MAb recognizes a heat-stable antigen which is expressed by most B cells and immature thymocytes and, thus, is useful for enriching for mature thymocytes.
23. M. K. Jenkins, J. D. Ashwell, R. H. Schwartz, *J. Immunol.* **140**, 3324 (1988); C. Havele and V. Paetkau, *ibid.*, p. 3303.
24. A. M. Kruisbeek *et al.*, *J. Exp. Med.* **161**, 1029 (1985); S. Marusic-Galesic, D. S. Stephany, D. L. Longo, A. M. Kruisbeek, *Nature* **333**, 177 (1988).
25. A. R. Ready, E. J. Jenkinson, R. Kingston, J. J. T. Owen, *Nature* **310**, 231 (1984); H. von Boehmer and K. Hafen, *ibid.* **320**, 626 (1986).
26. E-K Gao, D. Lo, R. Cheney, O. Kanagawa, J. Sprent, unpublished observations.
27. A. Pierres *et al.*, *J. Immunol.* **132**, 2775 (1984).
28. W. L. Havran *et al.*, *Nature* **330**, 170 (1987).
29. R. Ceredig, J. W. Lowenthal, M. Nabholz, H. R. MacDonald, *ibid.* **314**, 98 (1985).
30. M. Sarmiento, A. L. Glasebrook, F. W. Fitch, *J. Immunol.* **125**, 2665 (1980).
31. O. Leo, M. Foo, D. H. Sachs, L. E. Samelson, J. A. Bluestone, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1374 (1987).
32. We thank C. Chen for technical assistance, B. J. Fowlkes for providing some of the staining reagents, helpful advice, and along with R. N. Germain and E. Shevach for critically reviewing the manuscript, and A. Hess and G. Santos for encouragement, advice, and for providing the cyclosporine A.

24 June 1988; accepted 26 August 1988

Identification of an Intracellular Peptide Segment Involved in Sodium Channel Inactivation

PETER M. VASSILEV, TODD SCHEUER, WILLIAM A. CATTERALL

Antibodies directed against a conserved intracellular segment of the sodium channel α subunit slow the inactivation of sodium channels in rat muscle cells. Of four site-directed antibodies tested, only antibodies against the short intracellular segment between homologous transmembrane domains III and IV slowed inactivation, and their effects were blocked by the corresponding peptide antigen. No effects on the voltage dependence of sodium channel activation or of steady-state inactivation were observed, but the rate of onset of the antibody effect and the extent of slowing of inactivation were voltage-dependent. Antibody binding was more rapid at negative potentials, at which sodium channels are not inactivated; antibody-induced slowing of inactivation was greater during depolarizations to more positive membrane potentials. The peptide segment recognized by this antibody appears to participate directly in rapid sodium channel inactivation during large depolarizations and to undergo a conformational change that reduces its accessibility to antibodies as the channel inactivates.

VOLTAGE-SENSITIVE SODIUM CHANnels mediate the rapid increase in Na^+ permeability during the rising phase of the action potential in many excitable cells (*1*). Their ion conductance is regulated on the millisecond time scale by two experimentally separable processes: voltage-dependent activation, which controls the rate and voltage dependence of the Na^+ conductance increase upon membrane depolarization, and inactivation, which mediates the Na^+ conductance decrease during a maintained depolarization (*1*). The principal protein component of Na^+ channels is the α subunit, a glycoprotein of approximately 260 kD (*2*). It is expressed in association with β1 (36 kD) and β2 (33 kD) subunits in nerve and β1 subunits in muscle (*2*). Messenger RNA encoding the α subunit is sufficient to direct the synthesis of functional Na^+ channels in *Xenopus* oocytes (*3*), although their inactivation is slower than native Na^+ channels (*4*). The primary structures of Na^+ channel α subunits from rat brain and eel electroplax have been inferred from the nucleotide sequence of cDNA clones (*5*). However, the relations between molecular structure and the mechanisms of ion permeation and channel gating are not yet understood. Here we identify a highly conserved intracellular segment of the α subunit that is involved in channel inactivation by use of site-directed antibodies.

