lar surface of the molecule by antibodies as well as by polypeptide neurotoxins from scorpion, sea anemone, coral, and snail (*12, 20*). Evidently, inactivation of Na^+ channels is a transmembrane conformational change involving both intracellular and extracellular segments of the channel protein.

REFERENCES AND NOTES

1. B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, 1984).
2. W. S. Agnew, *Annu. Rev. Physiol.* **46**, 517 (1984); R. L. Barchi, *Trends Biochem. Sci.* **9**, 358 (1984); W. A. Catterall, *Science* **223**, 653 (1984); *Annu. Rev. Biochem.* **55**, 953 (1986).
3. A. L. Goldin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7503 (1986); M. Noda *et al.*, *Nature* **322**, 826 (1986).
4. V. J. Auld *et al.*, *Neuron* **1**, 449 (1988).
5. M. Noda *et al.*, *Nature* **312**, 121 (1984); M. Noda *et al.*, *ibid.* **320**, 188 (1986).
6. C. M. Armstrong, F. Bezanilla, E. Rojas, *J. Gen. Physiol.* **62**, 375 (1973); E. Rojas and B. Rudy, *J. Physiol. (London)* **262**, 501 (1976); D. Eaton, M. S. Brodwick, G. S. Oxford, B. Rudy, *Nature* **271**, 473 (1978); G. S. Oxford, C. M. Wu, T. Narahashi, *J. Gen. Physiol.* **71**, 227 (1978); J. Patlak and R. Horn, *ibid.* **79**, 333 (1982).
7. D. Gordon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8682 (1987); D. Gordon, D. Merrick, D. A. Wollner, W. A. Catterall, *Biochemistry* **27**, 7032 (1988); D. Merrick and W. A. Catterall, unpublished results.
8. D. A. Wollner and W. A. Catterall, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8424 (1986).
9. O. P. Hamill, A. Marty, E. Neher, B. Sackmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981).
10. T. Gonoi, S. J. Sherman, W. A. Catterall, *J. Neurosci.* **5**, 2559 (1985).
11. The patch-clamp micropipettes were prepared from microhematocrit capillaries (VWR), and their tips were fire-polished to yield pipette tip resistances of 0.3 to 0.5 megohms. The pipette intracellular solution contained 105 m*M* CsF, 40 m*M* CsCl, 10 m*M* NaF, and 5 m*M* EGTA (*p*H 7.2), adjusted with CsOH. Affinity-purified antibodies were dialyzed against this intracellular solution and aliquots of the antibody-containing solution were introduced into the pipette immediately before the electrophysiological experiments. The affinity-purified antibodies were introduced into the pipette solution at concentrations that completely immunoprecipitate 0.5 n*M* Na^+ channels, as determined in previous studies (*7*). Sodium currents were recorded in the presence of reduced extracellular Na^+ to minimize series resistance errors and enhance voltage control (*10*). The extracellular solution contained 120 m*M* tetraethylammonium chloride, 35 m*M* NaCl, 1.5 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, 5 m*M* glucose, and 5 m*M* Hepes (*p*H 7.4). The whole-cell Na^+ currents were measured with a List L/M-EPC7 patch-clamp amplifier. Capacity and series resistance were compensated for with the patch-clamp circuitry. The remaining leakage and capacitive transients were compensated by an external analog circuit. The current signals were filtered at 1 kHz through an eight-pole Bessel filter. Voltage stimuli were applied and the currents were digitzed (20 μs per point) and analyzed with an IBM-XT computer, a Labmaster (Scientific Solutions Inc., Solon, OH) board, and programs based on the Fastlab (Indec Systems, Sunnyvale, CA) system. Recordings were made at room temperature, 21 to 23°C. Na^+ current traces were scaled by factors of 1.05 to 1.6 to allow direct comparison of current time courses. In some experiments (for example, the current-voltage curve illustrated in Fig. 2B) no scaling was required to make quantitative comparisons of Na^+ currents before and after antibody treatment. Similar antibody effects were observed regardless of the scaling factor used in individual experiments.
12. W. A. Catterall, *Annu. Rev. Pharmacol. Toxicol.* **20**, 15 (1980); G. Strichartz, T. Rando, G. K. Wang, *Annu. Rev. Neurosci.* **10**, 237 (1985); H. Meves, J. M. Simard, D. D. Watt, *Ann. N.Y. Acad. Sci.* **479**, 113 (1985).
13. W. A. Catterall, *J. Biol. Chem.* **252**, 8669 (1977); *J. Gen. Physiol.* **74**, 375 (1979).
14. H. R. Guy, *Molecular Biology of Ion Channels: Current Topics in Membrane Transport*, W. Agnew, Ed. (Academic Press, New York, in press).
15. V. L. Salgado, J. Z. Yeh, T. Narahashi, *Biophys. J.* **47**, 567 (1985).
16. R. W. Aldrich, D. P. Corey, C. F. Stevens, *Nature* **306**, 436 (1983); R. W. Aldrich and C. F. Stevens, *J. Neurosci.* **7**, 418 (1987); C. M. Armstrong and F. Bezanilla, *J. Gen. Physiol.* **70**, 567 (1977); F. Bezanilla and C. M. Armstrong, *ibid.*, p. 549; C. M. Armstrong, *Physiol. Rev.* **61**, 644 (1981).
17. C. A. Vandenberg and R. Horn, *J. Gen. Physiol.* **84**, 535 (1984); T. Gonoi and B. Hille, *ibid.* **89**, 253 (1987).
18. R. E. Weiss and R. Horn, *Science* **233**, 361 (1986); J. P. Ruppersberg, A. Schurz, R. Ruedel, *Neurosci. Lett.* **78**, 166 (1987).
19. H. Meiri *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5058 (1987).
20. W. A. Catterall, *ISI Atlas Sci. Pharmacol.* **2**, 190 (1988).

21. We thank D. Merrick and D. Gordon for providing the affinity-purified antibodies to the peptides and J. Offord and L. Maechler for help with preparation of cell cultures. Supported by research grant NS-15751 from the National Institutes of Health, research contract DAMD-17-84-C-4130 from the U.S. Army Research Office, and a grant from the Muscular Dystrophy Association to W.A.C.

