Mechanosensitive unpaired innexin channels in C. elegans touch neurons

Rachele Sangaletti, Gerhard Dahl, and Laura Bianchi

Department of Physiology and Biophysics, University of Miami, Miller School of Medicine, Miami, Florida

Submitted 14 July 2014; accepted in final form 18 September 2014

Sangaletti R, Dahl G, Bianchi L. Mechanosensitive unpaired innexin channels in C. elegans touch neurons. Am J Physiol Cell Physiol 307: C966–C977, 2014. First published September 24, 2014; doi:10.1152/ajpcell.00246.2014.—Invertebrate innexin proteins share sequence homology with vertebrate pannexins and general membrane topology with both pannexins and connexins. While connexins form gap junctions that mediate intercellular communication, pannexins are thought to function exclusively as plasma membrane channels permeable to both ions and small molecules. Undoubtedly, certain innexins function as gap junction proteins. However, due to sequence similarity to pannexins, it was postulated that innexins also function as plasma membrane channels. Indeed, some of the leech innexins were found to mediate ATP release as unpaired membrane channels with shared pharmacology to pannexin channels. We show here that Caenorhabditis elegans touch-sensing neurons express a mechanically gated innexin channel with a conductance of ~1 nS and voltage-dependent and K+ selective subconductance state. We also show that C. elegans touch neurons take up ethidium bromide through a mechanism that is activated and blocked by innexin activating stimuli and inhibitors, respectively. Finally, we present evidence that touch neurons’ innexins are required for cell death induced by chemical ischemia. Our work demonstrates that innexins function as plasma membrane channels in native C. elegans neurons, where they may play a role in pathological cell death.

innexin; C. elegans; electrophysiology

DIRECT INTERCELLULAR COMMUNICATION is achieved through gap junctions. Gap junctions are large intercellular channels that are permeable to both ions and small molecules up to ~1–2 kDa (16, 24, 51). In vertebrates, gap junctions are formed by two connexon hemichannels each consisting of six identical or homologous connexin subunits (19, 41). Each connexin subunit has four transmembrane domains, two extracellular loops and intracellular NH2 and COOH termini (61). In invertebrates, gap junctions are formed by homologous connexin subunits (19, 41). Each connexin subunit has four transmembrane domains, two extracellular loops and intracellular NH2 and COOH termini (61). In vertebrates, gap junctions are formed by connexins that share sequence homology with vertebrate pannexins and general membrane topology with both pannexins and connexins. While connexins form gap junctions that mediate intercellular communication, pannexins are thought to function exclusively as plasma membrane channels permeable to both ions and small molecules. Undoubtedly, certain innexins function as gap junction proteins. However, due to sequence similarity to pannexins, it was postulated that innexins also function as plasma membrane channels. Indeed, some of the leech innexins were found to mediate ATP release as unpaired membrane channels with shared pharmacology to pannexin channels. We show here that Caenorhabditis elegans touch-sensing neurons express a mechanically gated innexin channel with a conductance of ~1 nS and voltage-dependent and K+− selective subconductance state. We also show that C. elegans touch neurons take up ethidium bromide through a mechanism that is activated and blocked by innexin activating stimuli and inhibitors, respectively. Finally, we present evidence that touch neurons’ innexins are required for cell death induced by chemical ischemia. Our work demonstrates that innexins function as plasma membrane channels in native C. elegans neurons, where they may play a role in pathological cell death.

innexin; C. elegans; electrophysiology

The role of invertebrate innexins in gap junction formation is well documented both in C. elegans and Drosophila (3, 32, 66, 67, 74, 75). However, less is known about innexins as plasma membrane channels, although there is evidence suggesting that indeed invertebrate innexins can form plasma membrane channels. For example, C. elegans innexin nsy-5 is expressed in the distal tip cells of the gonad (1) and close inspection by electron microscopy has never revealed gap junctions between the distal tip cells and the underlying germ line at any stage (Hall DH and Hedgecock E, unpublished observations) (1). Similarly, in the medicinal leech innexins have been shown to be expressed in glia and to play a role in migration of microglia to sites of injury (5, 52). Nonjunctional innexin channels were demonstrated in insect cells (36). Finally, leech inv-3 can form plasma membrane channels when expressed in Xenopus oocytes (5).

