Toxin and kinetic profile of rat brain type III sodium channels expressed in *Xenopus* oocytes

Rolf H. Joho¹, J. Randall Moorman², Antonius M.J. VanDongen¹, Glenn E. Kirsch¹, Hanna Silberberg¹, Gabriele Schuster¹ and Arthur M. Brown¹

¹Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030 (U.S.A.) and ²Department of Medicine, University of Texas Medical Branch, Galveston, TX 77550 (U.S.A.)

(Accepted 15 August 1989)

Key words: Sodium channel; *Xenopus* oocyte; Toxin

Sodium (Na⁺) channels are members of a multigene family and are responsible for generation and propagation of the action potential in excitable cells. We have assembled, in a transcription-competent vector, a full-length cDNA clone encoding the rat brain type III Na⁺ channel. *Xenopus* oocytes microinjected with in vitro synthesized mRNA expressed functional rat brain Na⁺ channels from such ‘cloned’ RNA transcripts. We found that type III Na⁺ currents in whole cell microelectrode voltage clamp and in cell-attached patch recordings decayed much more slowly than any other reported Na⁺ current. In addition, we saw typical and additive effects of α- and β-scorpion toxins, suggesting that the Na⁺ channel α-subunit itself contains functional and distinct toxin binding sites.

INTRODUCTION

Voltage-gated Na⁺ channels are large transmembrane glycoproteins whose functional behavior has been studied extensively. Although the primary amino acid sequence is known, virtually nothing is known about the precise structures responsible for the two characteristic properties of these proteins: voltage sensitivity and ionic selectivity. Several cDNA clones encoding different rat brain Na⁺ channels have been isolated using a cDNA probe for the Na⁺ channel of *Electrophorus* electroplax to detect cross-hybridizing sequences in a rat brain cDNA library. The sequences of two clones (types Ia and Ib) are identical with the exception of a 33 nucleotide insert/deletion corresponding to 11 amino acids in the coding region. Two other cDNA clones (types II and III) have been isolated from the rat brain. They are approximately 90% homologous to type I and to each other. The most recently reported clone differs from type II by 6 amino acid changes and has been named IIA. Northern blot analyses of mRNA from rat brain using Na⁺ channel cDNA clones as radioactively labeled probes show several large mRNA species migrating at 9–10 kb.

Full-length cDNA clones covering the entire coding region of the 3 brain channel types have been assembled in transcription-competent vectors and the resulting in vitro RNA transcripts have been injected into *Xenopus* oocytes. Currents through expressed voltage-gated Na⁺ channels could be detected with voltage-dependence and tetrodotoxin (TTX) sensitivity similar to native brain Na⁺ channels. However, only oocytes injected with Na⁺ channel type II-, IIA-, and III-specific mRNAs showed substantial inward sodium currents upon depolarization; oocytes injected with type Ia- and Ib-specific mRNA had only very small currents or none at all. The functional differences and the physiological significance of the different Na⁺ channel types encoded by these 3 mRNAs in the rat brain are not yet clear.

To begin studies of the structure-function relationship of the Na⁺ channel, we have isolated and assembled the rat brain type III Na⁺ channel in a transcription-competent vector, and we have used in vitro synthesized RNA transcripts to express type III Na⁺ currents in microinjected *Xenopus* oocytes. We show that the decay of macroscopic type III Na⁺ currents is much slower than for native neuronal Na⁺ currents or those expressed by oocytes injected by total brain RNA, or type II- or IIA-specific RNA. In addition, we show that both α- and β-scorpion toxins from *Tityus serrulatus* have characteristic effects on the gating of the expressed type III Na⁺ channel.
MATERIALS AND METHODS

Recombinant DNA technology

Unless otherwise specified, standard techniques were used to handle RNA, recombinant DNA, library screening and subcloning. After cloning in bacteriophage lambda vectors, the DNA inserts were subcloned either into M13 mp18 or 19 for DNA sequencing purposes, or into pBluescript (Stratagene) for bulk growth and assembly of full-length cDNA clones that could be used for in vitro transcription.