1 June 1988; accepted 2 August 1988.

Sequence and Expression of mRNAs Encoding the α_1 and α_2 Subunits of a DHP-Sensitive Calcium Channel

STEVEN B. ELLIS, MARK E. WILLIAMS, NANCY R. WAYS, ROBERT BRENNER, ALAN H. SHARP, ALBERT T. LEUNG, KEVIN P. CAMPBELL, EDWARD MCKENNA, WALTER J. KOCH, ANNA HUI, ARNOLD SCHWARTZ,* MICHAEL M. HARPOLD*

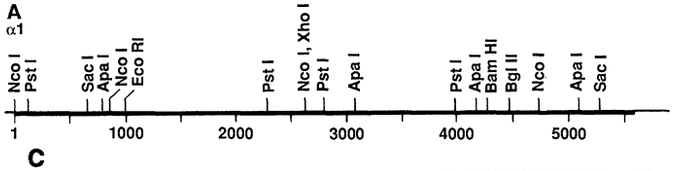
Complementary DNAs were isolated and used to deduce the primary structures of the α_1 and α_2 subunits of the dihydropyridine-sensitive, voltage-dependent calcium channel from rabbit skeletal muscle. The α_1 subunit, which contains putative binding sites for calcium antagonists, is a hydrophobic protein with a sequence that is consistent with multiple transmembrane domains and shows structural and sequence homology with other voltage-dependent ion channels. In contrast, the α_2 subunit is a hydrophilic protein without homology to other known protein sequences. Nucleic acid hybridization studies suggest that the α_1 and α_2 subunit mRNAs are expressed differentially in a tissue-specific manner and that there is a family of genes encoding additional calcium channel subtypes.

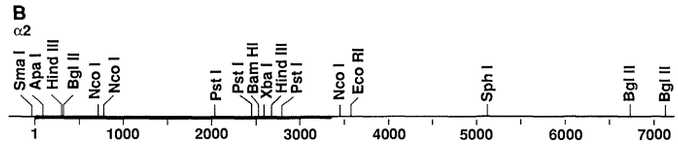
THE L-TYPE BUT NOT THE T-TYPE OR N-type Ca^{2+} channels are sensitive to "Ca^{2+} antagonist" drugs, including the dihydropyridines (DHPs). Skeletal muscle DHP-sensitive Ca^{2+} channels (L-type) comprise at least two large polypeptide subunits, α_1 and α_2, which copurify with equimolar stoichiometry (*2–5*). The α_1 subunit, which may be weakly glycosylated, contains receptor sites for at least two classes of Ca^{2+} antagonists, the dihydropyridines and the phenylalkylamines, and has a molecular mass of 155 to 170 kD under both reducing and nonreducing conditions. The α_2 subunit, which is extensively glycosylated, does not bind either DHP or phenylalkylamine Ca^{2+} antagonists and has a molecular mass of 165 to 175 kD under nonreducing conditions and of 135 to 150 kD under reducing conditions. Two additional subunits may also be present, a nonglycosylated β subunit (50 to 55 kD) and a glycosylated γ subunit (30 to 33 kD) (*2, 3, 6, 7*). In addition, the α_1 and β subunits are substrates for protein kinases, whereas the α_2 and γ subunits are not (*7, 8*). Although active DHP-sensitive Ca^{2+} channels have been reconstituted in vitro (*9*), it is unknown which subunits are required for a functional DHP-sensitive Ca^{2+} channel.

We describe here the sequences and tissue-specific expression of the α_1 and α_2 subunits of a DHP-sensitive Ca^{2+} channel determined by using cloned cDNA sequences. To isolate the cDNA clones, monoclonal antibody IIF7, specific for the α_1 subunit from rabbit skeletal muscle triads (*6*), and guinea pig polyclonal antisera, specific for the gel-purified α_2 subunit (*4*), were each used to screen 1.0×10^6 recombinant phage of a rabbit back skeletal muscle cDNA library. Overlapping cDNA clones were isolated to determine the DNA sequence encoding each subunit (Fig. 1).

S. B. Ellis, M. E. Williams, N. R. Ways, R. Brenner, M. M. Harpold, The Salk Institute Biotechnology/Industrial Associates, Inc. (SIBIA), 505 Coast Boulevard South, La Jolla, CA 92037.
A. H. Sharp, A. T. Leung, K. P. Campbell, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.
E. McKenna, W. J. Koch, A. Hui, A. Schwartz, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

*To whom correspondence should be addressed.





