Volume 309, number 3, 253–257 FEBS 11510 September 1992

Chimeric study of sodium channels from rat skeletal and cardiac muscle

L.-Q. Chen[b], M. Chahine[a], R.G. Kallen[b,d], R.L. Barchi[c,d] and R. Horn[a]

[a]*Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110, USA, Departments of* [b] *Biochemistry and Biophysics and* [c] *Neuroscience and* [d] *The David Mahoney Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104, USA*

Received 20 July 1992

Two isoforms of voltage-dependent Na channels, cloned from rat skeletal muscle, were expressed in *Xenopus* oocytes. The currents of rSkM1 and rSkM2 differ functionally in 4 properties: (i) tetrodotoxin (TTX) sensitivity, (ii) μ-conotoxin (μ-CTX) sensitivity, (iii) amplitude of single channel currents, and (iv) rate of inactivation. rSkM1 is sensitive to both TTX and μ-CTX. rSkM2 is resistant to both toxins. Currents of rSkM1 have a higher single channel conductance and a slower rate of inactivation than those of rSkM2. We constructed (i) chimeras by interchanging domain 1 (D1) between the two isoforms, (ii) block mutations of 22 amino acids in length that interchanged parts of the loop between transmembrane segments S5 and S6 in both D1 and D4, and (iii) point mutations in the SS2 region of this loop in D1. The TTX sensitivity could be switched between the two isoforms by the exchange of a single amino acid, tyrosine-401 in rSkM1 and cysteine-374 in rSkM2 in SS2 of D1. By contrast most chimeras and point mutants had an intermediate sensitivity to μ-CTX when compared with the wild-type channels. The point mutant rSkM1 (Y401C) had an intermediate single-channel conductance between those of the wild-type isoforms, whereas rSkM2 (C374Y) had a slightly lower conductance than rSkM2. The rate of inactivation was found to be determined by multiple regions of the protein, since chimeras in which D1 was swapped had intermediate rates of inactivation compared with the wild-type isoforms.

μ-Conotoxin; Sodium channel; Skeletal muscle; Complementary DNA; Expression; Oocyte; Tetrodotoxin; Inactivation kinetics; Single channel conductance

1. INTRODUCTION

Two isoforms of voltage-dependent Na channels are found in skeletal muscle of rat; both have been cloned, sequenced, and expressed in *Xenopus* oocytes [1–3]. One of these, rSkM1 (also known as μI), is the predominant Na channel found in adult innervated muscle, and is sensitive to block by tetrodotoxin (TTX) at nanomolar concentrations. The TTX-resistant isoform (rSkM2 or RH1; IC_{50}>1 μM) is present in both developing and denervated skeletal muscle as well as in rat heart [1,4–6]. Adult Na channels from skeletal muscle are also uniquely sensitive to μ-conotoxin (μ-CTX), a property not shared either with the TTX-sensitive Na channels of brain or with TTX-resistant Na channels of muscle [7,8]. Accordingly the currents of rSkM1, but not of rSkM2, are blocked by nanomolar concentrations of μ-CTX when the cRNA is expressed in *Xenopus* oocytes [2,3]. At least two other functional differences are observed between the Na currents of the wild-type channels expressed in *Xenopus* oocytes, namely the single-channel conductance is larger in rSkM1 than in rSkM2 [9], and the rate of inactivation is greater in rSkM2 [2,3].

We have constructed chimeras and mutations that exchange portions of rSkM1 and rSkM2 to try to identify the regions of the protein that are responsible for their functional differences. Most of the mutations were constructed in the loop between the putative transmembrane segments S5 and S6, either in the first (D1) or the fourth (D4) of the four homologous domains. Portions of the S5–S6 segment from all four domains have been shown to contribute to TTX sensitivity and single channel conductance of rat brain Na channels [10]. A recent report demonstrated that full TTX sensitivity can be restored to rSkM2 by exchange of a single amino acid between the two isoforms [11]. This amino acid, tyrosine in rSkM1 and cysteine in rSkM2, is located in the so-called SS2 region of the S5–S6 loop of D1. An abstract of our data has appeared [12].

