An Arrhythmia Susceptibility Gene in Caenorhabditis elegans*

Ki Ho Park and Federico Sesti

From the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Department of Physiology and Biophysics, Piscataway, New Jersey 08854

KCNEs are evolutionarily conserved ancillary subunits of voltage-gated potassium (Kv) channels (1, 2). They regulate the function, trafficking, modulation by signaling molecules, and pharmacology of their channel partners in multiple mammalian tissues including heart, stomach, skeletal muscle, auditory epithelium, and the central nervous system (2–9). In human left ventricle, KCNE subunits endow the Kv channels that conduct pharyngeal muscle contractility. MPS-4 modulates EXP-2 function in a similar fashion as KCNE proteins endow human channels. When defective, MPS-4, can induce abnormal repolarization by mechanisms that resemble the way KCNE proteins are thought to provoke arrhythmia in human heart. Mutation of a conserved aspartate residue associated with human disease (MPS-4-D74N) alters the functional attributes of the C. elegans current. Taken together these data underscore a significant conservation of KCNE activities in different pumps. This implies that C. elegans can develop into a system to study the molecular and genetic basis of KCNE-mediated muscle contractility and disease states.

MPS-4 is the homologue of human KCNE1 (Fig. 1A) were not known. In an effort to characterize the physiological role of MPS-4, we found that this gene is expressed in the alimentary system of the worm, the pharynx. Notably, the pharynx is a pump that is considered a prototype of the human heart (24). Both organs develop during early embryogenesis and exhibit similar electrical activity characterized by long action potentials with a broad plateau phase. The pharyngeal action potential resembles the ventricular action potential in magnitude (ΔV ~ 80 mV), duration (~200 ms), and shape (plateau). This is not coincidental. In fact the type of channels that generate this signal and their functional attributes resemble those of the channels that generate the action potential in human ventricle (25). The pharyngeal action potential is regulated by at least three major voltage-gated currents. A rapidly activating-inactivating calcium current, produced by T-type calcium channels, is responsible for the upstroke of the action potential. A slowly inactivating calcium current, produced by L-type calcium channels, maintains the membrane depolarized during the plateau phase. A potassium outward current that is produced by EXP-2 repolarizes the membrane and terminates the action potential. The molecular correlate of I,Kp is EXP-2, which was originally identified by Avery and colleagues (26). EXP-2 shares with cardiac I,Kr the same unique mechanism of conduction. These channels exhibit ultrafast inactivation and conduct during membrane repolarization (27, 28). Thus, when the membrane becomes positive, the channels quickly move into the inactivated state and do not interfere with the development of the plateau phase of the action potential. When the membrane begins to repolarize at the end of the plateau phase these channels conduct large K+ currents that sharply terminate their respective signals.

This study starts from the observation that the attributes of the EXP-2 current do not completely recapitulate those of I,KP (25) suggesting that EXP-2 might partner with MPS-4 in pharyngeal muscle. We found that the two subunits form a complex in specific parts of the muscle, namely anterior isthmus and terminal bulb. We further show that genetic knockout of MPS-4 causes abnormal repolarization by a mechanisms that is reminiscent of the way KCNE proteins are thought to induce arrhythmia in human heart. This remarkable conservation of KCNE activities makes C. elegans a promising system to study.

*This work was supported by National Institutes of Health Grant R01GM68581 (to F. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

To whom correspondence should be addressed. Tel.: 732-235-4032; Fax: 732-235-5038; E-mail: sestife@UMDNJ.EDU.
A Model for KCNE Arrhythmia

the mechanisms underlying KCNE-mediated muscle contractility and disease states.

EXPERIMENTAL PROCEDURES

Molecular Biology—The D74N MPS-4 mutation was produced in wild-type MPS-4 in pCI-neo by plaque-forming unit-based mutagenesis (Stratagene). The construct was confirmed by automated DNA sequencing and quantified with spectrophotometry.

RNA Interference—For double-stranded RNA (dsRNA)\(^2\) production, \(\sim 1\) kb of exp-2 and \(\sim 0.5\) kb of mps-4 genomic DNA were amplified by PCR with oligonucleotides that added 5’ T7 promoter sequence. dsRNA in vitro synthesis was with the MEGAscript kit (Ambion) using the PCR products as templates. The reactions were annealed at 37 °C for 30 min after denaturation at 68 °C for 10 min. RNA was analyzed by agarose gel electrophoresis to verify that it was double stranded. Approximately 100 pl of dsRNA (1.5 μg/μl in H₂O) was injected into both gonads of young adults. Worms were allowed to lay the eggs contained in the uterus for 2–3 h and then transferred separately onto fresh plates. F1 progeny of injected worms were analyzed.