Construction of rat brain cDNA libraries enriched for long inserts

Library construction is described in detail elsewhere. Briefly, total cellular RNA was isolated from frozen brains from adult Wistar rats using the uracil/LiCl method. Poly(A) RNA was selected by one cycle of oligo(dT)-cellulose chromatography, precipitated with ethanol, dissolved in water, and stored at -80 °C for subsequent use. To produce a cDNA library with large inserts, we size-fractionated poly(A) RNA on a 10–30% sucrose gradient. Starting with an oligo(dT) primer and an RNA template bigger than 5 kb, we were able to obtain cDNA up to 6–9 kb in length although the bulk of the first strand product migrated between 2 and 4 kb on an alkaline agarose gel. Second strand synthesis was done according to Gubler and Hoffman, and after methylation and linker addition, long cDNA was selected by preparative electrophoresis through a neutral agarose gel (BioRad). Using 50 pg cDNA of 2.5–6.5 kb, we obtained 50,000 recombinants in λ g110.

Partial cDNA libraries enriched for Na⁺ channel inserts were generated in λ ZAP (Stratagene) using two Na⁺ channel-specific oligonucleotides as primer. Forty μg of poly(A) RNA was mixed with 1.0 μg of each specific primer in 20 μl 10 mM HEPES-HCl (pH 6.9), 0.2 mM EDTA. The mixture was heated for 2 min to 90 °C, then cooled on ice. First and second strand syntheses were done as described, except that EcoRI linker was used for cloning into the EcoRI site of λ ZAP. cDNA larger than 1000 bp was selected on an alkaline agarose gel and 50 ng of purified cDNA yielded 10⁶ pfu in λ ZAP.

To isolate Na⁺ channel III-specific 5' end clones, we used an oligonucleotide specific for the type III sequence as a 3²P-labeled screening probe.

Clone 3 contains 3 inserts and constructs were propagated in pBluescript-SK(-). Clone 3 stopped 14 nucleotides short of the presumptive ATG initiation codon. For patch clamp recording, the vitelline membrane was removed with a razor blade. The cDNA isolates (clones 34, 14, 5 and 3) and the strategy used to generate a full-length construct are shown in the top part of the figure. The coding region is depicted with a box, the 5' and 3' untranslated regions are indicated by the heavy line. The zigzag lines at the beginnings and at the ends represent the polylinker of pBluescript (SK(-) (not to scale). The relevant restriction endonuclease sites used in the construction are shown and are abbreviated as: A = ApaI; E = EcoRI; EA = EcoRI-ApaI; NsiI = NsiI; X = XhoI. The next 14 nucleotides match the 5' untranslating region of channel III with the exception of a T to C transition (position -7) in order to change an ATG immediately upstream and out of frame with the initiation triplet. The synthetic fragment extends from the ATG initiation codon for another 53 nucleotides encoding the channel III sequence and ends with an XhoI site. This synthetic 5' end fragment was ligated into XhoI cut and phosphatase treated clone 3, and a hybrid clone X/3 carrying the 78 bp XhoI fragment in the correct orientation was selected. The Sall-NsiI fragment (2203 bp) from X/3 was isolated, ligated into Sall and NsiI opened clone 34 to generate the construct S34. S34 contains about 700 nucleotides of 3' untranslated region, followed by a unique Nol site in the polylinker of the cloning vector.