2. MATERIALS AND METHODS

2.1. *Materials*

Chemicals and protocols were obtained from Bethesda Research Labs, Sigma, VWR, Stratagene Cloning Systems, and Amersham.

2.2. *Construction of chimeric and site-specific mutants*

pS1S1 and pS1S2 were constructed from the full-length cDNAs of rSkM1 (*S1*) and rSkM2 (*S2*) by transfering *SalI–SacI* and *HindIII–EcoRI* fragments from pSK(+) and pSP64T expression vectors [1,3] into pSelect-1 (pS1; Promega). Chimeras were constructed from existing or newly created endonuclease restriction sites or with new sites.

Correspondence address: R. Horn, Department of Physiology, Jefferson Medical College, 1020 Locust Street, Philadelphia, PA 19107, USA. Fax: (215) 955-2073.

Published by Elsevier Science Publishers B.V.

Single-stranded DNA templates were prepared from phagemids with the helper phage R408 or VCS M13 (Stratagene).

pS1*S*1-1'2'3'4' and pS1*S*2-1'234 chimeras were constructed from the full-length cDNAs of rSkM1 (*S*1) and rSkM2 (*S*2 or denoted with prime symbol) by interchanging the *Spl*I-*Eco*RI fragments (rSkM1 1,778–6,957; rSkM2 1,453–7,076) between pS1S2 and pSK(+)S1. The plasmids were linearized for transcription by *Eco*RI and *Not*I, respectively. Site-specific mutations were introduced as follows:

*S2/Spl*I site (underlined) 5'-GCTCCTCGTACGCCATGGCCA-3' at position 1,453 in pS1*S*2 following transfer of the full-length cDNA from pSP64T-rSkM2 to pS1 to form pS1*S*2.

*S1/Sac*I site (underlined) 5'-CACTGAAGGAGCTCAGCAG-GA-3' at position 2,840 in pS1*S*1.

pS1*S*1-D1SS2' (block mutation of SS2 segment of domain I of rSkM1 to correspond to the sequence of rSkM2 produced in pS1*S*1): 5'-AAGAAGATCATGTAGATCTTGCCCGCA-GATCGTAGGGTCTGTTGGTAAAGGCGCTCC-CAGCAGTCCTGCGTCATGAG-3' (Oligo #2A, Y401C, N404R, F406Y, L408Q, A412S, T416I); transcription from *Sac*I linearized template.

pS1*S*1-D1SS1' (block mutation of SS1 segment of domain I of rSkM1 to correspond to the sequence of rSkM2 produced in pS1S1R/A containing the vector sequence downstream from the *Eco*RI site in the MCS and the cDNA sequence from 138 (initiation codon 451) through the *Sac*I site (2,840); 5'-AGCTGGTCATAGCCATGG-TCGGGGTTCTCCCCAGCCTTCAGGCATCG TAGCCCT-CAGGGCATGTCCCAGCATCACTGCT-3' (Oligo #4A, H361T, E367R, I369L, R373E, N376D, and Y377H). The mutated *Eco*RI-*Spl*I fragment (138–1,778) was returned to pSK(+)- rSkM1 and the plasmid was linearized by cutting with *Not*I).

pS1*S*1-D4SS2' (block mutation of SS2 segment of domain IV of rSkM1 to correspond to the sequence of rSkM2 produced in pS1*S*1B containing the *Hin*dIII-*Eco*RI fragment (4402–6273); 5'-GCAGAA-GAAACAGATGCCGACGGCCGGGCTGCCGCAGTTCCCC-CTGCTACCGTTACTGTTGGGCAAGTTCGGGTCACAGTA-TGGGGGCCCACTGTTGAGGT-3' (Oligo #6A, D1545Y, T1549N, E1551P, P1553S, G1554S, T1555N, N1556G, V1557S, D1560N, N1563S, S1665A, I1566V). The mutated *Sac*II-*Eco*RI (4,818–6,273) fragment was returned to pSP64T-rSkM1 prior to linearization with *Spe*I.

