Single Ionic Channels of Two Caenorhabditis elegans Chemosensory Neurons in Native Membrane

W.T. Nickell, R.Y.K. Pun1, C.I. Bargmann2, S.J. Kleene

Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati College of Medicine, P.O. Box 670667 Cincinnati, OH 45267, USA

1Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, P.O. Box 670576 Cincinnati, OH 45267, USA

2Howard Hughes Medical Institute and Programs in Developmental Biology, Neuroscience, and Genetics, Department of Anatomy, The University of California, San Francisco, CA 94143-0452, USA

Received: 28 January 2002/Revised: 20 May 2002

Abstract. The genome of Caenorhabditis elegans contains representatives of the channel families found in both vertebrate and invertebrate nervous systems. However, it lacks the ubiquitous Hodgkin-Huxley Na⁺ channel that is integral to long-distance signaling in other animals. Nematode neurons are presumed to communicate by electrotonic conduction and graded depolarizations. This fundamental difference in operating principle may require different channel populations to regulate transmission and transmitter release.

We have sampled ionic channels from the somata of two chemosensory neurons (AWA and AWC) of C. elegans. A Ca²⁺-activated, outwardly rectifying channel has a conductance of 67 pS and a reversal potential indicating selectivity for K⁺. An inwardly rectifying channel is active at potentials more negative than −50 mV. The inward channel is notably flickery even in the absence of divalent cations; this prevented determination of its conductance and reversal potential. Both of these channels were inactive over a range of membrane potentials near the likely cell resting potential; this would account for the region of very high membrane resistance observed in whole-cell recordings. A very-large-conductance (>100 pS), inwardly rectifying channel may account for channel-like fluctuations seen in whole-cell recordings.

Key words: Olfaction — Receptor neuron — Electrophysiology — Caenorhabditis elegans — Nematode

Introduction

The nervous system of C. elegans uses familiar ionic channels. The genome contains sequences representing all of the known families of K⁺ channels, voltage-gated Ca²⁺ channels, cyclic-nucleotide-gated cationic channels, and Cl⁻ channels (Wei, Jegla & Salkoff, 1996; Bargmann, 1998). There is one fundamental omission, however. The Hodgkin-Huxley voltage-gated Na⁺ channel is basic to signal transmission, coding, and processing in most nervous systems; the varied ionic channels familiar to neurophysiology largely serve to regulate the firing of Na⁺ action potentials. The Hodgkin-Huxley channel is omitted from the C. elegans genome, and electrophysiological recordings find no evidence of fast Na⁺ currents. In nematode nervous systems, graded depolarization and electrotonic conduction appear to serve functions handled by spikes in other animals. Presumably, the ionic channels of C. elegans are adapted to this unique mode of operation.

The only comprehensive electrophysiological studies of nematode neurophysiology are from the parasitic nematode Ascaris suum. The resting potential of Ascaris neurons and muscle cells is low (−40 mV; del Castillo & Morales, 1967; Brading & Caldwell, 1971; Davis & Stretton, 1989a). Muscle resting potential is nearly independent of K⁺, but is dependent on Cl⁻ and organic acids; a large-conductance channel that passes both Cl⁻ and carboxylic acids may determine the resting potential (Brading & Caldwell, 1971; Robertson & Martin, 1996). The ionic dependence of the membrane potential of Ascaris neurons has not been determined. The whole-cell resistance of Ascaris neurons is very high; this allows
communication along the length of the neuron by electrotoneic conduction (Davis & Stretton, 1989a). Transmitter release is graded around the low resting membrane potential (Davis & Stretton, 1989a). Motor neurons support Ca\(^{2+}\)-mediated active currents (Davis & Stretton, 1989b).

Because of the difficulty of access and the small size of the neurons, electrophysiological studies of *C. elegans* have been slow to appear. Lockery and co-workers (Goodman et al., 1998; Lockery & Goodman, 1998) described whole-cell recordings from ASER and other neurons in the nerve ring. They found low resting potentials, low resting conductance in the vicinity of the resting potential, and Ca\(^{2+}\)-mediated active potentials. Richmond and Jorgensen (1999) recorded cholinergic agonist-generated synaptic currents in muscle, but did not report membrane resting potential or membrane resistance.