In vitro RNA synthesis and microinjection of Xenopus oocytes

Plasmid templates used for RNA transcription were linearized by digestion with NotI. The DNA was treated with proteinase K, extracted with phenol, chloroform, and concentrated by precipitation with ethanol. Transcription reactions were performed in 25 μl volumes containing 40 mM Tris-HCl (pH 8.0), 80 mM NaCl, 8 mM MgCl₂, 30 mM DTT, 2 mM spermidine, 1.0 mM ATP, CTP and UTP, 0.4 mM GTP, 2 mM m7G(5')ppp(5')G, 40 U RNasin, 25 U T7 RNA polymerase, and 150 ng of linearized DNA template. After 30 min at 37 °C, 10 U of RNAase-free DNase I (Boehringer) were added, and the samples were incubated for an additional 30 min at 37 °C. The transcripts were extracted twice with phenol/chloroform, and once with chloroform. The samples were adjusted to 2 M ammonium acetate and precipitated twice with ethanol, dissolved in water, and stored at -80 °C. The integrity of the transcripts was tested on a glyoxal gel, and the appropriate concentrations of RNA were diluted into 100 mM KCl, and 75 nl samples were injected into Xenopus oocytes.

Oocyte preparation and injection

Our methods for oocyte preparation and injection have been described previously. Briefly, laboratoy-reared adult female Xenopus laevis (Xenopus One, Ltd., Ann Arbor, MI) were anesthetized by immersion in 0.1% tricaine methanesulfonate and portions of ovaries were removed aseptically. Stage V–VI oocytes were isolated manually and injected with RNA solutions using a micrometer-driven 10 μl micropipettor (Drummond Scientific Co., Broomall, PA). After 3–6 days incubation in modified Barth's solution at 19 °C, the follicular layer of cells was removed manually, and oocytes were tested for Na⁺ channel expression. Uninjected oocytes from the same ovary were assayed for endogenous Na⁺ channels. TTX was obtained from Calbiochem, and scorpion toxins were the gracious gift of Dr. L. D. Possani.

Electrophysiological recording

Expression of voltage-dependent Na⁺ currents in injected oocytes were monitored using a two-microelectrode voltage clamp (Axoclamp-2A, Axon Instruments, Burlingame, CA). De-folliculated oocytes were placed in a recording chamber and perfused with a solution containing 145 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 10 mM glucose, and 10 mM HEPES (pH 7.35) and had resistances of 1–5 MΩ. Experiments were carried out at room temperature (20–22 °C). Macroscopic currents were acquired and analyzed using pCLAMP (Axon Instruments, Burlingame, CA).

For patch clamp recording, the vitelline membrane was removed manually in a hyperosmolar solution. Patch-clamp electrodes
The bath solution contained 150 mM potassium aspartate, 10 mM KCl, 1.8 mM CaCl₂, 10 mM glucose, and 10 mM HEPES (pH 7.35). The bath solution contained 150 mM potassium aspartate, 10 mM KCl, 1.8 mM CaCl₂, 10 mM glucose, and 10 mM HEPES (pH 7.35). Gigaohm seals were obtained in the cell-attached configuration. Recordings of macroscopic currents from large electrodes were acquired and analyzed using pCLAMP. Single-channel recordings were stored on videotape and analyzed offline. The analog signal was filtered at 5–10 kHz (-3 dB, 4-pole Bessel), digitized with 12-bit resolution at 20 kHz, and digitally filtered at 1 kHz (-3 dB, 4-pole Bessel). The technique for single-channel idealization has been previously described.

RESULTS

Isolation of overlapping sodium channel cDNA clones

A Na⁺ channel clone bearing a nearly full-length coding region (clone 34 in Fig. 1) was isolated. The sequence of the 5.8 kb insert of clone 34 was identical except for 7 nucleotides to the Na⁺ channel type III gene recently isolated (Fig. 2). The DNA sequence starts in the first domain (after the S3 segment), shows a long open reading frame that ends at a position corresponding to the carboxyl-terminus in channels I, II, and III, and extends for approximately 700 nucleotides into the 3’ untranslated region.