pS1*S*1-N404R: 5'-AGCTGGAAAAGTCTCTCCCAGTAGTC-3'. Mutation produced in pS1*S*1 and the plasmid linearized with *Spe*I.

pS1*S*2-R377N: 5'-TGCTGGTATAGGTTTTCCCAGCAGTC-3'. Mutation created in pS1*S*2A containing the vector sequence downsteam from the *Hin*dIII site from the multiple cloning segment (MCS) and the cDNA sequence from 201 (initiation codon 205) through the *Kpn*I site (1,896). The mutated *Apa*I-*Kpn*I fragment (887–1,896) was returned to pSP64T-rSkM2; plasmid linearized with *Eco*RI.

pS1*S*1-Y401C: 5'-GGTTCTCCCAGCAGTCCTGCGTC-3'. Mutation generated in pS1S1R/A. The mutated *Eco*RI-*Spl*I fragment (138–1,778) was returned to pSK(+)-rSkM1; plasmid linearized with *Not*I.

pS1S2-C374Y: 5'-GGCGTTCCCAGTAGTCCTGTGTC-3'. Mutation produced in pS1*S*2A. The mutated *Apa*I-*Kpn*I fragment (887–1,896) was returned to pSP64T-rSkM2; plasmid linearized with *Eco*RI.

All recombinant DNA constructs were confirm by nucleic acid sequencing as described previously [1].

2.3. *Electrophysiology*

The preparation and cRNA injection of *Xenopus* oocytes were described previously [13]. Macroscopic Na currents from cRNA-injected oocytes were measured with a two-microelectrode voltage clamp [13]. Briefly, the bath Ringer's solution contained (in mM): 116 NaCl, 2 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 5 HEPES (pH 7.6). Tetrodotoxin (TTX, Sigma Chemical Co.) and synthetic μ-conotoxin (μ-CTX, a gift of the Roche Peptide Chemistry Department) were added from a 1 mM stock solution in H_2O. The holding potential was −100 mV. Test pulses were delivered at a rate of 0.5 Hz. The capacity transients were removed by subtraction of records obtained after perfusion of a Ringer's solution with choline substituted for Na. The IC_{50} values were calculated by measuring the fraction of peak current blocked (F_b) by a fixed concentration of toxin, either 100 or 500 nM, at −10 mV, using the equation $IC_{50}=[\text{toxin}](1-F_b)/F_b$. In our fits of the rate of inactivation, the number and relative amplitudes of exponential components varied from oocyte to oocyte, as seen by others for expression of rSkM1 [2,14]. We therefore measured the 'time constant' of inactivation as the time for the current to decay to 1/e (i.e. 0.37) of its value at the peak. All data are presented as mean ± S.E.M., using at least 3 oocytes for each measurement.

Single-channel measurements were obtained by recordings from outside-out patches containing multiple channels [9,15]. The bath solution was (in mM): 150 NaCl, 2 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4). The pipette solution was 130 CsF, 10 CsCl, 5 EGTA, and 10 Cs-HEPES (pH 7.3). Amplitudes of single-channel currents were determined by either of three methods with equivalent results: (i) fitting amplitude histograms to sums of Gaussians [16], (ii) non-stationary fluctuation analysis of currents from macropatches [17], and (iii) 'by-eye' fits of individual openings with cursors. All recordings were obtained at 21–24°C.

3. RESULTS

3.1. *Toxin sensitivity*

The Na current of rSkM1, expressed in oocytes, is blocked by both μ-CTX and TTX [2], whereas that of rSkM2 is resistant to both toxins [3]. An example is shown in Fig. 1, using a two-microelectrode voltage clamp. The currents were elicited at −10 mV, near the peak of the current–voltage relationship, from a holding potential of −100 mV. TTX was applied at 100 nM and μ-CTX at 500 nM. TTX and μ-CTX had IC_{50}'s of ~35 and 54 nM, respectively, in rSkM1.

