KCNQ-like Potassium Channels in Caenorhabditis elegans

CONSERVED PROPERTIES AND MODULATION

Received for publication, March 11, 2005, and in revised form, March 28, 2005
Published, JBC Papers in Press, March 29, 2005, DOI 10.1074/jbc.M502734200

Aguan D. Wei‡, Alice Butler, and Lawrence Salkoff§
From the Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

The human KCNQ gene family encodes potassium channels linked to several genetic syndromes including neonatal epilepsy, cardiac arrhythmia, and progressive deafness. KCNQ channels form M-type potassium channels, which are critical regulators of neuronal excitability that mediate autonomic responses, pain, and higher brain function. Fundamental mechanisms of the normal and abnormal cellular roles for these channels may be gained from their study in simple model organisms. Here we report that a multigene family of KCNQ-like channels is present in the nematode, Caenorhabditis elegans. We show that many aspects of the functional properties, tissue expression pattern, and modulation of these C. elegans channels are conserved, including suppression by the M1 muscarinic receptor. We also describe a conserved mechanism of modulation by diacylglycerol for a subset of C. elegans and vertebrate KCNQ/KQT channels, which is dependent upon the carboxyterminal domains of channel subunits and activated protein kinase C.

The human genome contains five KCNQ genes that encode a family of K+ channel α-subunits possessing six transmembrane domains and a single pore loop. Functional channels are assembled from four α subunits, and may be either homo- or heterotetramers, depending on cell type. These channels serve a wide range of physiological roles. In the heart, KCNQ1 (originally designated KvLTQ1) is co-assembled with the product of the KCNE1 gene (variously designated minK, IsK, and MiRP) to form the cardiac I(K,n) delayed rectifier-like K+ current present in colonic crypt cells (8, 9). KCNQ2/KCNQ3 heteromultimers are thought to underlie the prototypic M-current. Mutations in either of these genes cause an inherited neonatal epilepsys (Benign Familial Neonatal Convulsion, BFNC) (10–14). The KCNQ4 gene may encode the molecular correlate of I(K,n) in outer hair cells of the cochlea, and I(K,L) in Type I hair cells of the vestibular apparatus, mutations that lead to a form of inherited progressive adult deafness (Autosomal Dominant Non-syndromic Deafness, DFNA2) (15). The recently identified KCNQ5 gene is expressed in brain and skeletal muscle and can co-assemble with KCNQ3, suggesting it may contribute to M-current heterogeneity (16, 17), although no linkage to a hereditary disease has yet been reported. Thus, mutations in four of the five human KCNQ genes are associated with hereditary diseases, suggesting a uniquely important role for this class of channels in a variety of physiological functions.

In this article we show that a three-gene family of KCNQ-like channels is also present in the nematode worm, Caenorhabditis elegans, which we call KQT channels. Many aspects of their functional properties, tissue distribution, and modulation have striking parallels with their mammalian orthologues. For example, C. elegans pharyngeal muscles possess many cardiac-like properties including electrical coupling and rhythmic myogenic contractions generated by prolonged action potentials (18). We show that kqt-1 is expressed in pharyngeal muscles, where it may serve a role analogous to I(K,n) in mammalian cardiac muscle. Additional kqt expression was found in nematode mechanosensory neurons, chemosensory neurons, and intestinal cells that may mediate cellular functions analogous to those in vertebrate species.

We also show, that like all mammalian KCNQ channels, C. elegans KQT channels are modulated by the M1 muscarinic receptor that utilizes a Goq signaling pathway. We address one potential signaling pathway of receptor-stimulated inhibition of M-currents using kqt and KCNQ genes from both C. elegans and vertebrates. We find that a subset of these kqt/KCNQ genes encode channels that are potently suppressed by submicromolar concentrations of the water soluble diacylglycerol analog, 1-oleoyl-2-acetyl-sn-glycerol (OAG), implicating DAG as a signaling intermediary. By analysis of chimeric subunit constructs, we find that this OAG1 suppression is mediated through the carboxy-terminal “tail” domains of OAG-sensitive channel subunits. We observe that OAG-mediated suppression

* This work was supported by National Science Foundation Grant IBN-0117341 (to A. W.), National Institutes of Health Grants R01GM067154-02 and R24RR017342-02 (to L. S.), and a pilot grant from the Digestive Diseases Research Center Core and Clinical Nutrition Research Unit at Washington University School of Medicine (to A. W.). 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 1754 solely to indicate this fact.

The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank®/EBI Data Bank with accession numbers AY572974 and AY572975.

‡ To whom correspondence may be addressed: Dept. of Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110. Tel.: 314-747-3306; Fax: 314-362-3446; E-mail: awei@wustl.edu.
§ To whom correspondence may be addressed: Dept. of Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110. Tel.: 314-747-3306; Fax: 314-362-3446; E-mail: salkoff@wustl.edu.