To isolate the missing 5’ end of the cDNA, we synthesized two oligonucleotides complementary to the sense sequence of Na⁺ channel type III starting 661 base pairs (bp) and 1530 bp downstream from the beginning of clone 34. These oligonucleotides were used to prime cDNA synthesis, and a partial library, enriched for the 5’ ends of Na⁺ channel mRNA was constructed in λ ZAP. Using a type III-specific radiolabeled probe, the missing 5’ ends were cloned (Fig. 1). Two of the isolates (clones 5 and 14) carried 5’ untranslated regions of 223 bp and 625 p, respectively. The third isolate (clone 3) lacked the first 14 nucleotides of the coding region. In the areas of overlap the DNA sequences of clones 3 and 14 were identical to clone 34 (Fig. 2). There are ten base changes in our completed sequence compared to the previously reported sequence of Na⁺ channel III [18]. Two of the nucleotide changes lead to two conservative amino acid changes (Fig. 2). Comparison of clone 5 to the known channel III sequence showed three inserts. The two inserts closest to the 5’ end are 182 and 818 nucleotides in length (position shown in Fig. 2). Both inserts interrupt the open reading frame of channel III and lead to premature termination of translation of the corresponding mRNA. The third insert is 96 nucleotides long and maintains the reading frame (position and sequence shown in Fig. 2). Previously, Na⁺ channel I had been isolated as two isoforms that were identical to one another with the exception of a 33 nucleotide insert [28]. This insert is at the same position in channel I as the 96 nucleotide insert in clone 5. Both inserts maintain the reading frame. Therefore, the two isoforms could have arisen by alternate splicing and differ by 11 or 32 extra amino acids in channel I and III, respectively. In the channel models that have been put forward [11,13,14,20], these additional amino acids are located in the cytoplasmic region between domains 1 and 2. This interdomain region contains several sites that undergo cAMP-dependent phosphorylation in vitro and in vivo [32] and therefore may be of particular importance in channel function.

Construction of full-length Na⁺ channel cDNAs

Fig. 1 depicts the strategy followed to assemble a full-length cDNA (S34) encoding the rat brain type III Na⁺ channel. The construct was assembled in ρBLUESCRIPT-SK(−) with the NotI restriction site at the 3’ end of the coding region. RNA transcripts were made in vitro using T7 RNA polymerase, and the ‘cloned’ mRNA was microinjected into Xenopus oocytes for functional studies. The RNA sequence obtained from S34 corresponds to the DNA sequence shown in Fig. 2. The first 37 nucleotides (small letters in Fig. 2) are derived from the polylinker present in pBluescript, and are followed by an additional 14 nucleotides preceding the AUG initiation codon.

Characterization of Na⁺ currents in oocytes injected with Na⁺ channel type III mRNA

Up to 20 ng of Na⁺ channel transcript S34 were injected into each oocyte. Four to 6 days later, oocytes had expressed inward currents which we measured with two-microelectrode voltage clamp and cell-attached patch clamp. Identification of these currents as arising from voltage-dependent Na⁺ channels rests on the evidence that (a) the current was highly sensitive to tetrodotoxin (TTX); (b) the reversal potential was 43 mV, a value expected for Na⁺ current, after inactivation had been removed by TsvV-5, and α-scorpion toxin; (c) and the inward current peaked at −5 mV, at which point Cl⁻ currents would be outward (E_Cl = −24 mV).

Fig. 3A shows a family of macroscopic type III Na⁺ currents in oocyte under two-microelectrode voltage clamp. The most striking difference to Na⁺ currents endogenous to the oocyte [30] or those expressed after injection of total brain RNA [3,21] (Fig. 3B), total muscle RNA [34], total heart RNA [36], type II Na⁺ channel mRNA [35], type IIA Na⁺ channel mRNA [31], and to native Na⁺ currents in neuronal cells [19] is a 10-fold or greater prolongation in the decay of the current. In addition, time to peak current is prolonged. Since the microelectrode voltage clamp does not accurately clamp the large and convoluted oocyte membrane [35], we examined whether the large difference in macroscopic current...
Fig. 2. Nucleotide sequence of rat brain Na⁺ channel type III. The DNA sequence corresponding to the RNA sequence transcribed off S34 is shown in capital letters and the derived amino acid sequence in the single letter code. Position 1 is the start site of T7 RNA polymerase, and the transcribed polylinker is shown in small letters. Differences in the published type I/II sequence are shown on top of the nucleotide sequence in capital letters and the derived amino acid sequence in the single letter code. Position 1 is the start site of T7 RNA polymerase, and the transcribed polylinker is shown in small letters. Differences in the published type I/II sequence are shown on top of the nucleotide sequence in capital letters and the derived amino acid sequence in the single letter code.
decay was present in cell-attached patch recordings where isopotentiality is established. Fig. 3C shows the average current obtained by summing 4 records from a large patch containing more than 40 Na⁺ channels expressed after injection of total brain RNA. The slow decay of the type III Na⁺ current was clearly present in