C

```
5'      AGAAGGGAGGGCGAGCGTGGTGTGTGCGCGCTCGGGCGCCGGCGGCACCGCCGAGGTCTGTTGGCAAAAGT  -238
CGCCCTTGATGGCGGCGGAGGCGAGGCAGCCGCGGCGCCGAACAGCCGACGCGCGCTAGCGGGGTCCGCCCGCCCCTTT  -159
CCCAGAGCCCAGCGCCGCCGTTCGCCGCCGCCGCCGCCCGCCGCGCCGTTCGCCGCCGCCGCCCGCGGGTGGC  -80
AGCGCCGCTCGGTCCCCGGCCCCGGGGCCGGCTGGGGGGCGGTCGGGGCGTGTGAGGGGCTTGCTCCCAGCTCGCGAAG  -1
-26                                                                          -7
ATG GCT GCG GGC CGC CCG CTG GCC TGG ACG CTG ACA CTT TGG CAG GCG TGG CTG ATC CTG   60
Met Ala Ala Gly Arg Pro Leu Ala Trp Thr Leu Thr Leu Trp Gln Ala Trp Leu Ile Leu
                -1  +1                                                          14
ATC GGG CCC TCG TCG GAG↓GAG CCG TTC CCT TCA GCC GTC ACT ATC AAG TCA TGG GTG GAT  120
Ile Gly Pro Ser Ser Glu Glu Pro Phe Pro Ser Ala Val Thr Ile Lys Ser Trp Val Asp
                                                                            34
AAG ATG CAA GAA GAC CTG GTC ACA CTG GCA AAA ACA GCA AGT GGA GTC CAT CAG CTT GTT  180
Lys Met Gln Glu Asp Leu Val Thr Leu Ala Lys Thr Ala Ser Gly Val His Gln Leu Val
                                                                            54
GAT ATT TAT GAG AAA TAT CAA GAT TTG TAT ACT GTG GAA CCA AAT AAT GCA CGT CAG CTG  240
Asp Ile Tyr Glu Lys Tyr Gln Asp Leu Tyr Thr Val Glu Pro Asn Asn Ala Arg Gln Leu
                                                                            74
GTG GAA ATT GCA GCC AGA GAC ATT GAG AAG CTT CTC AGC AAC AGA TCT AAA GCC CTG GTG  300
Val Glu Ile Ala Ala Arg Asp Ile Glu Lys Leu Leu Ser Asn Arg Ser Lys Ala Leu Val
                                                                            94
CGC CTG GCT TTG GAA GCA GAG AAA GTT CAA GCA GCC CAC CAA TGG AGG GAA GAT TTT GCA  360
Arg Leu Ala Leu Glu Ala Glu Lys Val Gln Ala Ala His Gln Trp Arg Glu Asp Phe Ala
                                                                           114
AGC AAT GAA GTT GTC TAC TAT AAC GCG AAG GAT GAT CTT GAT CCT GAA AAA AAT GAC AGT  420
Ser Asn Glu Val Val Tyr Tyr Asn Ala Lys Asp Asp Leu Asp Pro Glu Lys Asn Asp Ser
                                                                           134
GAA CCA GGC AGC CAG AGG ATC AAA CCT GTT TTC ATT GAC GAT GCT AAC TTT AGA AGA CAA  480
Glu Pro Gly Ser Gln Arg Ile Lys Pro Val Phe Ile Asp Asp Ala Asn Phe Arg Arg Gln
                                                                           154
GTA TCC TAT CAG CAC GCA GCT GTC CAT ATC CCC ACT GAC ATC TAT GAA GGA TCG ACA ATC  540
Val Ser Tyr Gln His Ala Ala Val His Ile Pro Thr Asp Ile Tyr Glu Gly Ser Thr Ile
                                                                           174
GTG TTA AAC GAA CTC AAC TGG ACA AGT GCC TTA GAT GAC GTT TTC AAA AAA AAT CGA GAG  600
Val Leu Asn Glu Leu Asn Trp Thr Ser Ala Leu Asp Asp Val Phe Lys Lys Asn Arg Glu
                                                                           194
GAA GAC CCT TCA CTG TTG TGG CAG GTG TTT GGC AGT GCC ACT GGC CTG GCC CGG TAT TAC  660
Glu Asp Pro Ser Leu Leu Trp Gln Val Phe Gly Ser Ala Thr Gly Leu Ala Arg Tyr Tyr
                                                                           214
CCA GCT TCT CCA TGG GTT GAT AAT AGC CGA ACC CCA AAC AAG ATT GAT CTT TAT GAT GTA  720
Pro Ala Ser Pro Trp Val Asp Asn Ser Arg Thr Pro Asn Lys Ile Asp Leu Tyr Asp Val
                                                                           234
CGC AGA AGA CCA TGG TAC ATC CAA GGT GCT GCA TCC CCT AAA GAT ATG CTT ATT CTG GTG  780
Arg Arg Arg Pro Trp Tyr Ile Gln Gly Ala Ala Ser Pro Lys Asp Met Leu Ile Leu Val
                                                                           254
GAT GTG AGT GGA AGC GTT AGT GGA CTG ACA CTC AAA CTC ATC CGG ACA TCC GTC TCC GAA  840
Asp Val Ser Gly Ser Val Ser Gly Leu Thr Leu Lys Leu Ile Arg Thr Ser Val Ser Glu
                                                                           274
ATG TTG GAA ACC CTC TCA GAT GAT GAT TTT GTG AAC GTG GCT TCA TTT AAC AGC AAT GCT  900
Met Leu Glu Thr Leu Ser Asp Asp Asp Phe Val Asn Val Ala Ser Phe Asn Ser Asn Ala
                                                                           294
CAG GAT GTA AGC TGC TTT CAG CAC CTT GTC CAA GCA AAT GTA AGA AAT AAG AAA GTG TTG  960
Gln Asp Val Ser Cys Phe Gln His Leu Val Gln Ala Asn Val Arg Asn Lys Lys Val Leu
                                                                           314
AAA GAT GCA GTG AAT AAT ATC ACA GCA AAA GGA ATC ACA GAT TAT AAG AAG GGC TTT AGT 1020
Lys Asp Ala Val Asn Asn Ile Thr Ala Lys Gly Ile Thr Asp Tyr Lys Lys Gly Phe Ser
                                                                           334
TTT GCT TTT GAG CAG CTG CTT AAT TAT AAT GTA TCC AGA GCC AAC TGC AAT AAG ATT ATC 1080
Phe Ala Phe Glu Gln Leu Leu Asn Tyr Asn Val Ser Arg Ala Asn Cys Asn Lys Ile Ile
                                                                           354
ATG TTG TTC ACG GAC GGA GGA GAA GAG AGA GCC CAG GAG ATA TTT GCC AAA TAC AAT AAA 1140
Met Leu Phe Thr Asp Gly Gly Glu Glu Arg Ala Gln Glu Ile Phe Ala Lys Tyr Asn Lys
                                                                           374
GAC AAG AAA GTA CGT GTA TTC ACA TTC TCA GTT GGC CAA CAT AAT TAC GAC AGA GGA CCT 1200
Asp Lys Lys Val Arg Val Phe Thr Phe Ser Val Gly Gln His Asn Tyr Asp Arg Gly Pro
                                                                           394
ATT CAG TGG ATG GCT TGC GAA AAT AAA GGT TAT TAT TAT GAA ATT CCA TCC ATT GGA GCC 1260
Ile Gln Trp Met Ala Cys Glu Asn Lys Gly Tyr Tyr Tyr Glu Ile Pro Ser Ile Gly Ala
                                                                           414
ATA AGA ATT AAT ACT CAG GAA TAC CTA GAT GTT CTG GGA AGA CCG ATG GTT TTA GCA GGA 1320
Ile Arg Ile Asn Thr Gln Glu Tyr Leu Asp Val Leu Gly Arg Pro Met Val Leu Ala Gly
                                                                           434
GAC AAA GCT AAG CAA GTC CAA|TGG ACA AAT GTG TAC CTG GAT GCA CTG GAA CTG GGA CTT 1380
Asp Lys Ala Lys Gln Val Gln|Trp Thr Asn Val Tyr Leu Asp Ala Leu Glu Leu Gly Leu
                                                                           454
GTC ATT ACT GGA ACT CTT CCG GTC TTC AAC ATA|ACT GGC CAA TTT GAA AAT AAG ACA AAC 1440
Val Ile Thr Gly Thr Leu Pro Val Phe Asn Ile|Thr Gly Gln Phe Glu Asn Lys Thr Asn
                                                                           474
TTA AAG AAC CAG CTG ATT CTT GGA GTG ATG GGA GTT GAT GTG TCT TTG GAA GAT ATT AAA 1500
Leu Lys Asn Gln Leu Ile Leu Gly Val Met Gly Val Asp Val Ser Leu Glu Asp Ile Lys
                                                                           494
AGA CTG ACA CCA CGT TTT ACA CTC TGC CCC AAT GGC TAC TAT TTT GCA ATT GAT CCT AAT 1560
Arg Leu Thr Pro Arg Phe Thr Leu Cys Pro Asn Gly Tyr Tyr Phe Ala Ile Asp Pro Asn
                                                                           514
GGT TAT GTG TTA TTA CAT CCA AAT CTT CAG CCA AAG CCT ATT GGT GTA GGT ATA CCA ACA 1620
Gly Tyr Val Leu Leu His Pro Asn Leu Gln Pro Lys Pro Ile Gly Val Gly Ile Pro Thr
                                                                           534
