**The Neuronal β₄ Subunit Increases the Unitary Conductance of L-type Voltage-gated Calcium Channels in PC12 Cells**

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**β** subunits of voltage-gated calcium channels influence channel behavior in numerous ways, including enhancing the targeting of α₁ subunits to the plasma membrane and shifting the voltage dependence of activation and inactivation. Of the four β subunits that have been identified, β₄ is of particular interest because mutation of its α₁ subunit interaction domain produces severe neurological defects. Its differential distribution in the hippocampus prompted us to examine whether this subunit was responsible for the heterogeneity of hippocampal L-type calcium channels. To study the functional effects of the β₄ subunit on native L-type calcium channels, we transfected β₄ cDNA subcloned out of embryonic hippocampal neurons into PC12 cells, a cell line that contains the β₁, β₂, and β₃ subunits but not the β₄ subunit. Cell-attached single-channel recordings of L-type channel activity from untransfected and transfected PC12 cells compared with recordings obtained from hippocampal neurons revealed an effect of the β₄ subunit on single-channel conductance. L-type channels in untransfected PC12 cells had a significantly smaller conductance (19.8 pico siemens (pS)) than L-type channels in hippocampal neurons (22 pS). After transfection of β₄, however, L-type single-channel conductance was indistinguishable between the two cell types. Our data suggest that calcium channel β₄ subunits affect the conductance of L-type calcium channels and that native hippocampal L-type channels contain the β₄ subunit.

Voltage-gated calcium channels are multisubunit proteins that show considerable heterogeneity in neuronal and nonneuronal cell types. All voltage-gated calcium channels have a central α₁ subunit that contains the ion pore and the voltage sensor and is capable of conducting current on its own. In addition to the α₁ subunit, channels are composed of several auxiliary subunits. Each calcium channel consists minimally of an α₁, β, and α₂δ subunits in a 1:1:1:1 molar ratio. The auxiliary subunits are in direct contact with the α₁ subunit and influence several aspects of channel gating as well as targeting of the channel to the membrane (for review, see Ref. 1). Multiple genes have been identified that code for α₁, β, and α₂δ subunits (10, 4, and 3, respectively) and the numerous potential combinations are a key contributor to the functional diversity of voltage-gated calcium channels.

There are general relationships that exist between calcium channel type and function. In the central nervous system, P-type (Ca₂.1) and N-type (Ca₂.2) channels, formed from α₁A and α₁B subunits, respectively, are largely responsible for supplying the calcium influx required for release of neurotransmitter (for review, see Ref. 2). L-type channels (Ca₁.1/1.3), the subject of this study, are formed from α₁C and α₁D subunits, and have been shown to be involved in some types of hippocampal synaptic plasticity (3–9) as well as more global cell signaling such as initiation of gene transcription (for review, see Ref. 10).

Previous research in our laboratory and others (11–16) has provided evidence for distinct subtypes of L-type calcium channels in central nervous system neurons, referred to by us as Ls and Lp. The Ls channel resembles the “standard” cardiac L-type channel in its fundamental characteristics, whereas the Lp channel has a smaller single-channel conductance and shows a distinctive low voltage-dependent potentiation of its activity. In this study we focused on the potential role of a particular β subunit in producing this diversity.

Four different genes coding for β subunits have been identified (for review, see Ref. 17). β subunits have no hydrophobic domains and thus are not anchored in the cell membrane. Instead, they are located intracellularly and bind to the α₁ subunit through protein-protein interaction domains (for review, see Ref. 18). Each of the β subunits has a distinctive distribution in the brain (19–23), and predictions of α₁-β inter- actions have been made based upon co-localization of the subunits (21). The predominant β subunits that associate with L-type channels in the hippocampus are β₃ and β₄ with the two being present at approximately equal levels (23). The β₄ subunit is also distinguished by binding to an additional α₁ subunit interaction domain (24). To understand the potential contribution of the β₄ subunits to L-type channel heterogeneity, we utilized undifferentiated PC12 cells which contain β₁-β₃, but lack the β₄ subunit (25) and also do not contain Lp channels. We found that transfection of the neuronal β₄ subunit into PC12 cells increased L-type channel unitary conductance, but did not produce L-type channels that showed the voltage-de- pendent potentiation characteristic of Lp channels. Thus we conclude that the β₄ subunit associates with native hippocampal L-type channels and accounts for their larger conductance as compared with PC12 L-type channels.

