Mutant Analysis of the Shal (Kv4) Voltage-gated Fast Transient K⁺ Channel in Caenorhabditis elegans

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Shal (Kv4) α-subunits are the most conserved among the family of voltage-gated potassium channels. Previous work has shown that the Shal potassium channel subfamily underlies the predominant fast transient outward current in Drosophila neurons (Tsunoda, S., and Salkoff, L. (1995) J. Neurosci. 15, 1741–1754) and the fast transient outward current in mouse heart muscle (Guo, W., Jung, W. E., Marionneau, C., Aimon, F., Xu, H., Yamada, K. A., Schwarz, T. L., Demolombe, S., and Nernonne, J. M. (2005) Circ. Res. 97, 1342–1350). We show that Shal channels also play a role as the predominant transient outward current in Caenorhabditis elegans muscle. Green fluorescent protein promoter experiments also revealed SHL-1 expression in a subset of neurons as well as in C. elegans body wall muscle and in male-specific diagonal muscles. The shl-1 (ok1168) null mutant removed all fast transient outward current from muscle cells. SHL-1 currents strongly resembled Shal currents in other species except that they were active in a more depolarized voltage range. We also determined that the remaining delayed-rectifier current in cultured myocytes was carried by the Shaker ortholog SHK-1. In shl-1 (ok1168) mutants there was a significant compensatory increase in the SHK-1 current. Male shl-1 (ok1168) animals exhibited reduced mating efficiency resulting from an apparent difficulty in locating the hermaphrodite vulva.

Voltage-dependent potassium (K⁺) (Kv)² channels are key regulators of membrane excitability. Based on their inactivation kinetics, Kv currents can loosely be divided into two categories as follows: noninactivating or slowly inactivating K⁺ currents, and rapidly inactivating transient currents also known as “A-type” currents. A-type currents were first described in molluscan neurons by Hagiwara et al. (3) and later by Connor and Stevens (4). Classical A-type currents (Iₐ) are voltage-dependent, Ca²⁺-independent K⁺ currents that undergo rapid activation and inactivation. The classical A-type currents activate at subthreshold voltages and recover from inactivation quickly compared with other Kv currents. They are also characterized by strong steady-state voltage-dependent inactivation. Repolarization to potentials negative to −50 mV is typically required to restore channel availability. A-type currents have a widespread distribution and are abundantly expressed in neurons and cardiac and smooth muscle cells, where they are thought to play many important physiological roles (2, 7–11).

Connor and Stevens (12) proposed that the Iₐ may determine the interspike interval in repetitively firing neurons. Studies in cerebellar granule cells demonstrated a role for Iₐ in determining the latency to first spike (13). Iₐ has also been demonstrated to limit the back propagation of action potentials into the dendritic arbor (14), to impact long term potentiation in CA1 hippocampal neurons (15), to affect neuronal excitability in the visual cortex (16), and to modulate compartmentalization of membrane excitability in distal dendritic spines (17). Fast transient K⁺ currents in cardiac muscles contribute both to myocyte excitability (18) and to the fast repolarization phase of the cardiac action potential (2, 8, 19, 20). Finally, the pharmacological block of channels that carry fast transient currents in gastrointestinal smooth muscle in mice supports the conclusion that the window currents carried by these channels contribute to the resting membrane potential and cellular excitability of smooth muscle (21, 22).

The molecular identity of the A-type currents in different tissues has been the subject of extensive investigation. Experiments with transgenic animals carrying dominant negative Shal α-subunit constructs indicate that Shal family channels express the cardiac fast transient outward K⁺ currents in cardiac myocytes (23). However, the molecular composition of Iₐ currents in many neurons remains uncertain. In Drosophila, a synthetic deletion allowed the physiological analysis of embryonic neurons lacking the shal gene, which showed that the vast majority of neurons express Shal currents (1). It is noteworthy that other Kv channels, like the Kv1 family, are capable of forming A-type currents, which add more complexity in trying to establish the
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specific contribution of a particular type of K\(_v\) channel in a particular cell type.

Here we show that the *Caenorhabditis elegans* shal gene (*shl-1*) encodes a unique transient current in muscle cells. We also show that the fast transient current carried by Shal channels contrasts with Shaker channels (*shk-1*) from *C. elegans*, which express currents that are only slowly inactivating. Both Shal and Shaker channels are expressed in body wall muscle cells and in a variety of neurons. Shal currents recorded both from body wall muscle cells in culture and heterologously expressed in *Xenopus* oocytes have properties similar to Shal channels from other species with regard to their rapid activation and inactivation, but they differ in that they are active in a more positive voltage range. The phenotype of the *shl-1* deletion mutation was manifested by an increase in muscle excitability reflected in abnormal aldicarb sensitivity, abnormal thrashing behavior, and mating deficiencies.

