Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 10161–10165, December 1989
Pharmacology

Localization of the receptor site for α-scorpion toxins by antibody mapping: Implications for sodium channel topology

(electrical excitability/neurotoxin/ion channel)

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Contributed by William A. Catterall, September 14, 1989

ABSTRACT **Site-directed and monoclonal antibodies recognizing different extracellular regions of the R_{II} sodium channel α subunit have been used to determine the sequences that comprise the receptor for α-scorpion toxins by evaluating the effect of antibody on voltage-dependent binding of radiolabeled toxin isolated from *Leiurus quinquestriatus* to both reconstituted rat brain sodium channel and rat brain synaptosomes. Of six antibodies tested, two recognizing amino acid residues 355–371 and 382–400 located on an extracellular loop between transmembrane segments S5 and S6 of domain I and one recognizing residues 1686–1703 of a similar loop of domain IV inhibit binding by 30–55%. Inhibition is concentration- (EC_{50} = 0.4–2 μM) and time- ($t_{1/2}$ = 40–80 min) dependent. Five different monoclonal antibodies recognizing the same extracellular loop in domain I inhibit binding completely with similar EC_{50} values as observed for site-directed antibodies. Kinetic studies of the antibody effect are consistent with a slowly reversible competition for the toxin receptor site. Our results suggest that the extracellular loops between segments S5 and S6 of domains I and IV comprise at least part of the α-scorpion toxin receptor site and support the membrane topology models in which domains I and IV are adjacent in the tertiary structure of the channel protein and six transmembrane sequences are contained in each of the four homologous domains.**

The voltage-dependent sodium channel is an integral plasma membrane protein responsible for the increase in sodium ion permeability during the rapidly rising phase of the action potential of excitable cells (1). The sodium channel isolated from rat brain consists of three nonidentical glycoprotein subunits, a 260-kDa α subunit that is covalently associated with a 33-kDa β_2 subunit and noncovalently associated with a 36-kDa β_1 subunit (2, 3). Messenger RNA encoding the α subunit alone can direct the synthesis of functional sodium channels in *Xenopus* oocytes (4, 5) and mammalian somatic cells (6). Computer analysis and similarity comparisons of the primary amino acid sequence of sodium channel α subunits (3) has been used to construct topological models of the α subunit within the plasma membrane having four homologous domains containing six (3) or eight (7, 8) transmembrane segments per domain.

α-Scorpion toxin V isolated from the venom of the North African scorpion *Leiurus quinquestriatus* (LqTx) binds to neurotoxin receptor site 3 on the sodium channel in a voltage-dependent manner (9, 10) and slows or blocks sodium channel inactivation at an extracellular locus (11, 12). Photoreactive derivatives of LqTx covalently label the α subunits in neurons, synaptosomes, and purified and reconstituted sodium channels (13–16), and sodium channels expressed from α-subunit mRNA in somatic cells have a functional receptor site for LqTx (6). The site of covalent attachment of photoreactive derivatives to the α subunit has been localized by immunoprecipitation with site-directed antipeptide antibodies to the extracellular loop between proposed transmembrane segments S5 and S6 of domain I (16). In the present experiments, we have analyzed the effects of a series of site-directed antipeptide and monoclonal antibodies (mAbs) on the voltage-dependent binding of LqTx to sodium channels to define regions of the channel structure that are located on its extracellular surface and are required for toxin binding at higher resolution.

EXPERIMENTAL PROCEDURES

Materials. LqTx was purified from venom purchased from Sigma and was radiolabeled by lactoperoxidase-catalyzed iodination as described (16). Batrachotoxin was a gift from John Daly (Laboratory of Bioorganic Chemistry, National Institutes of Health). Antisera directed against synthetic peptides corresponding to different sequences of the α subunit of the R_{II} sodium channel (17, 18) and monoclonal antibodies directed against the sodium channel α subunit (S. Rossie, L. Maechler, and W.A.C., unpublished results) were prepared as described. IgG was isolated from antisera by protein A–Sepharose chromatography. Fab fragments were generated from IgG by using immobilized papain and were resolved from Fc fragments and unreacted IgG by protein A–Sepharose chromatography. All antibodies recognize native sodium channels in immunoprecipitation assays.

