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Molecular and functional characterization of a novel sodium channel TipE-like auxiliary subunit from the American cockroach *Periplaneta americana*

Céline M. Bourdin [a], Nathalie C. Guérineau [b, c], Laurence Murillo [a, d], Sophie Quinchard [a], Ke Dong [e], Christian Legros [b, *]

[a] Laboratoire Récepteurs et Canaux Ioniques Membranaires (RCIM), UPRES EA 2647/USC, INRA 1330, SFR QUASAV n° 4207, Université d'Angers, UFR Sciences, 2 boulevard Lavoisier, F-49045, Angers Cedex, France
[b] Laboratoire de Biologie Neurovasculaire et Mitochondriale Intégrée, CNRS UMR 6214, INSERM U1083, UFR de Sciences Médicales, Université d'Angers, rue Haute de Reculée, F-49045, Angers Cedex, France
[c] Institut de Génomique Fonctionnelle, UMR CNRS 5203, INSERM U661, Université de Montpellier, 141 rue de la Cardonille, F-34094, Montpellier Cedex 5, France
[d] Laboratoire LIttoral ENvironnement et Sociétés (LIENSs), UMR 7266 CNRS Université de La Rochelle, Institut du Littoral et de l'Environnement, 2 rue Olympe de Gouges, F-17000, La Rochelle, France
[e] Department of Entomology, Genetics and Neuroscience Programs, Michigan State University, East Lansing, 106 CIPS, MI 48824, USA

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ABSTRACT

In *Drosophila melanogaster*, the functions of voltage-gated sodium (Na_v) channels are modulated by TipE and its orthologs. Here, we describe a novel *TipE* homolog of the American cockroach, *Periplaneta americana*, called PaTipE. Like DmTipE, PaTipE mRNAs are ubiquitously expressed. Surprisingly, PaTipE mRNA was undetectable in neurosecretory cells identified as dorsal unpaired median neurons. Phylogenetic analysis placed this new sequence in TipE clade, indicating an independent evolution from a common ancestor. Contrary to previous reports, our data indicate that the auxiliary subunits of insect Na_v channels are very distant from the mammalian BKCa auxiliary subunits. To decipher the functional roles of PaTipE, we characterized the gating properties of $DmNa_v1$-1 channels co-expressed with DmTipE or PaTipE, in *Xenopus* oocytes. Compared to DmTipE, PaTipE increased Na^+ currents by a 4.2-fold. The voltage-dependence of steady-state fast inactivation of $DmNa_v1$-1/PaTipE channels was shifted by 5.8 mV to more negative potentials than that of $DmNa_v1$-1/DmTipE channels. $DmNa_v1$-1/PaTipE channels recovered 3.2-fold slower from the fast-inactivated state than $DmNa_v1$-1/DmTipE channels. In conclusion, this study supports that the insect Na_v auxiliary subunits share functional features with their mammalian counterparts, although structurally and phylogenetically distant.

1. Introduction

Voltage-gated sodium (Na_v) channels represent crucial plasma membrane components, that control depolarization-triggered fast Na^+ influx, allowing the generation and propagation of action potentials in excitable cells (Hille, 2001). Na_v channels consist of a large pore-forming α-subunit (~260 kDa) and one or more smaller auxiliary proteins (Catterall et al., 2005). The Na_v channel α-subunit constitute molecular targets of numerous compounds such as, clinically used drugs for treatments of various diseases (e.g., epilepsy, chronic pain and cardiac arrhythmia), and also various animal and plant neurotoxins (Denac et al., 2000). Insect Na_v channels are targeted by extensively used insecticides belonging to pyrethroid or pyrazoline families (Silver et al., 2010; Soderlund, 2008). However, these insecticides also impact the functions of mammalian Na_v channel α-subunits because of their high homology with insect counterparts.

In contrast, the Na_v channels auxiliary subunits are more divergent in sequences and structure in both insects and mammals.