**MATERIALS AND METHODS**

**Molecular Biology**—EST yk327e11 (Kohara) encodes a full-length *shl-1* cDNA and was obtained already cloned into a pBSc II KS+ plasmid. Three *shk-1* isoform (a, c, and d2) expression constructs were created using EST yk442g4, which encodes the tetramerization domain and core of the protein, and includes the entire 3’ end of the gene. yk442g4 was inserted into our Oocyte Express pOXM vector. The alternative splice isoform expression constructs were created by inserting an EST encoding the variable 5’ end of the gene into the yk442g4:pOXM using BbvC1. The ESTs used were yk813e07, yk753e1, and yk1164g03, and yk1168a01 and yk736e9 for *shk-1* isoforms a, c, and d2. All ESTs were obtained from the laboratory of Yuji Kohara and were sequenced. Intron/exon boundaries were determined by comparing verified EST and genomic sequence. The W363F dominant negative SHL-1 (GenBank™ accession number NM_068574) construct was created by site-directed mutagenesis of the full-length *shl-1* construct. The alternative splice isoform (a, c, and d2) expression construct was created using a standard voltage-step protocol stepping from −100 mV to a range from −120 to +10 mV in 10-mV steps for 9 s with a depolarizing step to +40 mV for 250 ms. Steady-state inactivation data for SHL-1 currents (n = 7) were obtained using a protocol stepping from a holding potential of −100 mV to a range from −120 to +10 mV in 10-mV steps for 38 s with a depolarizing step to +40 mV for 1 s. The dominant negative SHL-1 construct cRNA was examined in a 1:1 ratio co-injection with the SHL-1 cRNA using the same protocols described for SHL-1 cRNA oocyte expression. All protocols utilized a 5-s intersweep interval at −100 mV to allow for inactivated channel recovery. Current records were filtered at 1 kHz, acquired digitally using Clampex 9.0 (Axon Laboratories), and analyzed using Clampfit 9.2 (Axon Laboratories). G-V curves were obtained by converting the peak current values from the I-V relationships to conductances by using the equation: G = I/(V − E\(_{g}\)), where G is the conductance; I is the peak current; V is the command pulse potential; and E\(_{g}\) is the K\(^+\) reversal potential. Conductance values were normalized and fitted with a Boltzmann equation: G/G\(_{\text{max}}\) = (1 + exp((V − V\(_{0.5}\))/k\(_{a}\)))\(^{-1}\), where G is the peak conductance; G\(_{\text{max}}\) is the maximal peak conductance; V and V\(_{0.5}\) are the command potential and the midpoint of activation, respectively; and k\(_{a}\) is the activation slope factor. Steady-state inactivation analysis was performed using the normalized current during the test pulse plotted as a function of the prepulse potential. The data were fitted with the Boltzmann equation: I/I\(_{\text{max}}\) = (1 + exp(V − V\(_{0.5}\))/k\(_{i}\)))\(^{-1}\), where I is the peak current; I\(_{\text{max}}\) is the peak current when the prepulse potential was −80 mV; V and V\(_{0.5}\) are the prepulse potential and half-inactivation potential, respectively; and k\(_{i}\) is the inactivation slope factor.

**Electrophysiology; Patch Recordings from Xenopus Oocytes**—Macropatch and unitary recordings were conducted as described previously (25) using an Axopatch 200A or 200B amplifier (Axon Instruments, Foster City, CA). Patch pipettes were constructed from Corning Glass 7052 or 7056 (Warner Instrument Corp., Hamden, CT). For the recording of fast currents (e.g. tail current relaxations) and single channel currents, the pipettes (−0.5–1 and 5–10 meq/mos in the bath solution, respectively) were coated with Sylgard elastomer (Dow Corning Co., Midland, MI). Passive leak current and the capacitive transients were subtracted online using a P/4 procedure. The recordings were filtered at 0.5–8 kHz (−3 dB, 8-pole Bessel filter; Frequency Devices, Haverhill, MA) and digitized at 2–40 kHz. All experiments were recorded at room temperature (23 ± 1°C).

Data analysis was conducted using Clampfit 8–9 (Axon Instruments), SigmaPlot 8–9 (Systat Software Inc., Point Richmond, CA), and Origin 7.0 (OriginLab Inc., Northampton, MA). The voltage dependence of the peak chord conductance (Gp-V relationship), steady-state inactivation, and time-dependent current relaxations were analyzed as described elsewhere (25, 26). The unitary conductance was estimated by applying voltage ramp protocols to evoke the single channel currents (−100 to +100 mV; 0.9 ms/mV). In this case, passive leak current and the capacitive transients were subtracted by...
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using blank sweeps (no unitary currents). Results throughout are expressed as means ± S.E.

Constructions and Transgenic Animals—The expression construct for shl-1 was created using two overlapping PCR products encompassing the entire 10-kb upstream region of the gene. We designed an ~500-bp overlap between the PCR fragments (5’-PCR was 6.4 kb, and 3’-PCR was 7.3 kb), as well as similar overlap with the “core” protein coding genomic DNA fragment (genomic DNA positions 22316−24990 in YAC Y73B6BL) fused in-frame to GFP in the pPD95 expression plasmid. The construct was originally created in a pPD95 plasmid containing a nuclear localization sequence (NLS), and several lines were also created where the NLS had been removed from the same construct. The expression construct for shk-1 was created in part using one long 11-kb PCR fragment encompassing all of the upstream noncoding sequence except for ~800 bp until the next open reading frame. The remainder of the shl-1::GFP construct was the complete genomic shl-1 gene open reading frame fused in-frame to GFP in a pPD95 expression plasmid. No NLS was present in the construct.