^{125}I-Labeled LqTx (^{125}I-LqTx) Binding to Reconstituted Sodium Channel. Rat brain sodium channels purified through the step of chromatography on wheat germ agglutinin–Sepharose (19) at concentrations of 300–400 pmol/ml in a solution containing 25 mM Hepes·Tris (pH 7.4), 100 mM Na_2SO_4, 0.4 mM $MgSO_4$, 157 mM *N*-acetylglucosamine, 4 μM tetrodotoxin, 1.65% Triton X-100, 0.19% phosphatidylcholine, and 0.12% phosphatidylethanolamine were reconstituted in phospholipid vesicles as described (20, 21). After preincubation of reconstituted vesicles with preimmune IgG, site-directed antibody, or mAb, ^{125}I-LqTx binding assays were performed essentially as described (20). Briefly, assays were initiated by the addition of 40 μl of preincubation mixture to 160 μl of a solution containing 0.23 nM ^{125}I-LqTx, 105 mM Tris sulfate, 0.5 mM magnesium sulfate, 25 mM Hepes·Tris, 150 mM sucrose, and bovine serum albumin at 4 mg/ml (pH 7.4). The sucrose concentration was adjusted to maintain osmolarity equal to the intravesicular medium. The reaction mixture was then incubated for 5 min at 37°C, diluted with 3 ml of wash buffer containing 163 mM choline chloride, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5 mM Hepes (adjusted to pH 7.4 with Tris base), bovine serum albumin at 1 mg/ml, and sucrose sufficient to maintain osmolarity, filtered over GF/F

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Abbreviations: LqTx, toxin V isolated from *Leiurus quinquestriatus*; ^{125}I-LqTx, ^{125}I-labeled LqTx; mAb, monoclonal antibody; Ab, antibody.

filters under vacuum, and washed three more times with the same wash buffer. Nonspecific toxin binding was determined in the presence of 1 μM unlabeled LqTx and accounted for 20–40% of the total binding. Approximately 75% of the nonspecific binding is to GF/F filters alone.

^{125}I-LqTx Binding to Rat Brain Synaptosomes. Rat brain synaptosomes were prepared as described (22) and stored at −80°C until used. After preincubation of synaptosomes with IgG as described in each figure legend, ^{125}I-LqTx binding was quantitated as described for reconstituted sodium channel. Incubations containing mAbs were performed at 37°C for 30 min instead of 5 min. Nonspecific binding was determined in the presence of 1 μM LqTx and accounted for 10–25% of the total binding.

RESULTS

Inhibition of Binding of ^{125}I-LqTx by Site-Directed Antibodies. Six site-directed antibodies (IgG) that recognize different proposed extracellular regions of the R_{II} sodium channel α subunit were evaluated for their ability to inhibit ^{125}I-LqTx binding to sodium channels in reconstituted phospholipid vesicles and synaptosomes (Fig. 1). Preincubation of purified sodium channels reconstituted in phospholipid vesicles with three of the six antibodies, $Ab_{355-371}$, $Ab_{382-400}$, and $Ab_{1686-1703}$, inhibited ^{125}I-LqTx binding by 28%, 55%, and 54%, respectively (Fig. 1*A*). Similar levels of antibody-mediated inhibition of ^{125}I-LqTx binding were observed when partially purified sodium channel was preincubated for 4 hr at 4°C with the same quantity of these antibodies before reconstitution and toxin-binding assays (data not shown). $Ab_{317-334}$, $Ab_{1429-1449}$, and $Ab_{1729-1748}$ did not have significant effects on LqTx binding (Fig. 1*A*). Ab_{SP19}, an antibody recognizing the proposed intracellular segment of the α subunit between homologous domains III and IV (residues 1541–1558) associated with sodium channel inactivation (23), also had no effect on toxin binding (data not shown). Similar results were observed using rat brain synaptosomes (Fig. 1*B*). In this case, $Ab_{355-371}$, $Ab_{382-400}$, and $Ab_{1686-1703}$ inhibited ^{125}I-LqTx binding by 33%, 29%, and 27%, respectively, whereas the other three antibodies had no effect. $Ab_{355-371}$ and $Ab_{382-400}$ are directed against synthetic peptides corresponding to amino acid residues that are located on the proposed extracellular loop between transmembrane segments S5 and S6 of homologous domain I near the site previously identified by covalent attachment of LqTx derivatives (16). $Ab_{1686-1703}$ is directed against a synthetic peptide corresponding to amino acid residues located on the proposed extracellular segment between transmembrane segments S5 and S6 in homologous domain IV. Because these three antibodies inhibit LqTx binding, whereas other closely related antipeptide antibodies do not, our results suggest that the corresponding extracellular segments of domains I and IV interact in forming the receptor site for LqTx.

