Ca^{2+} channels: diversity of form and function

Terry P. Snutch and Peter B. Reiner

University of British Columbia, Vancouver, Canada

The past year has seen some significant advances in our understanding of the structural and functional properties of neuronal voltage-gated Ca^{2+} channels. Molecular cloning and protein purification studies have identified structural components, and expression studies are beginning to define the biophysical and pharmacological properties of the cloned channels. A number of studies of native Ca^{2+} channels show that the concept of channel modulation includes gating by both voltage and ligands.

Current Opinion in Neurobiology 1992, **2**:247–253

Introduction

The rapid entry of Ca^{2+} into excitable cells is mediated by a heterogeneous class of molecule, the Ca^{2+} channel. In the nervous system, Ca^{2+} channels play roles in mediating a wide variety of events including the patterning of neuronal firing, neurotransmitter release, and gene expression. To date, four major types of Ca^{2+} channel have been described in neurons (called T, L, N and P) [1,2]. Low-threshold Ca^{2+} channels (T-type) are transiently activated by relatively small depolarizations from hyperpolarized holding potentials and are completely inactivated at positive potentials. The other three Ca^{2+} channel types are activated at more positive potentials (high threshold) and display diverse kinetic properties. A major distinguishing characteristic of the different high-threshold Ca^{2+} channels is their sensitivity to various pharmacological agents: L-type channels are sensitive to dihydropyridine (DHP) agonists and antagonists, N-type channels are blocked by the *Conus geographus* peptide toxin, ω-conotoxin GVIA (ω-CgTx), and P-type channels are blocked by a crude venom fraction from the funnel web spider *Agelenopsis aperta*. Much research has been directed towards understanding Ca^{2+} channel diversity and it has been proposed that the distinct Ca^{2+} channel types are selectively localized in neurons and that each type may make unique contributions to neuronal physiology (for examples, see [3,4]).

The molecular dissection of neuronal Ca^{2+} channels has been made possible by the previous biochemical and molecular characterization of the skeletal muscle L-type Ca^{2+} channel (reviewed in [5,6]). This channel is a heteroligomeric complex consisting of five subunits (α_1, α_2, β, γ and δ), with the α_2- and δ-subunits being derived from the same gene and proteolytically cleaved *in vivo* [7,8]. In a number of test systems the α_1-subunits of several cloned L-type channels have been shown to function as both the voltage sensor and Ca^{2+}-selective pore [9–12]. This brief review will focus on recent studies addressing the molecular nature of neuronal Ca^{2+} channel diversity and on the modulation of Ca^{2+} channels by voltage and ligands.

Structural and functional diversity of cloned neuronal Ca^{2+} channels

While neuronal Ca^{2+} channel diversity is likely to be the result of a number of factors, including modulation by accessory subunits and second-messenger mechanisms (see below), it is now evident that a significant portion of Ca^{2+} channel heterogeneity is due to the expression of unique α_1-subunits. Currently, molecular cloning has identified five primary types of α_1-subunit and four of these are expressed in the mammalian nervous system (classes A, B, C and D), Table 1, [13]. The deduced amino acid sequences of the Ca^{2+} channel α_1-subunits show an overall conservation in structure, and are evolutionarily related to voltage-gated Na^+ and K^+ channels (Fig.1).

In addition to the five major types of α_1-subunit (Table 1), molecular cloning and polymerase chain reaction studies have revealed a previously unanticipated degree of Ca^{2+} channel heterogeneity. Within each of the class A–D α_1-subunits, multiple isoforms are expressed. Of potential functional significance is the observation that in a number of instances the isoforms found within a given class are the result of a nearly precise substitution of putative transmembrane segments [14•,15–17]. Furthermore, the expression of some isoforms appears to be spatially regulated and may result in qualitative differences between Ca^{2+} channels in different cell types. In the case of the rat class C α_1-subunit, the generation of distinct isoforms is clearly a result of alternate splicing from a single gene [14•]. Alternate splicing is also suggested, but not yet proven, to be responsible

Abbreviations

ω-CgTx—ω-conotoxin GVIA; **DHP**—dihydropyridine; **G protein**—GTP-binding protein.

 ISSN 0959-4388

Table 1. Properties of cloned full-length Ca^{2+} channel α_1-subunits.

Class	cDNA	Amino acids	Predicted molecular mass (kD)	Channel properties	Distribution	Notes	References
Skeletal muscle (L-type)		1,873	212	High threshold, DHP-sensitive, slow activation in L cells	Skeletal muscle	Rate of activation and inactivation increased by β-subunit	[9,11,34•,35•, 62]
A (P-type)	BI-1	2,273	257	High threshold, blocked by crude venom from *Agelenopsis aperta*, insensitive to nifedipine and ω-CgTx, 16pS single channel conductance	Brain, heart, pituitary GH4C1, PC12, C-cells	In oocytes expression increased by skeletal muscle $\alpha_2\delta$- and β-subunits High levels of expression in cerebellum Isoforms differ in carboxyl region	[19••,20]
	BI-2	2,424	273				
	rbA-I	2,212	252				
B	rbB-I	2,336	262	Not available	Brain, PC12, C cells	Antibodies against rbB-I immunoprecipitate brain ω-CgTx binding sites	[26•]
C (L-type)	pCARD3	2,171	243	High-threshold, DHP-sensitive	Brain, heart, lung, pituitary, kidney, aorta, GH4C1, PC12, C cells	Distinct isoforms generated by splicing Modulated by β-, $\alpha_2\delta$- and γ-subunits	[10,12] [14•,17] [32•,36•,37•]
	pSCaL	2,166	242				
	rbC-I	2,140	240				
	rbC-II	2,143	240				
	VSmα_1	2,169	244				
D (L-type)	α_1D	2,161	245	High-threshold, DHP-sensitive, reversible block by 10–15 μM ω-CgTx, little inactivation over 700 ms	Brain, heart, pituitary, pancreas, GH4C1, PC12, C cells	Expression in oocytes requires β-subunit	[18••,60,61]
	CACN4	2,181	248				
	RBα_1	1,634	187				

