Cloning and functional characterization of a putative sodium channel auxiliary subunit gene from the house fly (Musca domestica)

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Abstract

The functional expression of cloned Drosophila melanogaster and house fly (Musca domestica) voltage-sensitive sodium channels in Xenopus oocytes is enhanced, and the inactivation kinetics of the expressed channels are accelerated, by coexpression with the tipE protein, a putative sodium channel auxiliary subunit encoded by the tipE gene of D. melanogaster. These results predict the existence of a tipE ortholog in the house fly. Using a PCR-based homology probing approach, we isolated cDNA clones encoding an ortholog of tipE (designated Vsscβ) from adult house fly heads. Clones comprising 3444 bp of cDNA sequence contained a 1317 bp open-reading frame encoding a 438 amino acid protein. The predicted Vsscβ protein exhibited 72% amino acid sequence identity to the entire D. melanogaster tipE protein sequence and 97% identity within the two hydrophobic segments identified as probable transmembrane domains. Coexpression of Vsscβ with the house fly sodium channel α subunit (Vssc1) in oocytes enhanced the level of sodium current expression five-fold and accelerated the rate of sodium current inactivation 2.2-fold. Both of these effects were significantly larger in magnitude than the corresponding effects of the D. melanogaster tipE protein on the expression and kinetics of Vssc1 sodium channels. These results identify a second example of a putative sodium channel auxiliary subunit from an insect having functional but not structural homology to vertebrate sodium channel β subunits. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Voltage-sensitive sodium channel; Auxiliary subunit; Musca domestica

1. Introduction

Voltage-sensitive sodium channels mediate the transient increase in sodium conductance that underlies the action potentials of most neurons and of vertebrate skeletal and cardiac muscle (Hille, 1992). The sodium channel α subunit, a large (~260 kDa) transmembrane glycoprotein, forms the ion pore and is sufficient to form functional sodium channels (Catterall, 1995). In vertebrates, sodium channel α subunits in the brain form a heterotrimeric complex with two smaller auxiliary subunits, designated β1 and β2 (36 and 33 kDa, respectively), whereas skeletal muscle sodium channels exist as dimers of α and β1 subunits (Isom et al., 1994; Catterall, 1995). Coexpression experiments with cloned sodium channel subunit cDNAs demonstrate that the β1 and β2 subunits, both independently and jointly, enhance the expression and modify the kinetics and voltage dependence of the expressed rat brain IIA channels (Isom et al, 1992, 1995).

In Drosophila melanogaster, abundant and physiologically important sodium channel α subunits are encoded by the para gene (Loughney et al., 1989). However, injection of para cRNA into Xenopus laevis oocytes does not typically result in the robust expression of voltage-sensitive sodium currents unless very high cRNA concentrations and long incubation times are employed (Feng et al., 1995). Recently, a smaller protein encoded by the tipE locus of D. melanogaster has been shown to enhance the expression of para sodium channels in
oocytes and also modify the kinetics of para sodium channel inactivation (Feng et al., 1995; Warmke et al., 1997). These results suggest that the tipE protein may function as a sodium channel auxiliary subunit in a manner analogous to the vertebrate sodium channel β subunits.

In the house fly (Musca domestica), para-orthologous sodium channels are encoded by the Vssc1 (also called Msc) gene (Ingles et al., 1996; Williamson et al., 1996). The enhancement of Vssc1 sodium channel expression in Xenopus oocytes by coexpression with the D. melanogaster tipE protein (Smith et al., 1997) implies that a functionally homologous protein exists in the house fly. In this paper, we report the isolation and cloning of a cDNA from the house fly encoding a tipE-like protein (designated Vsscβ), and demonstrate both the enhancement of Vssc1 sodium channel expression in oocytes and the modification of the kinetic properties of Vssc1 sodium channels by coexpression with Vsscβ.

2. Materials and methods

2.1. Isolation and cloning of Vsscβ cDNAs

Total RNA was extracted from adult house fly (insecticide-susceptible NAIDM strain) heads using Tri Reagent® (MRC, Cincinnati, OH). Poly(A+)RNA was separated by hybridization to biotinylated oligo(dT) immobilized on paramagnetic beads (PolyATract mRNA isolation kit, Promega, Madison, WI). First-strand cDNA was synthesized from poly(A+)RNA using oligo(dT) as the primer and used as the template for initial screening using the polymerase chain reaction (PCR). A 797 bp fragment of house fly Vsscβ cDNA was amplified by PCR with a set of degenerate oligonucleotide primers (TM1 and TM2, Table 1) based on the amino acid sequences of the transmembrane domains of D. melanogaster tipE protein (Feng et al., 1995). Amplifications employed the standard conditions for PCR with Taq polymerase, except for the use of higher concentrations of primers (100–250 pmol/50 μl reaction), and 35 thermal cycles (94°C for 30 s, 42°C for 30 s, and 72°C for 1 min) followed by final extension (72°C for 10 min).