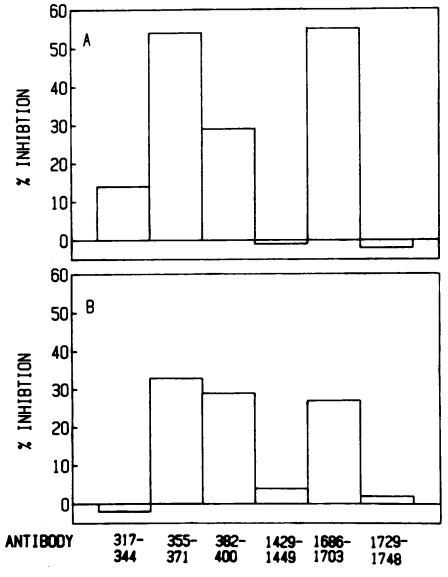

FIG. 1. Inhibition of ^{125}I-LqTx binding by site-directed antibodies. (*A*) Sodium channels in reconstituted phospholipid vesicles were preincubated with 5.5 μM preimmune IgG or 5.5 μM of the indicated site-directed antibodies (IgG) in a total volume of 300 μl containing 50 mM NaCl, 3 mM Hepes·Tris, 133 mM glycine hydrochloride, pH 7.4, and 1 μM batrachotoxin for 4 hr at 4°C with rotation, and ^{125}I-LqTx binding was measured as described. (*B*) Rat brain synaptosomes (50 μl of a 25-mg/ml suspension) were preincubated with 5.5 μM preimmune IgG or 5.5 μM of the indicated site-directed antibodies in a total vol of 300 μl of 25 mM NaCl, 2 mM Hepes·Tris, 133 mM glycine hydrochloride, pH 7.4, and 1 μM batrachotoxin, and ^{125}I-LqTx binding was measured as described. In both *A* and *B*, the data are expressed as the percent inhibition compared to control samples having only preimmune IgG.

Concentration–effect curves for inhibition of LqTx binding to either reconstituted rat brain sodium channel or synaptosomes by site-directed antibodies indicate that inhibition is concentration-dependent (Fig. 2). With reconstituted vesicles, EC_{50} values of 0.51 ± 0.21 μM, 0.39 ± 0.12 μM, and 0.57