**EXPERIMENTAL PROCEDURES**

*Primary Hippocampal Cell Cultures*—Hippocampal neurons were obtained from embryonic day 18 Sprague-Dawley rats. Hippocampi were removed bilaterally and maintained in cold phosphate-buffered saline during dissection. The tissue was triturated in 2 ml of MEM with 7.5% fetal bovine serum, and cells were plated on poly-d-lysine

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coated Petri dishes at a density of 10^6 cells/35 mm dish. Cultures were maintained at 37 °C in a 95% air/5% CO₂ humidified incubator in serum-free medium made up of MEM and Ham’s F12 (1:1) and containing 6 mg/ml glucose, 100 µg/ml transferrin, 25 mg/ml insulin, 20 mM progestrone, 60 µM putrescine, 30 mM selenium, and 0.5 units/ml and 0.5 mg/ml of penicillin and streptomycin, respectively. Cells were used between 3 and 14 days in culture.

Electrophysiological Recordings—Patch-clamp pipettes were pulled from borosilicate glass and filled with BaCl₂-solution containing (in mM): 20 BaCl₂, 90 choline chloride, 10 tetraethylammonium, and 10 HEPES (26). Cell-attached single-channel recordings were obtained using standard techniques (27) in a solution containing (in mM): 140 potassium glutamate, 10 HEPES-KOH, and 5 EGTA. The L-channel agonist FPL 64176 was included in all recordings. Recordings were made with an Axopatch 200 amplifier, and data was sampled with an INDEC 15125 analog-to-digital converter at 5 kHz and filtered at 1 kHz. Unless specified otherwise, the holding potential used was −70 mV. Voltage pulses were delivered at 5 s intervals. Leak and capacitative currents were subtracted digitally.

Data Analysis—Open times and open probabilities were obtained from sweeps idealized with the half amplitude crossing criterion and cubic spline interpolation (28). Overall open probability was calculated from all sweeps in response to a particular stimulus, including null-sweeps. Tail currents were included in the analysis. Results from open time analysis were plotted on square root-log coordinates, and mean open times were estimated from the maximum likelihood fitting (29). Data from all the experiments were summed and treated as a single distribution.

PC12 Cell Culture—PC12 cells were grown in culture flasks and maintained at 37 °C in a 95% air/5% CO₂ humidified incubator in Dulbecco’s modified Eagle’s medium with 7.5% fetal bovine serum, 7.5% horse serum, and 1 ml penicillin/Streptomycin (10,000 units/ml and 10 mg/ml, respectively). Once per week the cultures were fed and subcultured, using trypsin to lift cells off the bottom of the flasks, and as needed they were plated on 35 mm dishes coated with poly-L-lysine. Each batch of cells was passaged no more than 10 times before a new culture was started from the stock kept in liquid nitrogen. After plating, the cells were used for recordings within 1 week.

Molecular Biology—Messenger RNA was isolated from hippocampal and PC12 cell cultures using Oligotex Direct mRNA kit from Qiagen and eluted in a volume of 40 µl. cDNA was synthesized immediately following mRNA isolation with 6 µl of mRNA per reaction, using the SuperScript II reverse transcriptase enzyme from Invitrogen. Two different kinds of primers were used for the cDNA synthesis, oligo (dT) primers and random hexamer primers, and the two reactions were carried out separately. cDNAs synthesized from these two types of primers were mixed in a 1:1 ratio and used as templates for PCR reactions.

