Ionic Basis of the Resting Membrane Potential and Action Potential in the Pharyngeal Muscle of Caenorhabditis elegans

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Franks, Christopher J., Darrel Pemberton, Irina Vinogradova, Alan Cook, Robert J. Walker, and Lindy Holden-Dye. Ionic basis of the resting membrane potential and action potential in the pharyngeal muscle of Caenorhabditis elegans. J Neurophysiol 87: 954–961, 2002; 10.1152/jn.00233.2001. The pharynx of C. elegans is a rhythmic, actively contracting muscle that pumps bacteria into the gut of the nematode. This activity is maintained by action potentials, which qualitatively bear a resemblance to vertebrate cardiac action potentials. Here, the ionic basis of the resting membrane potential and pharyngeal action potential has been characterized using intracellular recording techniques. The resting membrane potential is largely determined by a K⁺ permeability, and a ouabain-sensitive, electrogenic pump. As previously suggested, the action potential is at least partly dependent on voltage-gated Ca²⁺ channels, as the amplitude was increased as extracellular Ca²⁺ was increased, and decreased by L-type Ca²⁺ channel blockers verapamil and nifedipine. Barium caused a marked prolongation of action potential duration, suggesting that a calcium-dependent pacemaker potential. In addition, the persistence of a prominent current that activates at negative membrane potentials and is blocked by Na⁺/K⁺ channel blockers procaine and quinidine, and the increase in action potential frequency caused by veratridine, a toxin that alters activation of voltage-gated Na⁺ channels, point to the involvement of a voltage-gated Na⁺ channel. Voltage-clamp analysis is required for detailed characterization of this current, and this is in progress. Nonetheless, these observations are quite surprising in view of the lack of any obvious candidate genes for voltage-gated Na⁺ channels in the C. elegans genome. It would therefore be informative to re-evaluate the data from these homology searches, with the aim of identifying the gene(s) conferring this Na⁺, quinidine, and veratridine sensitivity to the pharynx.

INTRODUCTION

The availability of the complete genome sequence for C. elegans presents the opportunity for a comprehensive investigation of the molecular determinants of cell excitability including the contribution of entire gene families (Bargmann 1998). For example, homology searches reveal more than 70 genes for voltage-gated Na⁺ channels. Surprisingly, many of these genes have been expressed and the resultant channel characterized in Xenopus oocytes (e.g., Kunkel et al. 2000; Wayne-Davis et al. 1999). The properties of the remainder are unknown. However, intracellular recordings have been made from excitable cells in C. elegans, from neurons (Goodman et al. 1998), pharyngeal muscle (Avery et al. 1995; Pemberton et al. 2001), and from somatic muscle (Richmond and Jorgensen 1999). This provides the opportunity, by comparison of wild-type currents with those recorded from mutant strains, to delineate the contribution of specific genes to native currents and cell excitability.

The currents recorded from C. elegans neurons consist of voltage-activated K⁺ and Ca²⁺ currents (Goodman et al. 1998). There is no evidence for a Na⁺ current, which would appear to corroborate the lack of an obvious candidate for a voltage-gated Na⁺ channel in the genome. The genetic determinants for the currents recorded from C. elegans neurons have not yet been investigated. More progress in this respect has been made from recordings of pharyngeal muscle. These muscles exhibit action potentials with a long plateau phase, qualitatively similar to vertebrate cardiac action potentials. Loss of function mutations in the gene egl-19, which encodes a putative voltage-gated calcium channel α subunit, decreases the slope of the initial phase of the action potential and the duration of the plateau phase (Lee et al. 1997). Furthermore, a K⁺ channel, with unusual kinetic properties, encoded by the gene exp-2 is implicated in the fast repolarization of the action potential (Wayne-Davis et al. 1999).

