Molecular and Cellular Neuroscience 59 (2014) 85–96

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Caenorhabditis elegans nicotinic acetylcholine receptors are required for nociception

Emiliano Cohen [a], Marios Chatzigeorgiou [b], Steven J. Husson [c,d], Wagner Steuer-Costa [e], Alexander Gottschalk [e], William R. Schafer [b], Millet Treinin [a,*]

[a] Department of Medical Neurobiology, Institute for Medical Research Israel–Canada, Hebrew University — Hadassah Medical School, Jerusalem 91120, Israel
[b] Cell Biology Division, MRC Laboratory of Molecular Biology, Hills Road, Cambridge UK
[c] Functional Genomics and Proteomics, KU Leuven, Naamsestraat 59, B-3000 Leuven, Belgium
[d] SPHERE — Systemic Physiological & Ecotoxicological Research, Department of Biology, University of Antwerp, Groenenborgerlaan 171/U7, B-2020 Antwerp, Belgium
[e] Buchmann Institute for Molecular Life Sciences and Institute of Biochemistry, Goethe-University Frankfurt, Max-von-Laue-Str. 15, D-60438 Frankfurt, Germany

ARTICLE INFO

Article history:
Received 16 September 2013
Revised 7 January 2014
Accepted 1 February 2014
Available online 8 February 2014

Keywords:
Caenorhabditis elegans
Polymodal nociceptors
Nicotinic acetylcholine receptors
Acetylcholine
Calcium

ABSTRACT

Polymodal nociceptors sense and integrate information on injurious mechanical, thermal, and chemical stimuli. Chemical signals either activate nociceptors or modulate their responses to other stimuli. One chemical known to activate or modulate responses of nociceptors is acetylcholine (ACh). Across evolution nociceptors express subunits of the nicotinic acetylcholine receptor (nAChR) family, a family of ACh-gated ion channels. The roles of ACh and nAChRs in nociceptor function are, however, poorly understood. *Caenorhabditis elegans* polymodal nociceptors, PVD, express nAChR subunits on their sensory arbor. Here we show that mutations reducing ACh synthesis and mutations in nAChR subunits lead to defects in PVD function and morphology. A likely cause for these defects is a reduction in cytosolic calcium measured in ACh and nAChR mutants. Indeed, overexpression of a calcium pump in PVD mimics defects in PVD function and morphology found in nAChR mutants. Our results demonstrate, for the first time, a central role for nAChRs and ACh in nociceptor function and suggest that calcium permeating via nAChRs facilitates activity of several signaling pathways within this neuron.

Introduction

In animals, injurious signals are detected by specialized sensory neurons called nociceptors. These neurons are often polymodal sensing both high-threshold mechanical stimuli and noxious temperatures, most also respond to chemical stimuli. These chemicals include protons, ATP, bradykinin, prostaglandins, other cytokines, and neurotransmitters (Woolf and Ma, 2007). Responses of nociceptors to noxious stimuli and the ensuing behavioral and physiological responses show a great degree of plasticity; evident at the sensory level by altered response threshold or intensity. Indeed, sensitization following injury is a unique property of polymodal nociceptors and is markedly different from the rapidly desensitizing responses of other sensory neurons. This sensitization is likely a result of chemicals, present in the environment following injury or inflammation, that act via signaling pathways within nociceptors to enhance responses to concurrent or future noxious stimuli (Fischer et al., 2010; Gold and Gebhart, 2010). For example, serotonin and bradykinin whose extra-cellular levels increase following inflammation are known to cause sensitization via regulation of activity or activation thresholds of nociceptor-expressed ion-channels (Beck and Handwerker, 1974; Loyd et al., 2013; Petho and Reeh, 2012).