While pannexins do not form gap junctions, the properties of the membrane channels formed by these proteins are very similar to those of the nonjunctional channels formed by leech innexins. Both pannexins and leech innexins form large channels with single channel conductances of 400–500 pS (4, 5, 20). The channels formed by both pannexins and innexins are characterized by a complex gating mechanism. The channels, when activated, dwell preferably in multiple subconductance states with rare and brief sojourns to the full open and full closed states. Such a gating pattern typically is not observed in ion channels and can be seen as a signature for innexin and pannexin channels. Pannexin and innexin membrane channels are permeable to ATP and tracer dyes (4, 5, 53, 52, 55). Innexin and pannexin channels also share activation stimuli. Both types of channels are activated by mechanical stress, by increased cytoplasmic calcium concentration, by increased extracellular potassium ion concentration, and by voltage (5, 13, 33, 55, 60). Furthermore, innexin and pannexin channels have similar pharmacologies; they are inhibited by carbenoxolone (CBX), probenecid, brilliant blue G (BBG), ATP, and arachidonic acid (5, 12, 20, 48, 59). So far characterization of nonjunctional innexin channels is restricted to leech innexins and a report on caterpillar cells (36). Furthermore, no electrophysiological characterization of plasma membrane innexin channels in native cells has been reported to date. The study of nonjunctional innexin channels in organisms that are genetically amenable such as C. elegans and Drosophila would help advance our understanding of the function of these channels in physio-pathological processes.

In this study, we characterized a large mechanosensitive channel that is expressed in C. elegans touch-sensing neurons both in situ and in culture. We found that it has an apparent conductance of ~1 nS, it displays a voltage-dependent K+− selective subconductance state, and it is blocked by the pannexin inhibitors CBX, BBG, and probenecid (12, 48, 52, 59, 72). We also showed that touch neurons display ethidium bromide (EtBr) uptake that is blocked by the same inhibitors.

Address for reprint requests and other correspondence: L. Bianchi, Rm. 5133 Rosenstiel Bldg., 1600 NW 10th Ave., Miami, FL 33136 (e-mail: lbianchi@med.miami.edu).
Based on the channel properties, we conclude that the mechanosensitive channel in touch neurons is an innexin and that it functions as a plasma membrane channel. Furthermore, we show that nonjunctional innexin channels in *C. elegans* touch neurons are required to execute cell death induced by chemical ischemia. Demonstration that innexins can function as plasma membrane channels in a tractable model organism such as *C. elegans* opens new venues for the study of the patho-physiological function of homologous vertebrate pannexins in the context of a whole organism.

**MATERIALS AND METHODS**

*C. elegans* strain. Nematodes were maintained at 20°C on standard nematode growth medium seeded with *Escherichia coli* strain OP50 (11). Growth conditions were switched to Na22 bacteria and 8P agar plates for the cell culture (see below). The strain used in all the experiments was ZB154, which expresses integrated transgene zdIs5[punc-4::GFP].

*C. elegans* cell culture. *C. elegans* embryonic cells were cultured following a published procedure (15, 56). Briefly, large quantities of gravid adults were grown on enriched peptone plates (8P plates) following a published procedure (11). Growth conditions were switched to Na22 bacteria and 8P agar plates for the cell culture (see below). The strain used in all the experiments was ZB154, which expresses integrated transgene zdIs5[punc-4::GFP].