1 The abbreviations used are: OAG, 1-oleoyl-2-acetyl-sn-glycerol; DAG, diacylglycerol; PMA, phorbol 12-myristate 13-acetate; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; PKC, protein kinase C; GFP, green fluorescent protein; PIP3, phosphatidylinositol 4,5-bisphosphate.
is mimicked by phorbol 12-myristate 13-acetate (PMA), a potent pharmacological activator of protein kinase C (PKC). Pretreatment with staurosporine, a PKC-specific inhibitor effectively blocks OAG inhibition. These results suggest that DAG-stimulated PKC may mediate receptor-coupled inhibition of a subset of M-currents through a mechanism involving the carboxyl-terminal tails of OAG-sensitive channel subunits. This mechanism is conserved for a subset of KCNQ/KQT channels in both C. elegans and vertebrates.

**EXPERIMENTAL PROCEDURES**

**Molecular Biology and C. elegans Transformed Strains**—Full-length cDNAs of kqt-1, kqt-2, and kqt-3 were generated by a combination of hybridization screens with 32P-labeled DNA probes, PCR from a commercial C. elegans cDNA library (number 90907, Stratagene, San Diego, CA), and reverse transcriptase-PCR from mRNA extracted from mixed staged wild-type animals, using oligonucleotide primer sequences based on predicted cDNAs from cosmid sequences C25B8 (kqt-1; GenBank™ accession number U41556), M60 (kqt-2; GenBank accession number U39995), and YAC sequence Y54G9a (kqt-3; GenBank accession number AL032648). PCR-generated cDNAs were sequenced with GenBank entries, as well as independently sequenced wild-type genomic fragments in some instances, to resolve sequence discrepancies with GenBank predictions. These cDNA sequences can be accessed with GenBank accession numbersAY572974 (kqt-1) andAY572975 (kqt-3). A full-length kqt-2 cDNA was contained within an EST (yk25b9) kindly provided by Yuji Kohara (National Institutes of Genetics, Mishima, Japan). C. elegans kqt cDNAs were modified by introduction of a Rozzo consensus sequence consensus sequence to each initiation methionine, and deletion of all 5' non-coding sequences. Full-length KCNQ4 was reconstructed from a partial cDNA (AK074957, obtained from the National Institute of Technology and Evaluation, Department of Biotechnology, Biological Resource Center, Chiba, Japan), with additional 5' fragments generated by PCR from cDNAs derived from human brain RNA and genomic DNA (Clontech, Palo Alto, CA). Additional cDNAs used in this study were generously provided by Jacques Barbanin (KCNQ1; Institut de Pharmacologie Moleculaire et Cellulaire, Valbonne, France), David McKinnon (KCNQ2, rat KCNQ3; State University of New York at Stony Brook, Klaus Steinmeier, Christian Lerche, Giscaard Seeböhm and Andreas Busch (KCNQ5; Aventis, Frankfurt, Germany), and Narasimhan Gautam (human M1 muscarinic receptor; Washington University School of Medicine). Chimeric KQT/KCNQ constructs were generated by the overlap-extension PCR technique (19). All cDNAs were subcloned into pOX (20) for Xenopus oocyte expression studies. To promote efficient heterologous expression in Xenopus oocytes, pOX incorporates 5' and 3' untranslated sequences from the Xenopus β-globin gene into transcribed cDNAs. All constructs were verified by sequencing. C. elegans transatlantic GFP fusion constructs for each kqt gene were generated using subclones of genomic cosmid (C25B8, M60) and YAC (Y54G9a) clones provided by the C. elegans Sequencing Consortium (Genome Sequencing Center, Washington University School of Medicine, and Sanger Institute, Hinxton, UK). Subclones were designed to encompass most or all exons for each kqt gene, and substantial lengths of 5' and 3' non-coding sequences. The kqt-1:GFP construct was created by first subcloning an 11.6-kb NruI fragment of C25B8, which includes the entire kqt-1 gene and ~9.0 kb of 5' untranslated and ~2.1 kb of 3' untranslated sequences, into pBluecriptII KS+ (Stratagene, San Diego). This genomic subclone was then modified by the insertion of a GFP cDNA sequence in-frame at a unique SpeI site within exon 16 (immediately 3' of the codon corresponding to T672), preserving the native 3' splice site of exon 16. The kqt-2:GFP construct was created by subcloning a 11.9-kb NsiI fragment from Cosmid M-60, encompassing the first 12 exons of kqt-2 and ~9.0 kb of 5' non-coding sequence, into pPD59.81 (gift of Andy Fire, Carnegie Institution of Washington), linearized with PstI. This construct thus produces a 3' translational fusion of KQT-2 at Ala-605 with GFP, lacking the last 70 predicted carboxyl-terminal amino acids encoded in exon 13. The kqt-3:GFP construct was created by subcloning a ~5.0-kb NsiI/NheI fragment amplified from Y54G9a by PCR, into pPD59.75 (gift of Andy Fire, Carnegie Institution of Washington). This construct encompasses all kqt-3 exons and ~1.5 kb of the non-coding sequence 5' of the initiation methionine, and creates a translational fusion of GFP with the C terminus of KQT-3. Two additional overlapping PCR-generated genomic fragments were made, encompassing sequences ~6.2 kb further 5' of the initial subcloned kqt-3 fragment. Coinjection of these PCR-generated fragments with the 5' linearized kqt-3:GFP construct yielded lines with stable labeling of chemosensitive neurons, because of transgenes presumably generated by efficient intragenomic recombinant (21). Transformed C. elegans strains were created by the standard germline injection technique (22), using ~20–50 ng/µl of each construct and rol-6(t-1107) as a selectable marker. GFP-positive chemosensitive neurons were identified in L1 staged larvae, assisted by co-labeling amphid sensory neurons (ASK, ADL, ASI, AWB, ASH, and ASJ) with DiI (Molecular Probes, Eugene, OR), as landmark cells (23).

