The seizure Locus Encodes the Drosophila Homolog of the HERG Potassium Channel

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Mutations in the seizure (sei) locus cause temperature-induced hyperactivity, followed by paralysis. Gene cloning studies have established that the seizure gene product is the Drosophila homolog of HERG, a member of the eag family of K⁺ channels implicated in one form of hereditary long QT syndrome in humans. A series of five null alleles with premature stop codons are all recessive, but viable. A missense mutation in the sei gene, which changes the charge at a conserved glutamate residue near the outer mouth of the pore, has a semidominant phenotype, suggesting that the mutant seizure protein acts as a poison in a multimeric complex. Transformation rescue of a null allele with a cDNA under the control of an inducible promoter demonstrates that induced expression of seizure potassium channels in adults rescues the paralytic phenotype. This rescue decays with a $t_{1/2}$ of $\sim 1-1.5$ d after gene induction is discontinued, providing the first estimate of ion channel stability in an intact, multicellular animal.

Key words: cloned potassium channel; Drosophila melanogaster; gene mapping; seizure gene; hyperexcitability; temperature-induced paralysis; sodium channel regulation; transformation; transgenic animals; HERG; $I_{Kr}$; eag gene family; ion channel turn over

The excitability properties of neurons are determined by the types, distribution, and density of ion channels they express. Changes in expressed ion channels can affect cell resting potentials, action potential threshold and duration, repetitive firing, and neurotransmitter release. For example, reduction of sodium channel function results in a less excitable cell, whereas reduction in potassium channel function results in hyperexcitability.

Temperature-sensitive paralytic mutations in Drosophila melanogaster, which affect sodium channel density or properties, provide a means to identify interesting gene products involved in ion channel structure or regulation. Four mutants have been implicated in sodium channel changes: $para^{ts}$, $nap^{ts}$, $tipE$, and $sei^{ts}$. The $para$ (paralytic temperature-sensitive) gene encodes the $\alpha$ subunit of a voltage-gated sodium channel (Loughney et al., 1989). The $nap$ (no action potential temperature-sensitive) locus encodes a nucleic acid binding protein likely to be involved in the expression or splicing of X-linked genes, including $para$ (Kernan et al., 1991). Recent cloning of the $tipE$ locus identified a novel membrane component that dramatically stimulates the expression of $para$ sodium channels but structurally is unrelated to previously identified sodium channel subunits (Feng et al., 1995a). The seizure ($sei$) gene is the last of these genes to be cloned.

The $sei$ mutants have been an anomaly among Drosophila mutations proposed to affect sodium channels, because the biochemical and electrophysiological phenotypes do not match the behavioral phenotypes. For example, both $sei^{ts1}$ ($ts1$) and $sei^{ts2}$ ($ts2$) homozygotes are hyperactive even at the permissive temperature (Jackson et al., 1984, 1985). Hyperactivity also is seen in electrophysiological recordings from dorsal longitudinal muscles (Kasbekar et al., 1987) and from the adult cervical giant fiber pathway (Elkins and Ganetzky, 1990). In addition, there is a reduction in membrane-bound sodium channels in the $sei^{ts1}$ allele, but not in $sei^{ts2}$ (Jackson et al., 1984, 1985). Similarly, whole-cell patch-clamp studies show reduced sodium currents in $ts1$, but not in $ts2$ (O’Dowd and Aldrich, 1988). This creates a paradox, because reduction in sodium channel density or activity should lead to hypoexcitability rather than the observed hyperexcitability. Furthermore, it is difficult to understand why the $ts1$ and $ts2$ alleles have similar effects on behavior and on cell excitability and yet have different effects on sodium channel density.

To resolve these inconsistencies, we used positional cloning to demonstrate that the $sei$ gene encodes a channel subunit most closely related to the human HERG potassium channel involved in the $I_{Kr}$ current in cardiac myocytes (Sanguinetti et al., 1995; Trudeau et al., 1995). Sequencing mutant $sei$ alleles shows that recessive hyperexcitability and temperature-induced paralysis are the null phenotypes for this locus. A missense mutation that forms a defective subunit has a semidominant phenotype, presumably because it interacts with wild-type subunits to form a poisoned channel. We use an inducible transgene in a null mutant background to estimate functional channel half-life in the intact animal.