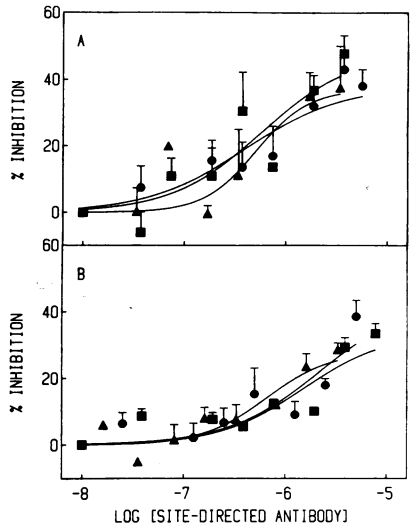

FIG. 2. Concentration-dependence of the inhibition of ^{125}I-LqTx binding by site-directed antibodies. (*A*) Inhibition of ^{125}I-LqTx binding to sodium channels reconstituted in phospholipid vesicles by $Ab_{382-400}$ (●), $Ab_{1686-1703}$ (■), and $Ab_{355-371}$ (▲). Sodium channels in reconstituted phospholipid vesicles (100 μl) were preincubated for 4 hr with the indicated concentrations of site-directed antibodies, 2 μM preimmune IgG, and 1 μM batrachotoxin in a total vol of 300 μl containing 50 mM NaCl, 3 mM Hepes·Tris, 133 mM glycine HCl, pH 7.4, with rotation, and ^{125}I-LqTx binding was measured as described. The percent inhibition at each antibody concentration was calculated from controls that contained only preimmune IgG. Each data point represents the mean ± SEM of three to five independent experiments where triplicate determinations were made at each antibody concentration. Curves represent fits of data to a sigmoidal function by a nonlinear-least-squares curve-fitting program (GRAPH PAD, Institute for Scientific Information). (*B*) Inhibition of ^{125}I-LqTx binding to rat brain synaptosomes by $Ab_{382-400}$ (●), $Ab_{1686-1703}$ (■), and $Ab_{355-371}$ (▲). Synaptosomes (50 μl of 25-mg/ml suspension) were preincubated with 7 μM preimmune IgG and the indicated concentrations of site-directed antibodies in a total volume of 300 μl of 25 mM NaCl, 2 mM Hepes·Tris, 133 mM glycine hydrochloride, pH 7.4, and 1 μM batrachotoxin, and ^{125}I-LqTx binding was measured as described. Each data point represents the mean ± SEM of three to four separate experiments in which triplicate determinations were made at each antibody concentration. Data were fit as described in *A*.

± 0.20 μM and maximal inhibition of 39%, 36%, and 47% were obtained for 5 μM $Ab_{355-371}$, $Ab_{382-400}$, and $Ab_{1686-1703}$, respectively (Fig. 2A). Higher EC_{50} values of 0.65 ± 0.15 μM, 2.0 ± 0.66 μM, and 1.35 ± 0.38 μM and similar maximal levels of inhibition of 29%, 43%, and 33% were observed with rat brain synaptosome preparations (Fig. 2B). It should be noted that the concentrations of antibody listed correspond to total IgG isolated from antisera, and, therefore, estimated EC_{50} values substantially exceed the true values for the active antibodies. Definition of maximal levels of inhibition was difficult because of limitations in the amount of antibody that could be added during the preincubation period.

The extent of inhibition of LqTx binding by site-directed antibodies was not increased by treatments designed to improve their access to their binding sites. Neuraminidase treatment of purified sodium channels to remove sialic acid residues before reconstitution did not enhance inhibition of toxin binding, and smaller Fab fragments generated by papain treatment of IgG produced similar levels of inhibition of ^{125}I-LqTx binding to reconstituted vesicles as native IgG. Moreover, the effects of maximal concentrations of $Ab_{355-371}$, $Ab_{382-400}$, and $Ab_{1686-1703}$ were not additive. These results suggest that the antipeptide antibodies completely inhibit LqTx binding to sodium channels to which they can gain access and bind, but that a fraction of sodium channels do not bind the antipeptide antibodies.

Inhibition of ^{125}I-LqTx Binding by mAbs. Our laboratory has recently characterized five independent mAbs that recognize epitope(s) in the extracellular loop of homologous domain I situated between transmembrane segments S5 and S6. The binding of these antibodies to sodium channels is prevented by peptides $SP_{317-334}$ and $SP_{382-400}$ but not by 11 other sodium channel peptides tested (S. Rossie, L. Maechler, W.J.T., and W.A.C., unpublished results). All five mAbs inhibit ^{125}I-LqTx binding to sodium channels in synaptosomes in a concentration-dependent manner (Fig. 3). Maximal levels of inhibition of 94%, 98%, 86%, 97%, and 89% were observed for mAbs 1G11, 1A9, 2E8, 3B2, and 3G4, respectively. EC_{50} values of 1.26 ± 0.10 μM, 0.56 ± 0.13 μM, 0.57 ± 0.06 μM, 0.77 ± 0.19 μM, and 0.39 ± 0.06 μM were obtained for these antibodies, respectively. Collectively, these results indicate that mAbs recognizing the same extracellular loop as $Ab_{355-371}$ and $Ab_{382-400}$ inhibit ^{125}I-LqTx binding to sodium channels in synaptosomes nearly completely at micromolar concentrations. These results provide additional support for the conclusion that this extracellular segment is required for formation of neurotoxin receptor site 3.