Na^+ channel α subunits consist of four homologous transmembrane domains that have approximately 50% amino acid sequence identity (Fig. 1A) (*5*). These are connected by hydrophilic segments that are predicted to be intracellular (Fig. 1A). Electrophysiological studies have shown that intracellular application of proteases and amino acid–specific reagents causes removal of Na^+ channel inactivation (*6*). These results indicate that regions of the Na^+ channel structure that are required for inactivation are located on the intracellular surface of the channel protein and are accessible to macromolecular reagents.

To identify functionally important regions on the intracellular surface of the Na^+ channel, antibodies were prepared (*7*) against synthetic peptides (SP1, SP11, SP19, and SP20) with amino acid sequences that correspond to both conserved and variable sequences (Table 1) of the intracellular segments between the four homologous domains of Type II rat brain Na^+ channel [(R_{II}) (*5*)] (Fig. 1A). These antibodies recognize the Na^+ channel purified from rat brain in native form, and their affinity for the native protein is comparable to their affinity for the peptide used as antigen (*7*). The functional effects of these antibodies, which were affinity-purified by adsorption to immobilized Na^+ channel α subunits (*8*), were analyzed by recording Na^+ currents of rat muscle cells in the whole-cell voltage clamp configuration (*9–11*). Rat skeletal muscle cells were dissociated from 20-day embryos, maintained in vitro for 4 days to allow fusion into multinucleated myotubes, treated with colchicine to obtain round "myoballs," and studied after a total of 8 to

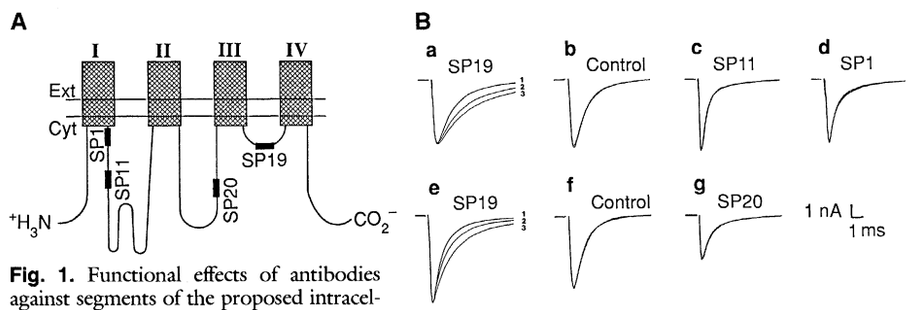

Fig. 1. Functional effects of antibodies against segments of the proposed intracellular domains of the Na^+ channel α subunit. (**A**) Schematic representation of the primary structure of Na^+ channel α subunit with designated peptide segments that are recognized by the four site-directed antibodies SP1, SP11, SP20, and SP19. (**B**) Na^+ currents during intracellular exposure to different sequence-directed antibodies. Na^+ currents were elicited from a holding potential of −70 or −110 mV by a 100-ms hyperpolarizing prepulse to −160 mV followed by a 9-ms test pulse to −5 mV. In each panel, two or three sequential Na^+ current traces are presented at increasing times of exposure to the antibody solution in the recording pipette. (a) Anti-SP19 for 6 (trace 1), 12 (trace 2), and 23 (trace 3) min at −110 mV; (b) control for 7 and 31 min at −110 mV; (c) anti-SP11 for 4 and 26 min at −110 mV; (d) anti-SP1 for 8 and 41 min at −110 mV; (e) anti-SP19 for 8 (trace 1), 25 (trace 2), and 61 (trace 3) min at −70 mV; (f) control for 8 and 68 min at −70 mV, and (g) anti-SP20 for 7 and 60 min at −70 mV. Na^+ current traces were normalized to allow direct comparison of time courses.