**RESULTS**

*C. elegans* touch neurons in situ and in culture express a large nonselective channel sensitive to mechanical stimuli. We undertook this study to look for mechanically gated channels in *C. elegans* body touch neurons. First, we conducted electrophysiological recordings on in situ *C. elegans* touch neurons, identified by expression of GFP under the control of mec-4 promoter (7). There are six body touch neurons in *C. elegans*, two anterior lateral microtubule cells (ALMs) and one anterior ventral microtubule cell (AVM) in the anterior part of the animal body and two PLMs and one posterior ventral microtubule cell (PVM) in the posterior. We used a previously described protocol to dissect the PLM touch neurons out of the animal body for patch-clamp experiments (38). Briefly, we glued a L4 animal onto a glass coverslip, released some of the positive hydrostatic pressure by nicking the cuticle near the vulva, and then made a small hole on the cuticle near a PLM.
touch neuron to release its cell body to the outside (Fig. 1A). In the inside-out configuration of the patch-clamp technique, we applied negative pressure to a patch of membrane and detected the activation of large channel that reversed at 0 mV in extracellular NaCl and intracellular KCl solutions (Fig. 1, B and C). Release of the negative pressure resulted in the closure of the channel (not shown). The channel conductances in four patches were 1.2, 2.3, 3.9, and 3.8 nS. We interpret these conductance levels as representing one and multiple channel openings, with possibly intermediate subconductance states.

Given the challenges of patch-clamp recordings in vivo in C. elegans, we continued our characterization of the mechanosensitive channel in cultured touch neurons (56). Embryonically derived ALM and PLM touch neurons are present in culture, represent ~0.5% of the population of cells, and differentiate recapitulating the properties they have in vivo (7, 56). Postembryonic neurons AVM and PVM are not present in culture. In touch neurons cultured in vitro for 4 days, application of negative pressure induced the activation of a mechanosensitive channel that resembled the one observed in situ (Fig. 2, A–D). Interestingly, application of positive pressure did not induce the activation of the large mechanosensitive channel (data not shown). The channel current reversed at 0 mV, and in the majority of the patches it had a conductance of 1 nS (Fig. 2E). In some patches we also detected a conductance of 2 nS, which may represent the conductance of two channels present in the same membrane patch (Fig. 2E). Interestingly, in several patches we observed that the channel appeared to transition to intermediate states. Moreover, in some patches we detected even larger conductance levels [2.7 ± 0.01 (n = 2), 3.2 ± 0.06 (n = 3), 3.9 ± 0.12 (n = 7), 4.9 ± 0.08 (n = 5), 5.9 ± 0.267, and 8.5 ± 0.177 (n = 3)], similarly to what we saw in situ, perhaps representing the opening of more than two channels. Taken together, these data show that C. elegans touch neurons in vivo and in culture express large mechanosensitive ion channels.

To investigate the selectivity properties of the large mechanosensitive channel, we perfused the intracellular and extracellular sides of the membrane patches with solutions of different ionic compositions. Thus we used both symmetrical and asymmetrical NaCl and KCl solutions as well as symmetrical Na-aspartate solutions. We found that the reversal potential of the large channel activated by negative pressure was 0 mV or a few mV away from 0 in all the experimental conditions (Table 1). These results suggested that the mechanosensitive channel we detect in touch neurons is poorly selective.

The mechanosensitive large channel is inhibited by the pannexin/innexin blockers. Channels formed by pannexins and connexins have a conductance ranging from 10 to ~450 pS, with the largest hemichannel being Cx50, which has a conductance of 470 pS (4, 5, 64). Recent electron microscopy studies performed on C. elegans innexin INX-6 heterologously expressed in insect Sf9 cells suggest that innexins are larger than pannexins and may have a larger conductance (39). Based on these published data, and on its single channel properties, we hypothesized that the large mechanosensitive channel that we identified in touch neurons, was an innexin channel. To test this hypothesis, we perfused inside-out patches with the pannexin/innexin blockers CBX, BBG, and probenecid. Note that even though these inhibitors have other targets (21, 28, 37, 70), the only common target is the pannexin channel (5, 12, 48, 52, 72). We found that application of 50 μM CBX reversibly inhibited