Animals were injected as described in Salkoff et al. (27). We utilized ~90 ng/µl of the Xhol-linearized plasmid DNA of the shl-1 or 100 ng/µl of the Sphl-linearized shk-1 transgene core constructs. For the shl-1 expression construct, 75 ng/µl of the Shal 5’-PCR and 91 ng/µl of the Shal 3’-PCR were added. For the shk-1 expression construct, 100 ng/µl of the Shaker PCR was added to the linearized plasmid and the expression construct, 100 ng/µl of the shk-1::GFP expression construct, 100 ng/µl of the shl-1::GFP and shl-1::GFP expression constructs were used as control strains: wildtype at the Oklahoma Medical Research Foundation and the Salkoff laboratory and the laboratory of Dr. Robert Barstead. The Knockout Consortium utilized a PCR-based knock out strategy to delete this region and within the region (primer sequences available on request) to sequence into the screened 3-kb region to identify the deletion breakpoints. The 10× outcrossed strain 2A056 shl-1 (ok1168) strain was obtained from Dr. Leon Avery. Aldicarb Assay—25 age-matched hermaphrodite worms from several strains (N2, unc-64 (OxIs34), integrated shl-1::W363F::GFP line 320, shl-1 (ok1168), integrated shl-1::W363F::GFP line 625, and pmyo-3::W363F::GFP line 22) were placed onto NGM-0.25 mM aldicarb plates with a small (1 cm diameter) spot of OP50 bacteria (31, 32). Animals were examined for paralysis every 30 min for 5 h, either by tap response or by lightly prodding the worm at the tail with a platinum wire pick. We assayed three plates per strain in each assay. All assays were performed blind at least three times.
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Turning behavior and vulva location were analyzed using the procedure defined in Loer and Kenyon (35) and Crowder et al. (36). 10 males of either N2 or DA2056 shl-1 (ok1168) were mated to either N2 hermaphrodites or unc-51 (e369) hermaphrodites and were analyzed for turning behavior, followed by location of the vulva. 9 of 10 N2 males were capable of exhibiting good turns (g) followed immediately by a slow search for the vulva using spicules (s). The other N2 male passed the vulva by more than 10% of the length of the hermaphrodite and proceeded to quickly swim back to search again for the vulva, or alternatively, they would turn around the hermaphrodite again for a new approach. None of the DA2056 shl-1 (ok1168) males exhibited any change in turning behavior compared with N2 males. DA2056 shl-1 (ok1168) males demonstrated variability in their ability to locate the vulva. These animals frequently exhibited a process of vulval location exhibited by the following pattern: g/f/g/f/g/p/g/o, where “g” is a good turn; “f” is a fast pass across the vulva without slowing; “p” is a slow pass over the vulva and a temporary pause but relatively quick (<10 s) continuation around the hermaphrodite again; and “o” indicates falling off the hermaphrodite without mating. Animals that mated exhibited a pattern similar to g/f/g/f/g/s/si, where “s” is a slowed approach to the vulva; “si” indicates spicule insertion. We examined 60 males per strain of both N2 and DA2056 shl-1 (ok1168).

RESULTS

Tissue Expression Patterns—Shal channels are the most highly conserved of all a-subunits of voltage-gated potassium channels. From the initiator methionine to amino acid residue 430, which encompasses all membrane spanning domains, Shal a-subunits have 75% identity with Drosophila and 69% identity with human orthologs (37). To examine native Shal (SHL-1) and Shaker (SHK-1) currents, we first determined the tissue expression patterns of these genes. We constructed a full-length cDNA translational fusion of shl-1 with ~5 kb of the upstream promoter region tagged to GFP, pshl-1::shl-1::GFP. Two versions of this expression plasmid were constructed, one with and one without a nuclear localization signal. Expression of shl-1 was observed in posterior intestine, body wall muscle, vulval muscle, male-specific diagonal muscles, and a variety of motor neurons, interneurons, and sensory neurons (Fig. 1, A and C; Table 1). Several neurons and muscle cells expressing SHL-1::GFP are known to be associated with a variety of behaviors, including thermosensation, chemosensation, dauer formation, egg-laying, male mating behavior, and locomotion. Expression in posterior intestine suggests a potential role for shl-1 in defecation. We also examined tissue expression for the...
most Shal-Kv4 channels are well described as an exponential time course, some results have hinted at the presence of bia-
 exponential prepulse inactivation kinetics (25). In C. elegans SHL-1 prepulse inactivation was clearly bia-
 exponential with both time constants decreasing with membrane depolarization (supplemental Fig. 1). SHL-1 channels also exhibit fast deact-
 ivation with modest voltage dependence (supplemental Fig. 2).