Department of Pharmacology, SJ-30, University of Washington, School of Medicine, Seattle, WA 98195.

18 days in cell culture (*10*). The large size and spherical cell shape of the myoballs allowed us to obtain adequate voltage-clamp control and to perfuse antibody-containing solutions inside the cell under stable recording conditions.

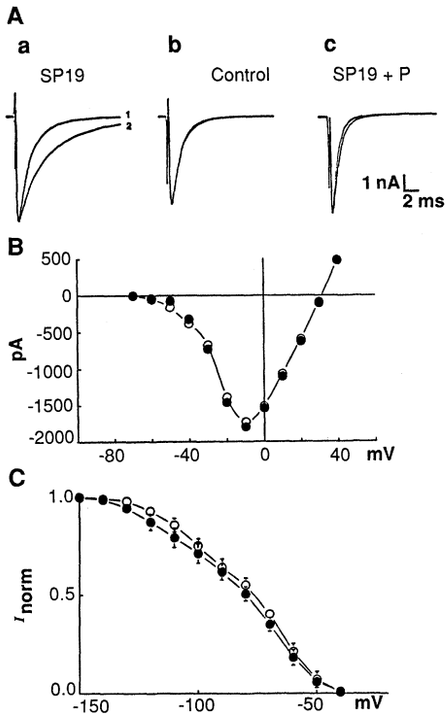

Fig. 2. Specificity of the effects of antibodies to SP19 on Na^+ currents. (**A**) Whole-cell Na^+ currents were recorded at the time intervals indicated below after the membrane was broken. The intrapipette solution contained no additions, Ab_{SP19}, or Ab_{SP19} that had been incubated with SP19 peptide (1 nmol per milliliter of original antiserum) for 30 min at 0°C before addition to the pipette (7). (a) Anti-SP19 antibodies for 5 (trace 1) or 24 (trace 2) min; (b) control solution for 5 and 33 min; and (c) anti-SP19 antibodies previously incubated with SP19 peptide for 6 or 28 min. Cells were maintained at a holding potential of −110 mV, hyperpolarized to −140 mV for 100 ms, and then stimulated by a 16-ms test pulse to −20 mV to elicit Na^+ currents. (**B**) Current-voltage relations of Na^+ currents in the presence of affinity-purified Ab_{SP19} in the recording pipette. Whole-cell Na^+ currents were measured after 7 (○) and 57 (●) min after breaking the cell membrane in the presence of antibodies to SP19 in the intrapipette solution. Cells were maintained at a holding potential of −70 mV, hyperpolarized to −120 mV for 40 ms, and depolarized to the indicated membrane potentials for 16 ms to elicit the Na^+ currents. Peak Na^+ currents are plotted as a function of the test pulse potential. (**C**) Mean Na^+ current inactivation curves in the presence of Ab_{SP19}. Whole-cell Na^+ currents were measured for five cells at 3 to 8 min (○) or 25 to 53 min (●) after breaking the cell membrane in the presence of Ab_{SP19} in the intrapipette solution. Cells were maintained at a holding potential of −70 mV, changed to the indicated prepulse potentials for 100 ms, and depolarized to −5 mV for 9 ms to elicit Na^+ currents. Mean values ± SEM from five experiments are plotted as a function of the applied prepulse potential.

Control experiments in the absence of antibodies (Fig. 1B, traces b and f) show no substantial changes in the kinetics of Na^+ currents in experiments that lasted as long as 180 min. However, when affinity-purified antibody to SP19 (Ab_{SP19}) was present in the pipette solution, a gradual slowing of Na^+ channel inactivation was observed over a period of several minutes as the antibody diffused into the cell (Fig. 1B, a and e). Intracellular application of antibodies to SP1, SP11, and SP20 had no effect on Na^+ currents under identical conditions (Fig. 1B, traces c, d, and g), suggesting that the effects of Ab_{SP19} were caused by its direct interaction with the SP19 segment of the Na^+ channel α subunit.