Despite the informative studies described above, there is still no detailed description of the properties of wild-type pharyngeal muscle. For example, the ionic dependence of the resting membrane potential and action potential has not been described. The study described here provides this information, and thus lays the foundation for further detailed comparisons with mutant strains to delineate the function of C. elegans ion channels. Surprisingly, we report that the pharyngeal action potential is dependent on the presence of extracellular Na⁺. The possibility that this may provide physiological evidence for the presence of a voltage-gated Na⁺ channel in C. elegans is discussed.

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METH ODS

Dissection procedures

C. elegans (N2 Bristol strain) were cultured and adult hermaphrodite animals picked from 3- to 5-day-old plates. The worms were placed in Dent’s saline (composition in mM: 140 NaCl, 6 KCl, 1 MgCl₂, 3 CaCl₂, 10 HEPES; and 5 d-glucose; pH 7.4) and transiently cooled to immobilize them. The anterior region was sectioned from the rest of the body at the level of the pharyngeal intestinal valve and transferred to a custom-built perfusion chamber (volume 500 µl) on a glass cover slip.

Electrophysiological recordings

The recording chamber was mounted on an inverted microscope and perfused via gravity feed with Dent’s saline at a rate of 5 ml/min. The preparation was secured by means of a suction electrode applied to the terminal bulb region of the pharynx and impaled with an aluminosilicate glass microelectrode (1.0-mm OD glass, with filament, pulled on a Sutter P-2000 microelectrode puller; 60–80 MΩ when filled with 4 M KAcetate, 10 mM KCl) connected to an AxoClamp 2B recording amplifier. The reference electrode was a silver chloride-coated silver pellet in 3 M KCl connected to the recording chamber by an agar bridge. All drugs were applied by addition to the perfusate. Data were acquired and analyzed using pClamp 7 (Axon Instruments). Values are expressed as means ± SE. The pharyngeal muscle action potentials were of variable frequency, amplitude, and duration (Fig. 1A) despite the fact that recordings were made from similar animals, i.e., adult hermaphrodites from 3- to 5-day-old plates. This is unlikely to reflect impalement of different muscle cells as all recordings were made from the terminal bulb muscle, either pm6 or pm7. Within the same animal, action potentials recorded from the terminal bulb did not vary greatly. Analysis of 16 recordings from individual animals, measuring the average properties of at least 12 action potentials in each, gave values of –68 ± 2 mV for resting membrane potential, 86 ± 4 mV for spike amplitude, and 0.26 ± 0.23 s for spike duration. Because of this variability in the wild-type action potential between animals, all comparisons between action potential shape under different experimental conditions were performed as “paired” experiments, on the same pharynx.

Ionic dependence of the pharyngeal muscle resting potential and action potential

The effect of changing the extracellular concentrations of ions that may contribute to the membrane potential and pharyngeal action potential shape was tested by switching the perfusate to a modified Dent’s saline, and then back to the control saline to check for reversibility of any observed effect. A summary of the results of the effects of changing the concentration of extracellular ion concentrations is shown in Table 1.

Increasing extracellular K⁺ concentration from 3 to 12 mM elicited a depolarization to –58 mV (n = 6; Fig. 1B). The membrane potential decreased by 39 mV for a 10-fold change in extracellular K⁺, which is less than the 58 mV expected from the Nernst equation if the membrane potential was entirely dependent on K⁺. In this respect, it should be noted that was in the range –80 to –65 mV. For 50 recordings the mean resting membrane potential was –74.0 ± 0.8 mV (mean ± SE). The pharyngeal muscle action potentials were of variable frequency, amplitude, and duration (Fig. 1A) despite the fact that recordings were made from similar animals, i.e., adult hermaphrodites from 3- to 5-day-old plates. This is unlikely to reflect impalement of different muscle cells as all recordings were made from the terminal bulb muscle, either pm6 or pm7. Within the same animal, action potentials recorded from the terminal bulb did not vary greatly. Analysis of 16 recordings from individual animals, measuring the average properties of at least 12 action potentials in each, gave values of –68 ± 2 mV for resting membrane potential, 86 ± 4 mV for spike amplitude, and 0.26 ± 0.23 s for spike duration. Because of this variability in the wild-type action potential between animals, all comparisons between action potential shape under different experimental conditions were performed as “paired” experiments, on the same pharynx.