The 5′ and 3′ end regions of the Vsscβ cDNA were isolated by employing a modified protocol of rapid amplification of cDNA ends (RACE) (Marathon™ cDNA Amplification Kit, Clontech, Palo Alto, CA). In both 5′ and 3′ RACE, double-stranded cDNA was synthesized from poly(A+)RNA, ligated with an oligonucleotide adapter, and used as the template for PCR. Gene-specific nested primer sets (5R1, 5R1Nest, 3R1 and 3R1Nest, Table 1) were designed from the sequence of the 797 bp internal Vsscβ cDNA fragment. Primary PCR was carried out using a set of adapter-specific and gene-specific primers in a 1:3 molar ratio. Authentic cDNA fragments were obtained by a second round of PCR using the nested primers (5R1Nest or 3R1Nest, Table 1) with agarose-gel-purified DNA bands obtained from the primary PCR reactions as templates. Both primary and nested PCRs were performed with the following two-step thermal program: five cycles of 94°C for 30 s and 72°C for 4 min, five cycles of 94°C for 30 s and 70°C for 4 min, and 27 cycles of 94°C for 20 s and 68°C for 4 min.

Following PCR or RACE, amplified products were isolated and concentrated using a Microcon-100 concentrator (Amicon, Beverly, MA) and cloned directly (TA Cloning Kit, Invitrogen, Carlsbad, CA). Insert-positive clones were identified by colony PCR. DNA sequences of the inserts were determined using the PCR sequencing kit with an Applied Biosystems 373 sequencer (Applied Biosystems, Foster City, CA). In all cases, at least three clones were sequenced to identify polymerase-induced nucleotide sequence polymorphisms. The full-length cDNA of the open-reading frame (ORF) of Vsscβ was generated by PCR with primers ST1 and SP1 (Table 1) and subcloned in pAlter-1 (Promega). The sequence integrity of the full-length ORF clone was confirmed by DNA sequencing.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequencea</th>
<th>PCR fragmentb</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM1</td>
<td>5′-CTTAGGCTTAACACATNATHTTYATGCGATT-3′</td>
<td>Partial ORF</td>
</tr>
<tr>
<td>TM2</td>
<td>5′-CTAGAACTCAARTANGCRTANTGATNARATA-3′</td>
<td>5′-UTR+partial ORF</td>
</tr>
<tr>
<td>5R1</td>
<td>5′-ACCACATGATTTGGGTGCCACCTGAAATAT-3′</td>
<td>5′-UTR+partial ORF</td>
</tr>
<tr>
<td>5R1Nest</td>
<td>5′-GGGGTGGATTTGGTGGGGTGGG-3′</td>
<td>Partial ORF+3′-UTR</td>
</tr>
<tr>
<td>3R1</td>
<td>5′-CCCCTGCTACTATTTCCCAAAGTATAGTCT-3′</td>
<td>5′-UTR+partial ORF</td>
</tr>
<tr>
<td>3R1Nest</td>
<td>5′-CCATGCGCATCAGGCTACCCCTCCC-3′</td>
<td>Full-length ORF</td>
</tr>
<tr>
<td>ST1</td>
<td>5′-CCCTGAGCTCATGATGATGAGAATTCCGAATCCCGCGGAT-3′</td>
<td></td>
</tr>
<tr>
<td>SP1</td>
<td>5′-TCCGCATGCACTTACCGCCGAGATCT-3′</td>
<td></td>
</tr>
</tbody>
</table>

a Designation of oligonucleotide mixtures: R=A+G; Y=C+T; S=G+C; H=A+C+T; N=A+G+C+T. The underlined sequences are the restriction sites incorporated to facilitate cloning.