sequence, and the corresponding amino acid changes are indicated below. The position and length of the first two inserts (in clone 5) are shown in small letters (pos. 3841-3900, 3901-3950, and 3951-4000). The position and length of the first two inserts (in clone 5) are shown in small letters (pos. 3841-3900, 3901-3950, and 3951-4000).
the cell-attached patch. Time to peak current was prolonged in type III Na⁺ channels (3.1 ms compared with 0.9 ms in the channels expressed by total brain RNA). At the single channel level, these effects were seen to be the result of prolonged bursts and delayed latency to first opening of the type III Na⁺ channels. An account of the single type III Na⁺ channel properties will be reported separately.

The level of expression of type III Na⁺ currents ranged up to 1500 nA per oocyte, which did not readily allow macroscopic current recordings from large cell-attached patches. We instead analyzed peak current-voltage (I–V) relationship, rates of current decay, and steady-state inactivation and inactivation gating in whole cell recordings. Since the rise time of the voltage step and resolution of the first 5–10 ms of membrane current are limited by the large capacitance of the oocyte, the peak current may not be measured accurately. We instead extrapolated the initial amplitudes of the currents from the decay phase to construct the peak I–V relationship and the steady-state activation and inactivation relationships. This technique has the advantage of analyzing the current trace well after the capacity current has subsided and the membrane voltage is maximally controlled. It is limited theoretically by the assumption that the processes of activation and inactivation are independent, uncoupled processes. This assumption was used by Stühmer and coworkers35 in studying expressed type II Na⁺ currents.

Fig. 4A shows the currents produced by 3-step depolarizations and the fit to these currents with biexponential decay functions. The dependence of the two rates of decay on the test potential is shown in Fig. 4C. Both fast and slow decay rates increased with increasing test depolarizations.

The I–V relationship (Fig. 4B) shows peak current at −5 to 0 mV and agrees well with the report of Suzuki et al.37. The steady-state activation curve (Fig. 4D) was constructed by plotting normalized conductance versus test potential and was fit by a Boltzmann function with midpoints of −10.7 mV and −4.7 mV. For activation, the values of A₀, A₁ and A₂ in nA are (−20 mV): 7.5, −82, −74; (−10 mV): −1.6, −394, −98; and (−5 mV): 1.6, −470, −81; and the values of t₁ and t₂ in ms are (−20 mV) 39.4, 168, (−10 mV) 24.9, 87 and (−5 mV) 20.0, 69. For B and D, the amplitude of the currents was measured as the sum of A₀, A₁ and A₂. In B–D, data points are the mean of 2–6 oocytes. Error bars are S.E.M. and are usually smaller than the symbols. B: current–voltage relationship. The finding of peak current amplitude at approximately −5 to 0 mV was confirmed in more than 50 other oocytes. C: time constants of decay as a logarithmic function of test potential in the same oocyte. D: steady-state inactivation as a function of 5 s (filled triangles) and 50 s (open triangles) prepulse potential, and normalized conductance from holding potential −100 mV as a function of test potential (open circles). The smooth lines are Boltzmann functions of the form f(t) = 1/(1 + [exp(Vm − V₅₀)/k]). After 5 s prepulses, V₅₀ = −36.1 mV and k = 7.8 mV; after 50 s prepulses, the values are −51.0 and 6.7 mV. For activation, V₅₀ = −10.7 mV and k = −4.7 mV.
The effects of toxins on type III Na$^+$ currents are shown in Fig. 5. A dose-response relationship to TTX. Data points are the mean of 3 experiments, and the S.D. is smaller than the symbols. The smooth line is the theoretical fit for a single binding site with an IC$_{50}$ of 1.8 nM. B-E show the response to α(TslV-5) and β(Ts-y) toxins. The I-V curves were obtained by changing the membrane potential from −100 to 50 mV in a continuous ramp lasting 1 s. Individual step depolarizations to −40 and −5 mV in control (C), α-toxin alone (5 μM) (D), and combined α- and β-toxins (5 μM) (E) are shown. α-toxin increased peak current without altering the I-V relationship, and β-toxin had the additive effect of shifting the threshold for current activation to more negative potentials. Calibration bars = 50 nA and 100 ms.