TABLE 1. Effect of extracellular ion concentration on the resting membrane potential and action potential of the pharyngeal muscle

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mM)</th>
<th>Resting Membrane Potential (mV)</th>
<th>Spike Amplitude (mV)</th>
<th>Spike Duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>3</td>
<td>–7.0 ± 0.5</td>
<td>10 ± 1</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>3</td>
<td>–7.0 ± 0.5</td>
<td>10 ± 1</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>K⁺</td>
<td>3</td>
<td>–73.0 ± 0.8</td>
<td>52 ± 2</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>K⁺</td>
<td>12</td>
<td>–58.0 ± 1.2</td>
<td>12 ± 1</td>
<td>0.2 ± 0.03</td>
</tr>
</tbody>
</table>

FIG. 1. Electrical properties of the pharyngeal muscle. A: examples of pharyngeal action potentials recorded from 4 wild-type animals. Resting membrane potentials for these cells were from top to bottom (from top to bottom) –80, –81, –78, and –76 mV. B and C: the effect of changing extracellular ion concentrations on the resting membrane potential. B: a Nernstian plot of the relationship between extracellular K⁺ concentration and the resting membrane potential. The solid line shows the regression line for a slope of 39 mV (R² = 0.98). C: a Nernstian plot of the relationship between extracellular Na⁺ and Cl⁻ concentration and resting membrane potential.
K elicited a transient excitation and burst of action potentials, but decreased from 1.5 to 10 mM (Fig. 2). There was an inverse relationship between extracellular Ca\(^{2+}\) concentration and spike duration (Fig. 2C). The mean action potential duration significantly decreased (measured from the 1st inflection from resting membrane potential to the return to resting membrane potential) when extracellular Ca\(^{2+}\) was increased from 1.5 to 3 mM (n = 8; P = 0.0195, paired Student’s t-test; Fig. 2C) and up to 10 mM (n = 4).

As action potentials persisted in zero Ca\(^{2+}\), we then tested the possibility that Na\(^{+}\) may play a role. Replacement of extracellular Na\(^{+}\), with the nonpermeant cation glucosamine, resulted in a depolarization (Fig. 1C) and a decrease in action potential slope followed by complete cessation of action potential generation (n = 8; Fig. 2D). It was more difficult to elicit a spike in zero Na\(^{+}\) by intracellular injection of depolarizing current than in zero Ca\(^{2+}\). Of six cells, only one elicited a spike in zero Na\(^{+}\) with injection of depolarizing current, with a threshold of about −40 mV. The dependence of the pharyngeal action potential on extracellular Na\(^{+}\) was studied in more detail, by determining the effect of partial replacement of Na\(^{+}\) by N-methyl-D-glucamine, for Na\(^{+}\) concentrations of 35, 50, and 70 mM. Reducing extracellular Na\(^{+}\) to 35 mM (n = 6), 50 mM (n = 8), and 70 mM (n = 6) all caused a reduction in the amplitude and slope of the spikes, rapidly followed by complete abolition of action potentials (Fig. 2E). The neurotransmitter, serotonin (5-HT), stimulates pharyngeal muscle by increasing action potential frequency (Franks et al. 1997), and application of 5-HT could reinstate action potentials in 50 mM Na\(^{+}\) (n = 4; data not shown) and in 70 mM Na\(^{+}\) (n = 6). The effect on the action potential slope was quantified by measuring the resting membrane potential to 0 mV giving a slope in millivolts per millisecond, comparing action potentials within 30 s before and after transfer to 70 mM Na\(^{+}\) perfusate, in the presence of 5-HT. There was a significant reduction from 5.6 ± 1.0 mV/ms in 140 mM Na\(^{+}\) to 2.7 ± 1.1 mV/ms in 70 mM Na\(^{+}\) (P = 0.0242; paired Student’s t-test; n = 6).