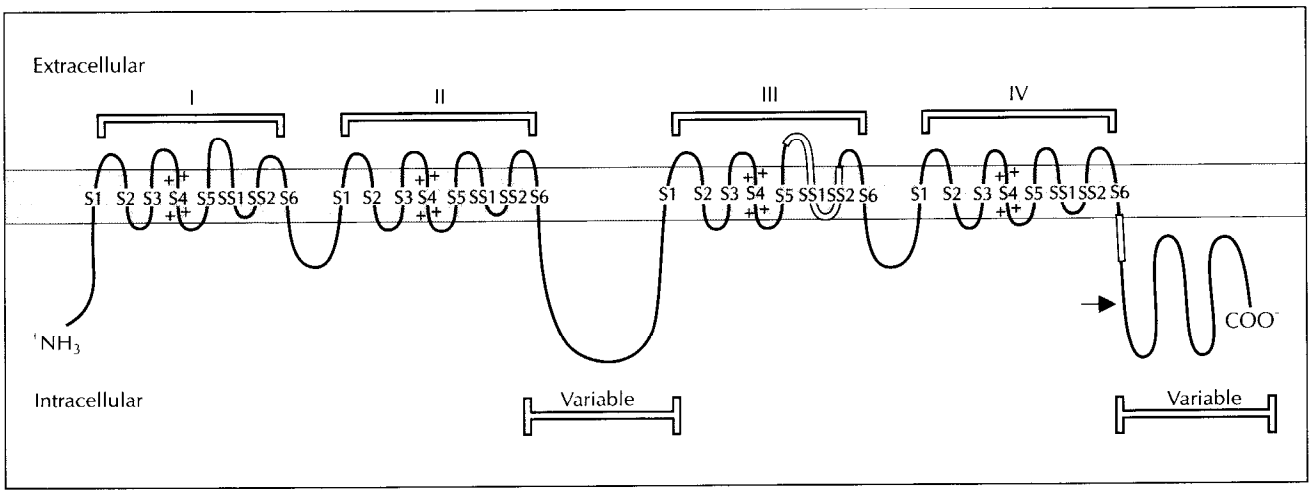

Fig.1. The predicted transmembrane folding model of the Ca^{2+} channel α_1-subunit. The five cloned types of Ca^{2+} channel α_1-subunit are between 210 kD and 270 kD in predicted molecular mass and possess four internal repeated domains (I–IV) that are modelled to contain six α-helical transmembrane regions (S1–S6), including one (S4) that is positively charged and is thought to form part of the voltage sensor. By analogy to K^+ channels the region separating segments S5 and S6 of each domain may contain two additional transmembrane segments (SS1 and SS2) that together form the pore of the channel [56]. The regions with the highest degree of amino acid identity among the five cloned types of α_1-subunit are the four repeated domains and a short segment immediately flanking S6 of domain IV. The latter region, together with the loop between segments S5 and S6 in domain III, is implicated in the formation of the binding site for dihydropyridines in L-type Ca^{2+} channels (white regions) [57–59]). Compared with other cloned Ca^{2+} channel α_1-subunits, the rat brain class D cDNA, RBα_1 [60], encodes a 187 kD protein truncated in the carboxyl terminus (arrow). The functional significance of this truncated α_1-subunit remains unclear, however, as both a second rat brain class D clone (H Kim *et al.*: *Soc Neurosci Abstr* 1991, 17:772), and the human brain [18••] and pancreas [61] class D homologues possess carboxyl segments that extend more than 500 amino acids past the end of RBα_1.

for generating heterogeneity within the other classes of α_1-subunits.

A further unexpected result from molecular studies is that two distinct classes of α_1-subunit encode L-type

Ca^{2+} channels in neurons (Table 1). Overall, the class C and D α_1-subunits are more closely related to each other (70–76% amino acid identity) than to the class A and B proteins (33–44%). The structural similarity between the class C and D proteins is also reflected in their functional properties. Antisense oligonucleotides against brain class C transcripts selectively block the expression of DHP-sensitive Ca^{2+} channels induced in *Xenopus* oocytes by rat heart RNA [14•]. Furthermore, microinjection of synthetic RNA derived from cardiac and lung class C α_1-subunits into oocytes results in the expression of high-voltage threshold Ca^{2+} channels that are sensitive to both DHP agonists and antagonists [10,12]. Although the human brain class D α_1-subunit alone is not functional in oocytes, co-injection of RNAs encoding the class D α_1-subunit and a brain β-subunit results in the expression of high-threshold Ca^{2+} channels that are also DHP-sensitive [18••]. The class C and D Ca^{2+} channels appear to differ in their current-voltage relations as well as pharmacologically, as the class D channels are partially and reversibly blocked by 10–15 μM ω-conotoxin [18••]. The class C and D Ca^{2+} channels are co-expressed in several cell lines (Table 1) and it will be particularly interesting to determine whether this co-localization extends to the mammalian nervous system, and if these distinct L-type Ca^{2+} channels make unique contributions to neuronal physiology.

