al., 1992*a*; Prystay, W., and T. P. Snutch, unpublished results). Thus, the values obtained by SDS-PAGE in 5% polyacrylamide gels likely underestimate the true molecular masses of these polypeptides. A similar discrepancy between the predicted and the observed molecular masses was described earlier for the skeletal muscle L-type and brain N-type calcium channel α1 subunits (De Jongh et al., 1989, 1991; Westenbroek et al., 1992), and is probably due to anomalous migration behavior of these polypeptides relative to molecular mass standards during SDS-PAGE.

We consistently observe similar ratios of long and short forms of both the class C and class D α1 subunits. Lowering the temperature to nearly 0°C by pre-cooling all vials and rotors on ice before use and applying high concentrations of a variety of protease inhibitors did not reduce the abundance of the lower molecular mass forms of the α1 subunits. Similarly, prevention of postmortem proteolysis by perfusion of the rats with the membrane-permeant protease inhibitors calpain inhibitor 1 and 2 and leupeptin prior to decapitation did not diminish the relative amount of the lower molecular mass forms of the α1 subunits as would have been expected if the α1 subunits were degraded by proteases during purification. Based on these observations, we conclude that two size forms of both class C and class D α1 subunits are likely to be present in situ, but we cannot exclude the possibility of additional proteolysis in vitro.

Generation of protein products of multiple sizes from genes encoding individual calcium channel α1 subunits is emerging as a common mechanism for generation of calcium channel diversity. Analogous to our present findings, two different size forms of the skeletal muscle L-type calcium