**Effects of toxins on type III Na$^+$ currents**

Fig. 5 shows the effect of Na$^+$ channel-specific toxins. TTX blocked the Na$^+$ currents reversibly with an IC$_{50}$ of 1.8 nM (Fig. 5A). Fig. 5B–E show the effects of toxins from the scorpion Tityus serrulatus. The current–voltage relationships in Fig. 5B were obtained by changing the membrane potential from −100 to 50 mV in a continuous ramp lasting 1 s. This technique appears acceptable for this analysis because of the very slow inactivation of type III Na$^+$ currents, especially after the addition of α-toxin.

TslV-5, an α-toxin$^{19,31}$, increased current without altering the I-V relationship (Fig. 5B). Step depolarizations to −40 mV did not evoke current either before or after this toxin was added to a final bath concentration of 5 μM (Fig. 5D); the current at test potential −5 mV was increased after toxin treatment compared to control, and a large non-inactivating component was present. This effect is similar to that in neuroblastoma cells$^{19}$ and in oocytes injected with high molecular weight RNA from brain$^{21}$. Ts-γ, a β-toxin from the same species, was added to the same oocyte after the α-toxin effect had stabilized. The ramp I-V showed activation at more negative potentials and a decrease in the peak current (Fig. 5B). This was confirmed in step depolarizations to −40 and −5 mV (Fig. 5E). Ts-γ alone shifted the peak of the I-V by −25 mV (not shown, n = 3). These toxin effects are similar to those seen in neuroblastoma cells$^{38}$ and heart cells$^{39}$.

**DISCUSSION**

*Slow inactivation of macroscopic type III Na$^+$ currents*

We isolated several overlapping cDNA clones of the rat brain Na$^+$ channel type III and used them to construct a full-length channel gene. RNA transcript from construct S34 leads to surface expression of Na$^+$ channels when injected into *Xenopus* oocytes.

Oocyte-expressed Na$^+$ currents encoded by total brain RNA$^{3,21}$ (Fig. 3B) decay at room temperature with a single exponential time course with a time constant of less than 10 ms. Stühmer and colleagues$^{35}$ likewise found oocyte-expressed type II Na$^+$ currents to decay with time constants less than 10 ms at all test potentials even at 15 °C. Currents encoded by high molecular weight brain RNA$^{21}$ and type IIA Na$^+$ channel mRNA$^{3}$ decay more slowly, but still with time constants less than 10 ms at room temperature. The decay of the expressed type III Na$^+$ current is markedly slower and occurs in two phases. Although Suzuki and coworkers$^{37}$ do not quantify the decay time constant in their report of expressed type III Na$^+$ currents, their published figures appear similar to ours, showing the time until 50% decay of peak currents to be 10–20 ms.