---

Fig. 1. Caenorhabditis elegans touch neurons express a large mechanosensitive channel. A: schematic representation of the experimental setup for patch-clamp experiments of touch neurons. The animal was glued onto a glass cover slip, part of the internal positive pressure was released by nicking the cuticle near the vulva with a glass rod, and the neuron cell body was exposed to the outside by puncturing the cuticle in the tail. B: representative family of currents recorded in an inside-out patch in a posterior lateral microtubule cell touch neuron. The membrane was held at 0 mV and stepped from -100 to +120 mV. C: same patch in B was subjected to 20 mmHg of negative pressure. Release of the negative pressure caused inactivation of the channel (not shown).
the large conductance mechanosensitive channel (Fig. 3, A–C). Similar results were obtained with 50 μM BBG and 50 μM probenecid. On average the pressure-induced current was inhibited 93.8 ± 6.7% (n = 10) by 50 μM CBX, 83.8 ± 6.9 by 50 μM BBG (n = 5), and 81.1 ± 9.4 by 50 μM probenecid (n = 5). Importantly, the mechanosensitive channel we detected in situ was also sensitive to CBX (59.7% ± 15.3 block; n = 3; Fig. 3D). To conclude, the pannexin/innexin inhibitors CBX, BBG, and probenecid block the large mechanically gated plasma membrane channel expressed in C. elegans touch neurons.

An innexin-dependent dye uptake pathway in touch neurons. Channels formed by both pannexins and innexins are permeable to small molecules and dyes, in addition to ions (52, 73). We thus wondered whether an innexin-dependent dye uptake occurred in C. elegans touch neurons. To test this idea, we incubated C. elegans cultured cells in a hypotonic solution containing the DNA-binding fluorescent dye EtBr. In hypotonic solution, EtBr was taken up by the cultured C. elegans cells including touch neurons identified by expression of GFP under the control of mec-4 promoter. Voltage steps were from −100 to +120 mV from a holding potential of 0 mV. C: 35 mmHg of negative pressure were applied to the same patch shown in B leading to the activation of large channels. D: release of the negative pressure leads to channel closing. E: current-voltage relationships of mechanosensitive channels similar to the ones shown in B. In the majority of patches we recorded channels of either −1 (●; n = 8) or −2 nS (□; n = 6). Different pressures were applied in each patch (from −20 to −50 mmHg). ●: Current-voltage relationship obtained from the same patches without application of negative pressure (n = 13).
Table 1. The mechanosensitive large channel is not selective

<table>
<thead>
<tr>
<th>Pipette/Bath</th>
<th>( E_{\text{rev}, \text{mV}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE1/CE2</td>
<td>(-9.5 \pm 1.9 \ (n = 4))</td>
</tr>
<tr>
<td>CE1/CE3</td>
<td>(-1.9 \pm 0.5 \ (n = 6))</td>
</tr>
<tr>
<td>CE6/CE5</td>
<td>(-6.1 \pm 4.3 \ (n = 2))</td>
</tr>
<tr>
<td>CE2/CE1</td>
<td>(+1 \pm 1 \ (n = 26))</td>
</tr>
<tr>
<td>CE4/CE4</td>
<td>(+8.4 \pm 1.8 \ (n = 12))</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; number of patches analyzed is shown in parentheses. Average reversal potentials \( E_{\text{rev}} \) of the mechanosensitive channel recorded in the inside-out configuration using the pipette and bath solutions are indicated. See MATERIALS AND METHODS for the ionic composition of the solutions.

experiments with at least 25 cells analyzed/condition), in touch neurons incubated in hypotonic solution (Fig. 4E). The partial effect of the blockers suggests the presence of an innexin-independent mechanisms of EtBr uptake in touch neurons in addition to an innexin-dependent one. Interestingly, we note that EtBr uptake that was insensitive to the blockers had the same amplitude of the EtBr uptake present in isotonic solution, further underscoring that this is mediated by mechanisms other than innexins (Fig. 4D and not shown). We also note that dye uptake by other cells was partially CBX sensitive (37.1 ± 14.2% inhibition; \( n = 3 \) experiments), suggesting that innexins are expressed on most \( C. \) elegans cells, as suggested by the overlapping and broad expression pattern of \( C. \) elegans innexin genes (1). Finally, dye uptake in touch neurons was unaffected by cell density, supporting that it occurred through hemichannels rather than gap junctions formed with neighboring cells. Thus the dye uptake experiments further support the idea that innexin plasma membrane channels are present in touch neurons and in most of the other \( C. \) elegans cells.