In contrast to shl-1 which appears to produce only one pro-
tein product, we cloned and expressed three splice variants of the
shk-1 locus termed a, c, and d (GenBank accession numbers, respectively, CAA88477.2, CAD57716.1, and
CAD57717.1). Of the three, only isoforms a and d2 expressed
 currents when cRNA from those forms was injected into Xenopus oocytes. Ion currents and basic channel properties for
SHK-1 isoform a are shown in Fig. 2, B, left, and C, left. The
SHK-1 isoform a expresses currents in a more depolarized volt-
 age range than isoform d2. The $V_{1/2a}$ for isoform a is 2.4 ± 0.8
 mV with a $k_a$ of 7.4 ± 0.7 mV, and the $V_{1/2a}$ is −19.5 ± 2.8 mV
with a $k_i$ of 3.7 ± 0.3 mV. For isoform d2 the $V_{1/2a}$ is −31.9 ± 1.2
mV; $k_{i,a}$ is 7.4 ± 1.1 mV; $V_{1/2a}$ is −62.5 ± 5.9 mV, and $k_i$ is 3.4 ±
0.5 mV. Currents of both splice variants inactivate very slowly
and can be characterized as delayed rectifiers (Fig. 2B, left).

Electrophysiology and Molecular Dissection of Native SHL-1
and SHK-1 in Cultured Myocytes—Because GFP promoter
experiments indicated that SHL-1 and SHK-1 were expressed
in body wall muscle cells, we recorded from these cells in cul-
 ture and analyzed the voltage-dependent current components.
Unlike adult body wall muscle cells, Ca$^{2+}$-dependent inward
 currents have not been seen in C. elegans myocytes in culture
(29). Cultured myocytes are easy to identify because of their
characteristic teardrop shape and also because they are signifi-
cantly larger (three times) than cultured neurons. In addition
they can be unequivocally identified by expressing GFP under
control of the body wall muscle-specific myosin heavy chain
promoter $pmyo-3$ (42). We sought to examine the native prop-
erties of SHL-1 and SHK-1 by isolating each current with three
different approaches.

By using cultured myocytes treated with RNAi, we were able
to selectively suppress either shl-1 or shk-1 mRNA translation
(28) and to record the currents remaining after such treatment
(7). Previously, it was shown that SLO-2 K$^+$ channels are abun-
dantly expressed in cultured myocytes but that they required
intracellular Ca$^{2+}$ and Cl$^-$ at relatively high levels to be active
(43). We thus took advantage of these special requirements of
the SLO-2 channels to eliminate the large SLO-2 current com-
ponent, and we performed all recordings with pipette recoring
solutions containing low physiological concentrations of Cl$^-$
and Ca$^{2+}$ (see “Materials and Methods”). Patch clamp record-
ings from wild type (N2) myocytes and from slo-2 (nf100) myo-
cytes demonstrated that two voltage-dependent current com-
ponents were present in both genotypes, a fast transient com-
ponent and a slowly inactivating component (7). Those
experiments showed that ion currents in N2 cells with low
intracellular Cl$^-$ and Ca$^{2+}$ are indistinguishable from slo-2
(nf100) myocytes. The properties we observed of SHL-1 and
SHK-1 currents expressed in Xenopus oocytes (Fig. 2, A–C, left)
combined with the GFP tissue expression data showing that
both shl-1 and shk-1 genes are expressed in body wall muscle

Electrophysiological Properties of SHL-1 Expressed in Xeno-
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er, and jellyfish showed that Shal potassium channels carry a
fast, transient outward current (37). Our results show that, in
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(Fig. 1) led us to hypothesize that the macroscopic voltage-dependent outward K⁺ currents expressed in C. elegans cultured myocytes were SHL-1 and SHK-1 currents. To investigate this hypothesis, we treated the slo-2 (nf100) myocytes with shk-1 RNAi. This resulted in the removal of the slowly inactivating current component, leaving a fast transient current (Fig. 2A, right). Notably, the native fast transient current strongly resembles the SHL-1 current observed when shl-1 cRNA is heterologously expressed in Xenopus oocytes. Conversely, treatment of myocytes with shl-1 RNAi removed the fast transient current component leaving only a slowly inactivating current (7). This slowly inactivating current is virtually identical to that seen in the shl-1 (ok1169) deletion mutant (Fig. 2B, right). This slowly inactivating current resembled the heterologously expressed SHK-1 current. Our data support the conclusion that there are only two normally expressed macroscopic voltage-dependent K⁺ currents in C. elegans myocytes in culture and that SHL-1 and SHK-1 channels carry these currents.

We characterized the conductance/voltage and steady-state inactivation relationships for the SHL-1 and SHK-1 currents recorded in vivo from myocytes (Fig. 2C, right). Typical of Shal currents in other systems, the V_{0.5} of activation for the fast transient SHL-1 current was more hyperpolarized than that observed for the slowly inactivating SHK-1 current (11.2 and 20.4 mV, respectively). The SHL-1 current was also less voltage-sensitive, with a slope factor k of 14.1 mV versus 7.7 mV for SHK-1. As expected, the V_{0.5} of steady-state inactivation was also more hyperpolarized for SHL-1 currents than for SHK-1 currents (−33 and −6.9 mV, respectively). Therefore, the native SHL-1 current is significantly more hyperpolarized in both activation and steady-state inactivation than the SHK-1 current, which is in agreement with the literature for the relative voltage ranges of currents for these channels (37, 44). The conductance/voltage and steady-state inactivation relationships for the SHL-1 and SHK-1 currents recorded in vivo from myocytes (Fig. 2C, right) were similar to those of the two currents expressed heterologously in Xenopus oocytes (Fig. 2C, left) (7).