Replacement of 120 mM NaCl with 120 mM LiCl also caused a depolarization from −70.2 ± 2.6 to −61.4 ± 2.4 mV (n = 8), a decrease in spike amplitudes and reduction in the slope of the rising phase of action potentials from 1.3 ± 0.2 to 0.6 ± 0.1 mV/ms. This effect was accompanied by an increase in the frequency of action potentials (data not shown).

### Effects of ion channel blockers

A summary of the results of the effects of ion channel blockers on the resting membrane potential, and action potential properties, is shown in Table 1.

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| **Table 1. Summary of the effects of extracellular ions and channel blockers on the resting membrane potential (E_M) and action potential of C. elegans pharynx** |
| **Condition** | **Δ E_M** (mV) | **Δ Duration** (%) | **Δ Overshoot** (%) | **Δ Frequency** (%) |
| 0 Na\(^{+}\) * | +5 | no APs | no APs | no APs |
| 0 Ca\(^{2+}\) | −5 | +85,765† | −51 | n.d. |
| 10 mM K\(^{+}\) | +21 | n.d. | −5 | −83 |
| 14 mM Cl\(^{−}\) ‡ | −2 | +20 | +2 | −30 |

For each “condition,” paired measurements of membrane potential, action potential (AP) duration, overshoot, and frequency were made from at least 3 cells, in Dent’s saline (control) and in the test solution as listed. The change in membrane potential is given in mV. For the other parameters the changes are expressed as a percentage of the control value. Tetrodotoxin (100 μM), saxitoxin (200 μM), and saxitoxin (400 nM) had no effect. *Na\(^{+}\) was replaced with N-methyl-D-glucamine. †Statistical significance from control was determined using the paired Student’s t-test with a significance level of P < 0.05. ‡NaCl was replaced with Nalsethionate. §Quinidine abolished action potentials after 20 s, therefore measurements of the action potential parameters were made immediately before their disappearance.

The Na\(^{+}\)-K\(^{+}\) ATPase blocker, ouabain (>100 μM), depolarized the muscle by 34 ± 9 mV (n = 4). Increasing extracellular K\(^{+}\) also resulted in a decrease in the amplitude of the pharyngeal action potential afterhyperpolarizations, and decrease in spike frequency (n = 6). Decreasing extracellular chloride elicited a transient excitation and burst of action potentials, but little change in membrane potential (n = 7; Fig. 1C).

Reducing extracellular Ca\(^{2+}\) from 3 mM to zero had little effect on resting membrane potential. However, the effect on the pharyngeal action potential was very marked (n = 30; Fig. 2A) and consistent with previous reports, suggesting that the pharyngeal action potential is Ca\(^{2+}\) dependent (Lee et al. 1997). There was a transient increase in action potential frequency, followed by a decrease in spike amplitude and a prolongation of the action potential plateau. Spike generation continued in 23 of 30 preparations, even after prolonged exposure (15 min) to 0 Ca\(^{2+}\) saline (the average exposure time was 5.9 min). In two cells in which spiking ceased, long-duration spikes, that overshoot 0 mV, could be induced by injection of depolarizing current, with a threshold of about −55 mV. The effects on action potential amplitude and duration were also observed when Ca\(^{2+}\) concentration was decreased from 3 to 1.5 mM, although less marked than with Ca\(^{2+}\) removal (Fig. 2, B and C). Conversely, as extracellular Ca\(^{2+}\) was increased, there was an increase in spike amplitude. The action potential overshoot (measured as the amplitude of the spike >0 mV), increased as extracellular Ca\(^{2+}\) was increased from 1.5 to 10 mM (Fig. 2B).

For each “condition,” paired measurements of membrane potential, action potential (AP) duration, overshoot, and frequency were made from at least 3 cells, in Dent’s saline (control) and in the test solution as listed. The change in membrane potential is given in mV. For the other parameters the changes are expressed as a percentage of the control value. Tetrodotoxin (100 μM), saxitoxin (200 μM), and saxitoxin (400 nM) had no effect. *Na\(^{+}\) was replaced with N-methyl-D-glucamine. †Statistical significance from control was determined using the paired Student’s t-test with a significance level of P < 0.05. ‡NaCl was replaced with Nalsethionate. §Quinidine abolished action potentials after 20 s, therefore measurements of the action potential parameters were made immediately before their disappearance.