Abbreviations: Na_v channels, voltage-gated sodium channels; BLAST, Basic Local Alignment Search Tool; RACE, Rapid Amplification of cDNA Ends.

* Corresponding author. Laboratoire de Biologie Neurovasculaire et Mitochondriale Intégrée, UMR CNRS 6214, INSERM 1083, UFR de Sciences Médicales, Université d'Angers, rue Haute de Reculée, F-49045, Angers Cedex, France.
E-mail address: christian.legros@univ-angers.fr (C. Legros).

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Table 1
Sequences of the oligonucleotides used in PCR and their corresponding region.

Primers name	Nucleotide sequence	Domain
1-Degenerated primer used to amplify PaTipE		
TDP-S1	5′-CCBGCNTTCACSACDATYTTCATG-3′	MS1-MS2
TDP-R1	5′-RTAVCCRCADCCYTTBACRTTBGG-3′	MS1-MS2
2-Specific primers used in RACE		
TP-R1	5′-CCACTCCGAGTCGTTCCCCAT-3′	5′UTR partial ORF PaTipE(PCR#1)
TP-R2	5′-TCTCTTGGACCGCAGATATTCGTG-3′	5′UTR partial ORF PaTipE(PCR#2)
TP-S1	5′-GTGGCGGAGCTCCACGAATATCTGC-3′	3′UTR partial ORF PaTipE(PCR#1)
TP-S2	5′-GGACTCATGGGGAACGACTCGGA -3′	3′UTR partial ORF PaTipE(PCR#2)
3-Primers used to amplify the full-length ORF		
TP-S3	5′-AAA*CCCGGG*CCAACCATGGACGAGCCGGAGATTGAGC-3′	Full-length ORF PaTipE
TP-S4	5′-GGGTGCATTCAAAGCACGATGACT-3′	Complete cDNA PaTipE
TP-R3	5′-CAAAA*TCTAGA*TCAGACTTCCGCTATCGGCCCAG-3′	Full-length ORF PaTipE
TP-R4	5′-CCACACAAGGTTAAAGCCTGTGGC-3′	Complete cDNA PaTipE
TP-S5	5′- AAAAGTTGTCAGCTGTTCGGCG-3′	Complete cDNA PaTEH1
TP-S6	5′-CAGAT*CCCGGG*ATGAGGAGCAGCAGCTCGGAG-3′	Full-length ORF PaTEH1
TP-R5	5′-(T)23CAAAATATAGGCCATGTATTTCTACC-3′	Complete cDNA PaTEH1
TP-R6	5′-CTGAT*TCTAGA*CCGAATCATGTTCTATCTTCT-3′	Full-length OFR PaTEH1

Designation of oligonucleotide mixtures: R = G + A; S = G + C; Y=C + T; M = A + C; B = G + T + C; D = G + A + T; V = G + A + C; N = G + A + T + C.
UTR, untranslated region; ORF, open-reading frame.
Restriction enzyme recognition sequences are underlined and correspond to *Xma*I site (CCCGGG) and *Xba*I site (TCTAGA).

In mammals, these auxiliary subunits, called β-subunits are glycoproteins containing a single transmembrane segment (Chahine and O'Leary, 2011). They modulate both trafficking and gating properties of Na_v channels (Patino and Isom, 2010). The *Drosophila melanogaster* genome analysis has revealed the absence of gene encoding proteins homologous to the vertebrate Na_v channel β-subunits (Littleton and Ganetzky, 2000). However, a family of five homologous genes encoding proteins (DmTipE, TEH1, TEH2, TEH3 and TEH4) have been previously reported in *D. melanogaster* with functions similar to those of mammalian β-subunits (Derst et al., 2006; Feng et al., 1995; Wang et al., 2015, 2013; Warmke et al., 1997). DmTipE is a glycosylated membrane protein (~65 kDa) that contains two membrane-spanning segments, encompassing a large extracellular loop and two intracellular extremities (Derst et al., 2006; Feng et al., 1995). This topological organization is similar to that of β-subunits (Slo-β) of the mammalian big conductance calcium-activated potassium channel (BK_{Ca}), suggesting a common ancestor (Derst et al., 2006).