Properties of a smaller conductance state of the large mechanosensitive channel. In 9 out of 54 inside-out patches, we observed the transition from a small outward rectifying channel to the large mechanosensitive channel following application of mechanical forces. Figure 5 shows one example of such occurrence. The channel started off as a voltage-dependent outward rectifying channel that was activated at voltages positive to \(+20 \text{ mV}\) (Fig. 5A). When \(-30 \text{ mmHg}\) of pressure was applied to the membrane patch, the channel displayed larger amplitude short openings (Fig. 5B), which became predominant with application of \(-50 \text{ mmHg}\), while the small conductance channel was no longer visible in the recordings (Fig. 5C). Release of the negative pressure caused the reappearance of the small conductance channel and the disappearance of the large conductance one (Fig. 5D). Given that when the large channel was fully activated, the small conductance one was no longer visible, we conclude that they represented two states of the same channel, similar to what shown for Panx1 (71).

To further characterize the smaller conductance state, we performed experiments in which we switched the solution bathing the intracellular side of the membrane patch from a KCl to a NaCl solution. Figure 6A shows a membrane patch perfused on the intracellular side with a KCl solution mimick-

---

Fig. 3. Innexin channel blockers block the touch neurons mechanosensitive large conductance channel. A: example of currents through the large mechanosensitive channel recorded in the inside-out configuration. Physiological NaCl (CE1) and KCl (CE2) solutions in the pipette and bath, respectively. The currents were activated by voltage steps from \(-100 \) to \(+120 \text{ mV}\) in \(20-\text{mV}\) increments. Holding potential was \(0 \text{ mV}\), and \(-30 \text{ mmHg}\) were applied to activate the channel. B: the same membrane was exposed to \(50 \mu \text{M}\) carbenoxolone (CBX; Ref. 12). C: after wash with control solution the channel was activated again by application of \(-30 \text{ mmHg}\) of pressure. The same experimental conditions used in A were used in both B and C. D: average percentage block of the mechanosensitive channel at \(-60 \text{ mV}\) caused by perfusion with CBX (50 \(\mu \text{M}\)), brilliant blue G (BBG; 50 \(\mu \text{M}\)), and probenecid (50 \(\mu \text{M}\)) in culture and in vivo as indicated. Data are expressed as the means \(\pm \text{SE}; n = 10, 5, 5, \) and \(3, \) respectively.
ing the intracellular milieu (CE2, see MATERIALS AND METHODS) and on the extracellular side with a NaCl solution mimicking the extracellular environment (CE1, see MATERIALS AND METHODS). In this patch, two voltage-dependent channels are activated at potentials positive to $+20 \text{ mV}$. These channels resemble the channel that switched conductance state upon application of negative pressure (Fig. 5, the conductance was 63 pS).

When the membrane patch was perfused on the intracellular side with a NaCl solution (CE1), the single channel currents were no longer present (Fig. 6B) and returned only after reperfusion with the KCl solution (Fig. 6C). In patches where we perfused the intracellular side of the membrane with the KCl solution, we measured an average single channel conductance of 69 pS (Fig. 6D). Taken together our data suggest that the smaller channel that transitions into the large conductance upon application of negative pressure is an innexin subconductance state.

Our results also show that this subconductance state conducts preferably K$^+$ ions or is gated by K$^+$ and is voltage dependent. Our results are in line with previous observations on pannexins subconductance states. Indeed, Panx1 can function in two major conductance states: a large conductance state permeable to small molecules including ATP and a small K$^+$-selective voltage-dependent state permeable to ions only. The transition from the small to the large conductance state is stimulated by extracellular K$^+$ concentration (71).

To further test the idea that the smaller channel is an innexin-related conductance (Fig. 6E–G). To conclude, the innexin mechanosensitive channel displayed voltage-dependent subconductance states that conduct K$^+$ ions. This is another feature shared by the channel we detected in touch neurons and pannexin channels.