Structurally, class A α_1-subunits differ significantly from DHP-sensitive Ca^{2+} channels in two hydrophilic regions predicted to be cytoplasmic: the segment separating domains II and III, and the carboxy-terminal region (Fig.1) [19••,20]. Of particular interest is that the class A domain II–III segment is much larger than that of DHP-sensitive Ca^{2+} channels (~430 versus ~130 amino acids) and shows no detectable homology to other proteins. The analogous region of the skeletal muscle α_1-subunit is involved in mediating excitation-contraction coupling [21], and it will be of interest to determine the functional role of this divergent region in Ca^{2+} channels expressed in the nervous system.

A significant development in the past year has been the demonstration that the rabbit brain class A α_1-subunit encodes a high-threshold Ca^{2+} channel that is insensitive to both DHP antagonists and ω-CgTx, but is blocked by crude venom from the funnel web spider *Agelenopsis aperta* [19••]. Together with the finding that class A transcripts are highly abundant in the cerebellum [19••,20], these results suggest that class A Ca^{2+} channels correspond to the P-type Ca^{2+} channels first described by Llinas and coworkers [22]. The physiological properties of the rabbit class A Ca^{2+} channel expressed in *Xenopus* oocytes do not correlate exactly with those described for P-type channels in cerebellar Purkinje cells (for a discussion, see [23]). Two pieces of evidence suggest that P-type Ca^{2+} channels may be more widespread than originally thought: first, a high-threshold Ca^{2+} current that is not blocked by ω-CgTx or DHP antagonists has recently been described in a variety of central nervous system neurons [24••,25•]; and second, class A transcripts have been detected in the heart, pituitary, and several neuronal and endocrine cell lines (Table 1).

Still actively being sought are cDNAs encoding T- and N-type Ca^{2+} channels. A possible candidate for N-type channels is the class B α_1-subunit. Analogous to class A Ca^{2+} channels, the class B α_1-subunit possesses a large hydrophilic segment linking domains II and III and also shares >80% amino acid identity in the four domains with class A α_1-subunits [26•]. Interestingly, there is little detectable identity between the class A and B α_1-subunits in either their domain II–III segments or in their carboxyl termini. Linking the class B protein to N-type channels are the results that antibodies directed against the class B α_1-subunit immunoprecipitate radiolabelled brain ω-CgTx binding sites, and that class B transcripts are selectively localized to the nervous system [26•]. Direct evidence of the cloning of an N-type channel awaits the expression of the class B protein, however. At present, there are no leading molecular candidates for T-type Ca^{2+} channels. One possibility is that T-type channels are derived by alternate splicing from one of the classes of α_1-subunit already cloned. Alternatively, T-type channels may be evolutionary quite distant from the high-threshold Ca^{2+} channels, and cloning may require the use of cell lines enriched for T-type channel expression and cloning strategies using alternative probes.

Subunit composition of neuronal Ca^{2+} channels

Neuronal N- and L-type Ca^{2+} channels are blocked with high-affinity by the *Agelenopsis aperta* peptide toxin ω-Aga-IIIA, indicating that they share some common structural features [27]. Furthermore, recent evidence suggests that neuronal N- and L-type channels are heteroligomeric complexes of roughly similar composition to the skeletal muscle DHP receptor. The purified brain ω-CgTx receptor is composed of a 230 kD α_1-like protein that binds ω-CgTx and four smaller proteins of between 60 kD and 140 kD [28•]. Two of the smaller proteins are likely to be $\alpha_2\delta$- and β-subunits as monoclonal antibodies against the skeletal muscle $\alpha_2\delta$- [29,30] and β- [31•] subunits immunoprecipitate both brain DHP and ω-CgTx binding sites. Indeed, molecular cloning results from a number of laboratories have identified β- and $\alpha_2\delta$-subunit isoforms that are expressed in the brain [18••,32•,33]. The existence of multiple brain β-subunit isoforms is also indicated by immunoprecipitation results that identify two distinct β-like subunits associated with the ω-CgTx receptor [31•].

Modulation of α_1-subunits

A number of new studies are providing evidence as to the roles of the other subunits in affecting the functional properties of the α_1-subunits. Perez-Reyes and co-workers [11] initially demonstrated that the skeletal muscle

α_1-subunit transfected into mouse L cells resulted in the expression of Ca^{2+} currents that were DHP-sensitive, but which showed an uncharacteristically slow activation. Two independent studies have now shown that the rate of activation and inactivation of the skeletal muscle α_1-subunit is dramatically increased by co-expression with the skeletal muscle β-subunit [34•,35•]. Co-expression of α_1- and β-subunits also increases the number of DHP binding sites in transfected L cells. Surprisingly, further combining the $\alpha_2\delta$- and γ-subunits seems to actually decrease the number of DHP binding sites found with α_1 and β together [35•]. In *Xenopus* oocytes the level of expression of brain class A and cardiac class C α_1-subunits is significantly increased by co-expression with $\alpha_2\delta$- and β-subunits [19••,32•,36•,37•]. In addition, the $\alpha_2\delta$-, β- and γ-subunits affect various functional properties of the class C α_1-subunit, including changes in kinetics and the voltage-dependence of activation and inactivation [32•,36•,37•]. No modulatory effects of the β- and $\alpha_2\delta$-subunits on the class A channel have been reported [19••]. While the modulatory effects of the other subunits seems to depend somewhat on the type of α_1-subunit and the expression system used, it is clear that both the level of expression and the kinetic properties of Ca^{2+} channel α_1-subunits are affected by the $\alpha_2\delta$-, β- and γ-subunits.