PCR primers used for amplification of β subunits and actin were designed based on published sequences. GenBank accession numbers for these sequences are as follows: rat β1, X61394, rat β2, M508545, rat β3, M88751, rat β4, L02315 and rat actin V01217. The sequences of the PCR primers were: β1 forward (nucleotide (nt) 1394–1413), AGG GAC CCT ACC TGT TTT CC; β1 reverse (nt 1899–1890), GAG CCT CTT CTC TCC CAG AAC; β2 forward (nt 1756–1781), GCT GAA GAA GAA CCT TGT GAA CC; β2 reverse (nt 2215–2204), AGA GAC ACG CAC GGT CAT TG; β3 forward (nt 1103–1122), TGG ATG AGA ACC AGC TGG AC; β3 reverse (nt 1392–1411), GAGCGTGTACACGTTCTG; β4 forward (nt 1464–1484), CCT TCT CAC GGT ATC CCA GAG; β4 reverse (nt 1813–1838), CGT GTT TAT TAT CTC ATG ACT ATG GC; actin forward (nt 262–281), TAC AAC CTC CTT GCA GCT CC; actin reverse (nt 294–313), CTT CGG CCA ACT GAT ATG AC.

For subcloning β1 was amplified by PCR using the following primers: GCA CGT CAG ACC ATG TCG TCC TCC GAC GCC TGC (3' primer), and GCA ACC GCG GTC AAT GCC TTC TGC TGC GGC AG (3' primer). The amplified β1 DNA was subcloned into the bistristrone plCMS-EGFP vector (Clontech).

LipofectAMINE 2000 (Invitrogen) was used to transf ect cDNA constructs into PC12 cells 24 h after plating. Cells were incubated with LipofectAMINE/DNA mix for 4 h (2 µg DNA + 5 µl LipofectAMINE in 1 ml Opti-MEM), in the absence of antibiotics. Following transfection, Opti-MEM was replaced with PC12 culture medium. Electrophysiological recordings were started 48 h after transfection.

RESULTS

We have shown previously that cultured hippocampal neurons possess two kinds of L-type voltage-gated calcium channels, Lp and Ls, that differ in their response to depolarizing voltage pulses (12, 13) and high frequency stimulation (16). In this study, cell-attached single-channel recordings from undifferentiated PC12 cells made with identical procedures revealed L-type channel activity that was highly reminiscent of Ls channels in hippocampal neurons (Fig. 1). None of the 28 recordings from PC12 cells, however, showed any sign of reopening activity, the defining characteristic of Lp channels. The recordings were typically greater than 30 min in duration (mean = 33.9 ± 3.0 min) and contained 3 channels (mean = 3.2 ± 0.3). Comparison of hippocampal Ls channel recordings and PC12 cell L-type recordings suggested that the two kinds of channels were very similar (Fig. 1, A and B). Measurement of the open time distributions confirmed this general impression (Fig. 1C). In the presence of 1 µM FPL 64176, two major open time components were observed that were not statistically different between hippocampal Ls channels and PC12 cell L-type channels (p > 0.05, Kolmogorov-Smirnov test). The two kinds of channels were not identical, however. Measurement of unitary conductance showed that hippocampal Ls channels had a significantly higher slope conductance (22.0 ± 0.2 pS; n = 8) than PC12 cell L-type channels (19.8 ± 0.3 pS; n = 18; p < 0.05; Fig. 1D). Thus, PC12 cells appear to have only a single kind of L-type voltage-gated calcium channel that has properties similar to Ls channels in hippocampal neurons. The only detected difference between the two lies in their single channel conductance. We used this observation to determine whether differential expression of the calcium channel β subunit genes in the two cell types could account for this difference in single channel conductance.

To assay for the presence of β subunits in hippocampal neurons and PC12 cells, mRNA was isolated from these cells and cDNA was synthesized by reverse transcription. There are four known genes for calcium channel β subunits (β1–β4), and four pairs of PCR primers were designed based on published sequences for these genes in rat. The primers were designed to amplify a C-terminal domain of these proteins. As a control for the absence of genomic DNA in the PCR reaction, PCR was also preformed using mRNA samples, which had not been subjected to reverse transcription. The PCR products were analyzed a 2% agarose gel.