### Ion channel blockers

- **4-AP 100 μM**: −2, +68, +17, +682†
- **Barium 1 mM**: −2, +127, −46, +329
- **Barium 1 mM**: −2, +115†, −57†, n.d.
- **Quinidine 100 μM**: +5, +134, n.d.
- **Quinidine 200 μM**: +8, +137†, −21, no APs
- **Verapamil 20 μM**: 0, −42†, −20, +25
- **Nifedipine 20 μM**: +2, −21†, −89†, −3
- **Veratridine 2 μM**: 0, 0, +27, +444†

4-Aminopyridine (4-AP) was tested from 10 μM to 1 mM (n = 3). At 10 μM, it increased action potential frequency, with no consistent effect on spike duration (Fig. 3). At 100 μM 4-AP, spikes occurred in bursts (Fig. 3, C and D). Up to 100 μM, there was no significant effect on resting membrane potential (control, −77 ± 2 mV; with 100 μM 4-AP, −79 ± 3 mV; n = 3; mean ± SE). However, at 1 mM 4-AP the membrane failed to repolarize completely during the bursts, leading to prolonged membrane depolarization (Fig. 3D).

Barium increased action potential duration with a threshold of around 100 μM (n = 4; Fig. 4, A and B), and action potential frequency was also increased in three of these cells. This was accompanied by a reduction in action potential amplitude as measured by the reduction of the overshoot (n = 4). However,
the most notable effect was the appearance of very extended duration action potentials, lasting in excess of 1 s, interrupted by brief repolarizations (Fig. 4C; n = 4). The effects of barium were reversed on washing.

Verapamil and nifedipine had similar effects on the pharyngeal action potentials. Both caused a decrease in spike duration and a decrease in spike amplitude (n = 8; Fig. 5, A and B). At higher concentrations (>100 μM), verapamil caused a complete and irreversible block of spikes. Small potentials of approximately 10 mV persisted (n = 3; Fig. 5C).

Iberitoxin had no effect at up to 400 nM (with a 30-min incubation; n = 3). Paxilline also had no effect at 10 μM.

High concentrations of tetrodotoxin (100 μM; n = 2) did not affect the pharyngeal action potentials. Saxitoxin (200 μM; n = 5) also had no effect on the shape of the pharyngeal action potentials. However, procaine, at concentrations above 1 mM, completely blocked spikes (n = 2; data not shown).

Addition of 5 mM Cs⁺ to the perfusate caused a depolarization from −72.9 ± 4.4 to −65.3 ± 5.6 mV and a decrease in overshoot from 29.3 ± 2.4 to 23.8 ± 2.6 mV (n = 5) but did not cause a significant change in any other parameters of the action potentials (data not shown).

Quinidine was tested at concentrations from 10 to 500 μM. At 200 μM a depolarization was observed, and cessation of action potentials occurred (Fig. 6; n = 3). Immediately prior to cessation of spiking, the action potentials were greatly extended in duration (Table 1). At lower concentrations, there was no consistent effect on spike amplitude, but spike duration was increased in all four cells at 100 μM quinidine (Fig. 6B).