The specificity of the antibody-induced modification of Na^+ currents was tested further by using the SP19 peptide to block the immunoreactivity of Ab_{SP19} (Fig. 2A). Affinity-purified antibodies to SP19 induced a significant slowing of the declining phase of Na^+ currents in about 20 min. At a test potential of −20 mV, the time constant for Na^+ channel inactivation increased from 2.3 to 4.4 msec (Fig. 2A, panel a). Under the same conditions, no time-dependent changes in Na^+ currents were found in control experiments in absence of antibody (Fig. 2A, panel b). After prior treatment of the affinity-purified Ab_{SP19} with the corresponding peptide (7), a nearly complete block of the effect of the antibody on Na^+ channel inactivation was observed (Fig. 2A, panel c). These experiments indicate that the effect of the Ab_{SP19} on Na^+ channel inactivation results from its binding to the corresponding segment of the Na^+ channel α subunit.

The effects of Ab_{SP19} on Na^+ channel function were quite specific. No reproducible changes in Na^+ current amplitude under the influence of Ab_{SP19} could be detected. No substantial shifts in current-voltage relations due to the influence of Ab_{SP19} were observed (Fig. 2B). In some individual experiments, slight (2 to 4 mV) shifts of the voltage dependence of steady-state Na^+ channel inactivation toward more negative membrane potentials were observed. However, statistical analysis of pooled data from five experiments did not reveal a significant shift of the inactivation curve in the presence of Ab_{SP19} (Fig. 2C).

The effect of the test-pulse potential used to elicit Na^+ channel activation upon the antibody-induced slowing of Na^+ channel inactivation was examined. Figure 3 shows the effect of affinity-purified Ab_{SP19} on Na^+ currents recorded at four different test potentials. At each potential, traces taken before and after the onset of the antibody effect have been superimposed. For small depolarizations (for example, to −60 mV), no change in the time course of the Na^+ current occurs, as indicated by the coincidence of the two current traces in Fig. 3D. Inactivation of Na^+ currents during test pulses to potentials equal to or more positive than −50 mV is slowed after application of antibody (Fig. 3C). For a test pulse to −30 mV, the time constant of Na^+ current decay is increased approximately twofold, from 3.5 to 7.2 ms (Fig. 3B). During test pulses to +90 mV (Fig. 3A), the antibody-induced slowing of inactivation is much more prominent than at negative test potentials. Such voltage-dependent effects are observed on the first depolarization after a prolonged period of inactivity, ruling out a dependence on stimulus frequency. The rising phase of the Na^+ current remained unaffected at all test potentials examined, indicating a high degree of specificity of the antibody effect for the inactivation process.

Na^+ channels bind α scorpion toxins and sea anemone toxins, which act at an extracellular site and specifically slow Na^+ channel inactivation (*12*). The binding of these toxins is voltage-dependent with higher affinity at more negative membrane potentials (*13*). To assess the voltage dependence of binding of the Ab_{SP19}, we have examined the rate of modification of the Na^+ current decay at

Table 1. Peptides used as antigens to generate antibodies. The amino acid sequences of the peptides used for development of antipeptide antibodies are presented in single letter code. They correspond to the indicated residue numbers of the R_{II} Na^+ channel from rat brain (*5*). Their positions in a proposed overall folding pattern of the α subunit (*5*) and the percentage of identical residues in Na^+ channels from eel electroplax and rat brain (*5*) are also given.

Peptide	Predicted intracellular segment	Residue number	Eel-rat conservation (%)	Amino acid sequence*
SP1	IS6/IIS1	427–445	84	AYEEQNQATLEEAEQKEAE
SP11	IS6/IIS1	467–486	8	ASAESRDFSGAGGIGVFSES
SP19	IIIS6/IVS1	1491–1508	100	TEEQKKYYNAnl†KKLGSKK
SP20	IIS6/IIIS1	1106–1125	40	PIALGESDFENLNTEEFSSE

*Abbreviations for the amino acid residues are: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr. †Norleucine (nl) was substituted for methionine.

two holding potentials, −70 and −110 mV (Fig. 4). The slowing of the Na^+ current decay occurs three times as fast at the more negative membrane potential. No changes in inactivation kinetics were observed in control experiments. Evidently, the binding of SP19 antibodies to their site of action on the Na^+ channel is more rapid at negative holding potentials at which the Na^+ channels are not inactivated.