Nomenclature

With the increasing number of full-length α_1-subunit cDNAs isolated from various laboratories, it has become increasingly difficult to keep track of the names assigned to them. The situation becomes even more complex when the large number of partial cDNAs generated by RNA polymerase chain reaction analyses are considered. Some clones are named after the tissue from which the cDNAs were first derived, some are named alphabetically, and some are named numerically. With the more recent demonstration of specific isoforms of $\alpha_2\delta$- and β-subunits, a further degree of complexity has been introduced into the nomenclature of Ca^{2+} channels. As future molecular cloning studies will undoubtedly define even further Ca^{2+} channel diversity, this may be an appropriate time to adopt a standardized Ca^{2+} channel nomenclature, perhaps analogous to that recently adopted for voltage-gated K^+ channels [38].

Both voltage and ligands can alter the kinetics of Ca^{2+} channels — facilitation

A subset of high-threshold Ca^{2+} channels exhibit facilitation—depolarizing pre-pulses enhance the amount of current evoked by subsequent depolarizations [39,40]. Facilitation involves a change in kinetic behavior of Ca^{2+} channels such that the facilitated channel exhibits openings of longer duration accompanied by an increased probability of opening [41]. In contrast, in the absence of facilitation the channel is characterized by brief openings. For example, in chromaffin cells facilitation involves a change in the kinetic behavior of a 27 pS channel sensitive to DHPs [42••]. It appears that more than one class of high-threshold Ca^{2+} channel may be subject to facilitation, however, as it is also exhibited by Ca^{2+} channels in rat sympathetic neurons sensitive to ω-CgTx [43].

The process underlying Ca^{2+} channel facilitation may be affected by receptor activation as well as by voltage. Neurotransmitters, acting through GTP-binding protein (G protein)-coupled receptors, have been shown to inhibit high-threshold Ca^{2+} currents in both sympathetic and dorsal root ganglion cells [44,45]. This inhibition appears to be due to a change in the voltage-dependence of the Ca^{2+} channel [46], and the agonist-induced inhibition can be overcome by depolarizing pre-pulses [47–49]. The available data suggest that under basal conditions the channels behave as if they had been facilitated by a depolarizing pre-pulse, i.e. they exhibit frequent long-duration openings. Agonist converts the channels to the mode in which openings of short duration predominate, and this can be overcome by depolarizing pre-pulses. Exactly the opposite situation prevails for those Ca^{2+} channels that are enhanced by agonists, i.e. the basal condition appears to be one in which the channels reside in the short-opening mode, and agonist converts them to a long-opening mode [50].

Because agonist-induced changes in Ca^{2+} channel behavior are relieved by depolarizing pre-pulses in a fashion essentially identical to that seen during the process of facilitation, it seems likely that the mode of Ca^{2+} channel behavior in any given cell is under constitutive control. An important question to be addressed is whether this phenomenon is a property of the Ca^{2+} channel complex or some as yet to be identified cellular process. A G-protein-dependent blocking molecule (either the G protein itself or a soluble intracellular messenger) coupled to Ca^{2+} channels has been proposed [46,51••]. During differentiation of neuroblastoma cells there arises a tonic inhibition of an ω-CgTx-sensitive Ca^{2+} channel, and the effect appears to be mediated by a G protein [52••]. Tonic modulation of Ca^{2+} channels provides a plausible explanation for both voltage- and agonist-mediated enhancement and diminution of Ca^{2+} channel activity.

From a functional point of view, facilitation may contribute to frequency-dependent Ca^{2+} entry in synaptic terminals. Ca^{2+} channels are facilitated not only by single long depolarizing pre-pulses, but also by a series of brief repetitive depolarizations. Moreover, the available data suggest that switching between two gating modes characterized by long- and short-duration openings is fundamental to modulation of Ca^{2+} channels. Dual control over this process by agonists and voltage provides a powerful and dynamic control over Ca^{2+} channel function in neurons.

An unexpected source of Ca^{2+}-dependent inactivation of Ca^{2+} channels

It has long been known that voltage-gated Ca^{2+} channels can be inactivated by a rise in intracellular Ca^{2+}. Classically, it has been thought that the source of such elevations in intracellular Ca^{2+} is the Ca^{2+} channel itself, an example of negative feedback. Using nystatin-perforated patch-clamp electrodes, which maintain the normal dynamics of intracellular Ca^{2+} levels, Kramer *et al.* [53•] have now shown that agonist-induced release of intracellular Ca^{2+}, via production of inositol 1,4,5-trisphosphate, can also result in inhibition of voltage-gated Ca^{2+} channels. Because studies employing the whole-cell patch clamp technique commonly buffer intracellular Ca^{2+}, this phenomenon had been previously overlooked.