A second way of separating and analyzing the currents present in myocytes in cell culture was by creating a transgenic animal carrying a dominant negative form of shl-1, which removed or greatly diminished the SHL-1 current. We constructed two transgenes translationally tagged with GFP fused to the shl-1 dominant negative construct that had a site-directed alteration in the pore of the channel (W363F, see “Materials and Methods”). One transgene was under control of the native shl-1 promoter (pshl-1), and the other was under control of the body wall muscle-specific promoter pmyo-3. The pshl-1::W363F::GFP transgenic line was then integrated to obtain a stable expression line. The effect of each of these transgenes on the native SHL-1 current was analyzed using the patch clamp technique in cultured myocytes. Cells carrying the dominant negative transgene were identified by their GFP fluorescence. In these cells we observed that the current consisted primarily of slowly inactivating currents. We compared the ratio of the normalized peak current to the sustained current to gauge the effectiveness of removing the SHL-1 component (Fig. 3). This analysis showed a significant reduction in the peak transient current in the animals carrying the dominant negative construct and also in RNAi-treated animals. In these cells a small amount of transient current remained, which may represent a residual component of SHL-1 current as well as a larger component of the slower inactivating SHK-1 current. In 1 of 9 and 1 of 7 cells, for pshl-1::W363F::GFP and pmyo-3::W363F::GFP, respectively, we observed a more hyperpolarized slowly inactivating current, which may represent expression of variable SHK-1 isoforms (data not shown). Control slo-2 (nf100) cells not carrying the transgene clearly have both the slowly inactivating SHK-1 and fast transient SHL-1 currents. However, cells carrying either of the dominant negative transgenes showed a significant reduction in the amount of the fast transient outward current (Fig. 3).

A deletion mutant for the Y73B6BL.19 shl-1 locus (International C. elegans Knockout Consortium) was the third method by which we characterized native physiological K⁺ currents in cultured myocytes. DNA sequence analysis showed that both the 5’ and 3’ breakpoints were within intronic sequence. The deletion removed two exons containing the terminal three amino acids of the fourth transmembrane domain and the entire fifth transmembrane domain (Fig. 4A). Furthermore, the deletion resulted in a frameshift adding 16 ectopic amino acid residues and an early stop codon. This early termination resulted in the elimination of membrane-spanning domains 5 and 6, together with the residues encompassing the pore of the channel, and all downstream sequence encompassing the carboxyl cytoplasmic portions of the channel (Fig. 4B). Clearly, the removal of protein domains required for ion conduction in the channel resulted in a loss-of-function. We cultured C. elegans myocytes from the shl-1 (ok1168) deletion mutant strain and examined the currents remaining in these cells using single-electrode whole-cell patch clamp. Our results showed that

* p<0.05
** p<0.0005

![Figure 3. Removal of SHL-1 current in cultured C. elegans myocytes.](image-url)
the fast transient current was completely absent from these cells (Fig. 2B, right, and Fig. 4C, bottom). The currents remaining in these shl-1 deletion cells appeared highly similar to those observed in the majority of our wild-type and slo-2 (nf100) myocytes after treatment with shl-1 RNAi (Fig. 4C, middle). This result is consistent with our molecular analysis of the shl-1 (ok1168) deletion, showing that it cannot form a functional channel and functions as an effective null when homozygous.

Definitive proof that SHL-1 and SHK-1 constitute the only voltage-dependent components in C. elegans myocytes results from the demonstration that selective elimination of both SHL-1 and SHK-1 currents produced cells having no macroscopic voltage-dependent outward current (43). To remove the SHL-1 component, we utilized myocytes from the transgenic dominant negative line (Fig. 5B) or the shl-1 deletion mutant (Fig. 5C). To remove the SHK-1 current, we treated both myocyte genotypes with shk-1 RNAi (Fig. 5, B and C). In such cells we observed a complete removal of all macroscopic outward current. Further examination of such cells at high gain revealed only single channel openings (Fig. 5, B and C, right traces). These single channel openings exhibited no detectable voltage dependence because their open probability was not noticeably different at different holding potentials (data not shown). We hypothesize that these single channel currents observed after the removal of all macroscopic currents could be due to any of several other genes encoding nonvoltage-dependent potassium channels that are expressed in C. elegans muscle (27). We therefore conclude that only three K+ channels produce the macroscopic outward K+ currents in cultured C. elegans myocytes, SHL-1, SHK-1, and the previously studied SLO-2 current (24).