Immunostaining—Immunolabeling was done following a freeze-cracking procedure modified by Shohei Mitani. To expose the pharynx, the head of a worm was chopped under a stereomicroscope using a 25-gauge needle in M9 buffer (22 mM KH₂PO₄, 22 mM NaH₂PO₄, 85 mM NaCl, 1 mM MgSO₄). The head was then transferred to a microscope slide coated with poly-L-lysine, covered with a coverslip, and immediately frozen at \(-80^\circ\)C for 30 min. The glass slide was put in \(-20^\circ\)C methanol for 5–15 min, washed 2 min with, respectively, 75% methanol, PBS; 50% methanol, PBS; 25% methanol, PBS. Then the sample was incubated with anti-pharynx was washed two times with PBS and then incubated in PBS + 2% bovine serum albumin at room temperature for 30 min. The bovine serum albumin/PBS solution was removed without washing and the sample was incubated with anti-MPS-4 + 0.1% Tween 20 at 4 °C overnight. The sample was washed three or more times with PBS and incubated with Cy3-conjugated goat anti-rabbit secondary antibody for 2 h at room temperature and washed three times with PBS. The sample was analyzed and photographed by an Olympus BX61 microscope equipped with a digital camera.

Co-immunoprecipitations—Chinese hamster ovary (CHO) cells were transiently transfected with cDNA using the Superfect kit (Qiagen) and studied 24–36 h post-transfection. CHO cells were washed with 10 ml of ice-cold PBS and lysed with 1% Nonidet P-40 buffer, 50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% IGEPAL CA-630, phosphatase and protease inhibitors (Calbiochem). Cell lysates were centrifuged for 60 min at 4 °C and the supernatant mixed with HA-conjugated beads (Roche) and rocked at 4 °C for 3 h. Beads were washed three times with ice-cold 1% Nonidet P-40 buffer and then incubated in SDS sample buffer at \(-90–95^\circ\)C for 5 min. Visualization was by polyclonal anti-MPS-4 (New England Peptides) and a horse-radish peroxidase chemiluminescence coupled secondary antibody (Jackson Laboratories).

Electrophysiology—Data were recorded with Axopatch 200B (Axon) PC (Dell) and Clampex software (Axon) and filtered at \(f_c = 1\) kHz and sampled at 2.5 kHz. Agar bridges were used throughout this study.

Current Clamp Recordings—The heads of the animals were chopped with a needle as described before. Heads were transferred to the recording chamber in the electrophysiological set up, using a Pasteur pipette and held in place with a suction electrode. The pharynx was continously perfused with a solution containing: 6 mM KCl, 140 mM NaCl, 3 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.5, with NaOH and 5 μM 5-HT to stimulate autonomic pharyngeal activity. A second intracellular electrode filled with 2 M KCl was used to record the electrical activity of the pharynx in current clamp.

Continuous recordings of pharyngeal electrical activity were analyzed using the half-threshold method (29). Histograms of the time between two consecutive action potentials and action potential duration were computed using Clampfit 9.2 software (Axon) and fitted to a single (N2) or double Gaussian distribution,

\[
\sum_{i=1}^{2} A_i \exp \left[ -\left( \frac{t-t_i}{\sigma_i} \right)^2 \right]
\]  

(Eq. 1)

where \(A_i\), are constants, \(\sigma_i\) are the variances and \(t_i\) the times in ms at which each Gaussian in Equation 1 is maximal.

Voltage-Clamp Recordings—CHO cells were transiently transfected with cDNA using the Superfect kit (Qiagen) and studied 24–36 h post-transfection. Bath solution was (in mM): 4 KCl, 100 NaCl, 10 HEPES (pH 7.5 with NaOH), 1.8 CaCl₂ and 1.0 MgCl₂. Pipette solution was 100 KCl, 10 HEPES (pH 7.5 with KOH), 1.0 MgCl₂, 1.0 CaCl₂, 10 EGTA (pH 7.5 with KOH).

The time course of deactivation was fitted to a double exponential function,

\[
l(t) = I_0 + I_1 e^{-t/\tau_1} + I_2 e^{-t/\tau_2}
\]  

(Eq. 2)

where \(I_0\), \(I_1\), and \(I_2\) are constants and \(\tau_1\) and \(\tau_2\) are the time constants of deactivation.

Isocronal open probability curves were fitted to the Boltzmann function,

\[
\frac{l}{l_{max}} = \frac{1}{1 + \exp[(V - V_0)/\Delta V]}
\]  

(Eq. 3)

where \(l/l_{max}\) is the normalized current, \(V\) is the membrane voltage, \(V_0\) is the value of the voltage at which Equation 3 is equal to 0.5 and \(\Delta V\) is the slope coefficient (in mV).

Dose-response curves were fitted to the Hill function,

\[
\frac{l}{l_{max}} = \frac{1}{1 + \left( \frac{D}{d_{50}} \right)^n}
\]  

(Eq. 4)

where \([D]\) is the dilution of the antibody, \(d_{50}\) is the dilution that halves Equation 4 and \(n_{50}\) is a coefficient.