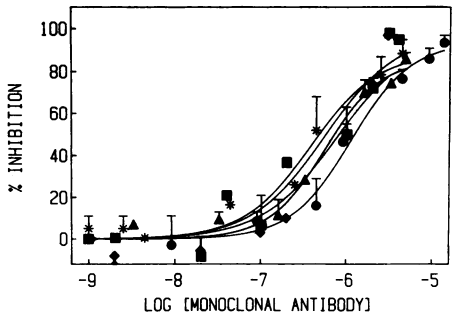

FIG. 3. Concentration-dependence of mAb-mediated inhibition of ^{125}I-LqTx binding to synaptosomes. (A) Inhibition of ^{125}I-LqTx binding to rat brain synaptosomes by mAbs 1G11 (●), 1A9 (■), 2E8 (▲), 3B2 (◆), and 3G4 (*). Synaptosomes (50 μl of 25-mg/ml suspension) were preincubated for 4 hr at 4°C with rotation with 7 μM preimmune IgG, and the indicated concentrations of mAbs in a total vol of 300 μl of 25 mM NaCl, 2 mM Hepes·Tris, 133 mM glycine hydrochloride, pH 7.4, and 1 μM batrachotoxin, and ^{125}I-LqTx binding was measured as described. Each data point is expressed as percent inhibition calculated from controls in which no antibody was added. Each point represents the mean ± SEM of two separate experiments in which triplicates were done at each antibody concentration. Data were analyzed as described for Fig. 2.

Time Course of Inhibition of ^{125}I-LqTx Binding. To examine the time course of antibody inhibition, toxin binding to either reconstituted vesicles or synaptosomes was measured after the indicated periods of preincubation with 1 μM $Ab_{382-400}$, 0.4 μM mAb3G4 (data not shown), or 6 μM mAb3G4 (Fig. 4A). The time courses for inhibition by $Ab_{382-400}$ are best described as monoexponential for both reconstituted vesicles and synaptosomes. Maximal levels of inhibition are obtained by 120–180 min of preincubation, and half-times of 78 and 38 min were observed using vesicles and synaptosomes, respectively. The time course for inhibition by 6 μM 3G4 is best described as biexponential with similar amplitudes of the fast (60%, $t_{1/2}$ = 1.2 and 0.2 min) and slow (30%, $t_{1/2}$ = 23 and 35 min) components in reconstituted vesicles and synaptosomes, respectively.