Low-threshold Ca^{2+} currents and action-potential generation

The central role of low-threshold Ca^{2+} channels (T-type) in the bursting behavior of neurons has been appreciated for nearly a decade [54]. Now, using a new technique called action-potential clamp, McCobb and Beam [55•] have shown that in dorsal root ganglion neurons the low-threshold Ca^{2+} current is the major Ca^{2+} current responsible for Ca^{2+} entry during a normal brief action potential, and is relatively insensitive to changes in action potential duration. In contrast, the contribution of high-threshold currents to Ca^{2+} entry increases markedly with action potential duration. These studies provide a new and intriguing glimpse into the functional roles of Ca^{2+} channels in neurons.

Perspective

A pressing objective of future molecular studies will be to correlate the functional properties of exogenously expressed Ca^{2+} channel cDNAs with those of Ca^{2+} channels described in neurons. The rather heterogeneous properties of Ca^{2+} channels found in the nervous system may make this task more difficult than it initially appears. In this regard, it may ultimately be necessary to compare the biophysical and pharmacological properties of Ca^{2+} channels found in defined neurons and neuronal cell lines with those of cDNAs isolated from the same cells. Together with the use of specific α_1-subunit antibodies to determine the cellular and subcellular localization of cloned Ca^{2+} channels, these studies will help address questions concerning the contributions of individual Ca^{2+} channel subtypes to neuronal function and the mechanisms of processes such as facilitation.

Acknowledgements

We are grateful to J Tomlinson for providing data concerning the expression of Ca^{2+} channel α_1-subunits in various cell lines shown in Table 1 and also to S Dubel for providing Fig. 1. Supported by research grants from the Medical Research Council of Canada (TP Snutch and PB Reiner) and the Howard Hughes Medical Institute International Research Scholars Program (TP Snutch). TP Snutch is a recipient of a fellowship from the Alfred P Sloan Research Foundation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- • of special interest
- •• of outstanding interest

1. BEAN BP: **Classes of Calcium Channels in Vertebrate Cells.** *Annu Rev Physiol* 1989, **51**:367–384.

2. HESS P: **Calcium Channels in Vertebrate Cells.** *Annu Rev Neurosci* 1990, **13**:337–356.

3. MILLER RJ: **Multiple Calcium Channels and Neuronal Function.** *Science* 1987, **235**:46–52.

4. TSIEN RW, LIPSCOMBE D, MADISON DV, BLEY KR, FOX AP: **Multiple Types of Neuronal Calcium Channels and Their Selective Modulators.** *Trends Neurosci* 1988, **11**:431–438.

5. CAMPBELL KP, LEUNG AT, SHARP AH: **The Biochemistry and Molecular Biology of the Dihydropyridine-Sensitive Calcium Channel.** *Trends Neurosci* 1988, **11**:425–430.

6. CATTERALL WA, SEAGAR MJ, TAKAHASHI M: **Molecular Properties of the Dihydropyridine-Sensitive Calcium Channel.** *J Biol Chem* 1988, **263**:3535–3538.

7. DE JONGH KS, WARNER C, CATTERALL WA: **Subunits of Purified Calcium Channels α2 and δ are Encoded by the Same Gene.** *J Biol Chem* 1990, **265**:14738–14741.

8. JAY SD, SHARP AH, KAHL SD, VEDVICK TS, HARPOLD MM, CAMPBELL KP: **Structural Characterization of the Dihydropyridine-Sensitive Calcium Channel α2-Subunit and the Associated δ Peptides.** *J Biol Chem* 1991, **266**:3287–3293.

9. TANABE T, BEAM KG, POWELL JA, NUMA S: **Restoration of Excitation-Contraction Coupling and Slow Calcium Current in Dysgenic Muscle by Dihydropyridine Receptor Complementary DNA.** *Nature* 1988, **336**:134–139.

10. MIKAMI A, IMOTO T, TANABE T, NIIDOME T, MORI Y, TAKESHIMA H, NARUMIYA S, NUMA S: **Primary Structure and Functional Expression of the Cardiac Dihydropyridine-Sensitive Calcium Channel.** *Nature* 1989, **340**:230–233.

11. PEREZ-REYES E, KIM HS, LACERDA AE, HORNE W, WEI X, RAMPE D, CAMPBELL KP, BROWN AM, BIRNBAUMER L: **Induction of Calcium Currents by the Expression of the α1-Subunit of the Dihydropyridine Receptor from Skeletal Muscle.** *Nature* 1989, **340**:233–236.

12. BIEL M, RUTH P, BOSSE E, HULLIN R, STUHMER W, FLOCKERZI V, HOFMANN F: **Primary Structure and Functional Expression of a High Voltage Activated Calcium Channel from Rabbit Lung.** *FEBS Lett* 1990, **269**:409–412.

13. SNUTCH TP, LEONARD JP, GILBERT MM, LESTER HA, DAVIDSON N: **Rat Brain Expresses a Heterogeneous Family of Calcium Channels.** *Proc Natl Acad Sci USA* 1990, **87**:3391–3395

14. • SNUTCH TP, TOMLINSON WJ, LEONARD JP, GILBERT MM: **Distinct Calcium Channels are Generated by Alternative Splicing**

and are Differentially Expressed in the Mammalian CNS. *Neuron* 1991, 7:45–57.

This paper demonstrates that class C α_1-subunits are encoded by a single gene and provides the first direct evidence that alternate splicing is responsible for generating Ca^{2+} channel isoforms.

15. PEREZ-REYES E, WEI X, CASTELLANO A, BIRNBAUMER L: **Molecular Diversity of L-Type Calcium Channels.** *J Biol Chem* 1990, 265:20430–20436.