\(^2\)The abbreviations used are: dsRNA, double-stranded RNA; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary; HA, hemagglutinin; RNAi, RNA interference; KO, knock out; DAD, delayed after depolarizations; GFP, green fluorescent protein.
Statistics—Data are indicated as mean ± S.E. The number of determinations is indicated by n. Student’s t test was used to evaluate the statistical significance of unpaired means. * and ** indicate p < 0.05 and p < 0.01, respectively.

RESULTS

MPS-4 Is Expressed in Pharyngeal Muscle—To determine MPS-4 expression in the pharynx, we employed immunocytochemical methods using a custom-made rabbit polyclonal antibody that recognizes an extracellular domain in MPS-4. Fig. 1B shows anti-MPS-4 staining of a permeabilized pharynx of a transgenic nematode expressing an exp-2:gfp reporter gene. The antibody could detect MPS-4 expression in terminal bulb and in anterior, but not in posterior isthmus or corpus (Fig. 1C). When the head of the worm is cut, the corpus remains covered by the cuticle because the body wall muscles contract (Fig. 1B). Thus, the cuticle might have prevented the antibody to stain the corpus even after robust permeabilization. However, in intact worms permeabilized by the same freeze-crack method we detected expression in the terminal bulb and isthmus (albeit less intense) but not in corpus (data not shown). Furthermore, in the cut head preparations such as the one shown in the figure, metacorpus and the region of corpus immediately adjacent to it are usually not covered by the cuticle and yet the antibody failed to detect MPS-4 (n = 3). This suggests that even if MPS-4 is expressed in corpus, the protein level is low. The antibody did not stain the pharynx of mps-4 KO nematodes (Fig. 1F) confirming its specificity. Notably, the expression pattern of MPS-4 partially overlapped with that of EXP-2 indicating that the two proteins colocalize in specific areas of the pharynx (Fig. 1, D and E).

MPS-4 Controls Pharyngeal Activity—We next investigated the physiological role of MPS-4 in the pharynx. In the presence of food for the nematode, bacteria, the pumping of mps-4 KO worms resulted in a slight (9%) but statistically significant decrease in the average number of pharyngeal contractions per minute compared with N2 animals (Fig. 2A). Analysis of the electropharyngeogram, an extracellular measurement of the electrical activity that controls the mechanical activity of the pharynx (30), indicated that the decreased pumping rate in mps-4 KO worms was due to protracted muscle excitation (compare Fig. 2, B and C). It must be emphasized that the electropharyngeograms were recorded in the presence of serotonin (5-HT) to induce autonomic myogenic contractions in the absence of food (30). Therefore pumping activity was more pronounced in these conditions than with bacteria (Fig. 2A). Epigenetic inactivation of exp-2 by RNA interference (RNAi) also decreased pumping frequency to the same extent as mps-4 KO (11%, Fig. 2A). The slowing down...
A Model for KCNE Arrhythmia

FIGURE 3. MPS-4 modulates the shape of the action potential. A, pharyngeal action potential recorded in a N2 animal. The head of the worm was chopped with a needle, transferred to the recording chamber, held in place with a suction electrode, and impaled with an intracellular electrode filled with 2 M KCl. B, pharyngeal action potential recorded in a mps-4 KO animal. C, pharyngeal action potential recorded in an exp-2 KO animal. Inset, full signal (scale bar, 50 ms). D, pharyngeal action potential recorded in a N2 animal treated with exp-2 RNAi.

TABLE 1
Action potential durations and intervals

For each single recording the mean action potential duration (ΔPd), action potential interval (ΔPi) and their respective S.D. for the indicated animals were calculated using the half-threshold method (29) and then averaged. n indicates the number of determinations. ΔPd and ΔPi distributions were fit to a single or double Gaussian function (Equation 1). t values are the values at which the Gaussian functions are maximal and s are the variances.

|          | N2             | mps-4 KO       | Anti_MPS-4 | exp-2 KO       | exp-2 RNAi |
|----------|----------------|----------------|------------|----------------|------------|
| ΔPd (ms) | 191 ± 23       | 249 ± 89       | 241 ± 78   | 367 ± 272      | 218 ± 77   |
| (APd) ms | 571 ± 87       | 632 ± 196      | 611 ± 203  | 688 ± 287      | 611 ± 178  |
| ΔPi (ms) |               |                |            |                |            |
| t1       | 179 ± 35       | 227 ± 35       | 251 ± 44   | 340 ± 93       | 215 ± 38   |
| s1       | 20 ± 3         | 69 ± 7         | 40 ± 6     | 65 ± 13        | 37 ± 4     |
| t2       | 619 ± 164      | 617 ± 125      | 821 ± 240  | 407 ± 56       |            |
| s2       | 63 ± 8         | 80 ± 8         | 293 ± 73   | 39 ± 5         |            |
| n        | 6              | 6              | 6          | 4              | 4          |

For APi (ms)...