**Electrophysiology**—Two-electrode voltage-clamp and patch clamp recordings were made from Xenopus oocytes injected with cRNAs, as previously described (20). Dose-response series were obtained with a recording chamber with a volume of ~150 µl, and solution changes with ~3 times the recording chamber volume, applied manually without plastic tubing to minimize potential error because of retention of lipophilic reagents to tubing. Two-electrode voltage-clamp measurements were made at steady-state, typically 2–3 min after drug application. Between drug series, all recording surfaces were flushed with 70% ethanol to remove residual drug samples, then with ND96 recording solution. Stock solutions of drugs were dissolved in MeSO4, stored at ~20 °C, and diluted in ND96 for each experimental series. In all instances, final MeSO4 concentrations never exceeded 1% (v/v). Control experiments showed that 1% MeSO4 had no effect on either endogenous or heterologously expressed currents in Xenopus oocytes. All measurements were made with 1.0 mM DIDS (Sigma) to block endogenous Ca2+-activated Cl− currents present in Xenopus oocytes. Composition of ND96 (in mM) was: 96 NaCl, 2.0 KCl, 1.8 CaCl2, 1.0 MgCl2, 5.0 HEPES (pH 7.2).

Excised inside-out patch-clamp recordings were made under symmetrical 160 mM K+ recording conditions, with zero Ca2+ and nearly CI−-free solutions. Recordings were obtained and low-pass filtered at 2.0 kHz, with either an Axopatch 200A (Molecular Devices, Sunnyvale, CA) or a model 2400 (A-M Systems, Carlsborg, WA) patch-clamp amplifier. Composition of pipette solution (in mM) was: 160 K-gluclate, 2.0 MgCl2, 5.0 HEPES (pH 7.2). Composition of cytoplasmatic bath/perfusion solution (in mM) was: 160 K-gluclate, 5.0 EGTA, 5.0 HEPES (pH 7.2).

Linopirdine was a gift from Larry Brown (DuPont Pharmaceuticals, Wilmington, DE). OAG, phorbol 12-myristate 13-acetate (PMA), and poly-lysine HCl (15–30,000 M) were purchased from Sigma. Staurosporine and oxtremorine were purchased from Tocris Cookson (Ellisville, MO).

**RESULTS**

A KCNQ-like Multigene Family in C. elegans—As in humans, KCNQ-like genes are represented in C. elegans as a multigene family. The C. elegans genome encodes 71 potassium channel genes (24), of which three have similarity to KCNQ genes, forming a family of genes we call kqt for “K” channel related to QT interval.” The three members of this family are kqt-1 (C25B8.1, Wormbase notation), kqt-2 (M60.2), and kqt-3 (Y54G9a.3). Primary sequence alignments revealed two domains of high conservation (~70% identity), defining the “core” transmembrane segments S1–S6, and an additional region of ~115 residues within the putative cytoplasmatic carboxyl-terminal tail, implicated in subunit multimerization (25, 26) (Supplemental Materials Fig. S1). Phylogenetic comparison of all C. elegans KQT and human KCNQ primary sequences identified two conserved subfamilies: C. elegans KQT-1 defining one subfamily with KCNQ2–5, whereas C. elegans KQT-3 defined a second subfamily with KCNQ1. KQT-2 did not group with either subfamily, nor did it possess a human ortholog. However, BLAST similarity searches clearly showed higher KQT-2 similarity in the vertebrate KCNQ gene family than to any other vertebrate potassium channel gene family (Fig. 1A).

Translational fusions of kqt genes with GFP revealed prominent expression in a variety of C. elegans tissues including pharyngeal muscles (kqt-1), intestinal cells (kqt-2, kqt-3), mechanosensory neurons (kqt-1, kqt-3), chemosensory neurons (kqt-3), and other head neurons (kqt-1, kqt-3) (Fig. 1, B–D). Some individual GFP-positive neurons were identified based on unique morphology and location, or double labeling with the
lipophilic tracer DiI, which reproducibly labels a set of chemosensory amphid and phasmid neurons (25). The mechanosensitive touch neurons PLM and ALM were clearly labeled by both kqt-1::GFP and kqt-3::GFP (Fig. 1E). In addition, a large subset of DiI-positive chemosensory amphid and phasmid neurons consistently labeled with kqt-3::GFP. These identified kqt-3::GFP positive neurons included the amphid neurons ADL, ASI, AWB, and ASH located in the head, and the phasmid neurons PHA and PHB in the tail (Fig. 1F). An additional DiI-negative neuron in the head labeled consistently with kqt-3::GFP, which we tentatively identified as the chemosensory neuron AWC. Additional unidentified head neurons were labeled by kqt-1::GFP and kqt-3::GFP, although expression was not reliably observed in neurons of the ventral or dorsal cord.