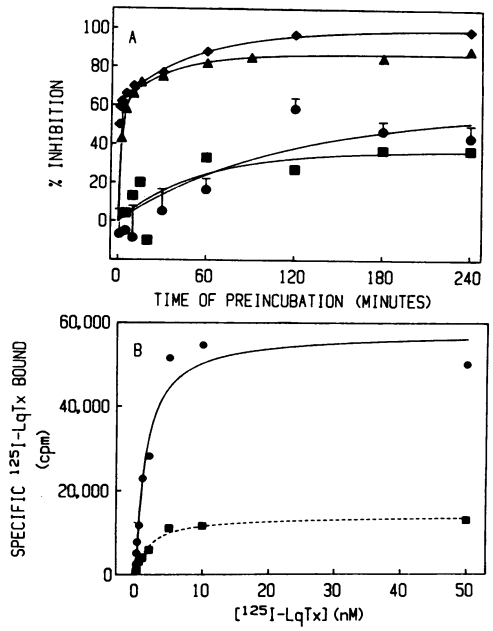

FIG. 4. Time course and saturation isotherm for inhibition of ^{125}I-LqTx binding. (A) $Ab_{382-400}$: rat brain synaptosomes were preincubated with 1 μM $Ab_{382-400}$ and 2 μM preimmune IgG (■) in 25 mM NaCl, 2 mM Hepes·Tris, 130 mM glycine hydrochloride, pH 7.4, and 1 μM batrachotoxin at 4°C. Sodium channels in reconstituted phospholipid vesicles were preincubated with 1 μM $Ab_{382-400}$ (●) and 2 mM preimmune IgG in 17 mM NaCl, 1 mM Hepes·Tris, 130 mM glycine hydrochloride, pH 7.4, and 1 μM batrachotoxin at 4°C. At the indicated times, aliquots were removed, and binding of ^{125}I-LqTx was measured as described. mAb 3G4: rat brain synaptosomes (◆) or sodium channels in reconstituted vesicles (▲) were preincubated with 6 μM mAb 3G4 in 15 mM NaCl, 1 mM Hepes·Tris, 67 mM sodium citrate, pH 7.4. At the indicated times, aliquots were removed, and binding of ^{125}I-LqTx was measured as described. Data are expressed as percent inhibition based on controls obtained at each time point. Each data point represents the mean ± SEM of three experiments for reconstituted vesicles studied with $Ab_{382-400}$ and one experiment for the other data. Triplicate determinations were made at each time point. Curves represent fits of data to a monoexponential ($Ab_{382-400}$) or a biexponential (mAb 3G4) function by a nonlinear least-squares curve-fitting program (GRAPH PAD). (B) Rat brain synaptosomes were incubated with 0.01–50 nM ^{125}I-LqTx in the absence (●) or presence (■) of 1 μM mAb 1A9 in 15 mM NaCl, 1 mM Hepes·Tris, 67 mM sodium citrate, pH 7.4, and ^{125}I-LqTx binding was measured as described. Points represent data from a single experiment in which triplicate determinations were made at each toxin concentration. Curves represent a nonlinear least-squares curve fit of data to a hyperbolic function (GRAPH PAD) that provided estimates of K_d and B_{max}.

Influence of mAbs on the Concentration Dependence of ^{125}I-LqTx Binding. The effect of the mAbs on LqTx binding is not reversible within the time of our experiments (data not shown). If the mAbs act as slowly reversible competitive ligands at the scorpion toxin receptor site, their effect on ^{125}I-LqTx binding should be seen as a reduction in the number of toxin-binding sites, and they should have no effect on the rate of dissociation of previously bound toxin. Fig. 4*B* shows saturation isotherms for ^{125}I-labeled LqTx binding to rat brain synaptosomes with and without 0.5 μM mAb 1A9. Similar K_d values of 1.5 ± 0.3 and 2.1 ± 0.3 nM were seen with and without mAb, respectively, but a 75% reduction in the number of binding sites was seen with mAb. Addition of 1 μM 1A9 had no effect on the rate of dissociation of previously bound ^{125}I-LqTx (data not shown). These results are consistent with an action of the mAbs as slowly reversible competitors for LqTx binding sites.

DISCUSSION

Interaction of Extracellular Segments of Domains I and IV in Forming the α-Scorpion Toxin Receptor Site. Photoreactive derivatives of LqTx were previously shown to be incorporated into the extracellular segment of the sodium channel between amino acid residues 317 and 400 in domain I (16). We show here that antipeptide antibodies recognizing residues 355–371 and 382–400 are effective inhibitors of LqTx binding, whereas antibodies recognizing the adjacent residues 317–335 and several other sodium channel segments are not. These results implicate the peptide segment from residues 355–400 immediately adjacent to proposed transmembrane segment S6 in α-scorpion toxin binding.

Five independent mAbs raised against purified rat brain sodium channels recognize epitope(s) formed by peptide segments between amino acid residues 317–400 (S. Rossie, L. Maechler, W.J.T., and W.A.C., unpublished results). Binding of these mAbs to sodium channels is blocked by synthetic peptides $SP_{317-335}$ and $SP_{382-400}$, but not by $SP_{355-371}$. Evidently, epitope(s) recognized by these mAbs are formed by interaction of two discontinuous peptide segments containing residues 317–335 and 382–400. We show here that these mAbs are effective inhibitors of LqTx binding and that their mechanism of inhibition is consistent with slowly reversible interaction at the α-scorpion toxin receptor site. Because $Ab_{317-335}$ does not inhibit LqTx binding appreciably, the combination of the results with antipeptide antibodies and mAbs points to amino acid residues 371–400 as playing an essential role in α-scorpion toxin binding.

Binding of α-scorpion toxins to the sodium channel is conformationally dependent (9, 20, 21), and it was predicted (16) that multiple polypeptide segments from different regions of the primary structure might contribute to formation of neurotoxin receptor site 3. Our present results show that $Ab_{1686-1703}$ is an effective inhibitor of LqTx binding, whereas several other antipeptide antibodies, including $Ab_{1729-1748}$, are not. Amino acid residues 1686–1703 are located in the extracellular segment between proposed transmembrane segments S5 and S6 of domain IV near the junction with S5. Thus, we propose that amino acid sequences from these two distant regions of the primary structure of the sodium channel α subunit (Fig. 5*A*) participate in formation of neurotoxin receptor site 3.