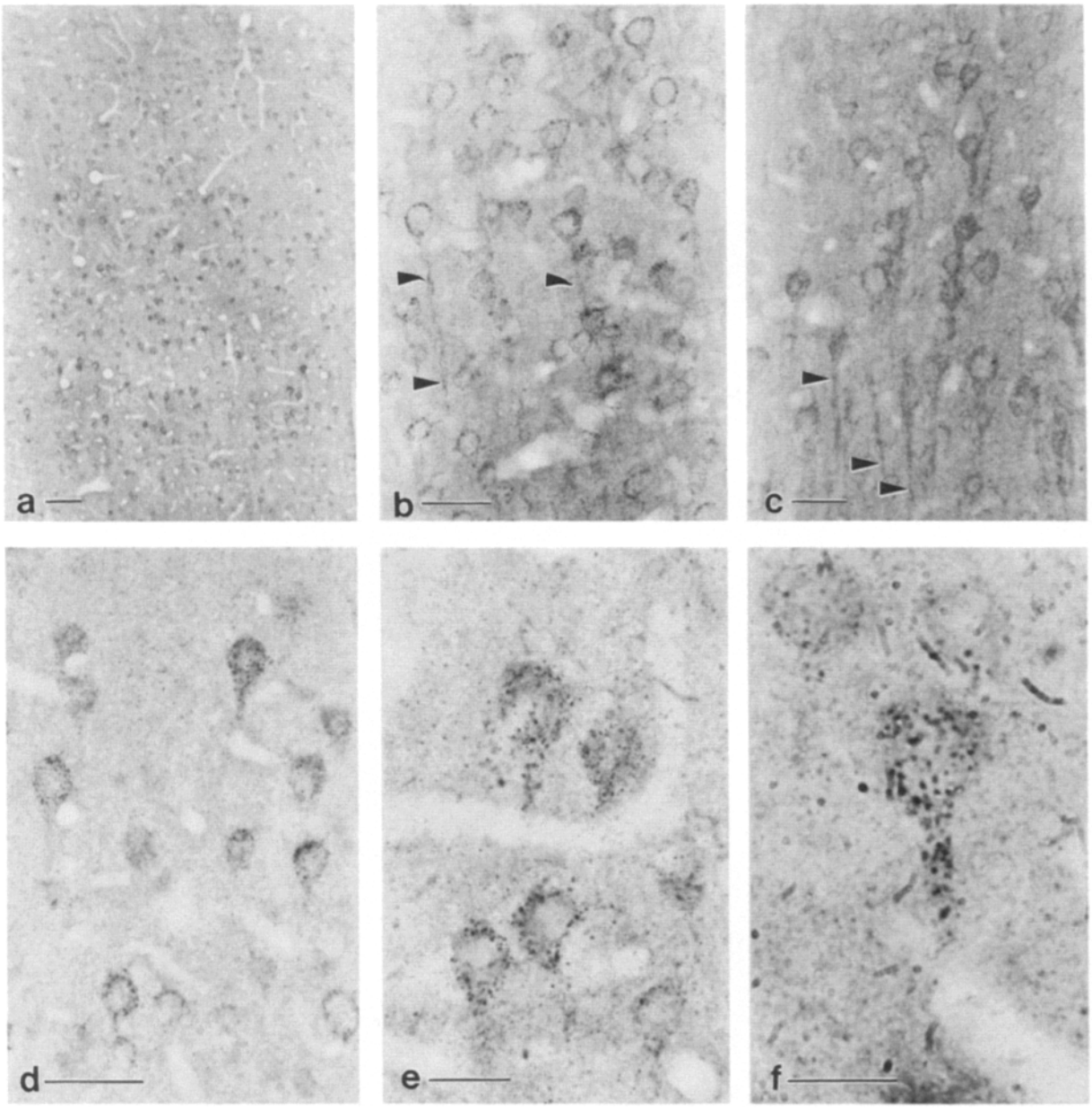


Figure 7. Localization of class C L-type calcium channels in the cerebral cortex. Sections were prepared and stained with anti-CNC1 as outlined in Materials and Methods. (*a*) Low magnification of the dorsal cerebral cortex illustrating anti-CNC1 staining in cells throughout all layers. (*b* and *c*) Higher magnification of neurons labeled with the CNC1 antibody to illustrate labeling in the cell body and the dendrites (*arrowheads*). (*d*, *e*, and *f*) Higher magnification of individual neurons in the dorsal cerebral cortex to illustrate the punctate pattern of CNC1 immunoreactivity in the cell body and proximal dendrites. Bars: (*a*) 100 μm; (*b–e*) 50 μm; (*f*) 25 μm.

channel α1 subunit with apparent molecular masses of 190 and 210 kD were described in purified preparations (De Jongh et al., 1989, 1991) and in intact skeletal muscle cells (Lai et al., 1990). In this case, the COOH-terminal end of the smaller form is truncated in comparison to the larger form which contains the complete sequence encoded by the cloned cDNA. The COOH-terminal region of the full-length form is rapidly phosphorylated by cAMP-dependent kinase suggesting that the two size forms might be differentially regulated by protein phosphorylation (Lai et al., 1990; Rotman et al., 1992). Since cDNAs encoding the major, truncated form have not been found, the two forms of the skeletal muscle L-type α1 subunit are likely to be produced by post-translational proteolytic processing in vivo. More recently, two different size forms of the neuronal ω-CgTx-sensitive N-type α1 subunit were observed (Westenbroek et al., 1992). These two forms might also possess different COOH termini, since recently isolated cDNA clones predict two or more size forms of class B N-type α1 subunits with C-terminal ends of different length (Dubel et al., 1992; Williams et al., 1992*b*; Dubel, S. J., and T. P. Snutch, unpublished observations). Similarly, cDNAs encoding two different forms of the class D L-type α1 subunits as well as the class A and class E α1 subunits have been identified by molecular cloning. In all cases, the two forms have COOH-terminal ends of different length (Hui et al., 1991; Mori et al., 1991; Starr