ATT AAT TTG AGA AAA AGG AGA CCC AAT GTT CAG AAC CCC AAA TCT CAG GAG CCA GTG ACA 1680
Ile Asn Leu Arg Lys Arg Arg Pro Asn Val Gln Asn Pro Lys Ser Gln Glu Pro Val Thr
                                                                           554
TTG GAT TTC CTC GAT GCA GAG TTG GAG AAT GAC ATT AAA GTG GAG ATT CGA AAT AAA ATG 1740
Leu Asp Phe Leu Asp Ala Glu Leu Glu Asn Asp Ile Lys Val Glu Ile Arg Asn Lys Met
                                                                           574
ATC GAT GGA GAA AGT GGA GAA AAA ACA TTC AGA ACT CTG GTT AAA TCT CAA GAT GAG AGA 1800
Ile Asp Gly Glu Ser Gly Glu Lys Thr Phe Arg Thr Leu Val Lys Ser Gln Asp Glu Arg
                                                                           594
TAT ATT GAC AAA GGA AAC AGG ACA TAC ACG TGG ACT CCT GTC AAC GGC ACA GAT TAT AGC 1860
Tyr Ile Asp Lys Gly Asn Arg Thr Tyr Thr Trp Thr Pro Val Asn Gly Thr Asp Tyr Ser
                                                                           614
AGT TTG GCC TTG GTA TTA CCA ACC TAC AGT TTT TAC TAT ATA AAA GCC AAA ATA GAA GAG 1920
Ser Leu Ala Leu Val Leu Pro Thr Tyr Ser Phe Tyr Tyr Ile Lys Ala Lys Ile Glu Glu
                                                                           634
ACA ATA ACT CAG GCC AGA TAT TCA GAA ACA CTG AAA CCG GAT AAT TTT GAA GAA TCT GGC 1980
Thr Ile Thr Gln Ala Arg Tyr Ser Glu Thr Leu Lys Pro Asp Asn Phe Glu Glu Ser Gly
                                                                           654
TAC ACA TTC CTA GCA CCA AGA GAT TAC TGC AGT GAC CTT AAA CCT TCA GAT AAT AAC ACT 2040
Tyr Thr Phe Leu Ala Pro Arg Asp Tyr Cys Ser Asp Leu Lys Pro Ser Asp Asn Asn Thr
                                                                           674
GAA TTT CTT TTA AAT TTC AAT GAG TTT ATT GAT AGA AAA ACT CCA AAC AAC CCA TCC TGT 2100
Glu Phe Leu Leu Asn Phe Asn Glu Phe Ile Asp Arg Lys Thr Pro Asn Asn Pro Ser Cys
                                                                           694
AAT ACA GAC TTG ATT AAT AGA GTC TTG CTG GAT GCA GGC TTT ACA AAT GAA CTT GTT CAA 2160
Asn Thr Asp Leu Ile Asn Arg Val Leu Leu Asp Ala Gly Phe Thr Asn Glu Leu Val Gln
                                                                           714
AAT TAC TGG AGT AAG CAG AAG AAT ATC AAG GGA GTG AAA GCA CGG TTT GTT GTG ACT GAT 2220
Asn Tyr Trp Ser Lys Gln Lys Asn Ile Lys Gly Val Lys Ala Arg Phe Val Val Thr Asp
                                                                           734
GGT GGG ATT ACC AGA GTT TAT CCC AAA GAG GCT GGA GAA AAT TGG CAG GAA AAC CCA GAG 2280
Gly Gly Ile Thr Arg Val Tyr Pro Lys Glu Ala Gly Glu Asn Trp Gln Glu Asn Pro Glu
                                                                           754
ACA TAT GAA GAC AGC TTC TAT AAA AGG AGC CTC GAT AAT GAT AAC TAC GTT TTC ACT GCT 2340
Thr Tyr Glu Asp Ser Phe Tyr Lys Arg Ser Leu Asp Asn Asp Asn Tyr Val Phe Thr Ala
                                                                           774
CCC TAC TTT AAC AAA AGT GGA CCT GGG GCC TAT GAG TCA GGC ATT ATG GTA AGC AAA GCT 2400
Pro Tyr Phe Asn Lys Ser Gly Pro Gly Ala Tyr Glu Ser Gly Ile Met Val Ser Lys Ala
                                                                           794
GTA GAA ATA TAT ATC CAA GGA AAA CTT CTT AAA CCT GCA GTT GTT GGA ATT AAA ATT GAT 2460
Val Glu Ile Tyr Ile Gln Gly Lys Leu Leu Lys Pro Ala Val Val Gly Ile Lys Ile Asp
                                                                           814
GTA AAT TCT TGG ATA GAG AAT TTC ACC AAA ACT TCA ATC AGG GAT CCG TGT GCT GGT CCA 2520
Val Asn Ser Trp Ile Glu Asn Phe Thr Lys Thr Ser Ile Arg Asp Pro Cys Ala Gly Pro
                                                                           834
GTT TGT GAC TGC AAA CGA AAC AGT GAT GTA ATG GAT TGT GTG ATT CTA GAT GAC GGT GGG 2580
Val Cys Asp Cys Lys Arg Asn Ser Asp Val Met Asp Cys Val Ile Leu Asp Asp Gly Gly
                                                                           854
TTT CTT TTG ATG GCC AAC CAT GAT GAT TAT ACC AAT CAG ATT GGA AGA TTC TTT GGA GAG 2640
Phe Leu Leu Met Ala Asn His Asp Asp Tyr Thr Asn Gln Ile Gly Arg Phe Phe Gly Glu
                                                                           874
ATT GAT CCA AGC TTG ATG AGA CAC CTG GTC AAT ATA TCA GTT TAT GCC TTT AAC AAA TCT 2700
Ile Asp Pro Ser Leu Met Arg His Leu Val Asn Ile Ser Val Tyr Ala Phe Asn Lys Ser
                                                                           894
TAT GAT TAT CAG TCG GTG TGT GAA CCT GGT GCT GCG CCA AAG CAG GGA GCA GGG CAC CGC 2760
Tyr Asp Tyr Gln Ser Val Cys Glu Pro Gly Ala Ala Pro Lys Gln Gly Ala Gly His Arg
                                                                           914
TCG GCT TAT GTG CCA TCA ATA GCA GAC ATA CTG CAG ATT GGA TGG TGG GCC ACT GCT GCT 2820
Ser Ala Tyr Val Pro Ser Ile Ala Asp Ile Leu Gln Ile Gly Trp Trp Ala Thr Ala Ala
                                                                           934
GCC TGG TCT ATT CTT|CAG CAG TTT CTG TTG AGT TTG ACT TTT CCA CGG CTC CTT GAG GCA 2880
Ala Trp Ser Ile Leu|Gln Gln Phe Leu Leu Ser Leu Thr Phe Pro Arg Leu Leu Glu Ala
                                                                           954
GCT GAT ATG GAG GAT GAC GAC TTC ACT GCC TCC ATG TCA AAG CAG AGC TGC ATC ACT GAG 2940
Ala Asp Met Glu Asp Asp Asp Phe Thr Ala Ser Met Ser Lys Gln Ser Cys Ile Thr Glu
                                                                           974
CAA ACC CAG TAT TTC TTC GAT AAT GAC AGC AAA TCG TTC AGT GGG GTA TTA GAC TGT GGG 3000
Gln Thr Gln Tyr Phe Phe Asp Asn Asp Ser Lys Ser Phe Ser Gly Val Leu Asp Cys Gly
                                                                           994
AAT TGT TCC AGA ATC TTT CAT GTA GAA AAG CTC ATG AAC ACC AAT TTA ATA TTC ATA ATG 3060
Asn Cys Ser Arg Ile Phe His Val Glu Lys Leu Met Asn Thr Asn Leu Ile Phe Ile Met
                                                                          1014
GTA GAG AGC AAG GGG ACA TGT CCC TGT GAC ACA CGG CTG CTC ATA CAA GCA GAG CAA ACT 3120
Val Glu Ser Lys Gly Thr Cys Pro Cys Asp Thr Arg Leu Leu Ile Gln Ala Glu Gln Thr
                                                                          1034
TCT GAT GGA CCA GAT CCT TGT GAT ATG GTT AAG CAA CCC AGA TAT CGA AAA GGG CCA GAT 3180
Ser Asp Gly Pro Asp Pro Cys Asp Met Val Lys Gln Pro Arg Tyr Arg Lys Gly Pro Asp
                                                                          1054
GTC TGC TTT GAC AAC AAT GTC CTG GAG GAT TAT ACT GAC TGC GGT GGG GTC TCT GGA TTA 3240
Val Cys Phe Asp Asn Asn Val Leu Glu Asp Tyr Thr Asp Cys Gly Gly Val Ser Gly Leu
                                                                          1074
AAT|CCT TCC CTG TGG TCC ATC ATC GGG ATA CAG TTT GTA CTG CTT TGG CTG GTT TCT GGC 3300
Asn|Pro Ser Leu Trp Ser Ile Ile Gly Ile Gln Phe Val Leu Leu Trp Leu Val Ser Gly
                                                1080
AGC|AGA CAC TGC CTG TTA TGA CCTTCTAAAACCAAATCTCCATAATTAAACTCCAGACCCTGCCACAACATG 3372
Ser|Arg His Cys Leu Leu

ATCCCTCCGTTATGTTAAAGTAGGGTCAACTGTTAAATCAGAACATTAGCTGGGCCTCTGCCATGGCAGAGCCCTAAGG 3451
CGCAGACTCATCAGGCACCCACTGGCTGCATGTCAGGGTGTCC ..... 3'
```