Prior results have suggested that certain instances of altered expression of Shal channels are accompanied by altered expression of other ion channel types (43). Our results in C. elegans seem to bear this out (Fig. 6). In our studies using the shl-1 deletion mutant, we found that the remaining sustained current, most likely due to SHK-1, may be up-regulated as a result of the complete removal of SHL-1. To test this hypothesis, we compared the sustained current amplitudes in the slo-2 (nf100) myocytes (12.5 pA/pF ± 1.9), N2 myocytes (12.6 pA/pF ± 1.5), and the shl-1 (ok1168) myocytes (25.5 pA/pF ± 8.5). All currents were normalized to account for variable cell size differences (45). There was no significant difference (p = 0.66 and p = 0.34, respectively) in the amplitudes of normalized sustained currents between N2 myocytes (12.6 pA/pF ± 1.5) and either the shl-1 RNAi-treated myocytes (14.9 pA/pF ± 6.8) or the pshl-1::W363F::GFP (16.2 pA/pF ± 7.4) transgenic myocytes. However, there was a significant (p < 0.05) increase in sustained current amplitude in shl-1 (ok1168) myocytes, which is most likely due to up-regulation of the SHK-1 current. Fig. 6 summarizes our findings and shows the relatively larger sustained current component in shl-1 mutant myocytes and control animals (Fig. 6A) and a statistical analysis of these data (Fig. 6B). These results parallel studies in lobster that have demonstrated that changes in Shal (Kv4) channel expression levels can result in changes in whole-cell currents because of the up-regulation of other channel types (46). Notably, in both studies up-regulation of compensatory currents depends on the complete absence of a channel protein product, even if the ion conduction pathway of the protein product is blocked. Therefore the Shal protein may have a functional role in regulation, which is distinct from ion conduction.
C. elegans Shal (Kv4) K⁺ Channel

**FIGURE 6.** Up-regulation of SHK-1 current in shl-1 (ok1168) mutant myocytes. A, current traces from shl-1 (ok1168) myocytes (top), N2 wild-type myocytes (middle), and slo-2 (nf100) myocytes (bottom) shown on the same scale normalized for cell capacitance. B, mean normalized sustained current amplitudes with standard errors for the slo-2 (nf100) myocytes (12.5 pA/pF ± 1.9), N2 myocytes (12.6 pA/pF ± 1.5), and the shl-1 (ok1168) myocytes (25.5 pA/pF ± 8.5). * indicates p < 0.05 compared with shl-1 (ok1168) myocytes.

**FIGURE 7.** Removal of SHL-1 current in body wall muscle is sufficient to confer aldicarb hypersensitivity with 0.25 mM aldicarb. A, both the shl-1(ok1168) deletion mutant and the pshl-1::W363F::GFP line 320 carrying the dominant negative construct displayed aldicarb hypersensitivity compared with N2 animals. B, we tested two lines carrying dominant negative constructs integrated on different chromosomes to ensure that the observed aldicarb hypersensitivity phenotype was not because of position effects in these transgenic animals. Both lines carrying integrated constructs (320 on chromosome X and 625 on chromosome IV) displayed aldicarb hypersensitivity. The aldicarb hypersensitivity of the dominant negative transgenic line was not influenced by position effects. C, expression of the dominant negative transgene under control of pmyo-3 (a muscle-specific promoter) resulted in aldicarb hypersensitivity similar to that observed for the dominant negative under the native shl-1 promoter. D, the heterozygous shl-1(ok1168)/+ animals had an intermediate phenotype which fell between N2 and shl-1(ok1168) homozygotes. The positive control for hypersensitivity to aldicarb for all assays was the strain unc-64 (Oxh34), a gain-of-function integrated transgenic syntaxin line. Physiological and Behavioral Analysis of SHL-1—A distinctive feature of C. elegans body wall muscle is that the resting potentials of cells are known to be significantly more positive than the resting potentials of muscle cells in mammalian species (47). This raises the possibility that SHL-1 channels in C. elegans, even though functioning in a more depolarized voltage range than their mammalian (or Drosophila) orthologs, may be active at or near the muscle cell resting potential. A significant Shal “window current” might then contribute substantially to cell resting conductance and thus basal muscle excitability and muscle responsiveness to neurotransmitter. If this were the case, eliminating the Shal current either by the deletion mutant or the dominant negative construct would increase muscle responsiveness to neurotransmitter and might manifest as an increase in sensitivity to aldicarb. Aldicarb is an acetylcholinesterase inhibitor that results in delayed clearance of acetylcholine (ACh) from the synaptic cleft of the neuromuscular junction. In C. elegans, the accumulated ACh results in rigid paralysis of animals with the severity of phenotype depending on the amount of ACh released into the cleft or the responsiveness of the postsynaptic muscle membrane to ACh (48). Thus, to explore the possibility of a heightened responsiveness of the postsynaptic membrane to ACh when the SHL-1 current was removed, we exposed both the shl-1 (ok1168) deletion mutant and the dominant negative transgenic line pshl-1::W363F::GFP to aldicarb (0.25 mM) (Fig. 7A). (Note that the expression of the dominant negative transgene was under control of the native shl-1 promoter.) We observed rigid paralysis in the animals with paralysis midpoints around 125 min for the dominant negative transgenic animals and about 132 min for the shl-1 (ok1168) animals (p < 0.0028 and p < 0.0025, respectively). These values were significantly different from those observed for wild-type N2 animals which was ~155 min. We also observed significant (p < 0.05) aldicarb hypersensitivity in both of our dominant negative transgenic lines under the native shl-1 promoter (Fig. 7B). We conclude that the SHL-1 current may well contribute to the postsynaptic muscle response to released ACh.