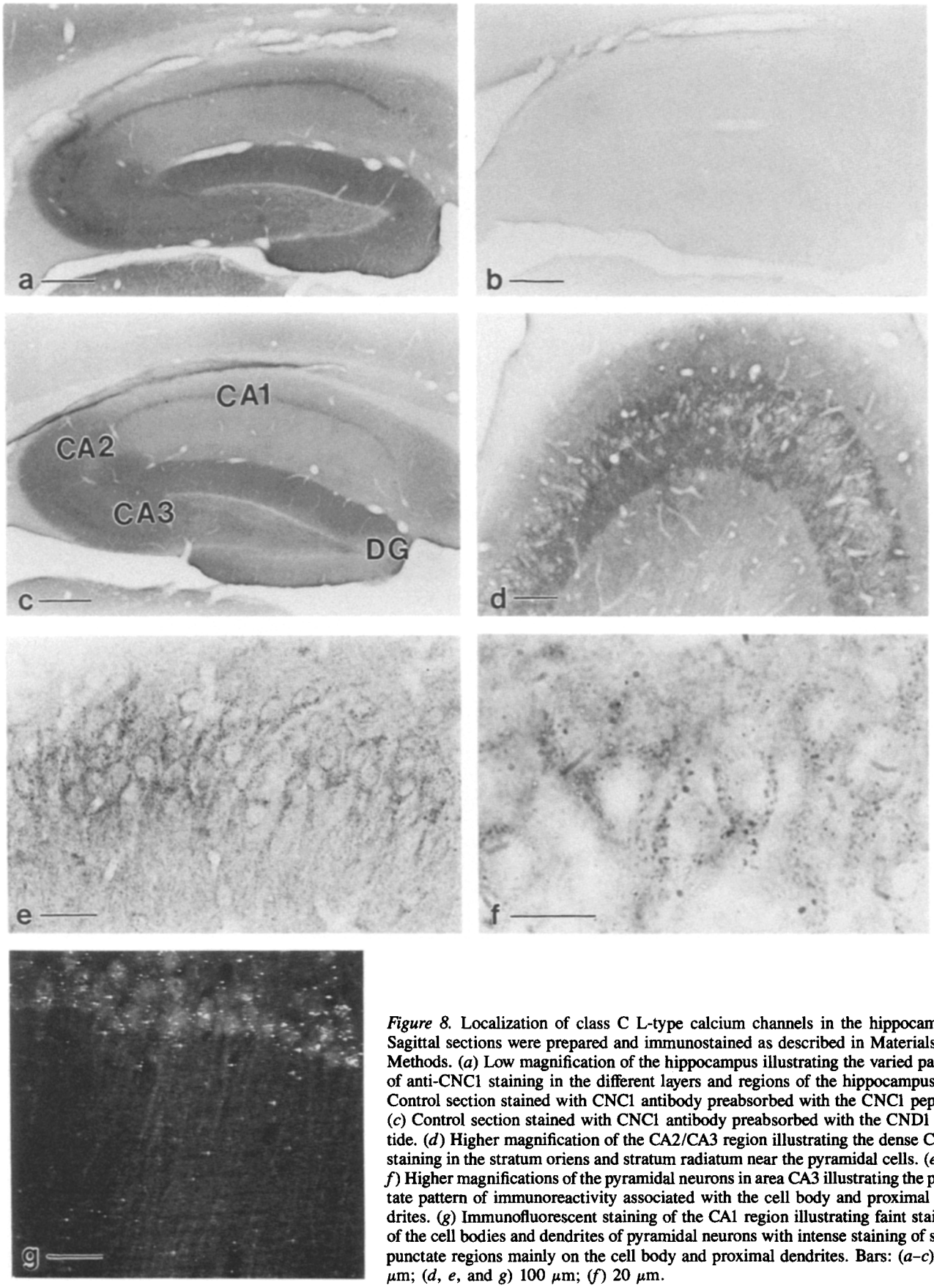


Figure 8. Localization of class C L-type calcium channels in the hippocampus. Sagittal sections were prepared and immunostained as described in Materials and Methods. (*a*) Low magnification of the hippocampus illustrating the varied pattern of anti-CNC1 staining in the different layers and regions of the hippocampus. (*b*) Control section stained with CNC1 antibody preabsorbed with the CNC1 peptide. (*c*) Control section stained with CNC1 antibody preabsorbed with the CND1 peptide. (*d*) Higher magnification of the CA2/CA3 region illustrating the dense CNC1 staining in the stratum oriens and stratum radiatum near the pyramidal cells. (*e* and *f*) Higher magnifications of the pyramidal neurons in area CA3 illustrating the punctate pattern of immunoreactivity associated with the cell body and proximal dendrites. (*g*) Immunofluorescent staining of the CA1 region illustrating faint staining of the cell bodies and dendrites of pyramidal neurons with