MATERIALS AND METHODS

Drosophila culture. Drosophila stocks were grown at 21°C on standard cornmeal medium (Lewis, 1960). The wild-type Canton-S strain was obtained from J. C. Hall (Brandeis University, Waltham, MA). The
deficiency stocks (Df(2)or883, Df(2)orBr11, Df(2)G10Br27, Df(2)bw–S46, and Df(2)bw–D23) were provided by B. Reed (M.I.T., Cambridge, MA). Df(2)Rp4, Df(2)Px, sepi1, and sepi2 were described previously (Lindsley and Zimm, 1992; Jackson et al., 1984, 1985). The bw ba and or883 stocks were provided by P. Bryant (University of California, Irvine, CA). Df(2)Rp4 was induced by γ-ray mutagenesis (M. Parisi and L. M. Hall, unpublished data).

Mutagenesis. The bw ba males were mutagenized with 4000 rads of γ-irradiation and mated in batches of 15 males and 30 sepi1 virgin females. The 106,457 F1 progeny were screened at 39°C for temperature-sensitive paralysis (Feng et al., 1995b), and eight new sei alleles were isolated. After recovery from paralysis at 21°C, individual flies were mated to CyO, cn bw/BL. Then brown-eyed (bw) offspring were mated to establish new sei lines over the CyO, cn bw chromosome.

Chromosomal walk. Fragments of yeast artificial chromosome (YAC) clone DYN13-25 (Cai et al., 1994) were used to initiate a chromosome walk by screening two Drosophila genomic cosmid libraries under high stringency conditions (Sambrook et al., 1989). Clones with an "M" as the first letter were from the KT3 cosmid library (a generous gift of Max Scott and John Lucchesi, Emory University, Atlanta, GA). Clones with an "S" as the first letter were from the iso-1 cosmid library in the Not-Bam-Not EcoRI vector (a generous gift of J. W. Tamkun, University of California, Santa Cruz, CA) (Tamkun et al., 1992).

Restriction fragment-length polymorphism (RFLP) mapping. Male or883 and female sei1 flies were mated to generate double heterozygote females or883sei1. Recombinants between or and sei were recovered by mating these females to Df(2)or883/M60ar, because this deletion uncovers both recessive or and sei genes. A total of 24 or sei recombinants were recovered and mated individually to Df(2)or883/M60ar to establish lines. Four lines failed to survive. RFLPs between or883 and sei1 were identified by extracting genomic DNA from each line (Jowett, 1986), digesting it with restriction enzymes, and comparing the resulting genomic Southern blots probed with labeled clones from the walk (Feng et al., 1995b). Each of 20 recombinant lines was analyzed by Southern blotting to determine the isofrom at each RFLP site.

Northern blotting. mRNA preparation from whole adults and Northern blot conditions were as described previously (Zheng et al., 1995). To standardize for RNA recovery differences, were probed Northern blots with probes identified by extracting genomic DNA from each line (Jowett, 1986), digesting it with restriction enzymes, and comparing the resulting genomic Southern blots probed with labeled clones from the walk (Feng et al., 1995b). Each of 20 recombinant lines was analyzed by Southern blotting to determine the isofrom at each RFLP site.

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RESULTS

Identification of the seizure transcript

Because the molecular nature of the seizure gene product was unknown, positional cloning was the method of choice to identify the transcript. As summarized in Figure 1A, the seizure gene was mapped first to the cytogenetic region 60A8–B10. Then a YAC clone that mapped between 60A10–A13 and 60B12–B13 (Cai et al., 1994) was used to identify a YAC clone that mapped between 60A10–A13 and 60B12–B13 (Cai et al., 1994) using sepi1 as the host.