Fig. 1. Restriction maps of the (**A**) α_1 and (**B**) α_2 subunit cDNA. The heavy line represents the coding sequence. The cDNAs were isolated from an oligo-dT–primed expression cDNA library constructed in λgt11 (*23*) from young adult rabbit back skeletal muscle poly(A)$^+$ RNA. Double-stranded cDNA was synthesized, Eco RI adapters (*24*) were added, and fragments >1500 nt were selected on a Sepharose CL-4B column and ligated into λgt11. Two α_1 cDNA clones were identified by screening with monoclonal Ab IIF7 (*6*). Each clone contained an ~500-nt insert and was related by cross-hybridization. One clone (λSkMCaCHα1.3) was sequenced (*11*). The 453-nt cDNA insert encodes amino acid residues 950 to 1100. On the basis of the identification of this clone with Ab IIF7, we conclude that the IIF7 epitope is encoded by this portion of the protein sequence. The cDNA library was rescreened with the λSkMCaCHα1.3 insert (*25*). (**C**) DNA sequence (*11, 26*) and predicted amino acid sequence of the rabbit skeletal muscle Ca^{2+} channel α_2 subunit. A presumptive amino acid signal peptide is shown as the first 26 amino acids (residues −26 to −1). An arrow identifies the proposed cleavage site and positive numbering starts with the proposed NH_2-terminal residue of the mature protein. The NH_2-terminal amino acid sequence previously determined (*4*) is shown in bold sequence; Thr^8, Trp^{12}, and Asp^{14} were not previously determined. An in-frame upstream stop codon is underlined, as well as the start and stop codons of an upstream short open reading frame. Three putative transmembrane regions are enclosed in boxes. Potential *N*-glycosylation and phosphorylation sites are identified by an asterisk (*) below Asn residues and P below the Ser and Thr residues, respectively. Three α_2 cDNA clones were identified by screening the λgt11 rabbit back skeletal muscle cDNA library with guinea pig antisera to α_2. Two clones, λSkMCaCHα2.2 (2.5 kb) and λSkMCaCHα2.4 (3.6 kb), overlapped to encode 4.75 kb of an ~8-kb transcript (Fig. 3B). An additional 14 overlapping clones encoding a total of ~7850 nt were isolated by DNA hybridization. Only 176 nt of ~4224 nt of 3′ untranslated sequence was confirmed in both directions and are reported.