Veratridine, with a threshold at 2 μM, increased both the action potential frequency (n = 5; Fig. 7A) and the slope of the spike rising phase from 1.4 ± 0.2 to 2.9 ± 0.6 mV/ms (n = 4), but it had no detectable effect on spike duration (control 232 ± 36 ms; with 2 μM veratridine 228 ± 31 ms; n = 5). The increase in frequency was associated with the appearance of bursts of spikes (Fig. 7B). In low Na⁺ (35 mM), which abolished spikes, veratridine (10 μM) was not able to reinstate action potentials (data not shown; n = 5). In contrast, in nominally Ca²⁺-free solutions, veratridine (20 μM) increased action potential frequency (n = 8; Fig. 7C) and decreased spike duration (control duration in Dent’s saline, 208 ± 49 ms; in zero Ca²⁺, 12,253 ± 3,607 ms; in zero Ca²⁺ with 20 μM veratridine, 1,843 ± 1,109 ms, P < 0.05 with respect to control; n = 6).
DISCUSSION

In this study intracellular voltage recordings were made from the spontaneously active terminal bulb of *C. elegans* pharyngeal muscle. The resting membrane potential was more negative than previously reported. For example, in a similar saline composition, a resting membrane potential of $-110 \text{ mV}$ was reported (Davis et al. 1995). The reason for this discrepancy is unclear but probably reflects the technical difficulty of obtaining a stable placement of an intracellular recording electrode in this small, rhythmically active muscle. The muscle contractions are coupled one-to-one with pharyngeal action potentials. There is evidence that this activity is myogenic, as pharyngeal pumping persists, albeit at reduced frequency, after all the pharyngeal neurons have been ablated (Avery and Horvitz 1989).

Experiments were conducted in which the extracellular concentrations of the major cations, Na$^+$, K$^+$, and Ca$^{2+}$, and the anion Cl$^-$ were either removed, or replaced with nonpermeant ions. All of the cells in the pharyngeal muscle preparation were subjected to these changes, including muscles and marginal cells coupled to the terminal bulb via gap junctions, and neurons with synaptic input onto the muscle of the terminal bulb. Therefore it is possible that some of the responses observed may be due to an indirect action. However, with this caveat in mind, these studies provide some insight into the ionic dependence of the resting membrane potential and the pharyngeal action potential. The membrane potential was de-
The resting properties of *C. elegans* pharynx described in this study are different from those for the pharynx of the large parasitic nematode *Ascaris suum* (Del Castillo and Morales 1967; Del Castillo et al. 1964). For *A. suum*, the resting membrane potential is around −40 mV and strongly dependent on the concentration of extracellular anions. However, qualitatively, the pharyngeal action potentials of *C. elegans* are similar to those of *A. suum*. Both have a long plateau phase and both muscles also generate unusual, K⁺-dependent, negative-going, potentials to a membrane potential near to −90 mV (Avery and Thomas 1997; Byerly and Masuda 1979; Del Castillo et al. 1964), which are particularly evident in recordings from *A. suum* because of the less negative resting membrane potential (Del Castillo et al. 1964). These hyperpolarizing potentials result in rapid muscle relaxation and thereby...
provide the “power-stroke” to move food into the intestine against the internal hydrostatic pressure of the worm.

Removal of external Ca\(^{2+}\) modified *C. elegans* pharyngeal action potentials. Amplitude, duration, and frequency were affected. Frequency transiently increased, and this can be explained by increased membrane excitability due to the loss of Ca\(^{2+}\) binding from the membrane. Amplitude was decreased as extracellular Ca\(^{2+}\) was decreased. However, in 23 of 30 cells, complete abolition of spikes was not observed. In those cells that did stop spiking, it was possible to elicit a spike by injection of depolarizing current. The threshold was relatively low, around −55 mV, and the duration of the spike was very extended. These experiments were carried out in “nominally” free Ca\(^{2+}\), as no chelator was included in the perfusate, and it is possible that sufficient Ca\(^{2+}\) was still present to support a Ca\(^{2+}\)-dependent action potential. Alternatively, Na\(^+\) may permeate voltage-gated Ca\(^{2+}\) channels when extracellular Ca\(^{2+}\) is reduced. The effect of decreasing extracellular Ca\(^{2+}\) on action potential duration was somewhat anomalous, as the duration increased as extracellular Ca\(^{2+}\) was decreased, i.e., the opposite effect to that expected if the plateau potential is determined by a Ca\(^{2+}\) current. One explanation is that the repolarization phase is partly determined by a Ca\(^{2+}\)-dependent K\(^+\) channel. Two genes encoding putative Ca\(^{2+}\)-dependent K\(^+\) channels have been identified in the *C. elegans* genome (Wei et al. 1996), and a Ca\(^{2+}\)-dependent K\(^+\) channel is expressed in pharynx (Yuan et al. 2000). However, neither iberiotoxin nor paxilline (both blockers of the high conductance Ca\(^{2+}\)-activated K\(^+\) channel) had any detectable effect on the pharyngeal action potentials. Barium, a weak activator of Ca\(^{2+}\)-dependent K\(^+\) channels (Meech 1974), caused a marked prolongation of the action potential, and this may be consistent with a role for these channels in repolarization. But the possibility that this is entirely caused by a barium block of delayed rectifier K\(^+\) channels cannot be discounted.

