Heterologous expression studies have shown that the activity of voltage-gated Ca\(^{2+}\) channels is regulated by their \(\beta\) subunits in a \(\beta\) subunit isoform-specific manner. In this study we therefore investigated if one or several \(\beta\) subunit isoforms associate with L-type Ca\(^{2+}\) channels in different regions of mammalian brain.

All four \(\beta\) subunit isoforms (\(\beta1b, \beta2, \beta3, \) and \(\beta4\)) are expressed in cerebral cortex as shown in immunoblots. Immunoprecipitation of (+)-\(^{3}H\)trisradipine-labeled L-type channels revealed that the majority of \(\beta\) subunit-associated L-type channels was associated with \(\beta3\) (42 \(\pm\) 8\%) and \(\beta4\) (42 \(\pm\) 7\%) subunits, whereas \(\beta1b\) and \(\beta2\) were present in a smaller fraction of channel complexes. \(\beta3\) and \(\beta4\) were also the major L-type channel \(\beta\) subunits in hippocampus. In cerebellum \(\beta1b, \beta2, \) and \(\beta3\) but not \(\beta4\) subunits were expressed at lower levels than in cortex. Accordingly, \(\beta4\) was the most prominent \(\beta\) subunit in cerebellar L-type channels. This \(\beta\) subunit composition was very similar to the one determined for \(^{125}\)I-\(\omega\)-conotoxin-GVIA-labeled N-type and \(^{125}\)I-\(\omega\)-conotoxin-MVIIC-labeled P/Q-type channel complexes in cerebral cortex and cerebellum.

Our data show that all four \(\beta\) subunit isoforms associate with L-type Ca\(^{2+}\) channels in mammalian brain. This \(\beta\) subunit heterogeneity may play an important role for the fine tuning of L-type channel function and modulation in neurons.

Voltage-gated Ca\(^{2+}\) channels control the depolarization-induced influx of extracellular Ca\(^{2+}\) into neurons and other electrically excitable cells. They exist as hetero-oligomeric complexes of different subunits (\(\alpha1, \alpha2\delta, \) and \(\beta\)). Different types of neuronal Ca\(^{2+}\) channels (termed L-, N-, P-, Q-, and R-type; 1) are discriminated by biophysical and pharmacological criteria (for reviews see Refs. 2–5). N- and P/Q-type channels are blocked by peptide toxins (\(\omega\)-CTx\(^{1}\)-GVIA and \(\omega\)-CTx-MVIIC or \(\omega\)-agatoxin-IVA, respectively), whereas L-type channels are modulated by drugs, such as dihydropyridines (6). These channel types are differentially distributed in the brain and even within a neuron (7, 8). Thereby they serve different physiological functions. N- and P/Q-type channels are abundant in nerve terminals and control Ca\(^{2+}\)-dependent neurotransmitter release (3). L-type channels are localized mainly on neuronal cell somata and proximal dendrites where they may control Ca\(^{2+}\)-dependent modulatory processes and excitation-transcription coupling (9).

The above Ca\(^{2+}\) channel types consist of different \(\alpha1\) subunit isoforms (class A–E) that also form their drug or toxin binding domains and therefore determine their pharmacological properties (1). In contrast, important biophysical and modulatory properties, such as voltage-dependent gating (10, 11) and channel modulation by G-proteins (12, 13) and kinases (14), are determined not only by \(\alpha1\) but also by associated \(\alpha2\delta\) and \(\beta\)-subunits. Whereas only one \(\alpha2\delta\) isoform is known, four different \(\beta\) subunit isoforms (\(\beta1–\beta4\)) are expressed in mammalian brain (15, 16). Heterologous expression studies revealed that \(\beta\) subunits can affect \(\alpha1\) function in a \(\beta\) subunit isoform-specific manner. For example, Ca\(^{2+}\) currents carried by \(\alpha1A, \alpha1E, \) and \(\alpha1C\) inactivate faster with coexpressed \(\beta3\) than with \(\beta2\) (14, 17, 18) subunits. \(\beta1, \beta3, \) and \(\beta4, \) but not \(\beta2\), are permissive for voltage-dependent facilitation of Ca\(^{2+}\) channels formed by \(\alpha1C\) (19). \(\beta3\) and \(\beta1\) subunits confer slightly different pharmacological properties to L-type channels (20). Therefore \(\beta\) subunit heterogeneity could participate in the fine-tuning of channel function. However, it is unclear if only one or several \(\beta\) subunit isoforms associate with these channels in mammalian brain. So far only the \(\beta\) subunit composition of L-type Ca\(^{2+}\) channels in skeletal muscle has been studied. In this tissue exclusively \(\beta1a\) subunits are associated with the channel complex (15, 21).