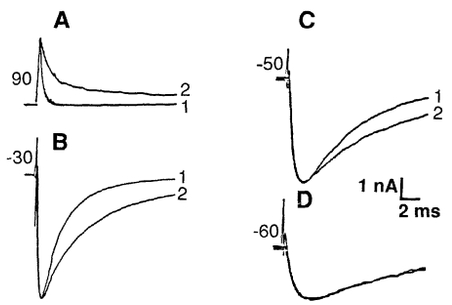

Fig. 3. Voltage dependence of the effect of antibodies to SP19 on Na^+ currents. Whole-cell Na^+ currents were recorded at 5 (trace 1) or 24 (trace 2) min after breaking the cell membrane in the presence of Ab_{SP19} in the recording pipette. The cell was maintained at a holding potential of −110 mV, hyperpolarized to −140 mV for 100 ms, and depolarized to the indicated membrane potentials for 16 ms to elicit Na^+ currents.

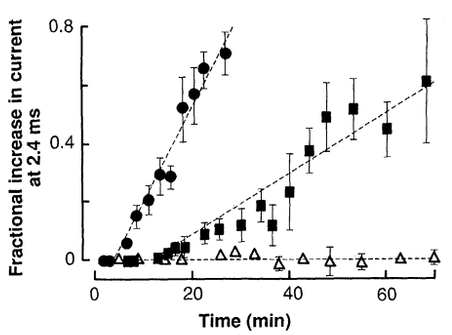

Fig. 4. Voltage dependence of the rate of action of Ab_{SP19}. Whole-cell Na^+ currents were recorded at the indicated times after breaking the cell membrane in the presence of control intrapipette solution (△) at a holding potential of −110 mV, in the presence of Ab_{SP19} in the recording pipette at a holding potential of −110 mV (●), or in the presence of antibodies to SP19 in the recording pipette at a holding potential of −70 mV (■). Cells were hyperpolarized to −160 mV for 100 ms and depolarized to −5 mV for 9 ms to elicit Na^+ currents. The rate of slowing of inactivation by Ab_{SP19} was assessed by measuring the fraction of the Na^+ current that had not inactivated 2.4 ms after the beginning of the test pulse as a function of the time (t) after breaking the cell membrane. This fraction, $f_{2.4}$, was calculated from each set of Na^+ current records and the fractional increase in Na^+ current at 2.4 ms $([f_{2.4}]_t/[f_{2.4}]_0 - 1)$ was plotted on the ordinate as a function of the time (t) of exposure to Ab_{SP19}. The value of $[f_{2.4}]_0$ was estimated from Na^+ current traces taken 1.5 to 5 min after breaking the cell membrane, before the antibody effect had begun. Mean values ± SEM from seven experiments in the presence of Ab_{SP19} and three experiments in the absence of antibody are presented.

Our results indicate that Ab_{SP19} can provoke a substantial slowing of Na^+ channel inactivation by binding to the corresponding amino acid sequence in the proposed intracellular segment between homologous domains III and IV (Fig. 1A). Because Ab_{SP19} is active from inside the cell, these results confirm the assignment of this segment of the α subunit to the intracellular side of the membrane. In addition, our experiments provide direct evidence for a role of this intracellular peptide segment in Na^+ channel inactivation. A molecular model of Na^+ channel function has been proposed that also assigns an important role in inactivation to this peptide segment (*14*). Further experimental evidence is required before the mechanism by which this peptide segment participates in channel inactivation can be defined.