Figure 1. Localization of the seizure gene. A. Deletion mapping. Heterozygotes for sei1 and each deficiency (Df) were tested for temperature-induced paralysis at 38°C. Only Df(2)or883 and Df(2)orBr11 failed to complement sei, locating the gene between 60A8 and B10. Deleted regions are indicated as black bars with regions of uncertainty shown as open bars. Other genes shown are eye color genes (bw, or), cloned genes for a muscarinyl acetylcholine receptor (mAChR) (Onai et al., 1989), and a sodium channel homolog (DSC1) (Salkoff et al., 1987). The regions deleted are as follows: Df(2)or883, 59D5–D10 to 60B3–B8; Df(2)orBr11, 59F6–F8 to 60A8–A16; and Df(2)G10Br27, 59F3 to 60A8–A16 (breakpoints defined in Reed, 1992); Df(2)bw–S46, 59D8–D11 to 60A7 (Simpson, 1983); Df(2)bw–D23, 59D4–D5 to 60A1–A2; Df(2)Px, 60B8–B10 to 60D1–D2; and Df(2)Rp4, 60B8–B10 to 60D1 (breakpoints defined in Lindsay and Zimm, 1992); and Df(2)Rp4, 60B8–B10 to 60D3–D8 (Paris and Hall, unpublished results). B–D. RFLP mapping and chromosome walking. Twenty recombinants between or883 and sei1 were analyzed for six RFLPs that distinguish the or883 chromosome from the sei1 chromosome. The positions of these RFLPs are indicated by an asterisk on the genomic DNA restriction maps (C). H. HpaI; X. Hox; E. EcoRI; B. BglII; O. XhoI. The number of recombinants with the or806 RFLP is plotted versus RFLP location on genomic DNA in B. The X intercept, determined by linear regression analysis, shows the approximate physical location of seizure. The small black bar in C shows the region in which genomic DNA aberrations were detected in seizure alleles. The cosmid clones from the chromosome walk are shown in D, relevant to the end of deletion Df(2)G10Br27. The heavy black bar represents the known end of this deletion, whereas the open bar shows a region of uncertainty. The dotted line indicates that the deletion extends to the left beyond the limits of this figure.
al., 1994) was used to initiate a chromosome walk (summarized in Fig. 1D) through this region.

Because many regions of the *Drosophila* genome are rich with transcripts (see, for example, Feng et al., 1995b), we used two approaches to identify the *seizure* transcript: RFLP mapping and analysis of γ-ray-induced chromosome aberrations that disrupt *seizure* function. Two γ-ray-induced *seizure* alleles (*G50* and *G87*) showed altered restriction patterns within a 10 kb segment of the chromosome walk (indicated by a small black rectangle in Fig. 1C). Thus, this 10 kb region was a candidate for at least part of the *seizure* gene.

RFLP mapping (Fig. 1B) was used to confirm, independently, the genomic location of *seizure* before cDNA cloning. The closer an RFLP is to *seizure*, the lower the frequency of recombination will be between them. The X intercept at position 118 kb of the chromosome walk (Fig. 1B) gives the approximate location of the *seizure* gene. Because the horizontal axes of Figures 1B–D are on the same scale, it can be seen that the position of *seizure* as defined by RFLP mapping coincides with the 10 kb region shown to contain either a small insertion or deletion in γ-ray-induced *seizure* alleles.

A genomic fragment from the region proposed to contain the *seizure* gene detected two candidate transcripts (3.4 and 3.0 kb in wild type) that were altered in size or amount in seven *seizure* alleles (Fig. 2). Some alleles (*ts1* and *G64*) showed a dramatic reduction in both transcripts, as compared with wild-type (*CS*). In others, there was a change in transcript size. For example, in *G87* both transcripts are 0.8 kb larger, plus there is an addition small transcript at 2.2 kb. *ts2* and *G85* show an increase in intensity of the larger 3.4 kb transcript relative to the smaller 3.0 kb one. Northern blots probed with single-stranded riboprobes or with the 0.8 kb genomic fragment originally used to probe the cDNA library each identify only the same 3.4 and 3.0 kb transcripts shown in this blot. Later the blot was reprobed with *Drosophila* ribosomal protein 49 (*rp49*) cDNA (O’Connell and Rosbash, 1984) to standardize for mRNA recovery (bottom panel).