We constructed chimeras in which all of domain I (D1) was exchanged between the isoforms. Table I shows that the sensitivity to TXX segregated with D1: i.e. rSkM2 became TTX sensitive if its D1 was replaced by that of rSkM1, and vice versa. The μ-CTX sensitivity of these chimeras did not, however, segregate exclusively with D1. Both chimeras had partial sensitivity to μ-CTX (Table I). These data show that D1 determines the TTX sensitivity and contributes to, but does not completely determine, the sensitivity to μ-CTX.

Table I also shows several block mutations in S5–S6 loops. In these mutants, segments of 22 amino acids were exchanged between rSkM1 and rSkM2. The block mutants were constructed in both D1 and D4. Our data show that the sensitivity to TTX segregated exclusively with a region of the S5–S6 loop close to segment S6 in D1, as expected from the results of Satin et al. [11]. This region is known as SS2. If SS2 of D1 contains the amino acid sequence of rSkM1, then the expressed currents of this channel have the high sensitivity to TTX characteristic of rSkM1, regardless of the origin of the rest of the molecule. Exchange of SS1, the adjacent region close to segment S5, did not affect the TTX sensitivity. The sensitivity of these block mutants to μ-CTX was intermediate between those of the two wild-type isoforms.

The SS2 region in D1, which is responsible for the

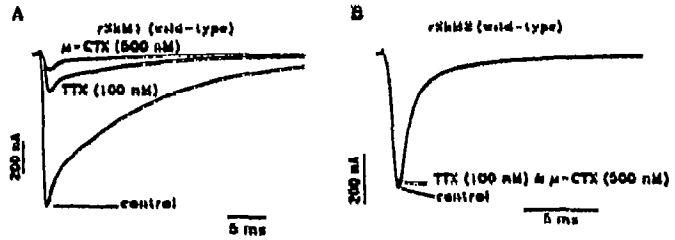

Fig. 1. Effect of TTX and μ-CTX on currents of rSkM1 and rSkM2. Current of (A) rSkM1 and (B) rSkM2 at −10 mV from a holding potential of −100 mV. Superimposed traces indicate the effects of 100 nM TTX and 500 nM μ-CTX.

difference in TTX sensitivity between rSkM1 and rSkM2, has the following amino acid sequence:

*

rSkM1: ^{396}LMTQDYWENLFQLTLRAAGKTY
rSkM2: ^{369}LMTQDCWERLYQQTLRSAGKIY

Each isoform contains a single glutamate in SS2 (*). This residue, when replaced by glutamine in rat brain Na channels, abolishes TTX sensitivity [18]. All but 6 amino acids (underlined) are identical in this region of D1. There is one difference in charge: a positively charged arginine (R377) in rSkM2 is replaced by a neutral asparagine (N404) in rSkM1. These residues, however, are not the primary determinants for the different TTX sensitivities of the wild-type isoforms. Satin et al. [11] showed that the point mutant rSkM2(R377N) has a lower, rather than a higher, sensitivity to TTX than the wild-type isoform. We have constructed the same mutation with equivalent results (data not shown). We have also constructed the complementary mutation in rSkM1. This mutant, rSkM1(N404R), had little effect on either TTX sensitivity, with an IC_{50} of 57±4 nM, or μ-CTX sensitivity (IC_{50} = 55±7 nM).

We also exchanged the tyrosine (Y401) in rSkM1 for the cysteine (C374) of rSkM2 [11]. Fig. 2 shows that the exchange of this residue switched the TTX sensitivity of the two isoforms. The IC_{50} for TTX block of rSkM1-(Y401C) was 1.5±0.3 μM, and for rSkM2(C374Y) was 5.2±0.4 nM. rSkM2(C374Y) was even more sensitive to TTX than wild-type rSkM1. By contrast the sensitivity to μ-CTX in rSkM1(Y401C) was reduced below that found in native rSkM1 (IC_{50} = 197±7 nM), but not to the level found in native rSkM2. Conversely the mutation rSkM2(C374Y) did not recover the sensitivity to μ-CTX found in native rSkM1.