was much marked in exp-2 KO animals (40%, Fig. 2A) probably reflecting the limited efficiency of exp-2 RNAi. We next applied the MPS-4 antibody (diluted 1:1,000 from the serum stock) to intact pharynxes in the presence of 1 μM 5-HT. The antibody caused a statistically significant reduction (~24%) in the number of contractions per minute. Furthermore, anti-MPS-4 was ineffective in the pharynxes of mps-4 KO worms that, notably, pumped at a slower pace at baseline (Fig. 2C). Taken together these data led us to conclude that MPS-4 is not only expressed, but it also necessary for the normal activity of the pharynx.

mps-4 KO Delays Repolarization—To elucidate the mechanism by which mps-4 KO slowed pharyngeal pumping we recorded the electrical activity of the muscle in the current-clamp configuration of the patch-clamp. Fig. 3A shows a typical wild-type action potential. EXP-2, which is active at the end of the plateau phase, sharply terminates the signal and produces repolarization (arrow) during which the membrane is electrically refractive. The effect of knocking out mps-4 induced several modifications in the signal (Fig. 3B). First, action potential duration was increased in mps-4 KO animals by 25% compared with N2 animals (Table 1). Second, the after hyperpolarization transient was significantly suppressed. The effects of knocking out exp-2 were qualitatively similar (prolongation of the signal and suppression of the after hyperpolarization transient, Fig. 3C) but more pronounced than in mps-4 KO animals. In contrast, the alterations exerted by exp-2 RNAi were consistent with a partial suppression of exp-2 and thus of I_{Kp} (Fig. 3D). Notably exp-2 RNAi caused effects that were qualitatively and quantitatively similar to those caused by mps-4 KO (Table 1) raising the possibility that the lack of MPS-4 produced partial inhibition of I_{Kp}. 
A Model for KCNE Arrhythmia

FIGURE 4. mps-4 KO induces abnormal repolarization. A, representative recording of the electrical activity of the pharynx of a N2 animal. B, representative recording of the electrical activity of the pharynx of a mps-4 KO animal. DAD events are clearly recognizable. The arrows mark periods of high frequency activity. C, representative recording of the electrical activity of the pharynx of a N2 animal in the presence of anti-MPS-4 (in a 1:1000 dilution) in the test solution. D, representative recording of the electrical activity of the pharynx of a exp-2 RNAi animal. E, representative recording of the electrical activity of the pharynx of a exp-2 KO animal. In all cases the test solutions contained 1 μM 5-HT.

A representative recording of the activity of a wild-type pharynx is shown in Fig. 4A. The activity of these pharynxes was remarkably regular (n = 6). Conversely, mps-4 KO pharynxes were characterized by action potentials of variable length and by delayed after depolarizations (DAD) (Fig. 4B). DADs, which are abnormal depolarizations that cause a second action potential to be fired before the first is terminated, were probably triggered by the short refractory period in mps-4 KO worms. Application of anti-MPS-4 perturbed the electrical activity of the pharynx in a similar fashion (Fig. 4C). exp-2 KO as well as animals treated with exp-2 RNAi also exhibited long action potentials and multiple DAD events (Fig. 4, D and E). To quantify these alterations we analyzed continuous recordings of activity (5–10 min) using the half-threshold method (29). This approach, which is employed in single-channel analysis, allows to translate original recordings into idealized traces of ion channel openings and closings and to compute all relevant statistical quantities. An example of an idealized trace as well as calculated histograms is illustrated in Fig. 5. We estimated the mean duration of the action potential (APi) and the mean duration of the interval separating two consecutive action potentials (APd) by formally treating them as dwell open and closed times. These quantities, which are listed in Table 1, reflect the qualitative impression that mps-4 KO and exp-2 KO led to prolonged action potentials, DADs, and irregular rhythm. Thus, mps-4 KO, exp-2 RNAi, or treatment with anti-MPS-4 increased the APd by, respectively, 21, 17, 47, and 21%. The standard deviations (S.D.) were significantly greater than in wild-type animals revealing increased variability in AP duration and the occurrence of DADs. With respect to the interval between action potentials, whereas the means were only slightly increased, the S.D. values were significantly greater in all cases compared with control, as expected. The APd and APi exhibited normal distributions (Fig. 5, B and C). However, whereas the APi histograms of N2 animals always exhibited a single Gaussian distribution, the histograms of mps-4 KO or exp-2 KO animals as well as of animals treated with exp-2 RNAi or anti-MPS4 contained two Gaussians underscoring the occurrence of DADs. The APi histograms were also double-peaked. A broad Gaussian peaked at around 600–900 ms reflected the irregular rhythm induced by suppression of mps-4 or exp-2. A second Gaussian, peaked at ~250 ms corresponded to periods in which firing occurred at high frequency (arrows in Fig. 4) probably representing pre-DAD states. In contrast, the APi distribution in exp-2 KO animals was single peaked but very broad.