![Figure 2. Northern blot of seizure alleles. Poly(A⁺) RNA (10 µg) from wild-type (CS) and sei mutant adult flies was loaded into each lane, as indicated. The blot was probed with a seizure cDNA, including bases +127 to +2668 of the ORF. (Northern blots of wild type probed with single-stranded riboprobes or with the 0.8 kb genomic fragment originally used to probe the cDNA library each identify only the same 3.4 and 3.0 kb transcripts shown in this blot.) Later the blot was reprobed with *Drosophila* ribosomal protein 49 (*rp49*) cDNA (O’Connell and Rosbash, 1984) to standardize for mRNA recovery (bottom panel).](image)

### Table 1. Effects of seizure alleles on transcript abundance

<table>
<thead>
<tr>
<th>Band (kb)</th>
<th>Wild-type</th>
<th>ts1</th>
<th>ts2</th>
<th>G43</th>
<th>G50</th>
<th>G64</th>
<th>G85</th>
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<td>3.9</td>
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</tr>
<tr>
<td>3.4</td>
<td>520 (100)</td>
<td>61.2</td>
<td>818</td>
<td></td>
<td>456</td>
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<tr>
<td>3.4</td>
<td>520 (100)</td>
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Band intensity relative to rp49 for each allele tested (cpm in indicated band)/(cpm in rp49 band) × 10⁻⁴.

*Italicized number in parenthesis = band intensity relative to the wild-type equivalent.*

*aBand intensity calculated relative to the 3.4 kb wild-type band.*

*bBand intensity calculated relative to the 3.0 kb wild-type band.*

*cAfter correction for loading differences.*

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Wang et al. • The *Drosophila* Seizure Potassium Channel
The seizure gene encodes a potassium channel subunit of the eag family

Sequencing wild-type cDNA corresponding to the disrupted transcripts revealed a long ORF encoding a deduced protein of 855 amino acids (Fig. 3A, “S” lines) with a predicted molecular mass of 97.5 kDa. The dendrogram (Fig. 4) derived from database comparisons shows that sei is a member of the eag class of potassium channels, which also have similarities to cyclic
nucleotide-gated cation channels (Guy et al., 1991). The complete sei protein shows a striking similarity (58% similarity; 47% identity) to HERG, a member of the eag family recently cloned from humans (Warmke and Ganetzky, 1994). The similarity and identity are even higher (84 and 72%, respectively) if the comparison is limited to the hydrophobic core. Identification of this new Drosophila potassium channel subunit, which is closer to a human potassium channel than to other Drosophila channels, establishes sei and HERG as a distinct potassium channel subfamily, as predicted previously (Warmke and Ganetzky, 1994).

Structural features of the sei protein

The hydropathy plot (Fig. 3B) and the alignment of sei- and HERG-deduced proteins (Fig. 3A) illustrates structural features that they have in common. First, each has six predicted transmembrane α-helices (Fig. 3, S1–S6) plus a pore region (P) between S5 and S6. The amphipathic S4 domain with positive amino acids (K, R) every third residue is important in voltage-sensing. The complete conservation of this domain between sei and HERG suggests they may have similar voltage-dependent properties. The sequence of the sei protein in the P region (SLTSVGFGN) is very similar to the potassium channel consensus sequence (tm1tvG[y/f]Gd) (Chandy and Gutman, 1995), providing strong evidence that sei is a subunit of potassium-permeable channels.

There is also a striking identity in the proposed cyclic nucleotide binding domain (cNBD) in the C-terminal cytoplasmic region. However, the rest of the C terminus and most of the N terminus of the sei protein have very little in common with HERG or with other members of the eag potassium channel gene family. Both the N and C termini of the sei protein are significantly shorter than HERG.

There is a consensus site for Nglycosylation 12 or 14 amino acids upstream of the P region, which is conserved in all eag family members (Warmke and Ganetzky, 1994). In addition, there are potential phosphorylation sites just downstream of S6 that are conserved in sei and in HERG as is a phosphorylation site within the cyclic nucleotide binding domain. In total, the sei protein has 9 possible protein kinase C phosphorylation sites, 6 CaM kinase phosphorylation sites, 1 protein kinase A phosphorylation site, and 11 casein kinase phosphorylation sites on predicted cytoplasmic domains. These sites, especially those conserved between sei and HERG, suggest the potential for regulation of this channel by phosphorylation.