Early analysis of chemical excitants of nociceptors identified ACh as a pain-producing substance (Armstrong et al., 1953). Further analysis showed that nAChRs mediate some of the effects of ACh on nociceptors (Bernardini et al., 2001). Indeed, mammalian nociceptors express multiple nAChR subunits (Damaj and Flores, 2001; Genzen et al., 2001; Rau et al., 2004; Spies et al., 2006). Analysis of the role of nAChRs in nociception, however, is hindered by the many roles and wide expression of nAChRs. For example, sites of action of analgesic drugs targeting nAChRs include targets in the central nervous system, sensory endings of nociceptors, and non-neuronal cells mediating inflammatory-responses (Damaj and Flores, 2001; Vincler et al., 2006).

Responding appropriately to noxious and injurious signals is essential for survival, as evidenced by conservation of polymodal nociceptors in evolution. Polymodal nociceptors are found in both vertebrates and invertebrates and are conserved for function, molecular determinants and morphology (Hall and Treinin, 2011). Recently, the *Caenorhabditis elegans* PVD and FLP neurons emerged as well-conserved models for polymodal nociceptors. These two pairs of neurons, FLP in the head and PVD in the midbody, sense high-threshold mechanical stimuli and temperature; PVD sense noxious cold while FLP sense noxious heat (Chatzigeorgiou and Schafer, 2011; Chatzigeorgiou et al., 2010). Like

* Corresponding author.
E-mail address: millet_t@cc.huji.ac.il (M. Treinin).

1044-7431/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2014.02.001

mammalian nociceptors PVD and FLP express several nAChR subunits (Smith et al., 2010; Treinin et al., 1998). These subunits, however, are not conserved in evolution; as DEG-3 and DES-2, shown to express in PVD, belong to a *C. elegans* specific group of nAChR subunits (Jones and Sattelle, 2003; Treinin et al., 1998).

Conservation between PVD and mammalian nociceptors enables analysis of the role of ACh and nAChRs in nociceptor function. We examined mutants affecting DEG-3, DES-2, and ACh synthesis for defects in PVD function or morphology and demonstrated roles of nAChR subunits and ACh in PVD function and development. Furthermore, this work shows that ACh and ACh-gated ion-channels enhance the response amplitude of PVD to noxious stimuli. We suggest that functions of nAChRs in PVD represent an evolutionarily conserved mechanism for facilitating responses of polymodal nociceptors.

Results

DEG-3 and DES-2 localize to PVD sensory dendrites

The DEG-3 and DES-2 nAChR subunits are together required for formation of a functional ACh- and choline-gated ion-channel. *deg-3* and *des-2* are encoded by a single operon that was shown to express in several neurons including PVD and FLP neurons (Treinin et al., 1998). Neurotransmitter gated channels are presumed to function in synapses, however, anti-DEG-3 staining did not show synaptic localization, instead it demonstrated localization of DEG-3 to sensory dendrites and revealed the multi-dendritic nature of PVD and FLP (Halevi et al., 2002; Yassin et al., 2001). Thus, suggesting a role for DEG-3 in sensory transduction.

PVD neurons are multi-dendritic neurons having a complex arborization pattern. Each of the two PVD neurons extends one axon to the ventral cord and two longitudinal primary dendrites, one extending to the tail and the other to the head. Secondary branches growing ventrally or dorsally from primary dendrites each form a menorah like structure whose terminal branches, quaternary branches, lie in between the body muscles and the hypodermis (Albeg et al., 2011; Oren-Suissa et al., 2010). Fig. 1A,B shows that DEG-3 decorates the entire dendritic arbor of PVD, primary, secondary, tertiary, and quaternary branches. Similarly, a full length DES-2::GFP fusion also localizes to the entire sensory arbor of PVD (Fig. 1C). Interestingly, a DES-2::GFP fusion lacking the C-terminus (129 amino acids starting at P421 within the large intracellular loop) does not localize to terminal branches (Fig. 1D). Absence of the truncated DES-2::GFP from PVD terminal branches (Fig. 1D) is not a result of transgene-induced defects in their development, as