Although domains I and IV of the sodium channel α subunit are distant from each other in the primary structure of the channel, the four homologous domains defined in the primary structure are proposed (5, 7, 8) to be organized in a square array around a central transmembrane pore, placing domains I and IV adjacent to each other in the three-dimensional structure of the channel protein (Fig. 5*B*). Our results provide experimental support for this widely accepted proposal.

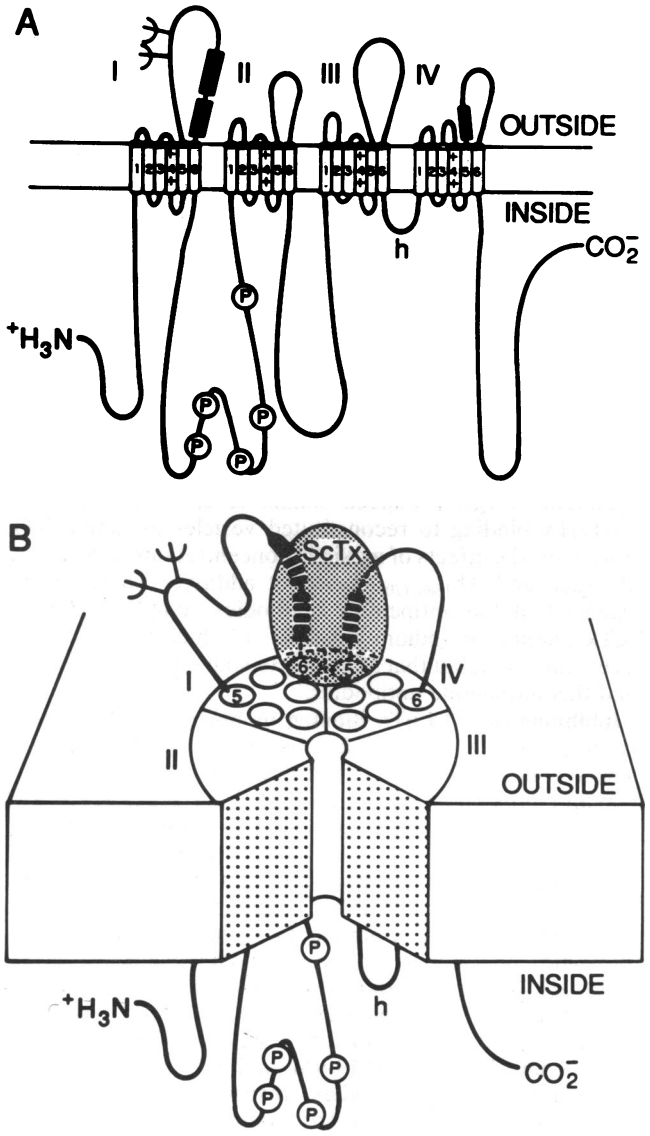

FIG. 5. Structure of the R_{II} sodium channel α subunit. (*A*) A two-dimensional view of the proposed transmembrane folding of the sodium channel α subunit depicting the sites of interaction of the site-directed antibodies ($Ab_{355-371}$, $Ab_{382-400}$, and $Ab_{1686-1703}$) that inhibit LqTx binding (dark boxes), known sites of protein phosphorylation (P) and glycosylation (Ψ), and site of inactivation gating (h). (*B*) A three-dimensional view of the sodium channel α subunit showing the proposed folding of the four homologous domains that brings domains I and IV into close apposition and allows formation of a transmembrane pore. An α-scorpion toxin molecule (ScTx) is depicted binding to the channel by interaction with the extracellular sequences between transmembrane segments S5 and S6 of domains I and IV that are recognized by $Ab_{355-371}$, $Ab_{382-400}$, and $Ab_{1686-1703}$.