b UTR, untranslated region; ORF, open-reading frame.
2.2. Expression

Oocytes were obtained surgically from female frogs (Nasco, Ft. Atkinson, WI) and defolliculated by incubation with Type 1A collagenase (Sigma, St. Louis, MO) followed by manual removal of remaining follicle cells. Stage IV–VI oocytes were incubated in ND-96 medium (Goldin, 1992) supplemented with 1% sodium pyruvate, 1% penicillin/streptomycin and 5% horse serum (Sigma) for 24 h at 18°C prior to injection. The cRNA used in expression experiments was synthesized from linearized plasmid (Vssc1 or Vsscβ) or purified PCR fragment (tipE) templates using a commercial kit (mMessage mMachine™, Ambion, Austin, TX). The integrity and approximate concentration of the RNAs obtained by these methods were determined by electrophoresis in agarose/formaldehyde gels. Oocytes were injected with 25–50 nl of an aqueous solution of cRNA (either Vssc1 alone or a 1:1 molar ratio of Vssc1 and either tipE or Vsscβ cRNAs, ~1 ng/ml) and incubated in supplemented ND-96 medium at 18°C for up to 24 days prior to electrophysiological analysis of sodium currents.

2.3. Electrophysiology

Electrophysiological recordings of sodium currents were obtained from oocytes in ND-96 at ambient temperature (16–20°C). Recordings were performed in oocyte recording chambers fabricated from Plexiglass with a glass coverslip bottom bonded with cyanoacrylate glue. Membrane currents of oocytes were recorded using two-microelectrode voltage clamp with a virtual ground (TEV-208, Dagan Corp., Minneapolis, MN or GeneClamp 500, Axon Instruments, Foster City, CA), filtered with a 2 kHz low pass (four-pole Bessel) filter, digitized at 10 kHz (inactivation experiments) or 20 kHz (all other experiments), and stored electronically (MacLab, AD Instruments, Milford, MA or pClamp, Axon Instruments). Borosilicate glass recording electrodes (0.3–2.0 MΩ) were filled with filtered 3 M KCl, coated with insulating resin, and shielded with grounded aluminum foil. Compensation circuitry (TEV-208, Dagan) was used to remove leakage current. Net sodium currents were obtained by subtracting traces from the same oocyte obtained in the presence of 1 γM tetrodotoxin (TTX; Sigma) or by the P/2 method (Bezanilla and Armstrong, 1977). Sodium currents obtained upon a 50 ms step depolarization from −100 mV to −10 mV were used for the determination of levels of sodium current expression and kinetics of inactivation. For activation experiments, families of sodium current traces were generated by step depolarizations to test potentials from −90 to 60 mV (in 10 mV increments) from a holding potential of −120 mV. To determine the voltage dependence of steady-state inactivation, families of sodium currents were generated using a two-pulse protocol beginning with a step from a holding potential of −100 mV to a conditioning potential ranging from −90 mV to 50 mV (in 10 mV increments) for 160 ms, followed by a second pulse to −10 mV for 50 ms after a brief 1 ms step to the holding potential. Five seconds separated each set of double pulses to prevent rundown. The short step to the holding potential between depolarizing pulses did not significantly affect inactivation and was inserted to facilitate the subtraction of capacitive transients. Data analysis was performed in Axograph (Axon Instruments, Burlingame, CA). Midpoint potentials for activation and steady-state inactivation (e.g., test potentials producing half-maximal activation or steady-state inactivation) were determined by least-squares fits of current–voltage data from individual experiments to the Boltzmann distribution (r>0.90). Student’s t-test or the Mann–Whitney non-parametric test was used to compare amplitudes of peak transient sodium currents and midpoint potentials for activation and steady-state inactivation obtained for Vssc1 sodium channels expressed alone or with tipE or Vsscβ.

3. Results

Our initial screen for tipE-like cDNA sequences in the house fly employed PCR on adult head first strand cDNAs with sets of degenerate oligonucleotide primers that were designed on the basis of the predicted amino acid sequences of the two putative transmembrane domains of the tipE protein. One set of primers (TM1 and TM2, Table 1) yielded a specific 797 bp amplification product that gave a predicted amino acid sequence having 73% identity with the corresponding segment of the D. melanogaster tipE protein. The use of RACE with nested gene-specific primers corresponding to internal sequences of the 797 bp amplification product (Table 1) facilitated the isolation a 3444 bp cDNA sequence (Fig. 1). The Vsscβ cDNA contained a 1317 bp open-reading frame, a 616 bp 5′-untranslated region and a 1511 bp 3′-untranslated region. The open-reading frame was preceded by multiple in-frame stop codons followed by a consensus D. melanogaster translation initiation sequence (AAAC) immediately upstream of the first ATG codon of the open-reading frame. The 3′-untranslated region contained two consensus polyadenylation sequences (AATAAA) following the first in-frame stop codon but did not contain a poly(A) tail.