intense staining of small punctate regions mainly on the cell body and proximal dendrites. Bars: (*a–c*) 500 μm; (*d*, *e*, and *g*) 100 μm; (*f*) 20 μm.

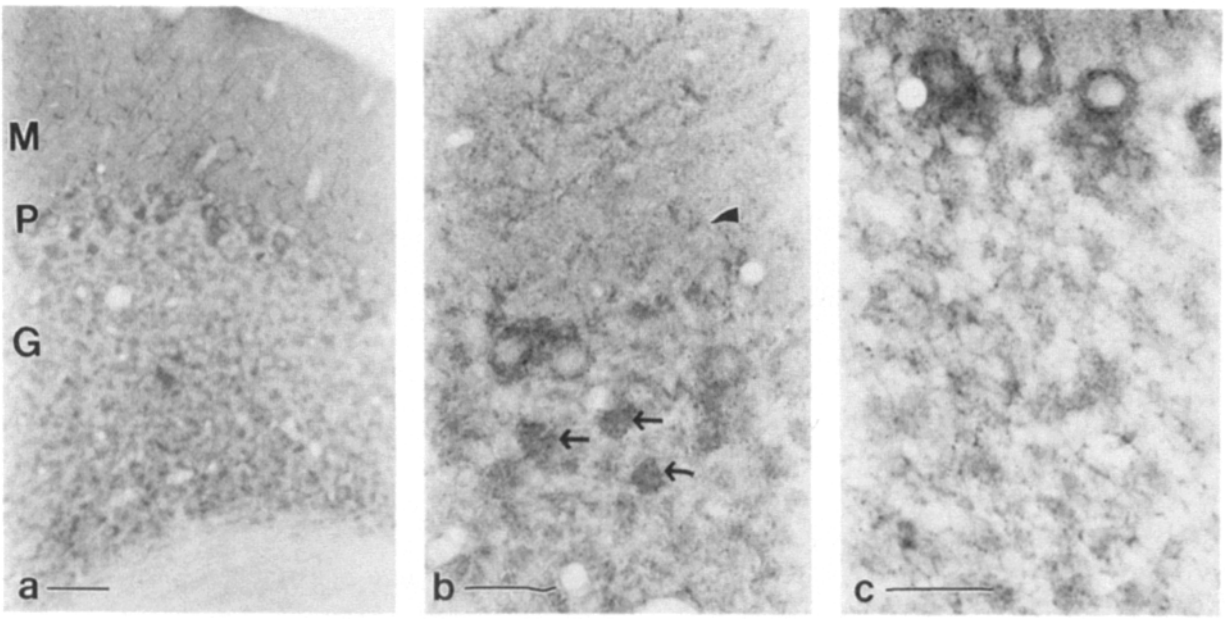


Figure 9. Localization of class C L-type calcium channels in cerebellum. Sagittal sections were prepared and stained as described in Materials and Methods. (*a*) Lower magnification of a region of the cerebellum illustrating the pattern of staining in the granular (*G*), Purkinje cell (*P*), and molecular (*M*) layers. (*b*) Higher magnification illustrating anti-CNC1 staining of Purkinje cell bodies and dendrites, especially at branch points. Faint staining of interneurons in the molecular layer (*arrowhead*) and of Golgi cells (*arrows*) in the granular layer were observed with the CNC1 antibody. (*c*) Higher magnification of the Purkinje cell layer and the granular layer to illustrate the intense anti-CNC1 immunoreactivity throughout the granular layer. Bars; (*a*) 100 μm; (*b* and *c*) 50 μm.