We are interested in understanding transduction mechanisms in two chemosensory neurons, AWA and AWC. In both of these neurons, odorant specificity and likely second-messenger pathways have been identified by genetic methods (Komatsu et al., 1996; Sengupta, Chou & Bargmann, 1996; Colbert, Smith & Bargmann, 1997; Troemel, Kimmel & Bargmann, 1997; Bargmann & Kaplan, 1998). However, there are substantial neurophysiological differences between *C. elegans* and organisms that are well characterized electrically. Thus, preliminary studies of basic *C. elegans* neural function are necessary before transduction mechanisms can be fully understood. Characterizing the ionic channels present in these neurons would be a good start.

Of the numerous ionic channels represented in the genome, several have already been localized to the membranes of neurons AWA and AWC. A cyclic nucleotide-gated cationic channel (TAX-2/TAX-4) is located primarily in the dendrite of AWC, although channels are also present in the soma (Coburn & Bargmann, 1996; Komatsu et al., 1996). In a heterologous expression system, this channel has a conductance of 56 pS and is best gated by cyclic GMP (Komatsu et al., 1999). OSM-9, a member of the TRPV family of channels, is present in both AWA and AWC, although it serves different functions in the two neurons (Colbert et al., 1997). The electrophysiological properties of this channel have not been reported. SLO-1 and SLO-2 BK K\(^+\) channels are present in numerous unidentified neurons of the nerve ring. When expressed in frog oocytes, these channels have large conductances; some are activated by Ca\(^{2+}\) and/or Cl\(^-\) (Yuan et al., 2000).

Heterologous expression provides a powerful means of determining the properties of genetically identified channels but cannot tell us how those channels function in their native environment. In the neuron, gene products may be altered before insertion into the membrane, combined with other subunits in unknown proportions, or regulated by internal messengers. Tissue culture of embryonic cells provides a closer approximation to in vivo conditions (Christensen et al., 2002). Embryonic neurons express important markers and exhibit currents similar to those observed in adult neurons. However, cultured neurons lack normal cell-cell interactions and morphological specializations. For these reasons, functional properties of the channels must be determined in the native membrane. We have chosen to record from identified chemosensory neurons AWA and AWC in adult worms. Cell-attached recording from these neurons is as close as we can come to an in vivo recording; it is likely that the soma and dendritic ending remain intact. In our preliminary survey, single channels appear to fall into a small number of classes. While it is presently impossible to match these channels with reported genes, more detailed studies of channel properties and continued progress in genetic analysis should make this an attainable goal. The properties of the common channels appear to account for the whole-cell properties of *C. elegans* neurons observed by ourselves and others. Less common large-conductance channels may also play important roles in neuronal function.

Materials and Methods

EXPOSURE OF NEURONS (OVERVIEW)

The somata of the 24 amphid chemosensory neurons are located in the anterior sensory ganglion, just rostral to the terminal bulb of the pharynx. Processes of these neurons extend anteriorly to join in the circumpharyngeal nerve ring (Ward et al., 1975; Ware et al., 1975). Each chemosensory neuron also extends one dendrite anteriorly to one of the two amphid sensilla, where it is exposed to the environment (Fig. 1A). We used strains of worms in which either the AWA (kyIs 17 (odh-10:GFP)) or AWC (kyIs 136 (str-2:GFP)) neurons express green fluorescent protein (GFP; Chalfie et al., 1994). Both the dendrites and axons of the labeled neurons are clearly visible under fluorescence microscopy (Fig. 1A).

To expose GFP-labeled AWA or AWC neurons, we cut the worm at about the level of the terminal bulb (near the dashed lines in Fig. 1, A and B). Contraction of body-wall muscles in the “head” then exposed the terminal bulb and some of the neurons (Fig. 1, C and D; Avery, Raizen & Lockery, 1995). In 5–10% of the cut worms, at least one GFP-labeled neuron was intact and exposed. Successful recordings required preparation of many worms to ensure an adequate number of accessible neurons.

Worms maintain a high turgor pressure; cutting them under standard saline (Dent’s Solution; Avery et al., 1995) results in explosive release of worm contents. GFP-labeled neurons exposed after this treatment often appeared swollen and typically disappeared within one hour after cutting. The internal pressure is probably due to a combination of osmotic pressure and tonic muscle contraction (Hobson, Stephenson & Beadle, 1952a; Hobson, Stephenson & Eden, 1952b; Harris & Crofton, 1957; Pax et al., 1995). To minimize damage to neurons, internal pressure was reduced by equilibrating worms in a low-tonicity medium before cutting them in a physiological saline containing an additional 100