Innexin inhibitors rescue touch neurons from chemically induced ischemia. Pannexins have been implicated in neuronal death in mammals (reviewed in Ref. 17). Panx1 is activated by molecules that are released by cells following mechanical, ischemic, or inflammatory injury of the central nervous system. These include high K$^+$, Zn$^{2+}$, ATP, glutamate, fibroblast growth factors, and proinflammatory cytokines. Activation of Panx1 by these molecules leads to neuronal death through
necrotic or inflammatory pathways. We wondered whether the homology between the pannexin and the *C. elegans* innexin channels we identified in touch neurons extended to cellular function. We thus tested the effect of the innexin inhibitors on chemically induced ischemia. To induce chemical ischemia, we incubated cultured touch neurons in a solution at pH 6.5 containing 5 mM azide in the presence and absence of probenecid or BBG for 16 h. CBX was not used because it was found to be toxic to cells in long-term incubations. We found that incubation with 5 mM azide at pH 6.5 induced cellular toxicity. The number of touch neurons in wells treated with azide was significantly lower than the number of touch neurons in control solution and neuronal processes appeared generally shorter (pH 6.5) (Fig. 7, A–D). In wells in which we added probenecid or BBG, touch neurons were significantly spared from death (Fig. 7, E and F). The percentage of rescue by probenecid and BBG was 102.2 ± 15.3 and 75.9 ± 16.5 in four and two independently performed experiments, respectively. Since the only known common targets of probenecid and BBG in invertebrates are innexins, our data support the idea that innexins expressed on *C. elegans* touch neurons are involved in the molecular mechanism of cell death induced by chemical ischemia. We note that similar effects of azide and probenecid and BBG were observed on the other *C. elegans* cells (not shown), again underscoring that innexins have a wide expression pattern in *C. elegans* cells (1). Taken together, our results suggest a parallelism between pannexin and innexin hemichannels both at the levels of single channel features and function in the context of a cell. Given the fact that *C. elegans* is a genetically amenable and powerful model system, we work sets the ground for exploiting this model system to further our understanding of innexin hemichannels and consequently vertebrate pannexins.

### DISCUSSION

In the present study we investigated the biophysical and pharmacological features of a mechanosensitive channel expressed in *C. elegans* touch neurons. We found that this channel is gated by application of negative pressure in inside-out patches. The mechanosensitive channel has an apparent conductance of ∼1 nS but also opens in subconductance states. Interestingly, the subconductance state of 69 pS appears to have some K⁺ selectivity, while the larger channel is nonselective. Moreover, we found that the large channel is blocked by the pannexin/innexin blockers CBX, BBG, and probenecid and the small conductance channel is blocked by CBX (other blockers were not tested). Taken together our work supports that the mechanically gated channel we detect in *C. elegans* touch neurons is formed by an innexin that functions as plasma membrane channel. The parallelism between the channel we detect in touch neurons and pannexins extends to its cellular function as well, as our data show its involvement in cell death induced by chemical ischemia.

Innexins as gap junction proteins. Expression in heterologous systems and in vivo experiments has shown that innexins form gap junctions. For example, *Drosophila* Shak-B and Inx-2 form electrically active gap junctions between paired *Xenopus* oocytes (47). Similarly, *C. elegans* inx-3 forms intercellular channels between paired *Xenopus* oocytes with properties that resemble those of connexons, including sensitivity to cytoplasmic acidification and to transjunctional voltage (31). Starich et al. (66) also provided evidence that *C. elegans* Inx-3 forms gap junctions in vivo. With the use of immunoelectron-microscopy, anti-INX-3 antibodies were shown to bind to structures that resemble gap junctions in the *C. elegans* pharynx (65). Finally, in vivo electrophysiological experiments...
have demonstrated that body-wall muscle cells of *C. elegans* are electrically coupled and that innexin *unc-9* is required for generation of cell-to-cell currents (32). It thus is established that invertebrate innexins, despite sharing no sequence homology with vertebrate connexins, are their functional homologs and form gap junction channels that mediate intercellular communication.