An alternative hypothesis to explain these results is that hypersensitivity is because of a presynaptic effect and that the SHL-1 current controls the release of ACh-containing vesicles in the presynaptic terminal. To differentiate between pre- or postsynaptic causes of aldicarb hypersensitivity, we expressed the dominant negative transgene under control of the body wall muscle-specific myo-3 promoter (49) to limit expression of the dominant negative construct to the muscle. The demonstration that the pmyo-3::W363F::GFP transgenic line was also aldicarb-hypersensitive would prove that the absence of the SHL-1 cur-
rent in muscle alone was sufficient to confer aldicarb hypersensitivity. Indeed, the pmyo-3::W363F::GFP transgenic line exhibited almost identical aldicarb hypersensitivity to that exhibited by the transgenic line pshl-1::W363F::GFP line 320 (Fig. 7C). Heterozygous shl-1(ok1168)/+ animals exhibited an intermediate level of aldicarb sensitivity (Fig. 7D). This result appears to validate the hypothesis that the increased sensitivity to accumulated ACh in the neuromuscular junction is a postsynaptic effect.

We examined a variety of behaviors in the shl-1 (ok1168) mutant animals in an effort to understand the shl-1 deletion mutant phenotype. shl-1 (ok1168) animals exhibited a defect in aldicarb sensitivity and a slight but significant alteration in thrashing behavior (data not shown). However, observed mating behavior presents a more rigorous behavioral assay that can reveal subtle defects in sensory processing; mating requires complex coordination of sensory information and intricate motor functions. Mating efficacy was determined by observing the number of mating plates possessing male progeny. Hermaphrodite C. elegans produce male progeny at very low penetrance unless mated by males (50). Two N2 or shl-1 (ok1168) males and two N2 hermaphrodites were placed on a small spot (~1 cm diameter) on mating plates. The number of successful mating events in these experiments, as indicated by the number of mating plates with male progeny, was significantly lower on plates with shl-1 (ok1168) male animals than with N2 males (48.8 and 85.4%, respectively) (Table 2). One possible complication in interpreting these results is the phenomenon of feminization whereby worms possessing an XO sex chromosome genotype exhibit female gonads and sexual behavior, instead of that of the male. Conceivably, the SHL-1 protein could be involved in sex determination turning males into phenotypic females. Conceivably, the SHL-1 protein could be involved in sex determination turning males into phenotypic females. Some measure of this deficit in fine motor control in mating may involve the fact that SHL-1 is expressed in male-specific diagonal muscles in the tail region.

**DISCUSSION**

SHL-1 and SHK-1 C. elegans voltage-gated potassium channels exhibit fast transient and delayed rectifier currents, respectively. In other species, Shal channels are typically described as operating in a hyperpolarized voltage range relative to other voltage-gated K+ channels (37, 52). However, in C. elegans, SHL-1 activates in a more depolarized voltage range than is typically observed for Shal channels in other species (37). Recent work has demonstrated that pyramidal neurons in the rat visual cortex also activate at more depolarized voltages than are typically observed in mammals (16). Nevertheless, SHK-1 currents operate in an even more depolarized voltage range and thus seem to maintain their relative functional relationship to SHL-1 channels, consistent with that found in other species (25, 38–41, 53, 54). Furthermore, there is evidence that C. elegans body wall muscle rests at a rather positive value relative to muscle cells of other species (approximately -20 to -30 mV (47, 55)) which suggests that SHL-1 channels have evolved to serve a specialized purpose in this species. A second gene encoding a transient K+ channel has been reported in C. elegans, kvs-1 (56). KVS-1 channels exhibit an even more depolarized activation and inactivation range than that observed for SHL-1 channels; it also exhibits significantly slower inactivation kinetics. kvs-1 is closer to the Shab (Kv2) family of voltage-gated K+ channels than to the Shal family (57), and this gene is not expressed in body wall muscle.

Native SHL-1 channels have properties similar to the heterologously expressed cloned channels in that they carry fast transient currents with rapid activation and inactivation. However, the native channels activate in a somewhat more hyperpolarized voltage range than the cloned channels (approximately -14 mV). Such differences could conceivably be due to accessory proteins or post-translational modification occurring in native cells. In mammals, modulatory subunits or post-translational modification affects Shal channel voltage sensitivity, and similar mechanisms are known to affect Shaker currents as

| Strain          | Vulva location behavior                  | Mating efficiency |
|-----------------|-----------------------------------------|-------------------|
| DA2056 shl-1 (ok1168) | Circles hermaphrodite and then stops at vulva | Poor (48.7805%) |
| N2              | Stops at vulva                           | High (85.3660%)  |

*Vulva location behavior is divided into two categories, shown as "approximate" and "precise." Approximate refers to the gross ability to locate the vulva. Precise refers to the process by which the vulva is located.*

*Mating efficiency is as described under "Materials and Methods." n = 10 for both the N2 and the DA2056 shl-1 (ok1168) strains.*
well. In *C. elegans* at least one protein similar to a mammalian modulatory subunit has been identified (58).