Mutations in seizure alleles

To determine the molecular basis for the recessive versus dominant phenotypes of different seizure alleles, we sequenced genomic DNA corresponding to the entire coding region from wild-type and homozygous mutant lines. We identified mutant changes in sei alleles, which is what we found for the EMS-induced ts1 and ts2 alleles. The tsha allele has a T to A change, which converts K282 (Fig. 3A) into a premature stop codon. The resulting truncated protein completely lacks the hydrophobic core that forms the transmembrane channel (Fig. 5).
Barring read-through of the stop codon, this nonsense allele should be a functional null.

In sei\(^{82}\) there is a G to A change, which changes a negatively charged group to a positively charged one (E490K in Fig. 3A) in a region close to the extracellular side of the S5 domain. This charge change is near the channel pore (Fig. 5) and could disrupt channel function. Because this is a missense mutation, sei\(^{82}\) should still make a full-length subunit.

The four γ-ray-induced alleles diagrammed in Figure 5 are all recessive and show hyperactivity and temperature-induced paralysis phenotypes indistinguishable from the ts1 allele. Three (G50, G43, and G64) are frameshift mutations leading to a premature stop codon in the approximate positions shown in Figure 5. The G87 allele deletes a large region from just before the start of transmembrane 6 through to a point in the C terminus past the cyclic nucleotide binding domain (Fig. 5). Although they differ in the sites of change, none of the γ-ray-induced alleles would produce a functional channel, because they lack part or all of important transmembrane domains. Thus, sei\(^{82}\), plus the γ-ray-induced alleles, defines the null phenotype of this gene and illustrates the consequences to the animal of complete absence of the seizure gene product.

The entire ORF also was sequenced for a fifth γ-ray-induced allele (G85, transcripts shown in Fig. 2), but no changes were identified despite the failure of this allele to complement other seizure alleles. This allele may have alterations in the 5′ or 3′ untranslated regions or in an alternative exon not yet identified. Interestingly, G85 and ts2 both show an increase in the 3.4 kb transcript relative to the 3.0 kb transcript (Table 1). The reason for this shift remains to be determined.

**Expression of a seizure\(^{*}\) transgene: paralysis rescue and estimation of channel half-life**

Mutant strains that completely lack the seizure potassium channel provide a unique opportunity to define when expression of the gene product is needed to rescue the paralytic phenotype. To address this question, we made transgenic flies in a null mutant background. The transgene was wild-type seizure cDNA encoding the protein shown in Figure 3A and was under the control of an inducible heat–shock promoter. In the absence of induction (Fig. 6, circles), the transgenic flies show a paralytic phenotype indistinguishable from the null mutant without the transgene. This suggests that uninduced expression of the transgene is not at physiologically relevant levels, at least with respect to the paralysis phenotype. In contrast, repeated induction of this transgene in adults (Fig. 6, squares) over a 5 d period rescues the paralytic phenotype in most flies by the sixth day. This experiment suggests that synthesis of this channel subunit in adults only is sufficient to restore normal locomotor function.

When induction of the wild-type transgene is stopped, the return of the paralytic phenotype should reflect the net rate of loss of functional channels from membranes and thus will provide an estimate of apparent half-life of functional channels in membranes in an intact animal. To estimate this half-life, just before reaching 100% rescue, we stopped induction of the transgene and monitored the return of the paralytic phenotype. As shown in Figure 6B, return of paralytic phenotype was rather rapid. The time to return to 50% paralyzed flies is ~1.5 d after 94% rescue is achieved.