Biochemical evidence for \(\beta\) subunit heterogeneity in mammalian brain has recently been provided for N-type and P/Q-type channels (22, 23). Multiple \(\beta\) subunit isoforms were found to be associated to different extents with both channel types after extraction from whole rabbit brain.

Here we report that \(\beta\) subunit heterogeneity also exists within neuronal L-type channels. We found that regional differences in the \(\beta\) subunit expression pattern affect \(\beta\) subunit composition in different regions of mammalian brain.

A preliminary report of our findings has appeared previously (24).

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagents were obtained from the following sources: \(^{125}\)I-\(\omega\)-CTx-GVIA, \(^{125}\)I-\(\omega\)-CTx-MVIIC (2200 Ci/mmole), and (+)-\(^{3}H\)trisradipine from DuPont NEN (Vien na, Austria); unlabeled \(\omega\)-CTx-GVIA from Sigma (Vienna, Austria); unlabeled \(\omega\)-CTx-MVIIC from Saxon Biochemicals (Hannover, Germany); pretaained molecular weight markers from Bio-Rad (Vienne, Austria); glutathione-Sepharose from Pharmacia (Vienna, Austria); Protein A-Sepharose from Sigma; calpain inhibitors I and II from Boehringer Mannheim (Vien na, Austria); all other protease inhibitors from Sigma.

**Sequence-directed Antibodies**—For antibody production in rabbits peptides were coupled to bovine serum albumin with glutaraldehyde (25) or synthesized on a lysine branch (octavalent NovaSyn PA resin,
Novabiochem) for immunization. Anti-β2 was generated as described (26). For immunoblotting and immunoprecipitation experiments, antibodies were purified by affinity chromatography on Sepharose-4B derivatized with the antigenic peptide (25). Antigenic epitopes comprised the following amino acids (residue number is given according to the sequences in Ref. 27): β1b, 516–536; β2, 595–604; β3, 470–483; β4, 469–474. Anti-β-com was raised against residues 61–79 in β1a (28).

Membrane Preparation—Membranes were prepared from guinea pig or rabbit cortex, hippocampus, cerebellum, and heart muscle as described (29). Brain regions were rapidly removed from rabbit or guinea pig brains and immediately placed in ice-cold homogenization buffer containing 0.02 mM NaHCO₃ and a protease inhibitor mixture (2 mM EDTA, 0.2 mM PMSF, 0.5 mM benzamidine, 2 mM iodoacetamide, 1 μM pepstatin A, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 20 μg/ml calpain inhibitor I and II, 0.1 mg/ml trypsin inhibitor). The tissues were then homogenized by 10–20 strokes in a Dounce homogenizer, and microsomes were collected by centrifugation at 45,000 × g (10 min, 4 °C). Microsomes were then washed three times with 50 mM Tris-HCl, pH 7.4 (37 °C), containing the same protease inhibitor mix. Membranes were resuspended in the same buffer at a protein concentration about 5 mg/ml and stored at −80 °C until use.

Affinity Purification of β Subunits and Immunoblotting—Glutathione S-transferase (GST) and a GST fusion protein with the α1 subunit interaction domain of the α1a subunit (AIDA) were prepared as described (21). All further steps were carried out on ice or at 4 °C. Typically 20 mg of microsomal protein isolated from rabbit or guinea pig brain regions were solubilized in 9 ml of buffer A (50 mM Tris-HCl, pH 7.4, containing the protease inhibitors used for membrane preparation) supplemented with 1% (w/v) CHAPS and 1 mM NaCl according to Ref. 21. 30–μl aliquots of glutathione-Sepharose equilibrated in buffer B (buffer A containing 0.1% (w/v) CHAPS, 0.1 mM NaCl) were coupled with 10 μg of GST or GST-AIDA and washed three times with the above buffer. Solubilized membranes were diluted 10-fold in buffer A and 4 ml were mixed with the coupled glutathione-Sepharose beads for 4 h or overnight. The beads were washed three times with 1.5 ml of buffer B, mixed with SDS-polyacrylamide gel electrophoresis sample buffer (15 min, 56 °C or 3 min, 95 °C), and the eluted protein separated on 10% polyacrylamide gels.