**MPS-4 and EXP-2 Co-assemble in Pharyngeal Muscle**—The findings that mps-4 KO, exp-2 KO, or treatment with anti-MPS-4 altered the action potential in a similar fashion suggested that the lack of MPS-4 diminished I\(_{\text{Kp}}\), and that therefore MPS-4 and EXP-2 form a complex in the pharynx. To strengthen the notion that the two proteins assemble in the pharynx we assessed the effect that epigenetic inactivation of mps-4 by RNAi had on the expression of EXP-2 tagged by GFP. Generally, KCNE proteins are essential components of their respective complexes that become unstable and/or cannot traffic to the plasma membrane without them (22, 31). As expected, mps-4 RNAi selectively attenuated GFP signals in isthmus and terminal bulb (Fig. 6). We conclude that MPS-4 and EXP-2 form a complex in pharyngeal muscle. Thus, I\(_{\text{Kp}}\), is composed by at least two similar but not identical currents, both produced by the same pore-forming subunit, EXP-2, and differentiated by the KCNE accessory subunit MPS-4 in specific parts of the pharynx.

**MPS-4 and EXP-2 Form a Complex in CHO Cells**—To characterize how MPS-4 functionally modulates I\(_{\text{Kp}}\), we expressed EXP-2 alone or with MPS-4 in CHO cells and used biochemistry and electrophysiology to determine complex formation and function. Fig. 7A shows that EXP-2 and MPS-4 co-immunoprecipitated in CHO cells, as expected. Macroscopic currents produced by EXP-2 or EXP-2-MPS-4 channels are shown in Fig. 7B. The voltage protocols used to elicit these currents are illustrated in the insets of the figure. Because of the conduction mechanism of the EXP-2 channel, the membrane must be depolarized by a preconditioning pulse that primes the channel by moving it into the inactivated state. In cells transfected with EXP-2 cDNA, negative membrane voltages evoked large tail currents. Co-transfection with MPS-4 cDNA induced three major modifications in the current: a reduction in its magnitude along with accelerated deactivation and with a left shift in the
threshold for steady-state activation ($V_{1/2}$). Thus, the current density at $-120$ mV of EXP-2 channels alone, which gives a measure of the whole cell current normalized for cell size, was $370 \pm 35$ pA/pF ($n = 25$), roughly twice that of complexes formed with EXP-2 and MPS-4 ($194 \pm 22$ pA/pF, $n = 33$, $p < 0.02$, Fig. 7C). The fact that MPS-4 decreases the EXP-2 current is not in contradiction with the finding that $mps-4$ KO also diminishes $I_{Kp}$ because they traffic together to the membrane. The time course of deactivation was best fit to a double exponential function (Equation 2) with slow and fast time constants that were $35\%$ ($\tau_1 = 120 \pm 13$ ms, $n = 22$, versus $75 \pm 11$ ms, $n = 33$) and $50\%$ ($\tau_2 = 18 \pm 4$ ms, $n = 22$, $p < 0.05$).

**FIGURE 5.** Mean action potential durations and intervals. A, the trace shown in the figure was recorded in a wild-type pharynx. The idealized trace was computed by Clampfit 9.2 software. B, representative histograms of action potential duration (APd) in N2 and mps-4 KO worms. Histograms were fitted to a single or double Gaussian function (Equation 1). C, representative histograms of the time between two consecutive action potentials (APi).