**DISCUSSION**

Potassium channel mutations are the direct cause of seizure hyperexcitability and paralysis

RFLP mapping, identification of mutant changes in the coding region of genomic DNA from six independently isolated seizure alleles, and transformation rescue with cDNA under an inducible promoter provide conclusive evidence that the potassium channel sequence presented here is the seizure gene product. The sequence conservation between seizure and other ion channels shows that it is a potassium channel of the eag subfamily and is related most closely to the HERG inwardly rectifying potassium channel. Although sei has not yet been expressed in Xenopus oocytes, the virtually complete conservation of the ion selective pore region (P) leaves little doubt that sei encodes a potassium channel α-type subunit. These results provide an explanation for the hyperexcitable phenotype seen with all sei mutant alleles. In general, decreases in potassium channel activity cause hyperexcitability because of prolonged depolarization during an action potential and/or failure to maintain a normally negative resting potential. Decrease in activity of this sei potassium channel subunit leads to hyperexcitability similar to that observed for other potassium channel subunit loss of function mutants, including Sh, eag, and Hyperkinetic (Wu and Ganetzky, 1992).

A recent report suggests that a “set” of cloned potassium channels (including Sh, Shab, Shal, and Shaw) accounts for most embryonic potassium currents in Drosophila (Tsunoda and Salkoff, 1995). Given the dramatic effects that sei mutants have on
behavior, sei also should be included in the list of physiologically important potassium channel components in Drosophila.

**Genetic evidence that the seizure potassium channel is a multimer**

Null alleles of sei are completely recessive, indicating that the 50% reduction of gene product generally expected in a heterozygous null is without consequence to the behavior of the organism. This suggests that excess sei gene product is normally available in wild type. In contrast, the missense allele sei^{ab} has a partially dominant phenotype, although its overall hyperexcitability phenotype as a homozygote is less extreme than the null alleles (Kasbekar et al., 1987). This suggests that the potassium channel that contains the sei protein is a multimer, as are other potassium channels (Isacoff et al., 1990). The presence of one or more copies of a mutant subunit in a multimer could act as a dominant “poison” to disrupt the functional properties of the remaining wild-type subunits. It is interesting to note that missense mutations in HERG, the human homolog of sei, also cause a dominant phenotype, in this case, of cardiac arrhythmia (Curran et al., 1995).

This genetic analysis cannot predict whether the sei potassium channel is a homo- or heteromultimer. There is, however, indirect evidence that suggests an additional component is required. cRNA from several different sei cDNA constructs fails to make functional channels when injected into Xenopus oocytes. Mistakes or an incomplete sequence cannot be the cause for this failure, because the same cDNA sequence rescues the sei behavioral phenotype in transgenic animals. This suggests that there is a factor required for sei expression that is present in the fly and absent in Xenopus oocytes. This factor may be another pore-forming subunit, or it may be an auxiliary subunit like tipE, which stimulates expression of a Drosophila sodium channel (Feng et al., 1995a). Candidates for a component needed for sei expression include a potassium channel β subunit, such as the Hyperkinetic gene product (Chouinard et al., 1995), or an unidentified component, such as the enhancer of seizure gene product (Kasbekar et al., 1987).

**Premature nonsense codons affect seizure RNA abundance**

As reviewed by Maquat (1995), mRNAs containing a nonsense codon as a result of either frameshift or nonsense mutations often are reduced in abundance. The severity of the reduction is correlated with the closeness of the nonsense mutation to the normal initiation codon. The different sei nonsense alleles described here provide a unique opportunity to determine whether this relationship holds true for mRNAs that encode integral membrane proteins and are associated with the endoplasmic reticulum during translation. In general, sei transcripts show a similar effect of premature nonsense codons, as reported in other systems. Northern analysis of sei alleles (Fig. 2, Table 1) shows that the presence of a premature nonsense codon in the first half of the message (ts1 and G64) causes a marked decrease in both sei transcripts, as compared with wild type. The case of G50 is complicated by the appearance of an extra transcript, which may be the result of a cryptic transcription start site. However, even if all three transcripts are considered, there is an overall reduction in total transcript level, as compared with wild type (after correcting for loading differences).

**Rescue of paralytic phenotype with an inducible wild-type seizure transgene**

Expression of the seizure potassium channel subunit in adults only is sufficient to rescue paralysis. This stands in contrast to the requirement for the tipE gene product during pupal development to rescue adult paralysis (Feng et al., 1995a). Although mutations in both the tipE gene and the sei gene cause a temperature-sensitive paralysis, they seem to do so by different mechanisms that are distinct in time.