3.2. *Single channel conductance*

It has been postulated that the TTX binding site has negatively charged sites that could accumulate Na ions locally and thus increase the conductance of the Na channel at physiological ionic concentrations [15,19,20]. In accordance with this idea rSkM1 has a larger single channel conductance than rSkM2, either in developing muscle [16] or when expressed heterologously in oocytes [9,15]. This suggested to us that TTX-sensitive mutants might have a higher single channel conductance than those that are insensitive to TTX. Fig. 3 shows the single channel current–voltage relationship for the two point mutants that interconvert TTX sensitivity, rSkM1(Y401C) and rSkM2(C374Y), obtained from outside-out patches. Linear regressions for these data are shown as dashed lines. For comparison, the best-fit regression lines for the wild-type channels are shown as solid lines, based on the data in Gellens et al. [9]. Although the point mutation in rSkM2 had little effect on the amplitude and conductance of single channel currents, the complementary mutation in rSkM1 caused a significant decrease in the slope conductance, from 32 pS in the wild-type to 15 pS in rSkM1(Y401C). Our data therefore show that the TTX-sensitive mutant has a lower single channel conductance than the TTX-resistant mutant, contrary to our expectation.

3.3. *Inactivation kinetics*

rSkM1, but not rSkM2, has abnormally slow kinetics of inactivation when expressed in oocytes. The time constants of inactivation are presented in Table I for all the chimeras and block mutations. The wild-type isoforms in our experiments had ~9-fold differences in time constants of inactivation. Block mutations in the

Table I

Time constant of inactivation and IC_{50} of TTX and μ-CTX block

Name	Inactivation (ms)	TTX sensitivity	μ-CTX sensitivity
Wild-type rSkM1	10.65 ± 0.8	34.9 ± 9 nM	54 ± 6 nM
Wild-type rSkM2	1.2 ± 0.3	3 ± 1 μM	> 10 μM
Chimera			
pS1S1-1'2'3'4'	2.1 ± 0.3	8 ± 2 nM	1.3 ± 0.1 μM
pS1S2-1'234	2.1 ± 0.8	1 ± 0.1 μM	0.5 ± 0.3 μM
Block mutant			
pS1S1-DSS2'	11.9 ± 1.2	1 ± 0.1 μM	0.7 ± 0.4 μM
pS1S1-D1SS1'	13.9 ± 1	20 ± 6 nM	96.4 ± 17 nM
pS1S1-D4SS2'	10.9 ± 0.8	34.3 ± 4 nM	287 ± 89 nM

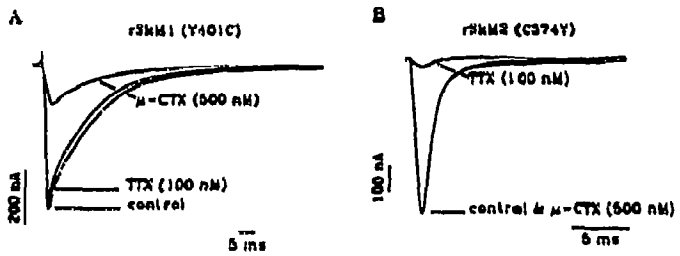

Fig. 2. Point mutants (A) rSkM1 (Y401C) and (B) rSkM2 (C374Y). The data were obtained under the same conditions as in Fig. 1. The time constant for inactivation of rSkM1 (Y401C) was 11.1±2 ms, and for rSkM2 (C374Y) was 0.97±0.1 ms.

SS2 region of either D1 or D4 had no significant effects on the kinetics of inactivation of rSkM1. The same was true for all the point mutations we examined, however, the two chimeras in which D1 was exchanged between the isoforms had intermediate rates of inactivation, each approximately 2-times slower than that of rSkM2 (Table I). Also, a block mutation in the SS1 region of D1 in rSkM1 caused a slower rate of inactivation than observed in the wild-type isoform. These data suggest a role of D1 in the rate of Na-channel inactivation in oocytes.