The α_1 subunit cDNA contains a 5619-nucleotide (nt) open reading frame that encodes a sequence of 1873 amino acids (Fig. 1A). This cDNA sequence is consistent with an ~6500 nt DHP receptor α_1 mRNA (see Fig. 3A). Although the nucleotide sequence of this cDNA is 99.4% identical to the cDNA sequence of the DHP receptor reported by Tanabe *et al.* (*10*), a number of differences were determined. Nucleotide differences were identified at 33 positions (*11*), of which three result in amino acid changes. The amino acid differences occur at residues 1808 (Thr to Met), 1815 (Ala to Val), and 1835 (Ala to Glu).

We also cloned approximately 7850 nt of α_2 cDNA (Fig. 1B), which is consistent with an ~8000-nt α_2 mRNA (see Fig. 3B). Three hundred and eight nucleotides of 5′ untranslated sequence, a 3318-nt open reading frame, and 176-nt of 3′ untranslated sequence are shown in Fig. 1C. The 5′ untranslated sequence of the α_2 cDNA is unusually long (*12*). The open reading frame encodes a sequence of 1106 amino acids (Fig. 1C). The deduced amino acid sequence yields a calculated molecular weight of 125,018 for the α_2 subunit, which contrasts with the observed molecular mass of 165 to 175 kD (under nonreducing conditions) determined by SDS–polyacrylamide gel electrophoresis (*2, 3, 6, 7*). However, the agreement between the sequence of amino acid residues 1 to 17 (Fig. 1C) with the partial amino-terminal sequence of the α_2 subunit (*4*) indicated that we cloned the α_2 subunit. We propose that the α_2 subunit has a 26–amino acid (residues −1 to −26) signal sequence. Although this proposed signal sequence is hydrophobic and of an appropriate characteristic length (*13*), it has an unusually short central hydrophobic region defined by Glu^{-1} and Gln^{-12}. The α_2 subunit contains 18 potential *N*-glycosylation sites (*14*) and two potential adenosine 3′,5′-monophosphate (cAMP)–dependent phosphorylation sites (*15*) (Fig. 1C).

The α_2 subunit lacks significant homology with any protein in the Dayhoff protein sequence database or with other sequences of ion channel and receptor proteins. An analysis of the α_2 subunit sequence for regional hydropathy (*16*) reveals that, in contrast to the α_1 subunit, this protein is substantially hydrophilic, although it does contain a number of hydrophobic regions that may represent transmembrane domains (Fig. 2). These segments are designated I (amino acid residues 422 to 445), II (residues 895 to 919), and III (residues 1056 to 1075) in Figs. 1C and 2. If the proposed α_2 subunit signal sequence is, in fact, cleaved between the Glu^{-1} and Glu^{+1} residues (Fig. 1C), the amino terminus would be extracellular. Furthermore, assuming that the three hydrophobic segments (Fig. 2) are transmembrane domains and that there are only three such domains, the carboxyl terminus would be intracellular. Such a transmembrane topography would result in 8 of the 18 potential *N*-glycosylation sites being extracellular and the two potential phosphorylation sites being intracellular (Fig. 2) and would be consistent with biochemical studies on the α_2 subunit (*2, 3, 6–8*).

To determine the tissue-specific expression of α_1 and α_2 mRNAs, total RNA was isolated from a variety of rabbit tissues for RNA blot analysis (Fig. 3). For the α_1 subunit, a prominent 6.5-kb transcript was detected in total RNA from skeletal muscle, while a much weaker hybridizing transcript of the same size was observed in aorta (Fig. 3A). Upon longer exposures of the autoradiograms, a weakly hybridizing 6.5-kb transcript was also observed in total RNA from heart; however, no α_1-specific hybridization was observed with total RNA from ileum or

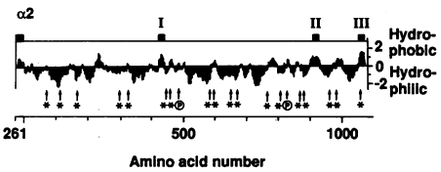


Fig. 2. Hydrophilicity profile of the α_2 subunit computed according to Kyte and Doolittle (*16*); the window size is 19 residues plotted at one-residue intervals. Potential glycosylation sites (*) and phosphorylation sites (P) are indicated. Three transmembrane domains are proposed: I, amino acid residues 422 to 445; II, amino acid residues 895 to 919; and III, amino acid residues 1056 to 1075. Transmembrane regions are proposed based on their hydropathy value, polarity index, and hydrophobic moment analysis (*16, 27*). The proposed signal sequence is indicated by the hatched box above residues −1 to −26.

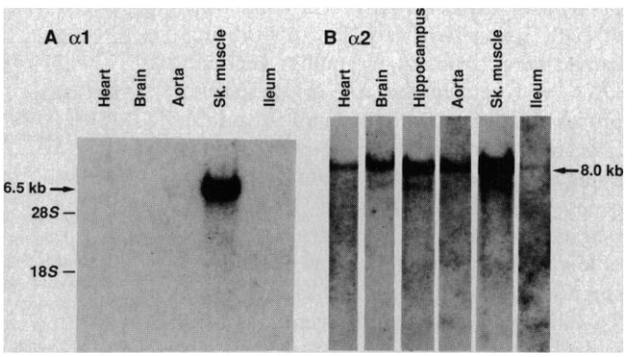


Fig. 3. RNA blot analysis of rabbit total RNA with (**A**) α_1 and (**B**) α_2 subunit cDNA probes. Total RNA was isolated from the tissues indicated, and 10 μg of RNA per lane was electrophoresed in a 1% agarose-formaldehyde denaturing gel and transferred to nitrocellulose filters (*28*). The filters were hybridized with the respective cDNA probes and washed in 0.015*M* NaCl, 0.0015*M* sodium citrate, 0.1% SDS, *p*H 7.0, at 37°C for α_1 and 50°C for α_2. The α_1 and α_2 cDNA probes, the Eco RI–Sac I fragment (nt 1006 to 5314) of pSkMCaCHα1.7, and the cDNA insert (nt 133 to 3494) of λSkMCaCHα2.3 were labeled by random priming. Autoradiography was for 7 days with an intensifying screen, except for the RNA from ileum and aorta hybridized with the α_2 cDNA probe, which was exposed to x-ray film for 14 days.