16. HUANG P, TEMIZER, QUERTERMOUS T: **Polymerase Chain Reaction Cloning of L-Type Calcium Channel Sequences from the Heart and the Brain.** *FEBS Lett* 1990, 274:207–213.

17. KOCH WJ, ELLINOR PT, SCHWARTZ A: **cDNA Cloning of a Dihydropyridine-Sensitive Calcium Channel from Rat Aorta.** *J Biol Chem* 1990, 265:17786–17791.

18. •• WILLIAMS ME, FELDMAN DH, MCCUE AF, BRENNER R, VELICELEBI G, ELLIS SB, HARPOLD MM: **Structure and Functional Expression of α1, α2 and β subunits of a Novel Human Neuronal Calcium Channel Subtype.** *Neuron* 1992, 8:71–84.

This thorough study reports the primary sequences of human brain class D α_1-, $\alpha_2\delta$- and β-subunits. Microinjection of synthetic RNA into *Xenopus* oocytes shows that in the presence of a brain β-subunit the class D α_1-subunit encodes an L-type Ca^{2+} channel. Also described is the intriguing result that microinjection of β-subunit alone into oocytes induces the expression of DHP-insensitive Ca^{2+} channels that resemble the endogenous oocyte Ca^{2+} channel.

19. •• MORI Y, FRIEDRICH T, KIM M-S, MIKAMI A, NAKAI J, RUTH P, BOSSE E, HOFMANN F, FLOCKERZI V, FURUICHI T, *et al.*: **Primary Structure and Functional Expression from Complementary DNA of a Brain Calcium Channel.** *Nature* 1991, 350:398–402.

This article reports the primary sequence and functional expression of two class A α_1-subunits from rabbit brain that differ in their carboxyl termini. The Ca^{2+} currents induced in *Xenopus* oocytes are insensitive to nifedipine and ω-CgTx, but are blocked by crude venom from the funnel web spider, strongly suggesting that they correspond to P-type Ca^{2+} channels.

20. STARR TVB, PRYSTAY W, SNUTCH TP: **Primary Structure of a Calcium Channel that is Highly Expressed in the Rat Cerebellum.** *Proc Natl Acad Sci USA* 1991, 88:5621–5625.

21. TANABE T, BEAM KG, ADAMS BA, NIIDOME T, NUMA S: **Regions of the Skeletal Muscle Dihydropyridine Receptor Critical for Excitation-Contraction Coupling.** *Nature* 1990, 346:567–569.

22. LLINAS R, SUGIMORI M, LIN JW, CHERKSEY B: **Blocking and Isolation of a Calcium Channel from Neurons in Mammals and Cephalopods Utilizing a Toxin Fraction (FTX) from Funnel-Web Spider Poison.** *Proc Natl Acad Sci USA* 1989, 86:1689–1693.

23. TSIEN RW, ELLINOR PT, HORNE WA: **Molecular Diversity of Voltage-Dependent Ca^{2+} Channels.** *Trends Pharmacol* 1991, 12:349–354.

24. •• REGAN LJ, SAH DWY, BEAN BP: **Ca^{2+} Channels in Rat Central and Peripheral Neurons: High-Threshold Current Resistant to Dihydropyridine Blockers and ω-Conotoxin.** *Neuron* 1991, 6:269–280.

An excellent study demonstrating that different types of dissociated rat neurons possess varying amounts of a high-threshold Ca^{2+} current that is insensitive to both DHPs and ω-CgTx.

25. • REGAN LJ: **Voltage-Dependent Calcium Currents in Purkinje Cells from Rat Cerebellar Vermis.** *J Neurosci* 1991, 11:2259–2269.

A companion study to [24••] showing that dissociated cerebellar Purkinje cells predominantly express a Ca^{2+} current that is insensitive to DHPs and ω-CgTx.

26. • DUBEL SJ, STARR TVB, HELL J, AHLIJANIAN MK, ENYEART JJ, CATTERALL WA, SNUTCH TP: **Molecular Cloning of the α-1 Subunit of an ω-Conotoxin-Sensitive Calcium Channel.** *Proc Natl Acad Sci USA* 1992, in press.

This article describes the primary structure of the class B α_1-subunit and demonstrates that polyclonal antibodies against this protein selectively immunoprecipitate rat brain ω-CgTx binding sites.

27. MINTZ IM, VENEMA VJ, ADAMS ME, BEAN BP: **Inhibition of N- and L-Type Ca^{2+} Channels by the Spider Venom ω-Aga-IIIA.** *Proc Natl Acad Sci USA* 1991, 88:6628–6631.

28. • MCENERY MW, SNOWMAN AM, SHARP AH, ADAMS ME, SNYDER SH: **Purified ω-Conotoxin GVIA Receptor of Rat Brain Resembles a Dihydropyridine-Sensitive L-type Calcium Channel.** *Proc Natl Acad Sci USA* 1991, 88:11095-11099.

The purified brain ω-CgTx receptor is a heteroligomeric complex consisting of a 230 kD subunit that binds ω-CgTx and four smaller subunits of 140 kD, 110 kD, 70 kD and 60 kD.

29. AHLIJANIAN MK, WESTENBROEK RE, CATTERALL WA: **Subunit Structure and Localization of Dihydropyridine-Sensitive Calcium Channels in Mammalian Brain, Spinal Cord, and Retina.** *Neuron* 1990, 4:819–832.