Conserved Functional Properties and Muscarinic Modulation of C. elegans KQT Channels—Potassium currents with functional properties resembling vertebrate M-currents were expressed by full-length kqt-1 or kqt-3 cDNAs in Xenopus oocytes. Functional expression of kqt-2 was not observed. KQT-1 and KQT-3 potassium currents displayed unusually slow activation and deactivation kinetics, under two-electrode voltage-clamp (Fig. 2, A and B). Normalized conductance-voltage plots revealed voltages for half-maximal conductance similar to vertebrate KCNQ orthologs (Fig. 2C) (KQT-1, V50 = −16 mV; KQT-3, V50 = −13 mV, versus −19 to −8 mV reported for vertebrate KCNQ channels (27); but others report −40 to −20 mV (28)). Both KQT-1 and KQT-3 potassium currents were reversibly blocked by the KCNQ-specific compound linopirdine (KQT-1 IC50 = 44 μM; KQT-3, IC50 = 79 μM), with ~4–8-fold lower sensitivity compared with vertebrate KCNQ channels (Fig. 2D).

All vertebrate KCNQ channels can be modulated through the M1 muscarinic receptor (17, 27). Significantly, we observed that this mode of regulation was conserved with C. elegans subunits, by coexpressing KQT-1 or KQT-3 with the human M1 muscarinic receptor in Xenopus oocytes. KQT-1 or KQT-3 currents expressed in these experiments were rapidly suppressed by bath application of the muscarinic agonist, oxotremorine (10 μM) (Fig. 2, E and F). M1 mediated suppression was more effective for KQT-1 (~90% inhibition) than for KQT-3 (~16% inhibition) (Fig. 2G).
These experiments demonstrate that *C. elegans* KQT channels encode M-currents, which are functionally similar to M-currents encoded by vertebrate KCNQ channels.

**A Subset of *C. elegans* KQT and Vertebrate KCNQ Channels Are Suppressed by Submicromolar OAG**—The lipid metabolite diacylglycerol generated by phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) has been described as a potential signaling intermediary for receptor-coupled inhibition of native M-currents in gastric smooth muscle (29) and perhaps other tissues (30). To test if DAG may mediate inhibition of cloned KQT channels, we applied the water soluble DAG analog, OAG, to oocytes expressing KQT-1 or KQT-3 channels. Potent suppression of KQT-1 currents was observed with submicromolar concentrations of OAG (IC$_{50}$ = 0.2 µM), resulting in ~80% maximal inhibition at saturating concentrations, within 2 min (Fig. 3, A and B). KQT-3 currents were also suppressed by OAG, but with ~1000-fold lower sensitivity (IC$_{50}$ = 201 µM) (Fig. 3B).

To test whether OAG-mediated inhibition observed with *C. elegans* KQT channels is conserved with vertebrate KCNQ channels, we examined the OAG sensitivity of currents carried by all the vertebrate KCNQ channels (KCNQ1–5) expressed as homomeric channels in Xenopus oocytes. Of these vertebrate KCNQ subunits, KCNQ5 currents were uniquely sensitive to inhibition by OAG at submicromolar concentrations (IC$_{50}$ = 0.1 µM), similar to *C. elegans* KQT-1 (Fig. 3C). Other KCNQ currents examined were far less sensitive to OAG inhibition, similar to *C. elegans* KQT-3, requiring ~1000-fold greater OAG concentrations for inhibition (KCNQ1, IC$_{50}$ = 717 µM; KCNQ3, IC$_{50}$ = 0.8 µM; (Fig. 3C) (KCNQ2 and KCNQ4 were similarly uninhibited by 125 µM OAG (data not shown)). Because KCNQ5 forms heteromeric channels with KCNQ3 in heterologous expression systems (16, 17), and most likely in vivo (31), we examined the OAG sensitivity of currents produced by co-expression of KCNQ5 and KCNQ3 cRNAs. In agreement with these previous reports, we observed that KCNQ3/5 co-injected oocytes produced currents with amplitudes 5–8 times greater than that predicted by the linear sum of individually injected KCNQ3 and KCNQ5 oocytes, consistent with efficient heteromeric channel formation. Moreover, heteromeric KCNQ3/5 currents, like homomeric KCNQ5 currents, were effectively suppressed by OAG at submicromolar concentrations (IC$_{50}$ = 0.6 µM) (Fig. 3D). Vertebrate KCNQ5 and *C. elegans* KQT-1 subunits thus define a conserved molecular subclass of M-channels with high sensitivity to OAG. Furthermore, KCNQ5 confers high OAG sensitivity to heteromeric channels formed with KCNQ3 subunits.