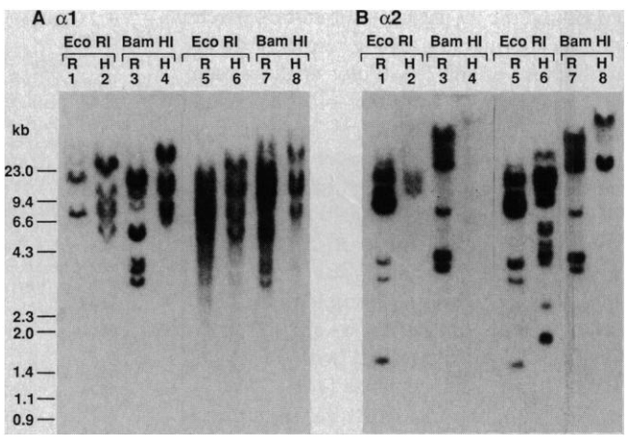


Fig. 4. DNA blot analysis of rabbit and human genomic DNA with (**A**) α_1 and (**B**) α_2 subunit cDNA probes. DNAs were digested with the indicated restriction enzymes and 15 μg of digested DNA was electrophoresed in a 0.8% agarose gel and transferred to nitrocellulose. Blots were hybridized at 42°C in 0.25 m*M* EDTA, 0.05*M* $NaH_2PO_4 \cdot H_2O$, 0.9*M* NaCl, *p*H 7.0 [5× saline sodium phosphate (SSPE)], 5× Denhardt's solution, 50% deionized formamide, 0.2% SDS, and sonicated herring sperm DNA (200 μg/ml) (*29*). (**A**) α_1; hybridization with pSkMCaCHα1.7 (*25*) cDNA probe. (**B**) α_2; hybridization with cDNA insert of λSkMCaCHα2.3 (*26*) subcloned into pIBI24 as probe. High stringency washing buffer consisted of 0.1× SSPE, 0.1% SDS, 65°C (lanes 1 to 4). Low stringency washing buffer consisted of 1× SSPE, 0.1% SDS, 50°C (lanes 5 to 8). R, rabbit genomic DNA; H, human genomic DNA.

brain. In contrast, an 8.0-kb transcript specific for the α_2 subunit was detected in total RNA from each of the tissues examined (Fig. 3B). In addition, a weakly hybridizing α_2 7.0-kb transcript was observed in skeletal muscle and the hippocampal region of the brain. The intensity of the α_2 hybridization signals also varied among the different tissues. This variability of the α_1 and α_2 hybridization signals may result from the absence or lower concentrations of the α_1 and α_2 transcripts in these tissues relative to skeletal muscle. Alternatively, the lower hybridization signals, or lack thereof, in certain tissues may be due to the presence of transcripts with varying degrees of sequence homology encoding α_1 and α_2 subunit–related subtypes.

To investigate the existence of gene sequences related to those encoding the skeletal muscle α_1 and α_2 subunits, rabbit and human genomic DNAs were digested with various restriction enzymes and DNA blots of these DNAs were hybridized with radiolabeled cDNA clones specific for the α_1 or the α_2 subunits (Fig. 4). Under conditions of high stringency, very few hybridizing bands were observed in rabbit genomic DNA with either the α_1- or α_2-specific probes (Fig. 4, A and B, lanes 1 and 3) indicating a low copy number, perhaps only a single copy each, of the α_1 and α_2 subunit genes. DNA blots of the same DNA preparations were also probed under conditions of low stringency with the same α_1- and α_2-specific probes (Fig. 4, A and B, lanes 5 and 7). Although more bands were observed with the α_1 cDNA probe than in the high stringency condition, no additional bands were observed with the α_2 cDNA probe. In addition, similar results were obtained with human genomic DNA (Figs, 4A and 4B, lanes 2, 4, 6, and 8), which suggests substantial conservation of the DNA sequences encoding the α_1 and α_2 subunits between rabbits and humans. These results, together with those of the RNA blot analyses, suggest that the α_1 subunit of the skeletal muscle DHP-sensitive Ca^{2+} channel may share homologous sequences with rabbit and human genes encoding distinct subtypes of Ca^{2+} channels. Additionally, these results confirm and extend those of recent peptide mapping and immunological studies that indicate that significant homology exists between the skeletal muscle α_2 subunit and a similar subunit associated with heart and brain L-type Ca^{2+} channels (*17*).

The sequence and structural homologies of the DHP receptor α_1 subunit with the α subunit of Na^+ channels (*18*) and a region of *Drosophila* K^+ channel proteins (*19*) support the conclusion that the α_1 subunit is part of a voltage-dependent Ca^{2+} channel. Biochemical and ultrastructural evidence demonstrates that the α_2 subunit interacts with the α_1 subunit, and perhaps also with the β and γ subunits, with equimolar stoichiometry as a complex localized in the T tubules (*3, 5–7*). The apparent inability of mRNA derived from an α_1 subunit cDNA to induce the expression of voltage-dependent Ca^{2+} channel activity in *Xenopus* oocytes (*20*) suggests that, in contrast to Na^+ (*18*) and K^+ (*19*) channels, the skeletal muscle DHP-sensitive Ca^{2+} channel may function only as a multiple subunit complex. Ion transport proteins and ligand-gated ion channels, such as Na^+,K^+-ATPase (*21*) and the nicotinic acetylcholine receptors (*22*), require multiple, distinct subunits for functions. For example, the α_2 subunit of the DHP-sensitive Ca^{2+} channel may be analogous to the β subunit of the Na^+,K^+-ATPase, an extensively glycosylated protein with a single putative membrane spanning region, which has been proposed to be required for the insertion of the α subunit into the membrane (*21*).