4. DISCUSSION

We examined four functional properties of Na currents that differ in the isoforms rSkM1 and rSkM2, when expressed in oocytes. These properties are TTX sensitivity, μ-CTX sensitivity, single channel conductance, and inactivation kinetics. Our results show that a single residue accounts for most of the difference in the TTX sensitivity between rSkM1 and rSkM2. The other three properties, however, cannot be explained so simply, suggesting that more extensive differences between the proteins, or differences at multiple sites, must account for the differences in function.

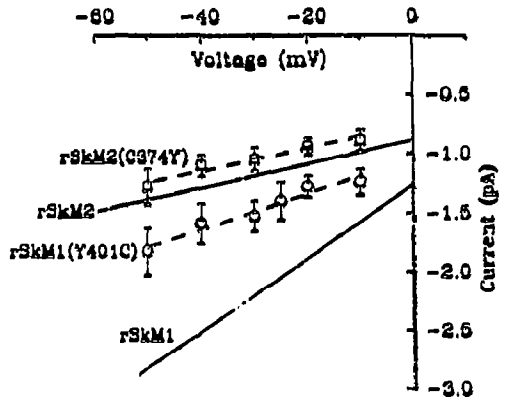

Fig. 3. Single channel amplitudes. Data were obtained from outside-out patches of the indicated mutations, and are plotted as mean ± S.E.M., along with best-fit linear regression lines. Solid regression lines were for the wild-type isoforms, as found in Gellens et al. [9].

4.1. *Toxin block*

The difference in TTX sensitivity between these two muscle isoforms is primarily explained by a single residue in the S5–S6 loop of D1 (Fig. 2). Our data do not show whether a cysteine at this position inhibits TTX block, or whether the tyrosine is necessary for block by TTX, however, other TTX-sensitive Na channels of rat brain or electroplax have either a phenylalanine or a tyrosine at this position [21–23], suggesting that an aromatic group here may be necessary for the action of TTX. The mutation of the cysteine (C374) of rSkM2 to phenylalanine also confers TTX sensitivity to this isoform [11]. The aromatic group may contribute directly to the TTX binding site, as suggested by the role of aromatic residues in the block by tetraethylammonium in K channels [24] or in the binding of acetylcholine to acetylcholinesterase [25]. The nature of interaction between the cationic toxin and the aromatic group is unknown, but electrostatic interactions are believed to occur between cationic amino groups and aromatic side chains [26]. The SS2 regions of the four domains of Na channels each contain from one to three aromatic residues, any of which may contribute to toxin binding. Tyrosine-401 in rSkM1 is the only SS2 residue which does not have a corresponding aromatic residue at the equivalent position in rSkM2. Since rSkM2 has all of the above-mentioned negative residues, as well as all-but-one of the aromatic residues in SS2 regions, it apparently has all of the structural requirements for TTX binding, with the exception of the one aromatic residue in D1. Further mutagenic experiments are required to understand the roles of the other aromatic residues of SS2 regions in sensitivity to TTX block.

Although TTX and μ-CTX show competitive binding [27], the mutations that restore TTX sensitivity to rSkM2 do not do the same for μ-CTX. This is not surprising, since all isoforms of TTX-sensitive Na channels, except those of skeletal muscle, are insensitive to μ-CTX. Also μ-CTX is a much larger molecule than TTX [28], and experiments using analogs of μ-CTX suggest that widely spaced regions of the molecule contribute to its ability to block Na channels [29,30]. Table I shows, in fact, that mutations in both D1 and D4 affect μ-CTX sensitivity. It seems likely, therefore, that the μ-CTX docking site is comprised of more extensive parts of the Na channel than the TTX binding site. The competitive interaction between the binding of the two compounds indicates that their binding sites may overlap, but not necessarily that the site are identical. Our data do suggest, however, that the two sites share specific amino acid residues, because the point mutation that renders rSkM1 TTX resistant also causes a 3.6-fold increase in the IC_{50} for μ-CTX.