An alternative explanation for the failure of repolarization during the action potential in low Ca\(^{2+}\) may be that this process depends on neurotransmitter release. In fact, this mechanism was proposed some years ago by Avery (1993). He suggested that the glutamatergic M3 neuron is activated by muscle activity during the plateau phase of the action potential. The subsequent glutamate release would be predicted to hyperpolarize the muscle when it is near to 0 mV, activating the K\(^+\) channel EXP-2 (Wayne-Davis et al. 1999) and thereby facilitating repolarization.

Previous studies have suggested that a major determinant of the pharyngeal action potential is an L-type Ca\(^{2+}\) channel (Lee et al. 1997). Gain-of-function mutations in *egl-19*, which encodes a putative α subunit of an L-type Ca\(^{2+}\) channel, resulted in a prolongation of the action potential plateau, whereas loss-of-function resulted in a decrease in the initial slope of the action potential. In agreement with this, we found that the L-type Ca\(^{2+}\) channel blockers, verapamil and nifedipine, both reduced action potential amplitude and duration. However, a complete block of action potentials was only observed at high concentrations of verapamil. Interestingly, barium also decreased spike amplitude, suggesting that this Ca\(^{2+}\) channel does not have a large conductance for barium, unlike mammalian L-type Ca\(^{2+}\) channels but similar to some invertebrate voltage-gated Ca\(^{2+}\) channels (e.g., Jeziorski et al. 1998).

Ion replacement produced the surprising result that the generation of the pharyngeal action potential is dependent on external Na\(^{+}\). Removal of extracellular Na\(^{+}\) caused a complete abolition of action potentials, which reversed when Na\(^{+}\) was returned to the perfusate. Three different cations, glucoamine, *N*-methyl-D-glucamine and lithium, were used to replace Na\(^{+}\). The reduction in external Na\(^{+}\) also caused a depolarization of about 5–10 mV, and it could be argued that this results in a depolarized block of the muscle, preventing action potential generation. However, this seems unlikely as the depolarization is relatively small, and furthermore, it was possible to reinstate action potentials in low Na\(^{+}\) if 5-HT was introduced into the perfusate. A comparison of the rise time of the action potential in normal and low Na\(^{+}\), in the presence of 5-HT to drive action potential generation, showed that there was a significant reduction in the slope in low Na\(^{+}\). A reduction in rise time was also observed with replacement of external Na\(^{+}\) with Li\(^{+}\). The simplest interpretation of the effect of zero, and low, external Na\(^{+}\) on the action potential is that activation of a voltage-gated Na\(^{+}\) channel is essential for the rising phase. If so, this must be a tetrodotoxin (TTX)-insensitive channel, as neither TTX nor saxitoxin had an effect. Further, indirect, evidence for the role of a voltage-gated Na\(^{+}\) channel is provided by the actions of pharmacological agents on the action potential. For example, the local anesthetic, procaine, blocked action potentials, as did the cardiac anti-arrhythmic drug, quinidine. Part of the anti-arrhythmic action of quinidine has been shown to be due to its ability to block cardiac Na\(^{+}\) channels (Grace and Camm 1998). Quinidine also blocks delayed rectifier K\(^+\) channels, and this provides an explanation for the increase in action potential duration observed with this drug. Veratridine, a toxin that has the well-characterized action of shifting Na\(^{+}\) channel activation (Ohta et al. 1973; Ulbricht 1969), increased spike frequency and induced bursting activity. This effect was observed in zero Ca\(^{2+}\), but not in low Na\(^{+}\), supporting the conclusion that this is a Na\(^{+}\)-dependent effect. Finally, in zero Ca\(^{2+}\), veratridine also decreased spike duration.