The level of expression in our experiments was 5- to 10-fold lower than that reported by Suzuki and coworkers$^{37}$, despite our injecting up to 4 times as much mRNA (20 ng compared with 5 ng per oocyte). This difference may reflect geographical variation in oocyte expression or differences in the 5′ untranslated ends. It is unlikely to affect our analysis of macroscopic inactivation, as (a) our results were the same for 200 and 1500 nA currents, (b) as noted above, the rate of current decay appears similar in the figures of Suzuki and colleagues$^{37}$, and (c) reducing current amplitude with TTX did not affect inactivation rate (Fig. 5A).

We found further evidence of very slow inactivation of type III Na$^+$ currents in two-pulse protocols. The amount
of type III Na⁺ current remaining at a test potential of 0 mV depended greatly on prepulse duration. In experiments using 50 s prepulses, the midpoint of the fitted Boltzmann function (~51.0 mV) was 15 mV more negative than for the 5 s prepulse experiments (~36.1 mV). The first value is similar to that found in type II and III Na⁺ currents using 2 s prepulses at room temperature and in Na⁺ currents expressed by total brain RNA using 50 ms prepulses at room temperature. This midpoint value, however, is about 15 or more mV more positive than the value reported for type II Na⁺ currents using 32 ms prepulses at 15 °C and in native Na⁺ currents under various experimental conditions in heart cells, neuroblastoma cells, and rat peripheral nerve. It is noteworthy that Stühmer and colleagues found a small slowly or non-inactivating component of current in their two-pulse experiments, which may correlate with our slowly inactivating current. Slowly inactivating TTX-sensitive components of neuronal Na⁺ currents have been only rarely reported.

The expected result of very slowly decaying neuronal Na⁺ currents would be prolonged neuronal action potentials. Such, however, have not been reported. The action potential of a neuron containing type III Na⁺ channels need not be prolonged if (a) neurons modulate the channel in such a way as to hasten inactivation or (b) other, faster inactivating Na⁺ channels were more numerous in the membrane. Long-term modulation of neuronal function might be achieved by varying the level of expression of fast and slowly inactivating Na⁺ channels.

Despite the shift of inactivation gating to more positive potentials, the overlap of type III Na⁺ current activation and inactivation curves after 5 s inactivating prepulses is small. The overlap is further reduced when the prepulse lasted 50 s. This is due to the large shift in activation gating to more positive potentials in our experiments. We found the midpoint of the normalized conductance curve to be ~10.7 mV compared with ~40.5 mV for type II Na⁺ currents at 15 °C. Some of the difference may lie in the inaccuracy of the microelectrode clamp compared with cell-attached patch recordings or in the temperature difference.

Scorpion toxin effects

The site of action of scorpion toxins has been unclear. Data from radiation inactivation experiments pointed to the large α-subunit as the site of toxin binding. However, photoactivatable derivatives of α- or β-scorpion toxins predominantly label one of the small subunits of the Na⁺ channel. Krafte and co-workers have shown that α-scorpion toxin prolongs Na⁺ currents expressed by oocytes injected with high-molecular weight mRNA from rat brain. Our results show that a cloned α subunit expressed in oocytes contains both functional α- and β-scorpion toxin binding sites. The intermediate effect of the mixture of α- and β-scorpion toxin is in keeping with the concept of different toxin binding sites. We cannot rule out the possibility that the oocyte provides its own β-subunit-like component. A definite answer to this problem requires the cloning and expression of the gene(s) encoding the β-subunits.

Acknowledgements. We thank Dr. M.E. Herrero-Zabaleta and Stacey Gouzene for excellent help in the initial phases of this work, Georges Frech for constructing one of the cDNA libraries, and Dr. Lourival D. Possani, Universidad National Autonoma de Mexico, for scorpion toxins. This work was supported by grants from the American Heart Association, Texas Affiliate (R.H.J. and G.E.K.), the Sealy Memorial Endowment (J.R.M.), and National Institutes of Health Grants KLO1858 (J.R.M.) and HL36930 and HL37044 (A.M.B.).

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