We showed that the major component of voltage-dependent current remaining in the *shl-1 (ok1168)* deletion mutant background is encoded by the Shaker gene, *shk-1*, and has the properties of a delayed rectifier current. We characterized two isoforms produced by the *shk-1* locus, isoform a and isoform d2. Both express slowly inactivating currents, but the d2 isoform of SHK-1 functions in a more hyperpolarized voltage range than SHK-1 isoform a and may inactivate slightly faster. When the Shal component was removed either by RNAi in cell culture or in cells of the strain carrying the dominant negative form of Shal, the remaining current seemed to have the properties of the SHK-1 functions in a more hyperpolarized voltage range than SHK-1 isoform a and may inactivate slightly faster. When the Shal component was removed either by RNAi in cell culture or in cells of the strain carrying the dominant negative form of Shal, the remaining current seemed to have the properties of the SHK-1 isoform d2. However, the current remaining in the *shl-1* deletion strain had properties more similar to the SHK-1 isoform a. In this latter case, the SHK-1 currents appeared to be larger. Precedents for this exist in the literature, which show that a second current component may be up-regulated to compensate for the removal of a first component. The compensating component may differ radically in its properties from the original component, which was removed (59, 60). For example, changes in HCN ion channel expression in response to increased Shal subunit expression have been observed previously in lobster stomatogastric ganglion cells (46, 61). In our case, the up-regulation of the SHK-1 current was not observed in either the *shl-1* RNAi-treated myocytes nor the dominant negative transgenic line *pshl-1::*W2636F. Because only the deletion mutant exhibited the increased SHK-1 current, the compensatory expression of this current may be responsive to the lack of all SHL-1 protein, whether functional or not.

Although a complete knockout of SHL-1 and SHK-1 in the *slo-2 (nf100)* background eliminated all macroscopic outward currents, some unidentified unitary currents remained. We hypothesize that these single-channel currents are likely due to the many two-pore *twk* channels expressed in body wall muscle (27). However, the macroscopic outward currents in *C. elegans* cultured myocytes seem to be entirely carried by SLO-2, SHK-1, and SHL-1 channels.

Detailed analysis of *shl-1-GFP* cell-type expression patterns showed channel expression in cells involved with a variety of behaviors. However, mutant phenotypes were not observed for chemosensation, thermosensation, defecation, or pharyngeal pumping. On the other hand, we observed a significant *shl-1* mutant phenotype with regard to the response to aldicarb. The total elimination of the SHL-1 current in all tissues by the deletion mutation, as well as the specific inhibition of SHL-1 current in body wall muscle by selective muscle-specific expression of the dominant negative construct, resulted in aldicarb hypersensitivity. This latter result strongly suggests that the muscle membrane itself is the site that confers aldicarb hypersensitivity. An attractive hypothesis that takes into account the unique properties of the SHL-1 current is that the mutant membrane is more susceptible to depolarization by acetylcholine than the wild-type membrane, because an SHL-dependent component of membrane resting conductance has been removed. This could come about via the SHL-1 window current, which may contribute to the resting membrane K⁺ conductance controlling basal resting membrane excitability. The fact that the SHL-1 current contributes to the K⁺ conductance at rest does not necessarily mean that it significantly changes the resting membrane potential. Indeed, if the membrane resting potential is close to $E_K$, the driving force on K⁺ is so low that a small but significant change in K⁺ conductance would not have a measurable effect on the resting potential. However, current components that are active at rest need not be very large to have a major impact on the overall excitability of the cell, even if they do not significantly change the resting membrane potential. This is because total cell resting conductance is usually small and represents only a tiny fraction of the overall cell membrane conductance during peak electrical activity. A crucial factor is their contribution to the critical balance of inward and outward currents at the threshold of active responses. Many models show that a change of only ~10% of resting cell conductance can have a major effect on the excitable properties of a cell.

We demonstrated a significant reduction in mating efficiency in *shl-1 (ok1168)* deletion mutant males, which at least partially involves a reduced ability to locate the vulva of the hermaphrodite. Male copulatory behavior includes input from neurons common to both hermaphrodite and male, as well as male-specific neurons (note that we did not specifically examine male-specific neurons for SHL-1 channel expression). The difficulty of *shl-1 (ok1168)* animals to effectively mate appears to be due to a deficit in fine motor control or spicule insertion (62, 63), which may result from a mutant alteration of membrane excitability in muscle or neurons or both. The ether-a-go-go-related potassium channel, UNC-103, is known to impact male-specific muscle contraction leading to failure of spicule insertion into the vulva of the hermaphrodite that reduces mating efficiency (62). Conceivably, SHL-1 functions in a similar manner, and removal of SHL-1 channels results in hypersensitivity of the muscle to ACh, lowering the efficiency of spicule insertion or impeding sensory perception of the vulva.

Loss of the *shl-1* gene product produces neither a cell nor an organismal lethal. Nevertheless, its role in fine-tuning complicated behavior such as mating may offer essential survival benefits and may be one factor contributing to the extraordinarily high conservation of the Shal potassium channel.

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