Immunoblot experiments were carried out as described (30). Prestained molecular weight markers (Bio-Rad) were run on the same gels. The apparent molecular masses of each batch were provided by the supplier.

Solubilization and Immunoprecipitation—Membrane-bound channels were prelabeled with (+)[^3]H]isradipine (1–2 nM) for 60 min at 37 °C in 50 mM Tris-HCl, 0.1 mM PMSF, 1 mM CaCl₂. All subsequent steps were carried out on ice or at 4 °C. Prelabelled membranes were collected, solubilized on ice for 60 min in 50 mM Tris-HCl, pH 7.4, 0.15 mM NaCl, 1% (w/v) digitonin, 0.2 mM PMSF, 0.5 mM benzamidine, 2 mM iodoacetamide, 1 μM pepstatin A, and nonsoluble proteins removed by centrifugation (10 min, 10,000 × g, 60 min). The digitonin extracts were used for immunoprecipitation or were further affinity-purified by chromatography on wheat germ agglutinin (WGA)-Sepharose (2 ml of packed resin for 5–20 mg of solubilized protein). Channel-associated activity was eluted from the resin in equilibration buffer (50 mM Tris-HCl, pH 7.4, 0.15 mM NaCl, 0.1 mM PMSF) containing 6% (w/v) N-acetylgalosamine. Active fractions were directly used in immunoprecipitation experiments or quickly frozen in liquid nitrogen and stored at −80 °C until use.

For immunoprecipitation of labeled channels, affinity-purified antibodies were coupled to Protein A-Sepharose. The Protein A-Sepharose-antibody complex was washed three times with 1.5 ml of ice-cold RIA buffer (solubilization buffer containing 0.1% digitonin) and then incubated for 12–16 h at 4 °C with 0.15–0.55 ml of solubilized extracts (prelabelled N- and P/Q-type channels) or WGA-Sepharose-purified channel preparations (L-type channels). Unbound radioactivity was removed by four 1.5-ml washes with RIA buffer. Bound radioactivity was determined by liquid scintillation of (+[^3]H]isradipine) or gamma-counting ([125]I-o-CTX-GVIA, [125]I-o-CTX-MVIIC).

High affinity binding of (+[^3]H]isradipine to solubilized L-type Ca²⁺ channels was determined using a filtration assay as described (29). This assay underestimated the total specific (+[^3]H]isradipine binding activity by about 20%. This was taken into account to calculate the binding activity employed for immunoprecipitation assays.

Statistics—Data are given as means ± S.D. for the indicated number of experiments.

FIG. 1. Affinity purification of β subunits. The results of Western blot analysis are shown. A, skeletal muscle β1a subunits. 2.3 mg of partially purified rabbit skeletal muscle transverse-tubule membranes were solubilized in 0.9 ml of buffer containing 1% CHAPS. 0.1 ml of the solubilized material was subjected to affinity chromatography on 5 μg of GST-AIDA (lanes 1–3) or GST alone (lanes 4–6) coupled to glutathione-Sepharose (0.03 ml) as described under “Experimental Procedures.” Identical aliquots (40 μl) of the extracted starting material (lanes 1 and 4) and the supernatant after incubation with the resins (lanes 2 and 5) and resin-bound protein (lanes 3 and 6) were separated by SDS-polyacrylamide gel electrophoresis and analyzed for β immunoreactivity in immunoblots employing anti-β-com. 52–56-kDa bands were stained as expected for β1a staining in skeletal muscle membranes (21). The same bands were stained in purified channel preparations (>90% pure, not shown). B, enrichment of β3 (left panel) and β4 (right panel) immunoreactivity from guinea pig cortex membranes. 20 mg of membrane protein were solubilized in a total volume of 9 ml and diluted 10-fold as described under “Experimental Procedures.” 4-ml aliquots were subjected to affinity chromatography on GST-AIDA (10 μg) coupled to glutathione-Sepharose. Immunostaining was with affinity-purified anti-β3 (lanes 1–3, left) or anti-β4 (lanes 1–3, right). Lanes 1 and 4, starting material (40–μl aliquots); lanes 2 and 5, supernatants (40 μl aliquots); lanes 3 and 6, GST-AIDA resin-bound protein. No other bands were specifically enriched. The electrophoretic mobilities of the stained bands were indistinguishable from those in Fig. 2. One of two experiments yielding similar results is shown.