30. AHLIJANIAN MK, STRIESSNIG J, CATTERALL WA: **Phosphorylation of an α1-Like Subunit of an ω-Conotoxin-Sensitive Brain Calcium Channel by cAMP-Dependent Protein Kinase and Protein Kinase C.** *J Biol Chem* 1991, 266:20192–20197.

31. • SAKAMOTO J, CAMPBELL KP: **A Monoclonal Antibody to the β Subunit of the Skeletal Muscle Dihydropyridine Receptor Immunoprecipitates the Brain ω-Conotoxin GVIA Receptor.** *J Biol Chem* 1991, 266:18914–18919.

This study demonstrates that the partially purified brain ω-CgTx receptor contains 58 kD and 78 kD polypeptides that are homologous to the skeletal muscle L-type Ca^{2+} channel β-subunit.

32. • PEREZ-REYES E, CASTELLANO A, KIM HS, BERTRAND P, BAGGSTROM E, LACERDA AE, WEI X, BIRNBAUMER L: **Cloning and Expression of a Cardiac/Brain β Subunit of the L-Type Calcium Channel.** *J Biol Chem* 1992, 267:1792–1797.

Co-expression of a β-subunit found in heart with the cardiac class C α_1-subunit shows that the β-subunit affects the current-voltage relationship and rate of activation.

33. PRAGNELL M, SAKAMOTO J, JAY SD, CAMPBELL KP: **Cloning and Tissue-Specific Expression of the Brain Calcium Channel β-Subunit.** *FEBS Lett* 1991, 291:253–258.

34. • LACERDA AE, KIM HS, RUTH P, PEREZ-REYES E, FLOCKERZI V, HOFMANN F, BIRNBAUMER L, BROWN AM: **Normalization of Current Kinetics by Interaction Between the α1 and β Subunits of the Skeletal Muscle Dihydropyridine-Sensitive Ca^{2+} Channel.** *Nature* 1991, 352:527–530.

Transfection into L cells of the skeletal muscle α_1- and β-subunits demonstrates that the β-subunit increases the rate of activation over that of the α_1-subunit alone.

35. • VARADI G, LORY P, SCHULTZ D, VARADI M, SCHWARTZ A: **Acceleration of Activation and Inactivation by the β Subunit of the Skeletal Muscle Calcium Channel.** *Nature* 1991, 352:159–162.

Similar to [34•], this paper shows that co-expression of skeletal muscle α_1- and β-subunits affects Ca^{2+} channel activation and inactivation properties. Co-expression of the $\alpha_2\delta$- and γ-subunits has minimal effects on the α_1-subunit properties.

36. • WEI X, PEREZ-REYES E, LACERDA AE, SCHUSTER G, BROWN, AM, BIRNBAUMER L: **Heterologous Regulation of the Cardiac Ca^{2+} Channel α1 Subunit by Skeletal Muscle β and γ Subunits.** *J Biol Chem* 1991, 266:21943–21947.

The cardiac class C α_1-subunit expressed in *Xenopus* oocytes is modulated by skeletal muscle β- and γ-subunits. The β-subunit increases the rate of activation of the α_1-subunit and also alters the current-voltage relationship. The γ-subunit further enhances the rate of activation and peak current.

37. • SINGER D, BIEL M, LOTAN I, FLOCKERZI V, HOFMANN F, DASCAL N: **The Roles of the Subunits in the Function of the Calcium Channel.** *Science* 1991, 253:1553–1557.

Similar to [36•], this article shows that the macroscopic properties of the cardiac class C α_1-subunit can be modulated by other subunits. Co-expression of the γ-subunit has a significant effect on the voltage-dependence of inactivation.

38. CHANDY KG: **Simplified Gene Nomenclature.** *Nature* 1991, 352:26.

39. FENWICK EM, MARTY A, NEHER E: **Sodium and Calcium Channels in Bovine Chromaffin Cells.** *J Physiol* 1982, 331:599–635.

40. HOSHI T, SMITH SJ: **Large Depolarization Induces Long Openings of Voltage-Dependent Calcium Channels in Adrenal Chromaffin Cells.** *J Neurosci* 1987, 7:571–580.

41. HOSHI T, ROTHLEIN J, SMITH SJ: **Facilitation of Ca^{2+}-Channel Currents in Bovine Adrenal Chromaffin Cells.** *Proc Natl Acad Sci USA* 1984, 81:5871–5875.

42. •• ARTALEJO CR, MOGUL DJ, PERLMAN RL, FOX AP: **Three Types of Bovine Chromaffin Cell Ca^{2+} Channels: Facilitation Increases the Opening Probability of a 27 pS Channel.** *J Physiol* 1991, 444:213–240.
This analysis of single Ca^{2+} channels in chromaffin cells represents the most complete and thorough analysis of facilitation to date.

43. IKEDA SR: **Double-Pulse Calcium Channel Current Facilitation in Adult Rat Sympathetic Neurones.** *J Physiol* 1991, 439:181–214.

44. DUNLAP K, FISHBACH GD: **Neurotransmitters Decrease the Calcium Conductance Activated by Depolarization of Embryonic Chick Sensory Neurons.** *J Physiol (Lond)* 1978, 317:519–535.

45. MARCHETTI C, CARBONE E, LUX HD: **Effects of Dopamine and Noradrenaline on Ca Channels of Cultured Sensory and Sympathetic Neurons of Chick.** *Pflugers Arch* 1986, 406:104–111.