**FIGURE 6.** MPS-4 and EXP-2 form a complex in pharyngeal muscle. A, representative pictures (merge of phase contrast and fluorescence and fluorescence) of a exp:gfp transgenic nematode. These worms display GFP fluorescence in the pharynx. B, GFP fluorescence in an exp:gfp nematode obtained from eggs laid 12–36 h after injection of the progenitor (P0) with MPS-4 dsRNA (day 1). C, normalized fluorescence ratios in control (filled) and exp-2:gfp nematodes treated with mps-4 RNAi 12–36 (day 1), 36–50 (day 2), and 50–74 (day 3) h after P0 injection (open). Fluorescence was quantified by ImageJ software available (rsb.info.nih.gov/nih-image/Default.html). Fluorescence ratio, FR, was normalized to control nematodes at day 1. Data are from groups of 10 or more worms. Statistically significant differences from control animals are indicated with *, $p < 0.05$. threshold for steady-state activation ($V_{1/2}$). Thus, the current density at $-120$ mV of EXP-2 channels alone, which gives a measure of the whole cell current normalized for cell size, was $370 \pm 35$ pA/pF ($n = 25$), roughly twice that of complexes formed with EXP-2 and MPS-4 ($194 \pm 22$ pA/pF, $n = 33$, $p < 0.02$, Fig. 7C). The fact that MPS-4 decreases the EXP-2 current is not in contradiction with the finding that $mps-4$ KO also diminishes $I_{Kp}$ because they traffic together to the membrane. The time course of deactivation was best fit to a double exponential function (Equation 2) with slow and fast time constants that were $35\%$ ($\tau_1 = 120 \pm 13$ ms, $n = 22$, versus $75 \pm 11$ ms, $n = 33$) and $50\%$ ($\tau_2 = 18 \pm 4$ ms, $n = 22$, $p < 0.05$).
versus 10 ± 3 ms, n = 33, at −120 mV) smaller in channels formed with MPS-4 (Fig. 7D). A voltage protocol consisting of a series of preconditioning voltages from −80 to +20 mV followed by a test pulse at −100 mV (Fig. 7E, inset) was used to determine the voltage dependence of the two channel types in symmetrical potassium. Fig. 7F shows characteristic EXP-2 and EXP-2-MPS-4 currents elicited by this protocol; isochronal open probability curves for EXP-2 channels alone or with MPS-4 are illustrated in Fig. 7F. Fit of the data to the Boltzmann function (Equation 3) gave a half-maximal activation voltage, $V_{1/2} = −15.5 ± 2.5$ mV and a slope coefficient $V_z = 5.3 ± 0.4$ mV for EXP-2 channels alone (n = 18). Co-expression with MPS-4 induced a 10-mV left shift in the midpoint for activation ($V_{1/2} = −25.1 ± 3.1$ mV, n = 15) without significantly affecting the slope coefficient of the curve ($V_z = 6.3 ± 0.2$ mV, n = 15). Fig. 7G shows representative current-dose relationships for EXP-2 and EXP-2-MPS-4 currents. The antibody did not induce any inhibition or any apparent modification in the current produced by EXP-2 channels alone (data not shown, n = 3), over a wide range of dilutions. In contrast anti-MPS-4 in a 1:1000 dilution inhibited EXP-2-MPS-4 currents by ~45% ($d_1/2 = 0.0011 ± 0.00002$; $n_{1/2} = 1.61 ± 0.2$, n = 4) without altering steady-state activation or deactivation kinetics ($V_{1/2} = −27.5 ± 5.1$ mV and $V_z = 5.5 ± 0.3$ mV; $\tau_1 = 83 ± 16$ ms and $\tau_2 = 7 ± 3$ ms, n = 4, data not shown). Taken together these data explain how MPS-4 contributes to pharyngeal contractility. $I_{Kp}$ becomes activated at the end of the plateau phase when L-type calcium channels begin to close and the membrane starts to repolarize (25). Hence, by decreasing the voltage by which EXP-2 channels are activated, MPS-4 ensures that they are all primed and ready to depolarize the muscle in a timely fashion. In addition, by speeding recovery for inactivation (deactivation), MPS-4 makes the muscle ready to undergo the next excitatory cycle.

**Mutation of a Conserved Aspartate Residue Associated with Human Disease Alters the Functional Attributes of the C. elegans Current**—A congenital mutation of aspartate to asparagine to yield D74N KCNE1 is linked to cardiac arrhythmia and deafness (9, 15, 32); the analog D74N MPS-4 (Fig. 1A) was studied. Channels formed with EXP-2 and D74N MPS-4 (Fig. 8A) were found to deactivate slower than heteromeric complexes containing wild-type MPS-4 subunits and (slightly) EXP-2 channels alone (Fig. 8B). The $V_{1/2}$ was shifted by ~25 mV toward depolarizing potentials (Fig. 8C, $V_{1/2} = 1.4 ± 0.4$ mV; $V_z = 6.1 ± 0.5$ mV, n = 11). This means that the mutant channel activates at potentials that are even more positive (~10 mV) than those of EXP-2 alone. Current density was slightly increased in the mutant compared with wild-type but not in a statistically significant fashion (244 ± 35 pA/pF, p < 0.067, data not shown). These findings further demonstrate that KCNE peptides share common mechanisms (33). Considering the important physiological role of voltage activation and recovery from inactivation these data strongly suggest that genetic mutations associated with arrhythmia in *Homo sapiens* might similarly affect pharyngeal contractility in *C. elegans*.
A Model for KCNE Arrhythmia

**FIGURE 8.** D74N MPS-4 alters the current. A, representative whole cell currents conducted by channels formed with EXP-2 and D74N MPS-4. Voltage protocol as described in the legend to Fig. 7A. B, deactivation rates for EXP-2, MPS-4 (dotted line) and EXP-2-D74N MPS-4 (triangles, n = 11). Time constants were obtained by fitting macroscopic currents to a double-exponential function (Equation 2). C, isochronal macroscopic curves for EXP-2-MPS-4 (dotted line) and EXP-2-D74N MPS-4 (n = 11). The theoretical line was computed using the Boltzmann function (Equation 3) with, respectively, \( V_{1/2} = 1.0 \text{ mV} \) and \( k = 5.9 \text{ mV} \).

**DISCUSSION**

We have analyzed the physiological role of MPS-4, a *C. elegans* kcne-related gene, by biochemistry, genetics, and electrophysiology. A custom made polyclonal antibody detected MPS-4 in the pharynx. Genetic and biochemical manipulations including knock out of *mps-4* or *exp-2*, treatments with anti-MPS-4 and *exp-2* RNAi altered the electrical activity of the pharynx in a fashion consistent with the diminished repolarizing potassium current. Moreover, *mps-4* RNAi in *exp-2;gfp* transgenic animals suppressed GFP signals in the isthmus and terminal bulb. EXP-2 and MPS-4 co-immunoprecipitated in CHO cells. When MPS-4 was co-transfected with EXP-2 it altered multiple attributes of the current. This body of evidence supports the notion that 1) MPS-4 operates in the pharynx of *C. elegans* and 2) it assembles with EXP-2 to form a complex that controls the repolarization of the muscle.