**Innexins are pannexin homologs.** Pannexins were identified in vertebrates in an in silico search for homologs of invertebrate innexins (13, 29, 40). Besides sharing a similar membrane topology (4 transmembrane domains and intracellular NH2 and COOH termini), pannexins share some homology with innexins (25–34% identity, 36–46% homology).

While under extreme experimental conditions (48-h pairing period) in heterologous expression systems pannexins can form gap junctions at low rate (13), their ability to form gap junctions in vivo has not been demonstrated. Indeed, pannexins have been suggested to function exclusively as channels in the nonjunctional plasma membrane. Evidence that support that pannexins function as plasma membrane channels includes the following: 1) electron microscopy and fluorescence showing that pannexins are not strictly localized at cell-to-cell contacts, rather they are uniformly distributed on the cell surface in unpolarized cells (62); 2) immunolocalization of pannexin proteins at the apical surface of polarized cells including erythrocytes, macrophages, and T cells (33, 42, 57); 4) glycosylation of the first and second extracellular loops of Panx1 and Panx3, respectively, with large sugar moieties most likely to hinder the formation of gap junctions (8, 9, 45); and 5) pannexin-dependent ATP release from a variety of cell types (4, 25, 33, 49). Moreover, while Panx1 can form gap junctions when expressed in oocytes, the coupling currents are small and the time required for gap junction formation between two oocytes is unusually long (8, 9, 13). Taken together, these data indicate that pannexins most likely function physiologically as plasma membrane channels. As plasma membrane channels, pannexins display large conductance, are permeable to ions and small molecules up to 1–2 kDa (4, 73), and are activated by voltage, extracellular K+, a low oxygen environment, and mechanical forces (4, 33, 60, 63). In addition, pannexin channels can be activated by ATP and glutamate through their respective receptors (34, 35, 69). Studies in heterologous expression systems and native cells including neurons and astrocytes have shown that pannexin 1 functions as ATP release channel (4, 25,
and associates with ATP-gated channels P2X7 to create a positive feedback molecular mechanism that can lead to cell death when activated (27, 34, 44). Cell death proceeds through activation of the inflammasome, a molecular platform that leads to caspase-1 activation and release of IL-1β (44, 60). These data support that pannexins function as plasma membrane channels in patho-physiological processes such as cell death and inflammation.

Are innexins plasma membrane channels? Some innexins are expressed in cells that migrate, suggesting that they may function as plasma membrane channels rather than gap junction channels (1). Also, innexins have been experimentally shown to have gap junction-unrelated functions. For example, C. elegans innexin unc-7 functions cell autonomously to promote the development of the active zones in GABAergic motoneurons (76). Furthermore, unc-7 functions with the NCA cation leak channel to modulate neuronal activity, and this activity is independent from gap junction formation (10). Taken together these data suggest that at least some innexins function as plasma membrane channels. However, no previous electrophysiological studies in native cells have demonstrated innexin plasma membrane channel activity. Our results show that the innexin channel expressed in C. elegans touch neurons has a conductance that is larger than that of Panx1 (1 vs. 0.5 nS) or connexins (270 pS). These data are consistent with the larger molecular structure of innexins (39). Interestingly, we found that the channel opens also to a subconductance state that is voltage dependent and more K+ selective. Similar subconductance states have been observed for Panx1 under certain experimental conditions including lack of mechanical