et al., 1991; Williams et al., 1992*a*; Niidome et al., 1992; Snutch et al., unpublished results). The higher and lower molecular mass forms of neuronal L-type α1 subunits may be created by differential splicing of mRNAs encoding their COOH-terminal ends or by a specific posttranslational proteolytic cleavage step.

The α1 Subunits of Class C and Class D L-Type Calcium Channels Are Differentially Localized within the Cell Bodies and Proximal Dendrites of Central Neurons

Previous immunocytochemical studies with the monoclonal antibody MANC1 raised against the α2 subunits of skeletal muscle calcium channels showed that most L-type calcium channels in the brain are localized in cell bodies and proximal dendrites of central neurons (Ahlijanian et al., 1990; Westenbroek et al., 1990). Whole cell voltage clamp recording detects L-type calcium currents in varying densities in the cell bodies of acutely dissociated hippocampal, cerebellar, and cerebral cortical neurons (Regan et al., 1991). Our immunocytochemical studies using anti-peptide antibodies that specifically recognize the class C and class D L-type α1 subunits demonstrate directly the presence of these two distinct calcium channels in the cell bodies and proximal dendrites of hippocampal pyramidal neurons, dentate granule neurons, cortical neurons, cerebellar Purkinje cells, many interneurons, and many other classes of central neurons. Chin et al. (1992) reported expression of mRNA encoding class D calcium channels in cortical, hippocampal, and cerebellar neurons. Evidently, the class C and class D α1 subunits are expressed by most brain neurons.

Within individual neurons, we observed a differential distribution of class C and class D α1 subunits. Class D L-type calcium channels are distributed evenly over most of the surface of the cell body with accumulations at the base of major dendrites of some neurons and a diminishing density on more distal regions of dendrites. Class C L-type calcium channels were generally distributed on the cell bodies and proximal dendrites at low density in many classes of central neurons, usually with a diminishing density on more distal dendritic regions. However, we also observed clusters of class C L-type calcium channels, mainly or exclusively on cell bodies and proximal dendrites. Class D calcium channels were not concentrated in clusters of Class C channels, but it is likely that they were present at a similar density in these clusters to other regions of the cell surface because we did not observe blank spots in the distribution of Class D channels that would correspond to the clusters of Class C channels. Based on our light microscopic studies, we hypothesize that these clusters are comprised of L-type calcium channels in the cell surface membrane, possibly at postsynaptic sites, rather than calcium channels in a discrete subset of presynaptic nerve terminals which form synapses primarily on the cell body and proximal dendrites. The clusters of class C calcium channels may also be associated with submembrane cisternae which are found in the cell bodies of many classes of central neurons and may represent specialized compartments involved in intracellular calcium regulation (Rosenbluth, 1962; Siegesmund, 1968; Henkart et al., 1976). Preliminary results of analysis of the localization of these clusters at high resolution in the electron microscope confirm that they are primarily in the cell surface membrane of cell bodies and dendrites and are often in a subsynaptic location. A

more definitive analysis of the nature of these clusters of the class C L-type calcium channels and their association with specific subcellular structures will require a combination of electron microscopic immunocytochemical studies and stereologic analysis of cellular compartments.