46. BEAN BP: **Neurotransmitter Inhibition of Neuronal Calcium Currents by Changes in Channel Voltage Dependence.** *Nature* 1989, 340:153–156.

47. ELMSLIE KS, ZHOU W, JONES SW: **LHRH and GTP-γ-S Modify Calcium Current Activation in Bullfrog Sympathetic Neurons.** *Neuron* 1990, 5:75–80.

48. POLLO A, TAGLIALATELA M, CARBONE E: **Voltage-Dependent Inhibition and Facilitation of Ca Channel Activation by GTP-γ-S and Ca-Agonists in Adult Rat Sensory Neurons.** *Neurosci Lett* 1991, 123:203–207.

49. LOPEZ HS, BROWN AM: **Correlation Between G Protein Activation and Reblocking Kinetics of Ca $^{2+}$ Channel Currents in Rat Sensory Neurons.** *Neuron* 1991, 7:1061–1068.

50. ARTALEJO CR, ARIANO MA, PERLMAN RL, FOX AP: **Activation Facilitation Calcium Channels in Chromaffin Cells by D_1 Dopamine Receptors Through a cAMP/Protein Kinase A-Dependent Mechanism.** *Nature* 1990, 348:239–242.

51. •• BERNHEIM L, BEECH DJ, HILLE B: **A Diffusible Second Messenger Mediates One of the Pathways Coupling Receptors to Calcium Channels in Rat Sympathetic Neurons.** *Neuron* 1991, 6:859–867.
This paper shows that there are two pathways leading to Ca^{2+} channel inhibition in sympathetic neurons, and at least one of them is due to activation of a soluble second messenger. Surprisingly, this intracellular message is not carried by either Ca^{2+}, cGMP, cAMP or protein kinase C.

52. •• KASAI H: **Tonic Inhibition and Rebound Facilitation of a Neuronal Calcium Channel by a GTP-Binding Protein.** *Proc Natl Acad Sci USA* 1991, 88:8855–8859.
Using differentiation of neuroblastoma cells as a model system, this study provides strong support for the notion that tonic modulation of Ca^{2+} channels is mediated by a G-protein-coupled process.

53. • KRAMER RH, KACZMAREK LK, LEVITAN ES: **Neuropeptide Inhibition of Voltage-Gated Calcium Channels Mediated by Mobilization of Intracellular Calcium.** *Neuron* 1991, 6:557–563.
Perforated patch recordings, which do not perturb intracellular Ca^{2+}, reveal that Ca^{2+} release from internal stores is capable of inhibiting voltage-gated Ca^{2+} channels.

54. LLINAS RR, JAHNSEN H: **Electrophysiology of Mammalian Thalamic Neurons In Vitro.** *Nature* 1982, 297:406–408.

55. • MCCOBB DP, BEAM KG: **Action Potential Waveform Voltage-Clamp Commands Reveal Striking Differences in Calcium Entry via Low and High Voltage-Activated Calcium Channels.** *Neuron* 1991, 7:119–127.
Capitalizing upon the new technique of action-potential clamp, this study examines the contribution of different classes of Ca^{2+} channel to action potential generation.

56. MACKINNON R: **New Insights into the Structure and Function of Potassium Channels.** *Curr Opin Neurobiol* 1991, 1:14–19.

57. REGULA S, SCHNEIDER T, NASTAINCZYK W, MEYER HE, HOFMANN F: **Identification of the Site of Interaction of the Dihydropyridine Channel Blockers Nitrendipine and Azidopine with the Calcium-Channel α1 Subunit.** *EMBO J* 1991, 10:45–49.

58. NAKAYAMA H, TAKI M, STRIESSNIG J, GLOSSMANN H, CATTERALL WA, KANAOKA Y: **Identification of 1,4 Dihydropyridine Binding Regions within the α1 Subunit of Skeletal Muscle Ca^{2+} Channels by Photoaffinity Labeling with Diazipine.** *Proc Natl Acad Sci USA* 1991, 88:9203–9207.

59. STRIESSNIG J, MURPHY BJ, CATTERALL WA: **Dihydropyridine Receptor of L-Type Ca^{2+} Channels: Identification of Binding Domains for [^{3}H](+)-PN200-110 and [^{3}H]Azidopine within the α1 Subunit.** *Proc Natl Acad Sci USA* 1991, 88:10769–10773.

60. HUI A, ELLINOR PT, KRIZANOVA O, WANG J-J, DIEBOLD RJ, SCHWARTZ A: **Molecular Cloning of Multiple Subtypes of a Novel Rat Brain Isoform of the α1 Subunit of the Voltage-Dependent Calcium Channel.** *Neuron* 1991, 7:35–44.

61. SEINO S, CHEN L, SEINO M, BLONDEL O, TAKEDA J, JOHNSON JH, BELL GI: **Cloning of the α1 Subunit of a Voltage-Dependent Calcium Channel Expressed in Pancreatic β Cells.** *Proc Natl Acad Sci USA* 1992, 89:584–588.

62. TANABE T, TAKESHIMA H, MIKAMI A, FLOCKERZI V, TAKAHASHI H, KANGAWA K, KOJIMA M, MATSUO H, HIROSE T, NUMA S: **Primary Structure of the Receptor for Calcium Channel Blockers from Skeletal Muscle.** *Nature* 1987, 328:313–318.

TP Snutch, Biotechnology Laboratory and Division of Neuroscience, Rm 237, 6174 University Blvd, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z3.

PB Reiner, Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z3.