Scorpion toxins are thought to have a small binding surface consisting of approximately 13 amino acid residues that are contiguous in the three-dimensional structure of the toxin (24). The inhibition of LqTx binding by antipeptide and mAbs described here indicates that this small binding surface may interact with a receptor site formed by the close apposition of residues 371–400 of domain I and residues 1686–1703 of domain IV of the sodium channel α subunit (Fig. 5*B*).

Transmembrane Organization of Amino Acid Sequences Between Transmembrane Segments S5 and S6. Proposals for the transmembrane organization of sodium channel α subunits (5, 7, 8) all suggest that the NH_2 and COOH termini and interdomain segments are cytoplasmic in orientation. Direct

support for this orientation has come from localization (Fig. 5B) of antibody-binding sites (25, 26), cAMP-dependent phosphorylation sites (27), and sites of channel inactivation (23). These models all propose that hydrophobic segments S1–S6 are transmembrane segments, although no direct experimental evidence supports these proposals. In addition, two models (7, 8) propose that amino acid sequences between transmembrane segments S5 and S6 form two additional complete or partial transmembrane segments. Because scorpion toxins clearly bind to the extracellular surface of the sodium channel, localization of their receptor sites will contribute to definition of its transmembrane orientation. Our previous work showed that at least part of the amino acid sequence between transmembrane segments S5 and S6 of domain I was extracellular and could be covalently labeled by photoreactive LqTx derivatives (16). Our present studies define this extracellular region more clearly.

High-affinity binding of α-scorpion toxins requires incorporation of the channel into an appropriate phospholipid environment and establishment of an internal negative membrane potential (21). Therefore, we can assume that all sodium channels to which we measure binding of LqTx are appropriately incorporated into sealed reconstituted phospholipid vesicles or synaptosomes with their extracellular surface facing outwards. Under these conditions, we find that antipeptide and mAbs recognizing amino acid residues 371–400 can bind to sodium channels and inhibit LqTx binding. These results provide strong evidence that these amino acid sequences in domain I are available to antibodies on the extracellular surface of the channel protein and are not buried deep within the protein or the phospholipid bilayer. Thus, our results do not support the proposals (7, 8) that these amino acid residues form partial or complete transmembrane segments in domain I. On the basis of these data, we favor the hypothesis that there are six transmembrane segments in domain I (5) and probably in each homologous domain. Thus, the amino acid sequences between transmembrane segments S5 and S6 in each domain would be fully extracellular (Fig. 5B) and available to participate in formation of receptor sites for the many other neurotoxins that act on the extracellular surface of the sodium channel.

Scorpion Toxin Action and the Site of Coupling of Activation to Inactivation. α-Scorpion toxins slow sodium channel inactivation specifically (11, 12), but the voltage-dependence of their binding closely follows that of channel activation (10). Based on these results, we have previously proposed that these toxins bind to a receptor site whose conformation changes upon channel activation and slow inactivation by preventing normal coupling of activation to subsequent inactivation (9, 10). Therefore, we expect that the site of α-scorpion toxin binding is located near regions of the channel that are critical for the coupling of activation to inactivation. Voltage-dependent activation of sodium channels is thought to involve sequential voltage-driven conformational changes initiated by the force of the electrical field acting on highly conserved gating charges located in the S4 transmembrane segments in each domain (3, 8). Neutralization of these charges in the S4 segment of domain I by site-directed mutagenesis causes a progressive reduction of activation gating charge (28), indicating that this segment plays an important role in initiating channel activation. Fast inactivation of the sodium channel is thought to be mediated by an inactivation gate formed by the intracellular segment between domains III and IV (23, 28, 29). The location of the α-scorpion toxin receptor site at a point of interaction between the extracellular segments of domains I and IV places the toxin in a position to interact with the S4 gating charges in these two domains and to slow or prevent the coupling of activation gating charge movements in domain I to conformational changes in domain IV that initiate closing of the inactivation gating segment. Further probing of the toxin receptor site may help to define the mechanism of activation–inactivation coupling.

We thank Ms. Dawn Merrick for sequence-directed antibodies, Mr. Carl Baker for preparation of sodium channels and synaptosomes, and Dr. Neil Nathanson for comments on the manuscript. This work was supported by National Institutes of Health Research Grant RO1-NS15751 and U.S. Army Research and Development Command Research Contract DAMD-84-C-4130 to W.A.C.

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