Possible Functions of L-type Calcium Channels in Cell Bodies and Proximal Dendrites

The distribution of L-type channels suggests that they exert greater influence on cytosolic calcium levels in the cell bodies and proximal dendrites than in the more distal dendrites or presynaptic nerve terminals. We hypothesize that the relatively dense localization of calcium channels serves to increase calcium influx at the base of major dendrites and in cell bodies in response to a summation of the excitatory synaptic inputs to the dendritic trees of central neurons. The relatively high concentration of L-type channels in cell bodies suggests an involvement of these channels in general cellular functions like regulation of cellular signaling pathways and gene expression. Elevation of the intracellular calcium concentration by activation of L-type calcium channels has been shown to directly activate Cl^- or K^+ channels and to modulate the activity of other proteins via Ca^{2+}-dependent phosphoprotein phosphatases and protein kinases (Kennedy, 1989). In addition, the expression of several genes depends on the activity of L-type channels in dissociated neurons and neural cell lines (Murphy et al., 1991; Sheng and Greenberg, 1990; Morgan and Curran, 1986). Thus, these L-type calcium channels may serve primarily to couple cell surface electrical signals to intracellular biochemical processes in the cell bodies of neurons.

What function might be served by the clustering of class C L-type calcium channels? L-type calcium channels in skeletal muscle cells are clustered at the junctions between transverse tubules and sarcoplasmic reticulum and are thought to initiate calcium release from the sarcoplasmic reticulum through physical interaction with the ryanodine receptor, the calcium release channel of the sarcoplasmic reticulum (Adams and Beam, 1990). L-type calcium channels may also be clustered at transverse tubule/sarcoplasmic reticulum junctions in cardiac cells, where they initiate calcium-induced calcium release by the cardiac form of the ryanodine receptor (Fabiato and Fabiato, 1979; Fleischer and Inui, 1989). Ryanodine receptors are expressed in neurons (McPherson and Campbell, 1990; Padua et al., 1991; Ellisman et al., 1990) and the cardiac form of the ryanodine receptor is localized to neuronal cell bodies (Kuwajima et al., 1992). Thus, it is possible that the clustered class C L-type calcium channels, like their counterparts in skeletal and cardiac muscle, may participate in localized release of intracellular calcium through direct interactions with intracellular calcium release channels or through calcium-induced calcium release. This activity might have an important impact on local calcium concentrations in cell bodies and proximal dendrites of neurons. In contrast to the clustered class C calcium channels, the more uniformly distributed class D L-type channels and the fraction of class C L-type calcium channels which are not clustered may serve as more general integrators of incoming information over the entire cell soma in response to action potentials and local depolarizations from the dendrites.

While we have emphasized that the majority of L-type calcium channels are localized in cell bodies and proximal dendrites, L-type channels are not absolutely restricted to these locations, but are also present in distal dendrites to varying degrees in different classes of neurons. For example, the weak dendritic staining for class C calcium channels was more prominent in the cerebral cortex, cerebellum, and CA2 and CA3 areas of the hippocampus relative to the CA1 area. In this location, L-type calcium channels may participate in calcium-dependent action potentials and intracellular calcium transients in dendrites. Thus, the class C and class D calcium channel subtypes are differentially distributed in different subcellular compartments, but their restricted localization does not appear to be all-or-none in nature. There is overlap in their distribution within individual neurons, different distributions of individual channel subtypes among different types of neurons, and varying densities within individual subcellular compartments indicating a very complex picture for the control of calcium influx into individual neurons through L-type calcium channels.

Comparison with the Localization of Other Calcium Channels

Compared to the class C and class D L-type calcium channels, N-type calcium channels encoded by the class B gene have a different, largely complementary distribution in neurons. Their density is highest in dendrites, suggesting that they play an important role in controlling calcium influx in this subcellular localization, and they are also present in some nerve terminals suggesting that they contribute to the rapid calcium influx which initiates release of neurotransmitters (Westenbroek et al., 1992). The differential expression and localization of calcium channels is likely to contribute to the specialized functional properties of the different subcellular compartments of neurons.

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