4.2. *Single channel conductance*

Models in which TTX blocks Na channels by entering and plugging the pore require that the TTX binding

site is intimately associated with the extracellular mouth of the channel. Our data show that the point mutation, rSkM1(Y401C), that reduces TTX sensitivity also decreases single channel conductance. Also, mutations in homologous regions of D3 and D4 of brain Na channels affect the monovalent/divalent selectivity of the channel [31]. Finally, the point mutant rSkM2(C374Y) changes the IC_{50} for Cd^{2+} block [11], which is believed to occur within the permeation pathway [32]. The accumulated data, therefore, provide strong support that SS2 regions from part of the pore, and that TTX binds to a site within it.

4.3. *Inactivation*

The rate of inactivation of rSkM1 Na current is abnormally slow when expressed in oocytes, by comparison both with the situation in native tissue and with heterologous expression in mammalian cells [2,14,33,34]. We have found that the two isoforms have comparable rates of inactivation when expressed in TSA-201 cells, a human kidney cell line (unpublished observation). It is not clear why the inactivation in oocytes is abnormal for rSkM1. It may depend on the absence in oocytes of a necessary cofactor, such as a subunit of the Na channel found in mammalian cells [35]. Alternatively, the oocyte may not correctly process the rSkM1 protein. It is also not clear why rSkM2, by contrast, has rapid inactivation kinetics in oocytes. Nevertheless our data suggest that D1 has a moderate influence on the inactivation rate, since chimeras in which D1 is swapped between the isoforms have intermediate rates of inactivation. This could be due to interdomain as well as intradomain interactions. The interpretation of these data will depend on better understanding of the influence of the oocyte expression system on the processing of Na channels.

Acknowledgements: We thank David Meyer and Calee B. Kallen for their assistance, Bob French and Lee Chabala for prepublication manuscripts, and Chris Miller for insightful discussions. The work was supported by American Heart Association, Muscular Dystrophy Foundation, Research Foundation of the University of Pennsylvania, Sterling Research Laboratory, and NIH Grant NS-18013 (RLB).