REFERENCES AND NOTES

1. R. J. Miller, *Science* **235**, 46 (1987); J. C. Venter and D. Triggle, Eds. *Structure and Physiology of the Slow Inward Calcium Channel* (Liss, New York, 1987).
2. W. A. Catterall, M. J. Seager, M. Takahashi, *J. Biol. Chem.* **263**, 3535 (1988); S. C. Froehner, *Trends Neurosci.* **11**, 90 (1988).
3. A. H. Sharp, T. Imagawa, A. T. Leung, K. P. Campbell, *J. Biol. Chem.* **262**, 12309 (1987); P. L. Vaghy *et al.*, *ibid.*, p. 14337.
4. N. Nakayama, T. L. Kirley, P. L. Vaghy, E. McKenna, A. Schwartz, *ibid.*, p. 6572.
5. A. T. Leung, T. Imagawa, B. Block, C. Franzini-Armstrong, K. P. Campbell, *ibid.* **263**, 994 (1988).
6. A. T. Leung, T. Imagawa, K. P. Campbell, *ibid.* **262**, 7943 (1987).
7. M. Takahashi, M. J. Seagar, J. F. Jones, B. F. X. Reber, W. A. Catterall, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5478 (1987).
8. T. Imagawa, A. T. Leung, K. P. Campbell, *J. Biol. Chem.* **262**, 8333 (1987).
9. B. M. Curtis and W. A. Catterall, *Biochemistry* **25**, 3077 (1986); J. S. Smith *et al.*, *ibid.* **26**, 7182 (1987).
10. T. Tanabe *et al.*, *Nature* **328**, 313 (1987).
11. Fragments of cDNA clones were subcloned into either pIBI24/25 (IBI, New Haven, CT) or M13 mp18/19. A sequential series of overlapping clones was generated prior to DNA sequencing [R. M. K. Dale, B. A. McClure, J. P. Houchins, *Plasmid* **13**, 31 (1985)]. The cDNA fragments were sequenced by the dideoxy chain–termination method [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)]. The positions of nucleotide differences and the sequence determined are as follows: 2001 (C), 2049 (T), 2081 (G), 2556 (A), 2631 (C), 2835 (C), 2874 (T), 3222 (G), 3450 (A), 3462 (T), 3486 (G), 3498 (T), 3720 (T), 4212 (C), 4449 (C), 4515 (A), 4803 (C), 4854 (A), 4950 (C), 5190 (A), 5238 (A), 5423 (T), 5444 (T), 5504 (A), 5514 (C), 5631–5632 (CA insertion), 5655 (G), 5739 (C deletion), and 5855–5858 (CTGG deletion). All nucleotide differences resulting in amino acid differences were confirmed by sequencing in both directions. In two cases, clones λSkMCaCHα1.4 and pSkMCaCHα1.6, significant alterations were found. The sequence of λSkMCaCHα1.4 diverges from the α_1 primary sequence at nt 3415, followed by a translational stop four codons farther in the carboxyl-terminal direction. The sequence of pSkMCaCHα1.6 contains an in-frame 57-nt deletion from nt 3610 to 3666.
12. M. Kozak, *Nucleic Acids Res.* **15**, 8125 (1987).
13. G. von Heijne, *J. Mol. Biol.* **184**, 99 (1985).
14. S. C. Hubbard and R. J. Ivatt, *Annu. Rev. Biochem.* **50**, 555 (1981).
15. J. R. Feramisco, D. B. Glass, E. G. Krebs, *J. Biol. Chem.* **255**, 4240 (1980).
16. J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
17. C. L. Cooper *et al.*, *J. Biol. Chem.* **262**, 509 (1987); M. Takahashi and W. Catterall, *Science* **236**, 88 (1987); *Biochemistry* **26**, 5518 (1987).
18. M. Noda *et al.*, *Nature* **312**, 121 (1984); M. Noda *et al.*, *ibid.* **320**, 188 (1986); M. Noda *et al.*, *ibid.* **322**, 826 (1986); A. L. Goldin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7503 (1986); W. Stühmer *et al.*, *Eur. Biophys. J.* **14**, 131 (1987).
19. B. L. Tempel, D. M. Papazian, T. L. Schwarz, Y. N. Jan, L. Y. Jan, *Science* **237**, 770 (1987); T L. Schwarz, B. L. Tempel, D. M. Papazian, Y. N. Jan, L. Y. Jan, *Nature* **331**, 137 (1988); L. C. Timpe *et al.*, *ibid.*, p. 143.
20. F. Hofmann, W. Nastainczyk, A. Röhrkasten, T. Schneider, M. Sieber, *Trends Pharmacol. Sci.* **8**, 393 (1987).
21. S. Noguchi, M. Mishina, M. Kawamura, S. Numa, *FEBS Lett.* **225**, 27 (1987).
22. J. Boulter *et al.*, *Proc. Natl. Acad. Sci. U.S.A* **84**, 7763 (1987).
23. U. Gubler and B. J. Hoffman, *Gene* **25**, 263 (1983); B. Lapeyre and F. Amabrie, *ibid.* **37**, 215 (1985); T. V. Huynh, R. A. Young, R. W. Davis, in *DNA Cloning: A Practical Approach*, D. M. Glover, Ed. (IRL, Oxford, U.K., 1985), vol. 1, p. 49.
24. W. I. Wood *et al.*, *Nature* **312**, 330 (1984).
25. λSkMCaCHα1.3 nt 2847–3300, numbering according to (*19*)] was used to rescreen the λgt11 library and 8 of 1×10^6 clones were positive. Clone λSkMCaCHα1.4 (1700 nt) extended the farthest 5′ to nt 2237. A fragment of this clone was used to screen 1×10^6 transformants of a rabbit back skeletal muscle cDNA library (constructed according to the method of H. Okayama and P. Berg). After an additional rescreening, the largest clone isolated, of eight positives, was pSkMCaCHα1.7 (5.3 kb) extending 5′ to nt ~450. Upon rescreening the λgt11 library, one clone, λSkMCaCHα1.8 (1.55 kb), was isolated that extended 78 nt 5′ of the start codon.
26. The nucleotide sequence shown was determined from clones λSkMCaCHα2.15 (nt −380–1123) and λSkMCaCHα2.3 (nt 133–3494). Five nucleotide differences among individual clones were observed, resulting in three amino acid changes. The nucleotides reported are from clone SkMCaCHα2.3. Differences occur in the sequence at positions 169, A(SkMCaCHα2.15); 347 and 348, AA(SkMCaCHα2.2); 984, T(SkMCaCHα2.4); and a deltion of nts 1858–1860 (SkMCaCHα2.2 and SkMCaCHα2.4). The resulting amino acid changes are Asn^{31}, Lys^{90}, and a deletion of Ser^{594}.
27. D. H. MacLennan, C. J. Brandl, B. Korezak, N. M. Green, *Nature* **316**, 696 (1985); D. Eisenberg, E. Schwarz, M. Komaromy, R. Wall, *J. Mol. Biol.* **179**, 125 (1984).
28. J. H. Han, C. Stratowa, W. Ruther, *Biochemistry* **26**, 1617 (1987); T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring, Harbor, NY, 1982).
29. J. Meinkoth and G. Wahl, *Anal. Biochem.* **138**, 267 (1984).

30. We thank L. Blonski and P. Prodanovich for preparation of the oligonucleotides; J. Robbins for RNA; D. MacLennan for providing the rabbit skeletal muscle Okayama-Berg cDNA library; P. Vaghy, T. Kirley, D. Slish, K. Kropp, J. Robbins, and G. Shull for assistance in some experiments, discussions, and manuscript review; R. Evans, J. Patrick, J. Sartor, G. Veliçelebi, and M. Wilson for discussions and reading the manuscript; and L. Wilson for typing the manuscript. Supported by SIBIA; the NIH and American Heart Association (A. S. and K.P.C.); and the Muscular Dystrophy Association (K.P.C.). E.M. and W.J.K. are NIH predoctoral trainees.

9 June 1988; accepted 10 August 1988