The open-reading frame of the Vsscβ cDNA encoded a 438-residue amino acid sequence (predicted molecular mass, 48.8 kDa; predicted isoelectric point, 4.23) that was 72% identical to the D. melanogaster tipE protein sequence [Fig. 2(a)]. Kyte–Doolittle analysis of the protein sequence [Fig. 2(b)] revealed two hydrophobic domains that aligned with the two putative transmembrane domains of the D. melanogaster tipE protein and
Fig. 1. Composite nucleotide sequence of the complete Vsscβ cDNA. The first nucleotide of the 1317 bp open-reading frame of the Vsscβ protein (shown in capital letters) is designated as sequence position 1. Underlined sequences in the 3′-untranslated region identify a 181 nt segment (sequence positions 1900–2080) with 82% nucleotide sequence identity to a segment in the 5′-untranslated region of the house fly CYP6A1 gene (Cohen et al., 1994) and a 502 nt segment (sequence positions 2327–2828) complementary to a sequence encoding a predicted peptide with 84–88% amino acid sequence identity to the carboxyl termini of D. melanogaster arrestin (Smith et al., 1990) and Calliphora erythrocephala arrestin (Plangger et al., 1994) together with associated 3′-untranslated elements. Consensus polyadenylation sequences on the sense and antisense strands are identified in boldface type. The GenBank accession number for the complete Vsscβ cDNA sequence is AF131734.
exhibited 97% amino acid sequence identity to those regions. Optimal alignment of the Vsscβ and tipE protein sequences [Fig. 2(a)] revealed that the Vsscβ sequence was truncated, lacking residues aligning with the last 20 amino acids of the tipE carboxyl terminal sequence. The Vsscβ protein contained one potential phosphorylation site in the amino terminus (Thr residue at position 9) and four potential glycosylation sites in the region between the transmembrane domains (Asn residues at positions 72, 102, 108 and 249) that were
Fig. 3. Sodium currents in oocytes expressing house fly sodium channels measured using the indicated pulse protocols. (a) Current from an oocyte injected with Vssc1 cRNA. (b) Current from an oocyte injected with Vssc1 and Vsscβ cRNAs.

conserved in position and sequence context with potential glycosylation sites in the tipE protein. However, Asn224 of the Vsscβ sequence, which aligned with a fifth glycosylation site in the tipE sequence, did not lie in an appropriate sequence context for nitrogen-linked glycosylation (Kornfeld and Kornfeld, 1985).

The effects of Vsscβ on the expression of Vssc1 sodium channels are illustrated in Fig. 3. In the absence of Vsscβ [Fig. 3(a)], currents recorded from oocytes expressing only Vssc1 were small in amplitude and exhibited relatively slow inactivation kinetics. Coexpression of Vssc1 and Vsscβ [Fig. 3(b)] enhanced the magnitude of the peak transient current [note difference in amplitude calibration bars between Figs. 3(a) and (b)] that inactivated more rapidly than currents observed upon the expression of Vssc1 alone. Data such as those illustrated in Fig. 3, obtained from multiple experiments using different oocytes, are summarized in Table 2. Despite efforts to minimize variations between experiments in the amounts of cRNAs injected and all other experimental conditions, we encountered significant variability in the level of sodium current expression between oocytes that was not correlated with the presence or absence of tipE or Vsscβ, or with the absolute mean level of sodium current expression. Comparisons between mean values obtained in multiple experiments showed that Vsscβ enhanced the mean peak transient sodium current recorded from oocytes expressing Vssc1 sodium channels five-fold and accelerated the inactivation of this current 2.2-fold. Both of these effects of Vsscβ were statistically significant ($P<0.01$). The enhancement of peak transient current and the acceleration of current inactivation by Vsscβ were also significantly greater than the corresponding effects observed when Vssc1 was coexpressed with tipE ($P<0.05$).