Fig. 7. Innexin inhibitors spare C. elegans cells from chemically induced ischemia. A–D: photographs of cultured touch neurons incubated in the indicated solutions. Touch neurons were identified by expression of GFP under the control of the mec-4 promoter. E: number of cells/field was averaged for the conditions indicated on the x axis. The same number of cells was plated in each well at day 0. Forty-eight hours later, cells were incubated with the indicated solutions, and after 16 h they were fixed and photographed with a ×63 objective. Touch neurons were identified by expression of GFP under the control of the mec-4 promoter. Thirty fields were scored per condition. Average numbers of touch neurons were as follows: 6.3 ± 0.4 at pH 7.3, 7.1 ± 0.4 at pH 6.5, 3.4 ± 0.2 at pH 6.5 + 5 mM azide (NaN₃), 5.9 ± 0.4 at pH 6.5 + 50 μM probenecid, 5.8 ± 0.3 at pH 6.5 + 5 mM azide + 50 μM probenecid, 5.8 ± 0.3 at pH 6.5 + 10 μM BBG, and 5.7 ± 0.3 at pH 6.5 + 5 mM azide + 10 μM BBG. **P < 0.01 comparing with pH 6.5 + 5 mM azide, by ANOVA, Tukey’s HSD test. F: percentage of rescue by probenecid and BBG was calculated as follows: number of touch neurons in azide plus probenecid (or BBG) minus number of touch neurons in azide divided by the number of touch neurons at pH 6.5 minus the number of touch neurons in azide. The percentage of rescue by probenecid and BBG was 102.2 ± 15.3 and 75.9 ± 16.5 in 4 and 2 independently performed experiments, respectively.
forces, although their physiological significance is still not understood (4, 33, 71).

What could be the role of a plasma membrane innexin channel in \textit{C. elegans} touch neurons? The chemically induced ischemia experiments support that touch neurons innexins are involved in pathological cell death, similarly to pannexins (17, 22). More experiments will have to be performed to identify the molecular pathway they operate in. It is also intriguing that the innexin channel we identified and characterized in \textit{C. elegans} touch neurons is mechanosensitive. \textit{C. elegans} touch neurons are specialized sensory neurons that detect mechanical forces applied onto the body of the worm. Genetic, imaging, and electrophysiological studies have shown that ion channels of the degenerin/epithelial Na channel (DEG/ENaC) family named MEC-4 and MEC-10 and DEGT-1 are expressed in these neurons and mediate sensitivity to “gentle” and to “harsh” touch stimulation, respectively (2, 14, 38, 68). However, the dependence on DEG/ENaC channels of the full range of mechanical stimulations has not been fully demonstrated. Thus it is possible that the innexin channel expressed in touch neurons mediates responses to higher intensity stimuli or other types of mechanical stimulations. Future experiments in which the innexin gene responsible for the innexin conductance in touch neurons is identified and deleted or altered will address this question. Our data show that the innexin channel expressed in touch neurons gates also independently from mechanical forces in a subconductance state at voltages higher than +20 mV. Thus the activity of this channel may, in addition to sensing mechanical force, be required for other basic neuronal functions. Note that, our in situ experiments support that the plasma membrane innexin channel is not a feature of embryonic cells, or cells cultured in vitro, but rather an intrinsic property of touch neurons. While we cannot exclude the possibility that dissection of the touch neuron cell body from the animal disrupts gap junctions located in the cell body, electron microscopy studies do not support the presence of gap junctions on the cell body of touch neurons (Hall D, personal communication).

We report here the electrophysiological characterization of an innexin channel that acts as a functional plasma membrane channel in \textit{C. elegans} touch neurons. Our data significantly extend our understanding of innexins at the functional level and suggest that \textit{C. elegans} touch neurons could be used as a model to advance our understanding of the physiological function of innexins in the context of an organism. Given the homology between innexins and pannexins, these type of studies are likely to shed light onto the still largely elusive role of pannexins in vertebrates.

ACKNOWLEDGMENTS

We thank M. Driscoll for providing the ZB154 \textit{C. elegans} strain and for support during the initial experiments and Peter H. Larsson for critical reading of the article.

GRANTS

This work was supported by American Cancer Society Grant RGS-09-043-01-DDC and National Institute of Neurological Disorders and Stroke Grant ROI-NS-070969 (to L. Bianchi) and a Craig H. Nielsen Foundation Grant (to G. Dahl).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.S. and L.B. performed experiments; R.S. and L.B. analyzed data; R.S., G.D., and L.B. interpreted results of experiments; R.S. and L.B. prepared figures; R.S. and L.B. drafted manuscript; R.S., G.D., and L.B. approved final version of manuscript; G.D. and L.B. conception and design of research; G.D. and L.B. edited and revised manuscript.

REFERENCES


AJP-Cell Physiol • doi:10.1152/ajpcell.00246.2014 • www.ajpcell.org