**Suppression by OAG Is Mediated Through the COOH-terminal Tail of OAG-sensitive KQT/KCNQ Subunits**—To investigate the structural regions necessary for OAG-mediated inhibition of channels formed by KCNQ and KQT subunits, we generated a series of chimeric constructs between subunits with high and low sensitivity. Other functional classes of potassium and non-selective cation channels gated by cytosolic
ligands (Slo, SK, and CNG) are encoded by subunits that appear to be modularly composed of a core voltage-sensing and pore-forming domain (S1–S6), linked to unique carboxyl-terminal tails fully confer either high or low OAG sensitivities, which are consistent with OAG sensitivity being determined by the carboxyl-terminal tails of KCNQ/KQT subunits. We first examined chimeric channels formed by exchanging carboxyl-terminal tail domains between C. elegans KQT-1 and KQT-3 subunits. Attaching the tail from the OAG-sensitive KQT-1 subunit to the core of KQT-3 created a functional chimeric subunit (K3CR/Q1TL) with high OAG sensitivity, essentially identical to wild-type KQT-1 channels (Fig. 3A). This result suggests that the structural requirement for inhibition by OAG may employ an evolutionarily conserved mechanism, an analogous chimeric subunit was generated attaching the tail from the vertebrate KCNQ5 subunit, KCNQ5, to the core of C. elegans KQT-3 (K3CR/Q5TL). As with K3CR/K1TL, the interspecies chimeric subunit also produced channels with high OAG sensitivity, and an OAG dose-response profile essentially identical to wild-type KQT-3 (Fig. 3F). High OAG sensitivity could thus be conferred to C. elegans KQT-3 by substituting the tail from the OAG-sensitive channel subunits from either vertebrate or C. elegans species.

Conversely, low OAG sensitivity was also conferred by attaching the tail from vertebrate KCNQ1 to the core of C. elegans KQT-1 (K1CR/Q1TL) (Fig. 3G), although the analogous interspecies chimera K1CR/K3TL failed to express (see construct 1.1, Fig. 4). Results from additional interspecies core and tail chimeric combinations were consistent with a determinative role of the tail in OAG sensitivity. Thus, chimeric subunits with the core of C. elegans KQT-1 and tail of vertebrate KCNQ5 (K1CR/Q5TL) exhibited high OAG sensitivity (IC50 = 0.032 μM), whereas chimeras with the core of C. elegans KQT-3 and tail of vertebrate KCNQ3 (K3CR/Q3TL) exhibited low OAG sensitivity (IC50 = 200 μM) (data not shown). Together, these results are consistent with OAG sensitivity being determined by the carboxyl-terminal tails of KCNQ/KQT subunits.

Although we determined that the full-length carboxyl-terminal tails fully confer either high or low OAG sensitivities, attempts to localize OAG sensitivities to a smaller domain within the tails were unsuccessful (Fig. 4). No single tail subregion was able to confer complete OAG sensitivity. Our results suggest that the structural requirements for OAG sensitivity are possibly complex and not mediated through a single subregion of the carboxyl-terminal tail, despite the apparent functional modularity of the entire tail domain.

Suppression Is Not Mediated by Direct Binding of OAG to the Channel Protein—Because DAG directly interacts to alter the gating of cyclic-GMP gated cation channels (34–36) and a subset of TRPC cation channels (37, 38), we tested the possible ability of OAG to directly inhibit KQT/KCNQ channels in ex-
DISCUSSION

Conservation of KCNQ Channels between Vertebrates and C. elegans—Human KCNQ genes encode M-type potassium channels active lipid phosphatases present in *Xenopus* oocyte membranes (41), run-down has been interpreted as evidence supporting the hypothesis that the loss of endogenous PIP$_2$ may mediate receptor-coupled inhibition of KCNQ channels (39, 40, 42). Our results suggest that KQT-1 and KCNQ5 channels may be particularly insensitive to a decrease of PIP$_2$ from endogenous levels, and that additional signaling mechanisms may be utilized by these channels. Consistent with this interpretation, OAG inhibits KQT-1 channels through a mechanism that does not involve direct binding of OAG to the channel protein. Furthermore, our observations suggest that fluctuations of PIP$_2$ levels near the endogenous membrane concentrations in *Xenopus* oocytes may not contribute significantly to modulating either KQT-1 or KCNQ5 channel activity, under our recording conditions.

**OAG Suppression Acts through Activated PKC**—To test the possible involvement of PKC in mediating suppression of KQT-1, a pharmacological activator and inhibitor of PKC were applied to oocytes expressing KQT-1, assayed by two-electrode voltage clamp. Bath application of the potent PKC activator PMA at 20 nM rapidly suppressed KQT-1 currents with kinetics that mimic those observed with 1.25 μM OAG (Fig. 6, A–D) (for OAG, 82% suppression of maximal current, τ = 54 s; for PMA, 86% suppression of maximal current, τ = 56 s). OAG-mediated suppression of KQT-1 current was blocked nearly completely by pretreating oocytes with the PKC-specific inhibitor, staurosporine (1 μM, 8-h soak) (Fig. 6, A and D). These results suggest that nearly all of the suppression of KQT-1 currents that we observe with OAG can be explained through the action of activated PKC.