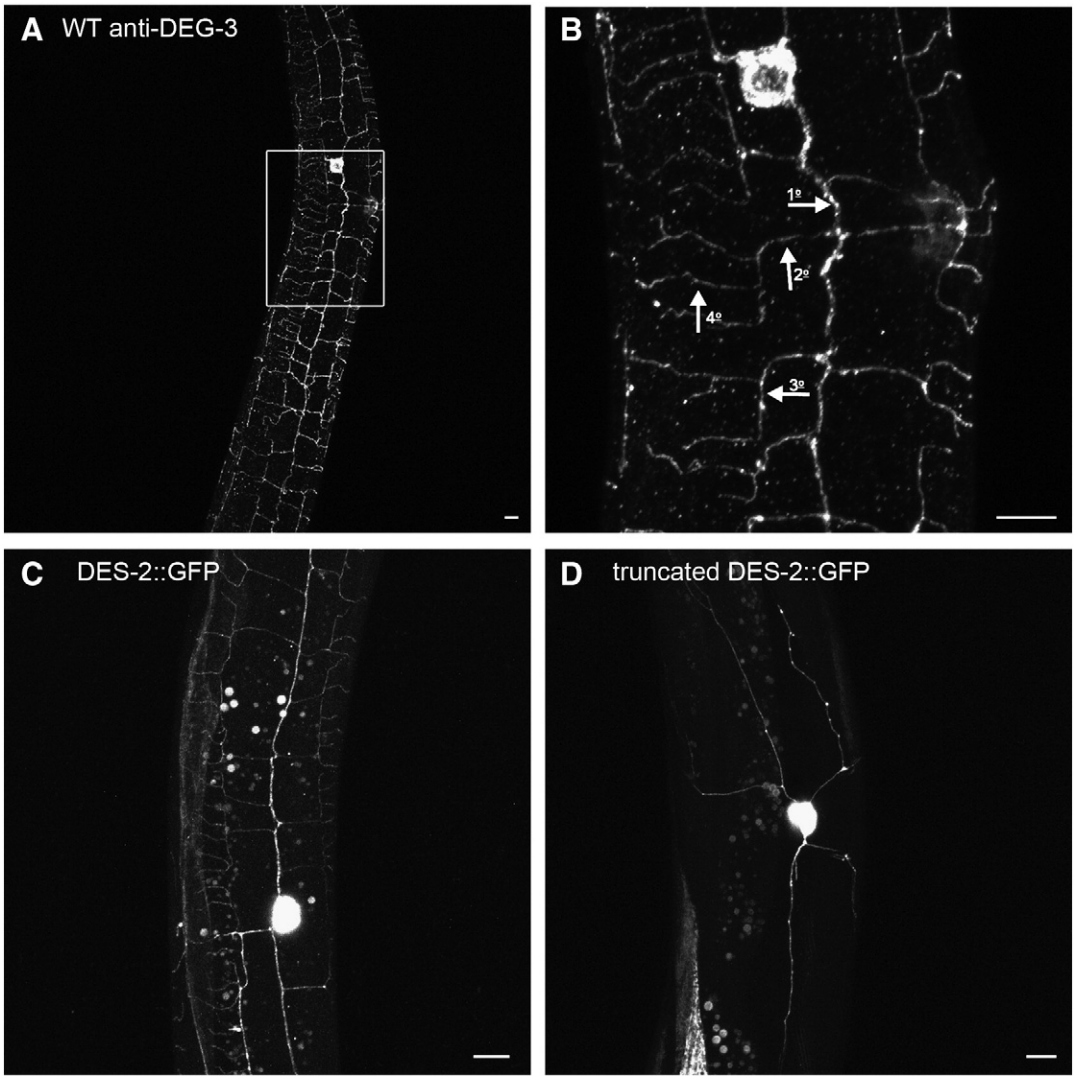

Fig. 1. DEG-3 and DES-2 localization in PVD's sensory arbor. A) Anti-DEG-3 antibody staining. B) Magnification of boxed area in (A). Arrows and numbers indicate representative primary (1°), secondary (2°), tertiary (3°), and quaternary branches (4°). C) Full-length DES-2::GFP fusion localization in PVD. D) PVD localization of a truncated DES-2::GFP fusion. Shown are images of late L4-young adults, scale bar 5 μm.