**Potassium channel turnover in the whole animal**

To our knowledge there has been no previous estimate of stability of sei/HERG potassium channels in an intact, multicellular organism. By inducing expression of the seizure + transgene and monitoring the paralysis phenotype, we were able to titrate expression to the point at which 94% rescue of the mutant phenotype was attained. Then induction was discontinued. The rate of return of the paralytic phenotype should reflect the net loss of functional channels from the membrane. With this method we estimate that the apparent half-life of functional sei channels is ~1–1.5 d. This is consistent with metabolic labeling studies of sodium channels that estimated the apparent half-lives of the α subunit and the αβ2 complex to be 30 and 50 hr, respectively, in cultured embryonic rat brain neurons (Schmidt and Catterall, 1986). It is also consistent with estimates of calcium channel lifetime of 5–8 d in Paramecium (Schein, 1976). There is one report (Zhao et al., 1995) suggesting that Shaker potassium channels in cultured “giant” Drosophila neurons in some genetic backgrounds turn over more rapidly than we report here. This could indicate differences in stability of different potassium channel types or could reflect differences in different mutant backgrounds.

**Relationship between seizure and sodium channel regulation**

How can the fact that seizure encodes a potassium channel subunit be reconciled with observations that the ts1 allele is associated with changes in levels of functional sodium channels (Jackson et al., 1985; O’Dowd and Aldrich, 1988) and that the ts2 allele has an altered pH dependence and altered K_D for saxitoxin binding (Jackson et al., 1984)? We propose that the reduction in channel levels in ts1 is a consequence of sei effects on cell excitability. There are previous reports that link electrical activity to density of sodium channels (Dargent and Couraud, 1990; Dargent et al., 1994) (for review, see Catterall, 1992), including a report of downregulation of sodium channels in nerve terminals of epileptic mice (Willow et al., 1986). The effects of the ts2 allele on saxitoxin binding sites are more difficult to understand. They may be an experimental artifact because of the high incubation temperatures used in these studies. Alternatively, they may reflect a ts2-induced shift in expression of sodium channels with different binding properties as a result of alternative splicing (Thackeray and Ganetzky, 1994, 1995) or expression of different α-subunit genes [para, Loughney et al. (1989) vs DSC1, Salkoff et al. (1987)].

There are multiple mechanisms by which sodium channel density is regulated by electrical activity of the cell. In cultured rat muscle fibers, spontaneous electrical activity causes a feedback downregulation of sodium channel density by changing levels of mRNA encoding sodium channel α subunits (Oloff and Catterall, 1989). Treatments that mimic increased electrical activity, such as elevation of cytosolic calcium with ryanodine or the ionophore A23187, also cause sodium channel downregulation...
REFERENCES


Dargent B, Paillart C, Carlier E, Alcaraz G, Martin-Eauclaire MF, et al. (1994) Rather than by channel synthesis, as reported for other genotypes, the different sei alleles described here, plus mutations in other potassium channel components (Wu and Ganetzky, 1992), provide a unique opportunity to dissect genetically the role of potassium channel activity in sodium channel regulation.

GenBank accession number

The GenBank accession number for the seizure cDNA reported here is U36925.

Tse2


Sodium channel downregulation in response to hyperexcitability also has been observed in neurons treated with sodium channel activators, including alpha-scorpion toxin, batrachotoxin, and veratridine (Dargent and Caudrow, 1990). In these studies, the mechanism of regulation is by sodium channel internalization (Dargent et al., 1994) rather than by channel synthesis, as reported for muscle (Oford and Catterall, 1989).

Although the mechanisms for sodium channel downregulation differ for the two cases summarized above, they have in common that the response to hyperexcitability is a decrease in the density of functional sodium channels in the plasma membrane. We propose that the decrease in functional sodium channels associated with the seI allele in Drosophila may be the consequence of hyperexcitability. To explain the normal sodium channel levels found in the ts2 missense allele, we postulate that there is a minimum hyperexcitability level required to induce the downregulation. This level is reached in the null tsI allele, but not in the missense ts2 allele. The different sei alleles described here, plus mutations in other potassium channel components (Wu and Ganetzky, 1992), provide a unique opportunity to dissect genetically the role of potassium channel activity in sodium channel regulation.