**C. elegans KCNQ-like Potassium Channels**

![Image](https://example.com/image.png)

**FIG. 4.** Chimeric constructs exchanging subdivisions of COOH-terminal tails between *C. elegans* KQT-1 and KQT-3, do not fully transfer OAG sensitivity. Top panels, diagram of chimeric constructs and summary of OAG dose-response data for functional chimera created with KQT-1 core (A) and KQT-3 “core” (B). OAG sensitivities (top panels) summarized by a plus sign (+) for high sensitivity (IC$_{S_0}$ > 1.0 μM), a minus sign (−) for low sensitivity (IC$_{S_0} >$ 100 μM), a plus/minus sign (±) for intermediate sensitivity, and zero (0) for non-expressing constructs. The full-length tail of KQT-1 attached to KQT-3 core (3.1 in this figure, identical to K3CR/K1TL in Fig. 3E), fully conferred high OAG sensitivity (not shown in this figure, see Fig. 3E). By contrast, only one chimeric construct, 1.4, transferred partial OAG sensitivity, whereas all others resembled either wild-type KQT-1 (A), or KQT-3 (B) OAG sensitivities. Fitted Hill parameters for 1.4 were: IC$_{S_0}$ = 0.32 μM, s = 0.78, I$_{max}$ = 0.58 (n = 10). N for the other constructs: 1.5 (n = 5), 1.6 (n = 4), 3.2 (n = 5), 3.3 (n = 3), 3.4 (n = 3), and 3.6 (n = 8). Hill function fitted as described in the legend to Fig. 2. All data were plotted with S.E., and fitted dose-response functions for KQT-1 (K1) and KQT-3 (K3).

**FIG. 5.** Excision inside-out patches. We reasoned that OAG may exert its inhibitory effect on OAG-sensitive KCNQ/KQT channels either by a direct interaction with carboxyl-terminal tail domains, or indirectly through the stimulation of PKC-dependent phosphorylation of the channel protein or other accessory proteins. To test the possibility of a direct action of OAG on the channel protein itself, we perfused OAG onto the cytoplasmic face of excised inside-out patches containing KQT-1 channels, in the absence of ATP to preclude any kinase activity possibly retained on the patch. Inhibition of KQT-1 activity was not observed after application of 100 μM OAG, when patches were subjected to either steady membrane depolarization, or a set of voltage step pulses (Fig. 5, A and B).

Under our recording conditions, KQT-1 channels in excised patches also exhibited no evidence of “run-down.” Similarly, excised patches containing KCNQ5 channels failed to exhibit run-down (Fig. 5E). These results are in contrast to the rapid run-down, which has been described for mammalian KCNQ channels (39, 40), which we also observed in excised patches containing KCNQ2/3 or KCNQ5/3 heteromeric channels under our recording conditions (Fig. 5, C and D). Because run-down for many channels is attributed to rapid PIP$_2$ turnover by active lipid phosphatases present in *Xenopus* oocyte membrane (41), run-down has been interpreted as evidence supporting the hypothesis that the loss of endogenous PIP$_2$ may mediate receptor-coupled inhibition of KCNQ channels (39, 40, 42). Our results suggest that KQT-1 and KCNQ5 channels may be particularly insensitive to a decrease of PIP$_2$ from endogenous levels, and that additional signaling mechanisms may be utilized by these channels. Consistent with this interpretation,
channels linked to diverse hereditary diseases (28). However, many aspects of their role in cellular functions and their characteristic modulation by G-protein receptor-coupled signaling remain to be clarified. Conserved mechanisms underlying these processes may be revealed by the study of KCNQ orthologs in model organisms. We describe here the characterization of a small family of KCNQ-like genes in *C. elegans* (kqt-1, kqt-2, kqt-3) with high functional conservation, and tissue expression profiles in many cell types that may be analogous to those expressing vertebrate KCNQ genes. Heterologous expression of kqt-1 and kqt-3 revealed currents similar to vertebrate KCNQ currents with respect to kinetics of gating, voltage dependence, pharmacology, and the ability to be suppressed by activation of the M1 muscarinic receptor.