RESULTS

Region-specific Expression of β Subunit Isoforms in Mammalian Brain—To investigate the association of all known subunit isoforms with neuronal voltage-gated L-type Ca²⁺ channels in mammalian brain, we raised anti-peptide antibodies against unique sequences of β1b, β2, β3, and β4 subunits. In addition, an antibody against an epitope highly conserved in all β subunit isoforms (anti-β-com) was generated. We used these antibodies to determine their association with neuronal Ca²⁺ channels solubilized from rabbit or guinea pig cerebral cortex, hippocampus, and cerebellum membranes in immunoprecipitation experiments. Their expression in these brain regions was analyzed in Western blots.

To determine their relative expression densities the four β subunit isoforms were extracted with CHAPS from microsomes prepared from brain (β1b, β2, β3, β4) or, for control purposes, from skeletal muscle (β1a). Extracts were affinity-purified on GST-AIDA-Sepharose (21) in the presence of protease inhibitors as described under “Experimental Procedures.” As shown in Fig. 1 for skeletal muscle β1a (anti-β-com staining) and neuronal β3 and β4 subunits, the enrichment of β subunit immunoreactivity was specific and absent when only GST was used as the affinity matrix (Fig. 1, lanes 4–6). In β subunit preparations from rabbit cerebral cortex anti-β-com specifically recognized a 63 ± 3/67 ± 3 kDa doublet and a 88 ± 4 kDa band (Fig. 2) (n = 4). A ~33-kDa band was also stained to a variable extent by β-com as well as all the other β antibodies and corresponded to the GST-AIDA polypeptide present at relatively high amounts (10 μg) in the β subunit preparations.

To assign the β-com-stained bands to individual β subunit isoforms, samples separated on the same gel were stained with isoform-specific antibodies. The 88-kDa band was composed of
anti-β1b (Fig. 2A) and anti-β2 staining (Fig. 2B). In contrast, bands stained by anti-β4 and anti-β3 accounted for the βcom immunoreactivity in the 63/67-kDa doublet. The majority of anti-β3 immunoreactivity was associated with the larger 67-kDa βcom band, whereas anti-β4 recognized both bands of the doublet to a variable extent (Figs. 1–3).

β subunit isoform staining was specific. It was completely suppressed in the presence of 1 μM of the respective antigenic peptides (not shown). As expected, only βcom but not the isoform-selective antibodies specifically recognized β1a extracted from partially purified skeletal muscle T-tubule membranes (Fig. 2A). βcom staining in rabbit heart represented β2 immunoreactivity (Fig. 2B) suggesting that other isoforms are absent or expressed at much lower levels in this tissue.

The same bands were also present in hippocampus (Fig. 2) and cerebellum extracts (Fig. 3). The relative abundance of the 88-kDa band was lowest in cerebellum because β1b and β2 expression density was lower in this region as compared with cerebral cortex (Fig. 2). When similar amounts of solubilized membrane protein from cerebral cortex and cerebellum were subjected to β subunit isolation and Western blotting (Fig. 3) similar βcom staining intensity was found for the 63/67-kDa doublet. β3-specific immunoreactivity was less abundant in cerebellum, whereas β4 was expressed at similar densities as in cerebral cortex (Fig. 3).

Taken together, the βcom staining pattern in mammalian brain can be explained by the presence of all four β subunit isoforms which are expressed in a region-specific pattern.