We found that all four pairs of β subunit primers amplified their respective cDNA targets from the hippocampal cultures, indicating that all four β subunit genes are expressed in hippocampal neurons (Fig. 2). In the case of PC12 cells, cDNAs coding for β1, β2, and β4, were amplified, but cDNA for β3 was not, confirming other reports (25) that the gene for this β subunit is not expressed in PC12 cells (Fig. 2). The band for β3 from PC12 cells was weaker than the bands for β1 and β3, indicating less amplification. The amount of amplification of β3 varied depending on the specific condition of the PCR reaction (temperature and magnesium concentration). However, there was never any hint of amplification of β4 even under very different PCR conditions. There was also no amplification in any of the samples when reverse transcription was omitted, demonstrating a lack of genomic DNA contamination (Fig. 2.—) in the PCR reactions. As a positive control for the presence of cDNA in both hippocampal and PC12 samples, actin primers were used. The size of each PCR product was as predicted by the published sequence for the four β subunits and actin (496 base pairs (bp) for β1, 438 bp for β2, 308 bp for β3, 374 bp for β4, and 813 bp for actin).

Because Ls channels in hippocampal neurons have a larger single channel conductance than L-type channels in PC12 cells, and because hippocampal neurons express a calcium channel β subunit that is not expressed by PC12 cells, it is possible that
β₄ Modulation of L-channel Conductance

**Fig. 1.** L-channels in PC12 cells are very similar to Ls channels in hippocampal neurons; the only difference is in their single channel conductance. A, example traces of a cell-attached recording from a cultured hippocampal neuron showing Ls channel activity in response to a step depolarization from −70 mV to −10 mV. Note the similarity to the PC12 cell L-channel in B. The L-channel agonist FPL 64176 (1 μM) was included in both recordings. B, example traces of a cell-attached recording from a PC12 cell showing L-channel activity. C, comparison of the open time distributions at −10 mV for L-channels in PC12 cells (dark bars in background) and Ls channels in hippocampal neurons (light bars in foreground). Extracted mean open times for L-channels in PC12 cells, 0.6 and 65 ms; and for Ls channels in neurons, 0.6 and 55 ms. The two distributions are not significantly different, p > 0.05 (Kolmogorov-Smirnov two-sample test). D, comparison of single channel conductance of L-channels in PC12 cells and Ls in hippocampal neurons. The conductance was 19.8 ± 0.3 pS in PC12 cells, n = 18, and 22.0 ± 0.2 pS in hippocampal neurons, n = 8, p < 0.05.

**Fig. 2.** Hippocampal neurons express all four calcium channel β subunits, whereas PC12 cells only express three. Results from PCR amplification of hippocampal neuron (HIPP) and PC12 cell (PC12) cDNA with primers for β₁, β₂, β₃, and actin. The PCR products were separated on a 2% agarose gel. Molecular weight standards are in the first and last lanes. Lanes with + signs represent PCR reactions in which cDNA was included, and lanes with − signs represents PCR reactions in which no reverse transcriptase was included in the cDNA synthesis, and thus serve as controls for the presence of genomic DNA. Note the absence of amplification in the β₄ PC12 + lane. Size of amplified PCR products were as expected from published sequences: 496 bp for β₁, 438 bp for β₂, 308 bp for β₃, 374 bp for β₄, and 813 bp for actin.

This calcium channel subunit contributes to the difference in single channel conductance. To test this hypothesis, we cloned the neuronal β₄ subunit into a bicistronic expression vector (pIRES) and transiently transfected PC12 cells to test if the presence of β₄ would change the conductance of L-type channels in PC12 cells. This vector allows for the co-expression of enhanced green fluorescent protein (EGFP) and the β₄ subunit as two separate molecules in the transfected PC12 cells (Fig. 3, A and B). When recording from PC12 cells transfected with β₄ DNA, we found an increased single channel conductance of L-channels compared with untransfected cells (Fig. 3, C–F). To confirm this result, three separate β₄ clones were tested. We consistently found an increase in the single channel conductance of transfected PC12 cell L-type channels in the three β₄ clones (Fig. 4A). The conductance of L-type channels in control untransfected PC12 cells was 19.8 ± 0.3 pS (n = 18). In cells transfected with clones 1–3, it was 22.6 ± 0.3 pS (n = 11), 22.2 ± 0.4 pS (n = 7), and 22.3 ± 0.6 pS (n = 6), respectively. The increase in conductance was statistically significant for all three of the β₄ clones tested (p < 0.01, t test). To examine the possibility that the change in conductance was a nonspecific consequence of β subunit overexpression, we transfected PC12 cells with the β₄ subunit instead of the β₄ subunit. In these cells, the mean unitary conductance of L-type channels was (20.3 ± 0.7 pS; n = 6) significantly different from cells transfected with the β₄ subunit (22.4 ± 0.24 pS; n = 24; p < 0.01, t test), but not significantly different from the native PC12 cell L-type channels (19.8 ± 0.3 pS; p > 0.39, t test). The conductance of L-type channels in the transfected PC12 cells was also compared with that of native Ls channels in hippocampal neurons and was found to not be statistically different (22.4 ± 0.24 versus 22.0 ± 0.21 pS; p > 0.3, Fig. 4B). This is consistent with the idea that the β₄ subunit is responsible for increasing the conductance of L-type channels in transfected PC12 cells, be-
cause neurons normally express the $\beta_4$ subunit, and the difference in conductance is the only detectable difference between Ls channels in neurons and L-channels in PC12 cells.