MPS-4 lowers the threshold for voltage activation and speeds recovery from inactivation (deactivation) thereby increasing the number of available channels at the end of the plateau phase and facilitating their quick reset. This may explain why *mps-4* KO causes diminished \( I_{Kp} \) and DADs. Notably, the functional modifications that MPS-4 exerts on EXP-2 are similar to those that KCNE2 induces in HERG even though the two pore-forming subunits exhibit low sequence homology (4). The only difference is that KCNE2 shifts the \( V_{1/2} \) by 10 mV toward more depolarizing potentials and thus delays the time at which \( I_{Kr} \) channels initiate to repolarize the muscle. This probably underlies local differences in the contractility of the two pumps. Abnormal repolarization is one cause of arrhythmia in vertebrates (19). KCNE1 and KCNE2 are thought to induce congenital and acquired Long QTs by prolonging the duration of the action potential through diminished \( I_{Ks} \) and/or \( I_{Kr} \) currents (4, 14–16). For example, functional studies in heterologous expression systems showed that channels formed with D76N KCNE1 and cardiac KCNQ1 exhibited lower unitary conductance, a requirement for larger depolarization to activate and more rapid deactivation (14). Abbott and Goldstein (33) further demonstrated that D76N analogs exert similar effects on their respective partners. The fact that also *mps-4* KO delays the duration of the action potential and shortens the refractory period by diminishing \( I_{Kp} \) and that the conserved D74N mutation in MPS-4 shifts the activation voltage toward depolarizing potentials and slows down recovery from inactivation underscores a remarkable conservation of KCNE activities in different pumps and suggests that *kcne* arrhythmia susceptibility mutations might act to impair pharyngeal muscle excitability when translated to *mps-4*.

Intriguingly, MPS-4 appears to assemble with EXP-2 only in local areas of the pharynx. Using anti-MPS-4 antibody we found that MPS-4 is expressed in terminal bulb and in anterior isthmus but it is absent in posterior isthmus for instance, where EXP-2 expression is clearly detectable. This qualitative impression was corroborated by the finding that the effect of knocking out *mps-4* affected the pharyngeal pumping phenotype to a lesser extent than knocking out *exp-2*, although one should consider that the terminal bulb is not electrically as active as the corpus (30) therefore the contribution of MPS-4 to the action potential might have been underestimated. The pharynx is a peristaltic pump and as such, different compartments contract and relax at different times. The relaxation of the isthmus and corpus are slightly phase-shifted, for example. Terminal bulb movements are rapid, whereas isthmus contractions are slow and not in synchrony with other compartments. Variations in ion channel expression and regulation may explain some differences in the function of pharyngeal compartments. Thus, it appears that MPS-4 is required to fine-tune pharyngeal muscle excitability by acting to locally differentiate the potassium repolarizing current.

One significant implication of the findings presented here is that *C. elegans* might be employed as a resourceful model system to study the mechanisms underlying KCNE-mediated arrhythmia susceptibility and pharmacology. We are currently characterizing the pharmacology of EXP-2-MPS-4 channels and the response of *C. elegans* to known proarrhythmic and anti-arrhythmic drugs. Preliminary results suggest that the pharmacological profiles of EXP-2-MPS-4 and HERG-KCNE2 are surprisingly similar. Hence, we expect that the unprecedented genetic and molecular tools offered by *C. elegans* can be used to reveal new insights into the mechanisms of arrhythmia, and to provide new models for pharmaceutical testing.

In summary, KCNE proteins, which modulate multiple Kv channels in human tissues, appear to be an essential feature also of invertebrate channels. These results underscore a high degree of functional conservation between mammalian and invertebrate channels and demonstrate that *C. elegans* is a valuable system for studying physiological aspects of K⁺ channels and their KCNE partner subunits.

**ACKNOWLEDGMENTS**—The exp-2::gfp transgenic strain was a gift of Dr. Leon Avery. The *mps-4* KO strain was a gift of Dr. Shohei Mitani. The *exp-2* KO was a gift of the Caenorhabditis Genetics Center (CGC). We thank Drs. John Lenard, Shi-Qing Cai, Geoffrey Abbott, and Thomas McDonald for critical reading of the manuscript and Fulvio Sesti for help with the graphic.