The voltage dependence of activation and steady-state

<table>
<thead>
<tr>
<th>Channel composition</th>
<th>Peak transient current (nA)$^b$</th>
<th>Inactivation time constant (ms)$^b$</th>
<th>Midpoint potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vssc1</td>
<td>$-217±207$ (10)</td>
<td>$6.4±3.2$ (7)</td>
<td>$-19.7±4.2$ (6)</td>
</tr>
<tr>
<td>Vssc1+Vsscβ</td>
<td>$-1085±5981$ (23)</td>
<td>$2.9±0.6$ (17)</td>
<td>$-16.9±7.9$ (14)</td>
</tr>
<tr>
<td>VSSC1+tipE</td>
<td>$-613±611$ (20)</td>
<td>$4.8±1.7$ (10)</td>
<td>$-19.6±1.3$ (8)</td>
</tr>
</tbody>
</table>

$^a$ Values are mean±standard deviations derived from the number of individual experiments, each performed with a different oocyte, shown in parentheses.

$^b$ Values from net sodium current traces obtained upon a 50 ms depolarization from $-100$ mV to $-10$ mV (see Fig. 3).

$^c$ Values obtained from plots of normalized current against test potential as in Fig. 4.

$^d$ Values obtained from plots of normalized current against holding potential as in Fig. 4.
inactivation of sodium channels obtained by the coexpression of Vssc1 and Vsscβ in oocytes are illustrated in Fig. 4, and the results of multiple experiments are summarized and compared with the results of experiments using Vssc1 alone or Vssc1 and tipE in Table 2. The mean midpoint potential for the activation of channels formed by the coexpression of Vssc1 and Vsscβ (−16.9 mV) did not differ significantly from the midpoint potential values obtained from channels formed by Vssc1 alone or by Vssc1 coexpressed with tipE. The mean midpoint potential for steady-state inactivation of channels formed by the coexpression of Vssc1 and Vsscβ (−32.5 mV) was also similar to the midpoint potential values for channels formed by Vssc1 alone or Vssc1 plus tipE; however, in these experiments the 3.3 mV depolarizing shift in the inactivation midpoint observed in the presence of Vsscβ was statistically significant (P<0.01).

4. Discussion

The ability of the D. melanogaster tipE protein to enhance the expression of house fly Vssc1 sodium channels in Xenopus oocytes (Smith et al., 1997) led us to hypothesize the existence of a functionally analogous and structurally similar protein in the house fly. We employed a PCR-based homology probing strategy using degenerate oligonucleotide primers that were designed to encode regions of the tipE protein sequence anticipated to exhibit the highest degree of sequence conservation. This approach yielded amplified cDNA sequences from house fly adult head mRNA pools that encoded a tipE-orthologous protein, which we have designated as “Vsscβ” (voltage-sensitive sodium channel, putative β subunit).

Optimal alignment of the tipE and Vsscβ protein sequences [Fig. 2(a)] revealed substantial sequence identity in the two proteins and a particularly high degree of identity in the two hydrophobic regions that are proposed to form transmembrane domains. However, the overall degree of amino acid sequence identity between Vsscβ and tipE (72%) is substantially lower than the 90% amino acid sequence identity between the orthologous Vssc1 and para sodium channel α subunit proteins of these two species (Loughney et al., 1989; Ingles et al., 1996). Other structural features conserved between the two proteins include a consensus phosphorylation site in the short amino-terminal domain and four consensus nitrogen-linked glycosylation sites in the large and relatively less conserved region lying between the two transmembrane domains. A fifth consensus glycosylation site in this region of the tipE protein was not conserved in the Vsscβ sequence. The existence of two putative transmembrane domains, and the location of consensus phosphorylation and glycosylation sites, support a predicted membrane topology of the tipE and Vsscβ proteins having intracellular amino and carboxyl termini and a large extracellular loop. This topology has been confirmed for the tipE protein by in vitro translation experiments (Feng et al., 1995).

The most notable difference in the structures of the Vsscβ and tipE proteins is the truncation of the Vsscβ sequence near the carboxyl terminal [Fig. 2(a)]. Because the point of truncation of the Vsscβ coding sequence was located at a position corresponding to an intron–exon junction identified in the tipE gene (Feng et al., 1995), we considered that the apparent truncation of the Vsscβ protein sequence might reflect the presence in the cDNA of an unspliced intron. However, translation in all reading frames of the entire 1.5 kb 3’-untranslated region of the Vsscβ cDNA failed to identify predicted amino acid sequence elements with identity or similarity to the 20 terminal amino acids of the tipE protein. Moreover, sequencing of PCR-amplified genomic DNA from this region of the Vsscβ locus confirmed the presence of a stop codon (TAA) at the position corresponding to the intron–exon boundary in the tipE gene (S.H. Lee and D.M. Soderlund, unpublished results). We therefore conclude that the truncated structure of Vsscβ relative to tipE reflects the native structure of the former protein and is not an artifact resulting from incomplete intron splicing of the cDNAs examined in this study.