The voltage dependence of the binding and action of Ab_{SP19} is of particular interest. The more rapid action of the antibody at a holding potential of −110 mV than at −70 mV may result from increased accessibility of the corresponding segment of the α subunit to the antibody in the resting state of the channel as compared to the inactivated state, because Na^+ channels in rat muscle cells are 80% inactivated over this voltage range (Fig. 2C). This suggests a direct participation of this peptide segment in the protein conformational changes that lead to channel inactivation. Similarly, the removal of Na^+ channel inactivation by intracellular perfusion with proteases is also more rapid at negative holding potentials (for example, −110 mV) compared to more positive potentials (for example, −30 mV) (*15*). The site of action of proteases in removing Na^+ channel inactivation may therefore be in or near the SP19 segment of the α subunit. It is noteworthy that Ab_{SP19} slows inactivation, but does not cause removal of inactivation as observed with proteases (*6*). Ab_{SP19} remains bound to Na^+ channels for several minutes to hours, as shown in biochemical experiments (*7*). Therefore, our findings are most consistent with the view that Ab_{SP19} may bind to the corresponding peptide segment of the α subunit and impede its movement, in contrast to the proteases, which damage the structure.

In contrast to the dependence of the binding of Ab_{SP19} on holding potential, the dependence of the action of Ab_{SP19} on the test potential occurs too rapidly to be due to voltage-dependent binding. It is more likely that the preferential slowing of Na^+ current decay at more positive membrane potentials reflects a specific action of the antibodies on pathways of Na^+ channel gating that are particularly important in determining Na^+ current time course at more positive membrane potentials. For example, both single channel recording and gating current experiments suggest that the time course of Na^+ current decay at more negative potentials is controlled primarily by a slow rate of channel entry to the activated state, whereas it is controlled by the inactivation process at more positive potentials (*16*). In any case, our results imply that there are multiple conformational pathways between the resting and inactivated states, as proposed in most recent models of Na^+ channel gating (*16, 17*), and suggest that Ab_{SP19} will be a valuable experimental tool to probe the involvement of the corresponding intracellular segment of the Na^+ channel in these pathways.

All of the antibodies that have been tested on Na^+ channels in rat muscle cells in this study are directed against segments of the α subunits of rat brain R_{II} Na^+ channels. At present, the amino acid sequence of rat skeletal muscle Na^+ channel α subunits is not known, and it is uncertain whether the SP1, SP11, SP19, and SP20 peptide segments of skeletal muscle Na^+ channels are identical to those of brain. The amino acid sequences of the SP19 segment of rat brain and eel electroplax Na^+ channels are identical (*5*), and Ab_{SP19} recognizes Na^+ channel α subunits in skeletal muscle, heart, electroplax, and insect nervous system (*7*), suggesting that the SP19 peptide segment is highly conserved in a wide range of Na^+ channel subtypes in different species. Moreover, the exponential time course of Na^+ current decay at −20 mV in the presence and absence of Ab_{SP19} suggests that the inactivation of both tetrodotoxin-sensitive and tetrodotoxin-insensitive Na^+ channels, which function in parallel in cultured rat muscle cells (*10, 18*), is slowed by Ab_{SP19}. The strong conservation of the amino acid sequence and functional effects of the SP19 peptide are consistent with an important role in rapid Na^+ channel inactivation, which is a conserved function of most Na^+ channels. The peptide segments corresponding to SP1, SP11, and SP20 are less well conserved and therefore may differ significantly in the α subunit of the skeletal muscle Na^+ channel.

In a previous study, it has been found that antibodies directed against a peptide corresponding to a proposed transmembrane segment containing amino acid residues 210 to 223 of the sequence of eel electroplax Na^+ channel increase the rate of activation and inactivation of slow Na^+ channels and shift the voltage dependence of inactivation of both slow and fast Na^+ channels when applied to the extracellular surface of dorsal root ganglion cells (*19*). Those results indicate that the process of Na^+ channel inactivation can be modified from the extracellu-

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.