![Figure 5](image1.png)

**FIG. 5.** OAG inhibition does not act through direct binding to KQT-1 channel protein, nor does KCNQ5 channel activity run-down in excised patches. A and B, excised inside-out patch recordings from oocytes expressing *C. elegans* KQT-1; C, vertebrate KCNQ2/3; D, KCNQ5; or E, KCNQ5 under symmetric 160 mM [K+] recording conditions. To preclude kinase activity, ATP was excluded from cytoplasmic recording solutions. A, application of 100 μM OAG (arrow) to the cytoplasmic face of a patch containing ~50 KQT-1 channels, failed to suppress channel activity evoked by constant depolarization (30 mV). Note tail current observed after repolarization to ~30 mV. B, currents from the same patch in response to a voltage step series (~80 to 70 mV, in 10-mV increments) before and after application of 100 μM OAG. Similar observations were made with additional patches, all showing no run-down upon excision (n = 5). C, rapid run-down of heteromeric KCNQ2/3 channels upon excision into bath solution (asterisk). The patch was held at 50 mV relative to resting potential prior to excision. D, similar run-down of heteromeric KCNQ5/3 channels upon excision. The patch was held at 40 mV relative to resting potential prior to excision. E, by contrast, KCNQ5 channel activity exhibited run-up or sustained activity upon excision. Two examples of patches held at 60 (top) or 70 mV (bottom) relative to resting potential prior to excision are shown. Similar observations made with additional KCNQ5 patches all failed to show run-down after excision (n = 4). Pipette recording solution contained (in mM), 160 K-glucuronate, 2 MgCl₂, 5 HEPES (pH 7.2). Bath and perfusion solutions contained (in mM), 160 K-glucuronate, 5 EGTA, 5 HEPES (pH 7.2).

![Figure 6](image2.png)

**FIG. 6.** OAG inhibition of KQT-1 is mediated by PKC. A, kinetics of KQT-1 current inhibition initiated by bath application of OAG (1.25 μM) (open circles, n = 8), PMA (20 nM) (filled circles, n = 7), or OAG (1.25 μM) after pretreatment of oocytes with staurosporine (1.0 μM, 8-h soak) (open triangles, n = 4), at time 0. Currents were evoked by 5-s voltage steps to 0 mV, repeated every 15 s, from a holding potential of ~90 mV. Peak currents are plotted and normalized to -15 s time points. Data sets fitted to a single exponential function, y = y₀ + Ae⁻ⁿᵗ, where y = normalized current, y₀ = asymptotic minimum current, τ = time constant of inhibition, and A = scaling factor. Fitted parameters for each data set are: OAG (y₀ = 0.18, A = 0.96, τ = 54 s), PMA (y₀ = 0.14, A = 0.89, τ = 56 s), and staurosporine + OAG (y₀ = 0.79, A = 0.30, τ = 72 s). All data were plotted with S.E.

B, example of current traces recorded before (-) and 150 s after (+) application of OAG. C, current traces before and 150 s after application of PMA. D, current traces from an oocyte pretreated with staurosporine, before and 150 s after application of OAG. For all current traces (B–D), tail currents were observed by repolarizing steps to ~100 mV; scale bars: 1.0 μA, 1.0 s.
Sequence comparison of the 3 C. elegans and 5 human members of this gene family revealed two distinctively conserved subfamilies: one defined by C. elegans KQT-3 and human KCNQ1, and a second defined by C. elegans KQT-1 and vertebrate KCNQ2–5. The functional correlates of this ancient subdivision of the KCNQ/KQT gene family are unclear. However, it is interesting to note that among the vertebrate subunits, functional heteromeric channels form only between KCNQ3 and other subunits of the second subfamily (KCNQ2–5) (28). By contrast, KCNE accessory subunits assemble only with KCNQ1 and not KCNQ2–5 (26, 28). It remains to be fully tested whether these functional distinctions among vertebrate KCNQ genes are conserved with the C. elegans channels. We have coexpressed KQT-1 with vertebrate KCNE1, and failed to detect any obvious changes in gating kinetics (data not shown). A distantly similar C. elegans KCNE-like gene (mps-1) has been reported (43) that modifies the gating kinetics of a C. elegans voltage-gated potassium channel (KVS-1), but its ability to modify KQT channel activity is unknown.

KQT::GFP translational fusions report prominent expression of kqt genes in a variety of C. elegans cell types, including pharyngeal muscle cells (kqt-1::GFP), intestinal cells (kqt-2::GFP, kqt-3::GFP), mechanosensory neurons (kqt-1::GFP, kqt-3::GFP), and chemosensory neurons (kqt-1::GFP, kqt-3::GFP). Many of these nematode cell types bear a functional resemblance to vertebrate tissues that are sensitive to diacylglycerol (DAG). We have observed that OAG-sensitive KQT channels in other vertebrate cell types including secretory neurons (44). Future analysis of native KQT-1 channels in C. elegans cells (56) may allow an examination of these issues in the context of specific cell types.

Receptor-coupled Inhibition of KCNQ/KQT (M-current) Channels—The ability of M-channels to act as robust effectors for a variety of G-protein-coupled receptors provides a linkage by which diverse extracellular ligands may modulate sustained changes in membrane excitability. A common requirement for G-protein-coupled receptor-coupled inhibition of M-channels appears to be receptor specificity for Gαq/11, mediating receptor-stimulated phospholipase C activity (45–47). Phosphatidylinositol metabolites have thus been implicated as important second messenger molecules required for further downstream signaling steps. PIP2 is one likely second messenger (39–42), however, other observations suggest that additional molecules may be employed. Other candidate second messengers include diacylglycerol (29, 30), Ca2+ (48), Ca2+-calmodulin (49), calcineurin (50), Src tyrosine kinase (51), and cyclic ADP-ribose (52).