Gapped BLAST searches (Altschul et al., 1997) of
nucleotide and protein sequence databases with the Vsscβ cDNA and all possible translation products identified unanticipated relationships between segments of the 3’-untranslated region of Vsscβ and other identified genes. Searching against nucleotide sequence databases identified a 181 nt segment of the coding strand sequence at positions 1900–2080 (Fig. 1) having 82% identity with a segment of the 5’-untranslated region of the house fly CYP6A1 gene, which codes for a cytochrome P450 isoform (Cohen et al., 1994). The possible significance of this conserved sequence element, which is not related to any identified regulatory or mobile element, remains obscure. Searching against protein sequence databases identified the terminal 502 nt of the Vsscβ cDNA sequence (Fig. 1) as the reverse complement of the 3’ end of a cDNA for arrestin, a regulatory protein in the visual transduction cascade. This segment contains coding sequence for a peptide with 84–88% amino acid sequence identity to the carboxyl terminal sequences of D. melanogaster arrestin (Smith et al., 1990) and C. erythrocephala arrestin I (Plangger et al., 1994), followed by a consensus polyadenylation sequence and a poly(A) tail. The polyadenylation sequence associated with the putative arrestin cDNA lies between the two polyadenylation sequences on the opposite strand that are associated with the Vsscβ sequence. The apparent overlap of these two distinct transcription units suggests a complex organization of this genetic locus similar to that documented for the TCP1 and ACAT2 genes of several vertebrate species (Shintani et al., 1999). However, additional sequence analysis of genomic DNA from this region will be required to confirm the linkage of these two genes that is implied by the structure of the cDNAs from this study, and to rule out the possibility of an artifact generated by tail-to-tail concatenation of mRNAs during the synthesis of the pool of first-strand cDNAs used for RACE.

We confirmed the functional homology of Vsscβ and tipE by assessing their relative abilities to modify the expression of Vssc1 sodium channels in Xenopus oocytes. In all respects, Vsscβ mimicked the actions of tipE to enhance the expression and accelerate the inactivation kinetics of Vssc1 sodium channels. Moreover, Vsscβ produced enhancement and acceleration of inactivation that were significantly greater than the effects of tipE determined under identical conditions. However, neither Vsscβ nor tipE produced large shifts in the voltage dependence of Vssc1 sodium channels (see Table 2). Because Vsscβ modified the functional properties of Vssc1 sodium channels as well as the level of expression, we have tentatively characterized this protein as a sodium channel auxiliary (β) subunit by analogy with the functional properties of mammalian β1 and β2 sodium channel subunits (Isom et al. 1992, 1995). This conclusion is supported by the preferential distribution of tipE in the D. melanogaster embryonic central nervous system, a pattern of expression similar to that found for the para sodium channel gene of D. melanogaster (Hong and Ganetzky, 1994; Feng et al., 1995). However, evidence for the specific coassembly of Vssc1 and Vsscβ in house fly neurons will be required to rule out the possibility that Vsscβ modifies the expression and kinetics of Vssc1 sodium channels by an effect on protein trafficking or some other indirect mechanism (Hall et al., 1999), rather than by specific incorporation into a heteromeric sodium channel complex.

Despite the functional similarity in oocyte expression assays between Vsscβ and mammalian β1 and β2 sodium channel subunits, there is no structural similarity between these proteins at the level of primary amino acid sequence. Further, a BLAST search of protein sequence databases using the Vsscβ protein sequence identified tipE as the only similar protein reported to date. Vsscβ and tipE therefore appear to be members of a novel class of nerve membrane glycoproteins. Recently, coexpression with tipE was shown to enhance the expression in oocytes of para-orthologous sodium channels from Blattella germanica (Hall et al., 1999), thereby implying the existence of tipE orthologs outside the Diptera. It will be of interest to identify genes orthologous to tipE and Vsscβ from other insect species and to determine whether orthologous genes are also present in other taxa.

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References