To determine whether transfection of the $\beta_4$ subunit altered channel properties in addition to unitary conductance, we compared the activation profile of L-type calcium channels in transfected and untransfected PC12 cells (Fig. 5). There was no difference between the half-maximal activation voltages for control and transfected PC12 cells (3.6 ± 1.1 versus 3.3 ± 1.2 mV; $p > 0.4$). Fig. 5A shows combined data for all the clones, but the individual clones were also tested separately ($n = 5$ for all the data points for untransfected cells, and $n = 9$ for all the data points for transfected cells). We also examined the open time distributions between transfected and untransfected PC12 cells (Fig. 5B). As with the activation voltages, no statistically significant differences were found ($p > 0.05$, Kolmogorov-Smirnov test). The extracted mean open times were 0.6 and 65 ms for untransfected cells and 0.6 and 70 ms for transfected cells. Thus the incorporation of the $\beta_4$ subunit into the L-channel complex appeared to affect unitary conductance selectively, without an obvious impact on channel kinetics.

The effect of $\beta_4$ transfection on the conductance of L-type channels in PC12 cells implies that this parameter can be used to gain insight into the subunit composition on native calcium channels. To this end we plotted frequency histograms of unitary conductance for Ls, Lp, L-type + $\beta_4$ and control L-type. Our prediction was that the native channels should show relatively narrow, unimodal distributions, whereas the transfected cells might show a bimodal distribution (reflecting channels that did, and did not, incorporate the exogenous $\beta_4$ subunit). Consistent with this, the conductance distributions for the Ls and Lp channels were narrow and non-overlapping, suggestive of the $\beta_4$ subunit being part of native hippocampal Ls, but not Lp, channels (Fig. 6, top two plots). The conductance distribution of L-type channels in control PC12 cells, although somewhat broader than that of the neuronal Ls and Lp channels, was still obviously more restricted than the distribution of L-type channel conductances from the transfected PC12 cells (Fig. 6, bottom two plots). Conductances of L-type channels from the transfected PC12 cells spanned a range from 19 to 26 pS, with a single peak. Our interpretation of this result is that the majority of L-type channels incorporate the exogenous $\beta_4$ subunit, although some do not, accounting for the small population of channels with relatively low conductances.

**DISCUSSION**

Voltage-gated calcium channels play important physiological roles in neurons and other cells, and several hereditary human genetic diseases are the result of mutations in voltage-gated calcium channels (for review, see Ref. 30). It is important, therefore, to understand how these channels function and how the different subunits interact to give channels their characteristics. We were interested in what role the $\beta_4$ subunit plays...
in the diversity of L-type voltage-gated calcium channels. To study this, we used two different cell types that are derived from different tissues in rat, hippocampal neurons and PC12 cells, the latter being a cell line originally derived from adrenal chromaffin cells (31). PCR analysis revealed that there is a difference in the expression pattern of one of the \( \beta_4 \) subunit gene in these two tissues. Consistent with previous reports, \( \beta_4 \) was not present in PC12 cells (25), but was expressed in hippocampus (19–23). This also agrees with the general finding that the \( \beta_4 \) subunit is expressed almost exclusively in the brain (19). Our cell-attached single-channel recordings revealed that PC12 cells contained only a single kind of L-type channel, whereas hippocampal neurons contained two, Lp and Ls. Examination of these two cell types thus provided an ideal system for examining the potential contribution of the \( \beta_4 \) subunit to the underlying molecular basis of the Lp/Ls differences in gating kinetics and unitary conductance.