Neuronal L-type Ca2+ Channels Are Associated with Different β Subunit Isoforms in Mammalian Brain—After having established the specificity of our antibodies, we investigated if L-type Ca2+ channels are associated with only one or several β subunit isoforms and if β subunit association varies in different brain regions. We reversibly labeled neuronal L-type Ca2+ channels complexes in cerebral cortex, hippocampus, and cerebellum membranes with the L-type Ca2+ channel-selective ligand (+)-[3H]isradipine and solubilized them in buffer containing 1% (w/v) digitonin. In cerebral cortex and hippocampus 74 ± 9% (n = 4) and 91 ± 22% (n = 4) of the solubilized (+)-[3H]isradipine labeling was immunoprecipitated with saturating concentrations of an antibody directed against α1C indicating that binding was associated with L-type channel complexes. 61 ± 18% (n = 5) and 80 ± 31% (n = 4) of the labeled L-type channels were immunoprecipitated by βcom. Therefore, most of the L-type channel complexes are associated with a β subunit which is accessible for βcom under non-denaturing conditions.

The results of Western blot analysis are shown. β subunits were extracted from equal amounts of membrane protein prepared from cerebral cortex (CTX) or cerebellum (CER) as described in Fig. 2 and separated on adjacent lanes. Immunostaining was carried out with the indicated affinity-purified antibodies. Molecular weight markers are as in Fig. 2.
Immunoprecipitation experiments with the isoform-selective antibodies revealed the association of more than one β isofrom with the channel complex. Affinity-purified anti-β3 and anti-β4 antibodies each immunoprecipitated 42% of the radioactivity recognized by anti-βcom (Fig. 4A). Smaller fractions were bound by anti-β1b and anti-β2 (Fig. 4A). Together our subunit-specific antibodies accounted for all (118%) βcom immunoprecipitable radioactivity in cerebral cortex.

Immunoprecipitation by these antibodies was saturable (see Fig. 5C). The nonspecific background signal observed with the same concentrations of control rabbit immunoglobulin was less than 10% (n = 3) of the radioactivity recognized by anti-βcom. In control experiments only βcom, but none of the isoform-specific antibodies, immunoprecipitated (+)-[3H]isradipine. Aliquots of the labeled channel preparation were diluted with RIA buffer to a final volume of 0.3 ml and subjected to immunoprecipitation as described for neuronal channels. One of two typical experiments is shown. Numbers denote the β isofrom to which antibodies were generated; C, βcom.

Similar β Subunit Composition of L-, N-, and P/Q-type Ca2+ Channels—Next we tested if the β subunit composition of L-type Ca2+ channels resembles the subunit composition of N- and P/Q-type Ca2+ channels in these regions (22, 23). For N- and P/Q-type Ca2+ channels it has been investigated before in digitonin extracts of whole brain membranes, but data on individual brain regions were unavailable. We therefore also subjected 125I-ω-CTx-GVIA- and 125I-ω-CTx-MVIIC-labeled channel complexes extracted from cerebral cortex and cerebellum to immunoprecipitation with our antibodies. We have previously shown that under our experimental conditions saturable high affinity 125I-ω-CTx-GVIA and 125I-ω-CTx-MVIIC binding occurs selectively to N-type and P/Q-type Ca2+ channels, respectively, with dissociation constants in the subpicomolar range (31).

In cerebral cortex and cerebellum saturating concentrations of anti-βcom recognized 85 ± 23% (n = 5) and 84 ± 13% (n = 4) of channels associated with 125I-ω-CTx-GVIA binding activity, respectively. The immunoprecipitation profile was very similar to L-type channels (Fig. 5, A and B). β3 and β4 subunits together immunoprecipitated >80% of βcom immunoprecipitable 125I-ω-CTx-GVIA binding in a saturable manner (Fig. 5C). As with L-type channels, a smaller fraction of N-type channel binding was associated with β1b and β2. In cerebellum again only β4 antibodies recognized substantial portions of N-type channel activity (Fig. 5B). Similar results as described for N-type and L-type channels were also obtained for 125I-ω-CTx-MVIIC-labeled P/Q-type channels in cerebral cortex (not shown). In cerebellum only β4 antibodies recognized significant
amounts of 125I-v-CTx-MVIIC-labeled P/Q-type channels (43 ± 18.5%, n = 3).

Isoform-selective antibodies completely accounted for the N-type (105%, Fig. 5A) and P/Q-type (>85%, not shown) channel binding recognized by anti-βcom in cerebral cortex but only for 40–50% in cerebellum. As for L-type channels this difference cannot be attributed to differences in membrane preparation because it was also found when the respective brain regions were isolated from the same animals in the same buffer and carried through the whole solubilization and immunoprecipitation procedure in parallel. It is therefore possible that in hippocampus and cerebellum immunoprecipitation by one or several of our antibodies was underestimated. At present we do not know if this is due to the expression of a yet uncharacterized β subunit isoform, which is immunoprecipitated by βcom but none of the other antibodies, or due to region-specific differences in proteolysis. C-terminal proteolysis could remove the C-terminal epitopes of our isoform-specific antibodies. However, we have obtained no evidence for extensive proteolytic breakdown of β subunits in immunoblots with our βcom antibody, which recognizes an epitope located near the N terminus of the β subunits.