If the only role of Na\(^{+}\) in the pharyngeal action potential is to act as the charge-carrier for the rising phase, then it would be predicted that a graded reduction in extracellular Na\(^{+}\) would cause a graded reduction in the spike amplitude. However, the effect of decreasing Na\(^{+}\) on action potentials was not graded, i.e., removal of 50% of external Na\(^{+}\) also abolished spikes. In low Na\(^{+}\), the pharyngeal action potentials could be reinstated when 5-HT was included in the perfusate. One possibility is that there is a Na\(^{+}\)-dependent pacemaker potential, the activity of which can be up-regulated by 5-HT. This pacemaker activity could be present either in a neuron, providing synaptic drive to the pharynx, or intrinsic to the muscle itself. Speculatively, 5-HT may act in a manner analogous to the role of norepinephrine on mammalian sino-atrial node, i.e., to increase the activity of hyperpolarization-activated pacemaker channels (Brown et al. 1979). Genes that encode these channels, HCN1 to HCN4, have been identified (Kaupp and Seifert 2001). However, homology searches fail to reveal any candidate genes for these channels in the *C. elegans* genome (Bargmann 1998). Furthermore, HCN channels are blocked by external Cs\(^{+}\) and Li\(^{+}\) (DiFrancesco 1982; Ho et al. 1994), but external Cs\(^{+}\) had little effect on the pharyngeal action potentials. Therefore, the mechanism underlying the pacemaker activity of the pharynx remains to be resolved.

Three of the blockers investigated in this study, barium,
quinidine, and 4-AP increased spike frequency. This may be
due to block of K_\text{A} channels, which control interspike intervals
in many rhythmically active cells. However, 4-AP is also
known to block delayed rectifier K\textsuperscript{+} channels (K_V), so this
would be expected to increase spike duration, whereas we
observed no consistent effect. Nonetheless, both barium and
quinidine, which are known to block K_V, increased spike
duration.

In conclusion, the pharyngeal action potential is generated
by a Na\textsuperscript{+}-dependent mechanism, and it is likely that this is
regulated by 5-HT. A verapamil and nifedipine-sensitive Ca\textsuperscript{2+}
channel contributes to the amplitude of the action potential and
the plateau potential, but in zero Ca\textsuperscript{2+} action potentials may
still be observed, suggesting that Na\textsuperscript{+} can also act as a charge
carrier through these channels. Intriguingly, there is indirect
evidence that a voltage-gated Na\textsuperscript{+} channel plays a role in the
pharyngeal action potential. Namely, that the rising phase of
the action potential is decreased in the absence of Na\textsuperscript{+}, and the
potentials are blocked by procaine and quinidine and increased
by veratridine. Further studies are in progress using voltage-
clamp techniques to further characterize the properties of the
Na\textsuperscript{+}-dependent, veratridine-sensitive, current in pharyngeal
muscle. The molecular identity of this channel would be of
interest as homology searches of the C. elegans
genome for voltage-gated Na\textsuperscript{+} channel genes have not re-
vealed any obvious candidates. Nonetheless, one of the genes
annotated as a Ca\textsuperscript{2+} channel may, in fact, be Na\textsuperscript{+} selective,
and this possibility remains to be tested.

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NOTE ADDED IN PROOF

Ren et al. (2001) have identified a prokaryotic ion selective chan-
nel, NaChBac, that has primary sequence similar to a voltage-gated
Ca\textsuperscript{2+} channel, but is selective for Na\textsuperscript{+}.

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