**REFERENCES**

1. Cai, S. Q., Park, K. H., and Sesti, F. (2006) *Cell Biochem. Biophys.* 46, 91–100
2. McCrossan, Z. A., and Abbott, G. W. (2004) *Neuropharmacology* 47, 787–821
3. Abbott, G., Butler, M., Bendahhou, S., Dalaks, M., Ptacek, L., and Goldstein, S. (2001) Cell 104, 217–231
4. Abbott, G., Sesti, F., Splawski, I., Buck, M., Lehman, M., Timothy, K., Keating, M., and Goldstein, S. (1999) Cell 97, 175–187
5. McCrossan, Z. A., Lewis, A., Panaghie, G., Jordan, P. N., Christini, D. J., Lerner, D. J., and Abbott, G. W. (2003) J. Neurosci. 23, 8077–8091
6. Roepke, T. K., Anantharam, A., Kirchhoff, P., Busque, S. M., Young, J. B., Geibel, J. P., Lerner, D. J., and Abbott, G. W. (2006) J. Biol. Chem. 281, 23740–23747
7. Yu, H., Wu, J., Potapova, I., Wymore, R., Holmes, B., Zuckerman, J., Pan, Z., Wang, H., Shi, W., Robinson, R., El-Maghrabi, M., Benjamin, W., Dixon, J., McKinnon, D., Cho, I., and Wymore, R. (2001) Circ. Res. 88, E84–87
8. Nicolas, M., Dememes, D., Martin, A., Kupershmidt, S., and Barhanin, J. (2001) Hear Res. 153, 132–145
9. Daggal, P., Vesely, M. R., Wattanasirichaigoon, D., Villafane, J., Kaushik, V., and Beggs, A. H. (1998) Circulation 97, 142–146
10. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) Nature 384, 78–80
11. McDonald, T., Yu, Z., Ming, Z., Palma, E., Meyers, M., Wang, K., Goldstein, S., and Fishman, G. (1997) Nature 388, 289–292
12. Sanguinetti, M., Curran, M., Zou, A., Shen, J., Spector, P., Atkinson, D., and Keating, M. (1996) Nature 384, 80–83
13. Sesti, F., Abbott, G. W., Wei, J., Murray, K. T., Saksena, S., Schwartz, P. J., Priori, S. G., Roden, D. M., George, A. L., Jr., and Goldstein, S. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10613–10618
14. Sesti, F., and Goldstein, S. A. (1998) J. Gen. Physiol. 112, 651–663
15. Splawski, I., Tristani-Firouzi, M., Lehmann, M. H., Sanguinetti, M. C., and Keating, M. T. (1997) Nat. Genet. 17, 338–340
16. Yang, Y., and Sigworth, F. J. (1998) J. Gen. Physiol. 112, 665–678
17. Curran, M., Splawski, I., Timothy, K., Vincent, G., Green, E., and Keating, M. (1995) Cell 80, 795–803
18. Wang, Q., Curran, M., Splawski, I., Burn, T., Millholland, J., VanRaay, T., Shen, I., Timothy, K., Vincent, G., and de Jaeger, T. (1996) Nat. Genet. 12, 17–23
19. Viskin, S. (1999) Lancet 354, 1625–1633
20. Keating, M., and Sanguinetti, M. (2001) Cell 104, 569–580
21. Anantharam, A., Lewis, A., Panaghie, G., Gordon, E., McCrossan, Z. A., Lerner, D. J., and Abbott, G. W. (2003) J. Biol. Chem. 278, 11739–11745
22. Bianchi, L., Kwok, S. M., Driscoll, M., and Sesti, F. (2003) J. Biol. Chem. 278, 12415–12424
23. Park, K. H., Hernandez, L., Cai, S. Q., Wang, Y., and Sesti, F. (2005) J. Biol. Chem. 280, 21893–21899
24. Olson, E. N. (2006) Science 313, 1922–1927
25. Shiono, B., and Avery, L. (2005) J. Exp. Biol. 208, 2177–2190
26. Davis, M., Fleischhauer, R., Dent, J., Joho, R., and Avery, L. (1999) Science 286, 2501–2504
27. Fleischhauer, R., Davis, M. W., Dzhura, I., Neely, A., Avery, L., and Joho, R. H. (2000) J. Neurosci. 20, 511–520
28. Smith, P., Baukrowitz, T., and Yellen, G. (1996) Nature 379, 833–836
29. Sakmann, B., and Neher, E. (eds) (1995) Single-channel Recording, 2 Ed., Vol. 1, Plenum Press, New York
30. Raizen, D. M., and Avery, L. (1994) Neuron 12, 483–495
31. Chandrasekhar, K. D., Bas, T., and Kobertz, W. R. (2006) J. Biol. Chem. 281, 40015–40023
32. Schulze-Bahr, E., Wang, Q., Wedekind, H., Haverkamp, W., Chen, Q., Sun, Y., Rubie, C., Hordt, M., Towbin, J. A., Borggreve, M., Assmann, G., Qu, X., Somberg, J. C., Breithardt, G., Oberti, C., and Funke, H. (1997) Nat. Genet. 17, 267–268
33. Abbott, G. W., and Goldstein, S. A. (2002) FASEB J. 16, 390–400