**DISCUSSION**

**β Subunit Heterogeneity within L-type Ca2+ Channels**—The major findings of our study are as follows. 1) All known β subunit isoforms participate in the formation of neuronal L-type Ca2+ channels in mammalian brain. 2) β3 and β4 subunits are most often found as part of the neuronal L-type channel complexes. 3) The fractional contribution of a particular β subunit isoform for channel formation varies among different brain regions. 4) The β subunit composition and regional differences are very similar to N- and P/Q-type channels in cerebral cortex and cerebellum.

This similarity of the β subunit composition between L-type channels and N- as well as P/Q-type channels is interesting because the subcellular distribution of L-type channels in neurons differs significantly from the distribution of N- and P/Q-type channels. L-type α1C and α1D subunits are predominantly found on the cell soma and proximal dendrites, whereas N-type α1B and P/Q-type α1A are also found along the length of dendrites and in presynaptic terminals (8, 32). Despite these differences in neuronal targeting, these channel types do not show major differences with respect to their β subunit composition. Obviously different β subunit isoforms can be targeted to different regions of a neuron.

Both α1C and α1D subunits participate in the formation of L-type Ca2+ channels in mammalian brain. We have made no attempts to determine if differences exist between the two L-type channels with respect to their β subunit composition. The fraction of channels associated with α1D is small (not more than 9–26%) in hippocampus and cerebral cortex as revealed by our immunoprecipitation experiments with α1C; see also Ref. 33) and therefore complicates such an analysis. We cannot exclude the possibility that β1b and β2, which are found only in a minor fraction of channels, are selectively associated only with α1D. However, based on our finding that α1C is associated with the majority of labeled channels in cerebral cortex and hippocampus, β-subunit heterogeneity must exist within class C L-type channels in these regions.

**Implications for Neuronal L-type Ca2+ Channel Function**—β subunits strongly affect the functional properties of the pore-forming α1 subunits of L-type (and non-L-type) channels. As shown by heterologous coexpression in X. laevis oocytes and mammalian cells, β subunits affect channel gating (10, 11), modulation by G-proteins (12, 13), and phosphorylation (14) as well as Ca2+ and drug (34, 35) interaction with L-type α1 subunits. Such studies also revealed that different β isoforms are able to confer different channel properties. For example, β3 confers a more rapid inactivation to currents mediated by α1C (17) than does β2. β isoform-specific effects on channel inactivation were also observed for non-L-type Ca2+ channel α1 subunits (11, 14, 18). Only β1, β3, and β4, but not β2, support voltage-dependent facilitation of α1C-mediated Ca2+ currents (19). Similarly, small differences in the sensitivity of the channel to the Ca2+-antagonist mibebradil and the modulation by protein kinase C were observed when different β subunits form part of the channel complex (14, 20). We now provide direct biochemical evidence that indeed different β subunits participate in the formation of neuronal L-type channels suggesting that these isoform-selective effects contribute to L-type Ca2+ channel plasticity in mammalian brain. β subunits could be involved in the fine tuning of L-type channel function in a region-specific manner. Based on our findings future coexpression studies should preferentially focus on the comparison of the properties of L-type channels containing β3 or β4 subunits, because these isoforms seem to be present in the majority of dihydropyridine-sensitive L-type channels in cortex, hippocampus, and cerebellum.

Taken together our data demonstrate that, like in other neuronal Ca2+ channel types, several β subunit isoforms contribute to the formation of neuronal L-type channels. Further studies must focus on the physiological and pathophysiological consequences of this heterogeneity and investigate if changes in β subunit expression could account for changes in L-type Ca2+ channel function also under pathophysiological conditions, such as neurodegeneration, cerebral ischemia, or aging (36).

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β Subunit Heterogeneity in Neuronal L-type Ca\(^{2+}\) Channels
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