Previous work from our laboratory (12, 13) has shown that Lp channels display a characteristic change in their gating behavior following a conditioning depolarization. Quiescent Lp channels do not typically open at voltages more negative than \(-40 \text{ mV}\), but following a depolarization to greater than \(-10 \text{ mV}\) these channels transiently show re-openings (not tail current openings) at voltages as negative as \(-90 \text{ mV}\). These re-openings are not observed in recordings of hippocampal Ls channels and were not observed in any of our recordings of PC12 cell L-type channels.

An additional difference between Ls and Lp channels is that the unitary conductance of hippocampal Lp channels is significantly smaller than that of hippocampal Ls channels. Our single-channel recordings indicate that the population of PC12 cell L-type channels is relatively homogeneous with properties that were generally identical to hippocampal Ls channels. Interestingly, however, the unitary conductance of the PC12 cell

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**Fig. 4.** Three separate clones confirmed that single channel conductance of L-channels in PC12 cells increase in cells transfected with hippocampal \( \beta_4 \) DNA. A, comparison of single channel conductance in PC12 cells transfected with three separate clones of hippocampal \( \beta_4 \) DNA and untransfected cells. In all three clones the conductance was significantly higher than in untransfected cells, \( p < 0.01, n = 18 \) for untransfected cells, \( n = 11 \) for clone 1, \( n = 7 \) for clone 2, and \( n = 6 \) for clone 3 asterisks. B, comparison of single channel conductance of L-channels in transfected and untransfected PC12 cells and Ls channels in hippocampal neurons. The conductance of L-channels in transfected PC12 cells and Ls channels in hippocampal neurons were not significantly different, \( p > 0.05, n = 18 \) for untransfected PC12 cells, \( n = 24 \) for transfected PC12 cells, and \( n = 8 \) for Ls channels.

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**Fig. 5.** There was no difference in the activation voltage or the open time distribution after transfection with \( \beta_4 \) in PC12 cells. A, activation curve showing open probability for L-channels in transfected and untransfected PC12 cells at voltages between \(-40 \text{ mV}\) and \(+40 \text{ mV}\), \( n = 5 \) for all data points from untransfected cells and \( n = 9 \) for all data points from transfected cells. B, open time distribution at \(-10 \text{ mV}\) for L-channels in untransfected PC12 cells (dark bars in background) and PC12 cells transfected with \( \beta_4 \) (light bars in foreground). Extracted mean open times for untransfected cells, 0.6 and 65 ms; and for transfected cells, 0.6 and 70 ms. The two distributions are not significantly different, \( p > 0.05 \) (Kolmogorov-Smirnov two-sample test).
channel behavior produced by transfection of the β₄ subunit is also consistent with work on lethargic mice (Lh) in which the β₄ subunit is absent, but in which P-type calcium current is not grossly disturbed, presumably because of other β subunits substituting for β₄ (32, 33).

The enhancement of the single channel conductance conferred by the β₄ subunit in this study was ~10%. This is in contrast to the much larger increase in L-type whole cell current seen when the cardiac muscle α₄ subunit is co-expressed with β₄ compared with α₁ alone (19). It has been established that intracellular calcium channel β subunits are involved in membrane targeting of L-type α₁ subunits (94–99). Thus a large part of the increase in the whole cell current seen when α₁ and β₄ are co-expressed may result from an increased number of functional channels in the plasma membrane. Although this chaperone function is a general feature of β subunits for all classes of voltage-gated ion channels (for review, see Ref. 40), our findings nevertheless leave open the possibility that part of the enhanced whole cell current can also be attributed to increases in the conductance of individual channels and that the four β subunits may have a differential effect in this regard.