The heterologous expression of insect Na_v channel α-subunits is a notoriously challenging task, limiting functional and pharmacological investigations. To date, the *Xenopus* oocyte expression system is the unique suitable system to heterologously express insect Na_v channels. Only tiny currents are obtained when the Na_v channel of *D. melanogaster* ($DmNa_v1$) is expressed alone in *Xenopus* oocyte (Feng et al., 1995; Warmke et al., 1997). By contrast, the co-expression of $DmNa_v1$ with the auxiliary subunit DmTipE generates high Na^+ current density that is associated with hastened inactivation kinetics (Derst et al., 2006; Feng et al., 1995; Warmke et al., 1997). The discovery of TipE in *D. melanogaster* has greatly improved the investigation of the molecular mechanisms of insecticides targeting Na_v channels (Dong, 2007). DmTEH1 and DmTipE robustly boost $DmNa_v1$ expression, resulting in 30-fold and 10-fold increases, respectively, in Na^+ current density. By comparison, DmTEH2 and DmTEH3 have weaker effects (5–8-fold increase in Na^+ currents), and DmTEH4 has no effect on $DmNa_v1$ channel expression (Derst et al., 2006). In addition, when $DmNa_v1$ is co-expressed with TipE or TEH1-4 subunits in *Xenopus* oocyte, elicited Na^+ currents display distinct inactivation properties (Derst et al., 2006).

Two distinct types of Na_v channels have been functionally characterized in the Dorsal Unpaired Median (DUM) neurons of the cockroach, *Periplaneta americana*, which is a commonly used neurophysiological model (Lavialle-Defaix et al., 2006, 2010; Zhao et al., 2005). We hypothesized that these Na_v channels differ by the composition of their auxiliary subunit. In this context, we cloned the Na_v channel α-subunit of *P. americana*, $PaNa_v1$ (Moignot et al., 2009). Unfortunately, $PaNa_v1$ is not expressible in *Xenopus* oocyte with DmTipE, DmTEH1 or PaTEH1 variants (Bourdin et al., 2013; Moignot et al., 2009). Then, $BgNa_v1$ (Na_v channel of *Blattella germanica*) and $DmNa_v1$, sharing high amino acid sequence identities with $PaNa_v1$ have been used in electrophysiological experiments in *Xenopus* oocytes. We have shown that the consequences of the interaction between α-subunit and the auxiliary subunits of *P. americana* (PaTEH1) or *Drosophila melanogaster* (DmTEH1) are similar, demonstrating the functional conservation of these homologous auxiliary subunits (Bourdin et al., 2013). Like DmTEH1, PaTEH1 variants strongly increase Na_v channel expression in *Xenopus* oocytes. PaTEH1 subunits also shift the voltage-dependence of Na^+ currents of both activation and inactivation to more negative potentials, compared to those elicited by $BgNa_v$ alone (Bourdin et al., 2013). Thus, both activation and inactivation properties, as well as the expression level of Na_v channels are modulated by co-expressed TipE-related proteins.

To extend the knowledge concerning insect Na_v channel auxiliary subunits, we isolated cDNAs encoding the DmTipE homolog, PaTipE, in the nervous system of *P. americana*. We report here a phylogenetic analysis showing that this novel subunit is evolutionary closely related to others TipE family members. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) experiments revealed a conserved tissue distribution of PaTipE and DmTipE both within the central nervous system and in non-neuronal tissues. Electrophysiological experiments were next performed to characterize the influence of PaTipE on $DmNa_v$ channels expression and gating properties in *Xenopus* oocytes.

2. Material and methods

2.1. Animals

American cockroaches (*P. americana*) were reared in our laboratory under standard conditions (29 °C, photoperiod of 12-h light/12-h dark). *Xenopus laevis* females used for oocytes preparations have been lab-bred. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of