REFERENCES

[1] Kallen, R.G., Sheng, Z.H., Yang, J., Chen, L.Q., Rogart, R.B. and Barchi, R.L. (1990) Neuron 4, 233–242.
[2] Trimmer, J.S., Cooperman, S.S., Tomiko, S.A., Zhou, J.Y., Crean, S.M., Boyle, M.B., Kallen, R.G., Sheng, Z.H., Barchi, R.L. and Sigworth, F.J. et al., (1989) Neuron 3, 33–49.
[3] White, M.M., Chen, L.Q., Kleinfield, R., Kallen, R.G. and Barchi, R.L. (1991) Mol. Pharmacol. 39, 604–608.
[4] Rogart, R.B., Cribbs, L.L., Muglia, L.K., Kephart, D.D. and Kaiser, M.W. (1989) Proc. Natl. Acad. Sci. USA 86, 8170–8174.
[5] Schotland, D.L., Fieles, W. and Barchi, R.L. (1991) Muscle Nerve 14, 142–151.
[6] Yang, J.S., Sladky, J.T., Kallen, R.G. and Barchi, R.L. (1991) Neuron 7, 421–427.
[7] Cruz, L.J., Gray, W.R., Olivera, B.M., Zeikus, R.D., Kerr, L., Yoshikami, D. and Moczydlowski, E. (1985) J. Biol. Chem. 260, 9280–9288.
[8] Gonoi, T., Ohizumi, Y., Nakamura, H., Kobayashi, J. and Catterall, W.A. (1987) J. Neurosci. 7, 1728–1731.
[9] Gellens, M.E., George Jr., A.L., Chen, L.-Q., Chahine, M., Horn, R., Barchi, R.L. and Kallen, R.G. (1992) Proc. Natl. Acad. Sci. USA 89, 554–558.
[10] Terlau, H., Heinemann, S.H., Stühmer, W., Pusch, M., Conti, F., Imoto, K. and Numa, S. (1991) FEBS Lett. 293, 93–96.
[11] Satin, J., Kyle, J.W., Chen, M., Bell, P., Cribbs, L.L., Fozzard, H.A. and Rogart, R.B. (1992) Science 256, 1202–1205.
[12] Chen, L.-Q., Chahine, M., Horn, R., Barchi, R.L. and Kallen, R.G. (1992) Biophys. J. 61, A133.
[13] Chahine, M., Chen, L.-Q., Barchi, R.L., Kallen, R.G. and Horn, R. (1992) J. Mol. Cell. Cardiol. (in press).
[14] Zhuo, J., Potts, J.F., Trimmer, J.S., Agnew, W.S. and Sigworth, F.J. (1991) Neuron 7, 775–785.
[15] Chahine, M., Chen, L.-Q., Kallen, R.G., Barchi, R.L. and Horn, R. (1992) Biophys. J. 62, 37–40.
[16] Weiss, R.E. and Horn, R. (1986) Science 233, 361–364.
[17] Sigworth, F.J. (1980) J. Physiol. 307, 97–129.
[18] Noda, M., Suzuki, H., Numa, S. and Stühmer, W. (1989) FEBS Lett. 259, 213–216.
[19] Sigworth, F.J. and Spalding, B.C. (1980) Nature 283, 293–295.
[20] Worley, J.F., French, R.J. and Krueger, B.K. (1986) J. Gen. Physiol. 87, 327–349.
[21] Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y. and Minamino, N. et al. (1984) Nature 312, 121–127.
[22] Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H. and Numa, S. (1986) Nature 320, 188–192.
[23] Kayano, T., Noda, M., Flockerzi, V., Takahashi, H. and Numa, S. (1988) FEBS Lett. 228, 187–194.
[24] Heginbotham, L. and MacKinnon, R. (1992) Neuron 8, 483–491.
[25] Sussman, J.L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L. and Silman, I. (1991) Science 253, 872–879.
[26] Burley, S.K. and Petsko, G.A. (1986) FEBS Lett. 203, 139–143.
[27] Moczydlowski, E., Olivera, B.M., Gray, W.R. and Strichartz, G.R. (1986) Proc. Natl. Acad. Sci. USA 83, 5321–5325.
[28] Lancelin, J.M., Kohda, D., Tate, S., Yanagawa, Y., Abe, T., Satake, M. and Inagaki, F. (1991) Biochemistry 30, 6908–6916.
[29] Sato, K., Ishida, Y., Wakamatsu, K., Kato, R., Honda, H., Ohizumi, Y., Nakamura, H., Ohya, M., Lancelin, J.M. and Kohda, D. et al. (1991) J. Biol. Chem. 266, 16989–16991.
[30] Becker, S., Prusak-Sochaczewski, E., Zamponi, G., Beck-Sickinger, A.G., Gordon, R.D. and French, R.J. (1992) Biochemistry (in press).
[31] Heinemann, S.H., Terlau, H., Stühmer, W., Imoto, K. and Numa, S. (1992) Nature 356, 441–443.
[32] Ravindran, A., Schild, L. and Moczydlowski, E. (1991) J. Gen. Physiol. 97, 89–115.
[33] Gonoi, T., Sherman, S.J. and Catterall, W.A. (1985) J. Neurosci. 5, 2559–2564.
[34] Ukomadu, C., Zhuo, J., Sigworth, F.J. and Agnew, W.S. (1992) Neuron 8, 663–676.
[35] Isom, L.L., DeJongh, K.S., Patton, D.E., Reber, B.F.X., Offord, J., Charbonneau, H., Walsh, K., Goldin, A.L. and Catterall, W.A. (1992) Science 256, 839–842.