We provided evidence that diacylglycerol can act as a potent second messenger for a molecular subset of M-channels encoded by kqt-1 in C. elegans and KCNQ5 in vertebrates. Using the water-soluble DAG analog, OAG, we found that among the channels formed by KQT/KCNQ subunits, only KQT-1, KCNQ5, and KCNQ5/3 heteromeric channels are uniquely sensitive to inhibition by submicromolar concentrations of OAG. Through an analysis of chimeric constructs, we demonstrated that high OAG sensitivity is dependent upon the carboxyterminal tail domains of OAG-sensitive subunit. The mechanism mediating inhibition through the tail is likely conserved because OAG sensitivity can be transferred in cross-species chimeric subunits. This mechanism acts in a dominant fashion, because OAG-sensitive KCNQ5 subunits conferred high OAG sensitivity to heteromeric channels composed of KCNQ5 and OAG-insensitive KCNQ3 subunits. However, we were unable to identify a more restricted subdomain of the carboxyl-terminal tail that could fully confer either high or low OAG sensitivity. We interpret from these results that OAG inhibition acts through a conserved mechanism requiring the carboxyl-terminal tail domain, and that the structural requirements for inhibition may be complex, unlikely transferable by any single subregion of the tail.

We also concluded from pharmacological experiments that the inhibitory action of OAG on KQT-1 acts through activated PKC. This interpretation is consistent with our inability to observe a direct inhibitory effect of OAG (100 μM) applied onto excised inside-out patches containing KQT-1 channels. The mechanism of PKC action is unaddressed by our findings, but we hypothesize that activated PKC may phosphorylate either the carboxyl tail domain of OAG-sensitive channel subunits, or carboxyl tail-associated accessory proteins to inhibit KQT-1 channel activity, and that a similar mechanism may underlie OAG-mediated inhibition for KCNQ5 channels. Sensitivity of KCNQ channels to activated kinases may also depend upon assembly into signaling complexes by scaffold molecules such as Yotiao and AKAP150 (53, 54). Although scaffolding molecules that bind either KQT-1 or KCNQ5 have not been identified, our interpretations based on expression in Xenopus oocytes could thus be modified in the context of other cell types.

Our results suggest that DAG mediates receptor-coupled inhibition for a molecular subset of M channels. This signaling mechanism may act in parallel to that proposed for PIP2 depletion (39–42). This OAG signaling mechanism may be particularly relevant for membrane environments with low endogenous levels of PIP2 (55), given the submicromolar potency that we observe for OAG. Our specific failure to observe run-down with excised patches containing KQT-1 or KCNQ5 suggests that fluctuations near endogenous levels of PIP2 may not be a prominent mechanism for inhibition of these channels, under our recording conditions. Multiple signaling intermediaries may thus mediate receptor-coupled inhibition of M-channels to allow flexibility for the modulation of this important class of potassium channels in a variety of cellular contexts. Future analysis of native KQT-1 channels in C. elegans cells (56) may allow an examination of these issues in the context of specifically engineered genetic backgrounds.

Acknowledgments—We thank Michael Nonet for generous use of equipment. This study also benefited from helpful discussions with Manuel Covarrubias, Michael Nonet, Maya Kunkel, Alex Yuan, Gonzalo Ferreira, Celia Santi, Gloria Fawcett, Matthew Schreiber, and David Thaler.

REFERENCES
1. Barhanin, J., Lesage, F., Guillemaire, E., Fink, M., Lazdunski, M., and Romney, G. (1996) Nature 384, 78–80
2. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Nature 384, 80–83
3. Wang, Q., Curran, M. E., Saplowski, I., Burn, T. C., Milliolland, J. M., Van-Raay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., de Jager, T., Schwartz, P. J., Toubin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Connors, T. D., and Keating, M. T. (1996) Nat. Genet. 12, 17–23
4. Neyroud, N., Tesson, F., Denjoy, I., Leibovici, M., Donger, C., Barhanin, J., Faure, S., Guy, F., Coumel, P., Petit, C., Schwartz, K., and Guicheney, P. (1997) Nat. Genet. 15, 186–189
5. Chouabe, C., Neyroud, N., Guicheney, P., Lazdunski, M., Romney, G., and Barhanin, J. (1997) EMBO J. 16, 5472–5479
6. Saplowski, I., Tristani-Firouzi, M., Lehmann, M. H., Sanguinetti, M. C., and Keating, M. T. (1997) Nat. Genet. 17, 338–340
7. Schulze-Bahr, E., Wang, Q., Wiedekind, H., Havemann, W., Chen, Q., Sun, Y., Rohrer, C., Hofert, M., Tewein, J. A., Bergkrete, M., Assmann, G., Qin, X., Somberg, J. C., Breithardt, G., Oberti, C., and Funke, H. (1997) Nat. Genet. 17, 267–268
8. Schroeder, B. C., Kuhisch, C., Stein, V., and Jentsch, T. J. (1998) Nature 396.