Although unitary conductance has been shown to be influenced by amino acid substitutions in the α₄ subunit (41, 42), and not by cardiac β subunits (43, 44), or β₁–β₃ (45), work by other investigators has provided indirect evidence that β subunits may be able modulate the conductance of voltage-gated calcium channels. First, β subunits have characteristic actions on L-type channel gating that can lead to differential effects on channel open probability (45). When coupled with the findings that L-type channels can display a variety of conductance states (46), and that unitary conductance can be modified by conditioning voltage pulses (47) and agonists that increase open probability (48), it is possible that conductance could also be affected as a consequence of changes in gating. In addition, single channel recordings from transfected COS7 cells revealed small as well as large conductance channels when α₄ subunits were expressed alone. Co-expression of β₄α₄ and α₄δ subunits with α₄ resulted in an increased proportion of large conductance channels, suggesting that the accessory subunits influence the conductance of the ion pore of α₄ (49).

There are, however, alternative mechanisms that could account for the increase in unitary conductance that do not involve a direct effect of β₄ on channel permeation. For example, transfection of the non-native β₄ into PC12 cells could recruit a different isoform of α₄ subunit that has a larger conductance than the native α₄ subunit. This could be consistent with work showing that the different β subunits can target α₄ subunits to characteristic locations (38), although targeting of α₁₄C is the same regardless of the type of β subunit (38).

With regard to the composition of native hippocampal Lp and Ls channels, our data suggest that β₄ is not part of the Lp channel complex because its conductance is smaller than Ls channels. Using the same logic, we predict that β₄ is a part of the hippocampal neuron Ls channel complex. This conclusion is supported by the relatively narrow distributions of Lp and Ls channel conductances in hippocampal neurons. Unlike the transfected PC12 cells, in which the distribution of conductances is rather broad, suggesting a mixed population of L-type calcium channels in which only a subset have incorporated the β₄ subunit, the hippocampal Ls channels may be composed exclusively of β₄-containing channels. An interesting implication of this is that the β₄ subunit, perhaps through a specialized binding domain (18, 50), may preferentially bind to the α₁₄C or α₁₁D subunits as compared with the other β subunits.

Our findings suggest that the different β subunits have differential effects on the current through the α₄ subunit. PC12

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Fig. 6. Distribution of Ls and Lp channel conductances suggest that the former contains the β₄ subunit, whereas the latter does not. Frequency histograms of unitary conductance measurements of Ls and Lp channels from hippocampal neurons (top two plots) plus β₄-transfected and control PC12 cells (bottom two plots). Unitary conductances from Ls and Lp channels show relatively narrow and non-overlapping distributions, especially as compared with the β₄-transfected PC12 cell L-type channels, which show a much broader distribution than the neuronal channels and the control PC12 cells. These data are consistent with β₄ being a normal component of native hippocampal Ls channels and with β₄ incorporation into the majority of L-type channels in transfected PC12 cells.

Ls channels was significantly smaller than the hippocampal Ls channels (Fig. 1) but was indistinguishable from hippocampal Lp channels when compared under identical ionic conditions (19.8 ± 0.3 versus 19.7 ± 0.31 pS).²

To determine whether the β₄ subunit was involved in regulation of calcium channel gating or unitary conductance, the hippocampal neuron β₄ gene was cloned out of hippocampal neurons and transfected into PC12 cells. Following transfection, the gating pattern of the L-type channels was unchanged, but the unitary conductance increased to a level not significantly different from Ls channels in hippocampal neurons. Thus, the key determinant of the conductance difference between the PC12 cell L-type channel, and the hippocampal Ls channel is likely to be the β₄ subunit. The particular subunit combination responsible for Lp channel gating, however, remains unknown. The relatively small alteration in calcium

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² J. M. Schjött and M. R. Plummer, unpublished observations.
cells expressed $\beta_1$-$\beta_3$, but an addition of $\beta_4$ still resulted in an enhanced conductance. There are other examples of the four $\beta$ subunits having differential effects on the current through the $\alpha_1$ subunit, $\beta_1$, $\beta_2$, and $\beta_4$, but not $\beta_3$, allow for voltage dependent facilitation when co-expressed with $\alpha_1C$ (51), and the four $\beta$ subunits affect inactivation to different degrees (for review, see Ref. 52). There is also evidence for differences in $\alpha_1$ subunit it is associated with (54). Thus, differential effects of the auxiliary subunits and differences in channel subunit composition in different tissues may serve as a mechanism to fine tune calcium channel function in the body.

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