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Molecular Cloning and Expression of a Kv1.1-like Potassium Channel from the Electric Organ of *Electrophorus electricus*

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Abstract. Electrocytes from the electric organ of *Electrophorus electricus* exhibited sodium action potentials that have been proposed to be repolarized by leak currents and not by outward voltage-gated potassium currents. However, patch-clamp recordings have suggested that electrocytes may contain a very low density of voltage-gated K^+ channels. We report here the cloning of a K^+ channel from an eel electric organ cDNA library, which, when expressed in mammalian tissue culture cells, displayed delayed-rectifier K^+ channel characteristics. The amino-acid sequence of the eel K^+ channel had the highest identity to Kv1.1 potassium channels. However, different important functional regions of eel Kv1.1 had higher amino-acid identity to other Kv1 members, for example, the eel Kv1.1 S4-S5 region was identical to Kv1.5 and Kv1.6. Northern blot analysis indicated that eel Kv1.1 mRNA was expressed at appreciable levels in the electric organ but it was not detected in eel brain, muscle, or cardiac tissue. Because electrocytes do not express robust outward voltage-gated potassium currents we speculate that eel Kv1.1 channels are chronically inhibited in the electric organ and may be functionally recruited by an unknown mechanism.

Key words: Potassium channels — Delayed rectifiers — Electric organ

Introduction

Potassium channels (K^+ channels) are a diverse class of membrane proteins that play critical roles in cellular function in both electrically excitable and non-excitable tissue (Rudy, 1988; Latorre et al., 1989; Hille, 2001). In nervous tissue, electrophysiological and pharmacological studies have shown a diverse class of ligand- and voltage-gated K^+ channels that play important roles in modifying electrical excitability and the action potential wave-form (Rudy, 1988; Latorre et al., 1989; Hille, 2001). In addition, K^+ channels are modulated by second-messenger cascades, activated by different neurotransmitter and neuropeptide receptors, which may further modify neuronal excitability and function (Kaczmareck & Levitan, 1987; Levitan, 1988).

Molecular cloning techniques have revealed a large family of voltage-gated K^+ channels in mammals, which may be divided into four subfamilies (*Shaker*, *Shab*, *Shaw*, and *Shal*). Some K^+ channel gene products were generated by alternative splicing, resulting in further variance in the K^+ channel family. The expression of cloned K^+ channel transcripts in *Xenopus* oocytes or cell lines have revealed channels with different voltage-gating properties, kinetics, and pharmacology, further attesting to the diversity of these channels on the functional level (for reviews, *see* Jan & Jan, 1990; Perney & Kaczmareck, 1991; Rudy, Kentros & Vega-Saenz de Miera, 1991; Pongs, 1992; Salkoff et al., 1992; Vega-Saenz de Miera et al., 1994).

The electric organ of *Electrophorus electricus* (EEO) has been used for both electrophysiological and biochemical characterization of ligand- and voltage-gated ion channels (Keynes & Martins-Ferreira, 1953; Nakamura, Nakajima & Grundfest, 1964; 1965; Ruiz-Manresa et al., 1970; Levinson, Duch & Thornhill, 1986). Two microelectrode voltage-clamp studies have suggested that electrocytes from the EEO do not exhibit an outward voltage-gated K^+ current (I_K or I_A) activated with depolarization, but that they display a large inward rectifying K^+ current

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(I_{IR}) upon hyperpolarization (Nakamura et al., 1964, 1965; Ruiz-Manresa et al., 1970). However, recent patch-clamp studies of electrocytes have revealed the presence of small outward voltage-gated K^+ currents in a small fraction of patches on the innervated membrane (Skenkel & Sigworth, 1991).

Given the preceding observations, the aims of this report were to clone the eel electrocyte outward rectifier K^+ channel(s), to express the channel in mammalian tissue culture cells, and to map the channel's mRNA distribution in eel tissue. We report the cloning and functional expression of an EEO Kv1.1-type K^+ channel and speculate on the possible role the channel may play in electrocyte physiology.

Materials and Methods

Cloning and Sequencing of EEO Kv1.1

Isolation of RNA from the main organ of the electric eel was by the guanidinium isothiocyanate method (Chomczyznski & Sacchi, 1987). Poly A RNA was purified by oligo(dT)-cellulose chromatography (Ausubel et al., 1990). The construction of a cDNA library from eel main organ poly A RNA in lambda GT10 followed standard protocols (Ausubel et al., 1990). The primary EEO cDNA library was screened at low stringency (10% formamide, 42°C, 2 × SSPE) with H5 pore probes from three of the subfamilies of K^+ channels, *Shaker* (Kv1.1 (RCK1)), *Shab* (Kv2.1 (DRK1)), and *Shaw* (Kv3.4 (KshIIIC)). The probes were labeled with ^{32}P-dCTP by the polymerase-chain-reaction (PCR) method (Ausubel et al., 1990). Positive clones were purified and the isolated cDNA inserts were subcloned in pBS vector for DNA sequencing of both strands by standard methods (Ausubel et al., 1990). A cDNA containing the complete open reading frame of eel Kv1.1 was subcloned into pcDNA3 and used for transfection into Chinese hamster ovary (CHO) pro5 cells (American Type Culture Collection, Rockville Maryland). The Lipofectamine Plus (Gibco/BRL) reagent was used for transient transfections per the manufacturer's protocol on cells plated on glass coverslips in a 35 mm dish. Cells were maintained in DMEM, or alpha-MEM, supplemented with 0.35 mM proline, with 10% FBS at 37°C under 5% CO_2.

Electrophysiological Recordings and Analysis

CHO cell lines were plated on glass coverslips and cotransfected with a plasmid containing eel Kv1.1 cDNA (1.0 microgram) and a plasmid containing a GFP cDNA (0.1 microgram) for viewing of transfected cells with a fluorescence microscope. Rat Kv1.1 was also analyzed to compare with eel Kv1.1. The bath recording solution contained (in mM) NaCl 150.0, KCl 5.0, $MgC1_2$ 1.0, $CaCl_2$ 2.0, glucose 5.0, HEPES 10.0 (pH 7.3, NaOH). Patch pipettes were fashioned from 8161 corning glass (Warner Instruments) and had tip resistances of 1.5–2.5 MΩ when filled with an intracellular solution containing (in mM) KCl 70.0, KF 65.0, Na 5.0, $MgCl_2$ 1.0, EGTA 10.0, glucose 5.0, HEPES 10.0 (pH 7.3, KOH).

Whole-cell currents were recorded and analyzed with an Axopatch 200B amplifier (Axon Instruments) and the pCLAMP8/Digidata 1200 data acquisition/analysis system (Axon Instruments) 20–30 hours post transfection at room temperature (23–25°C). Cells were held at −80 mV and depolarized to different voltages. Nontransfected CHO pro5 cells showed either no or at most 100 pA of endogenous K^+ current at maximum activating voltages of 40 mV (Thornhill et al., 1996). Mean linear membrane leak current was subtracted by the P/4 protocol. Membrane capacitance and series resistance (R_S) were estimated by the Clampex software from a transient capacitance current elicited by a 10 mV hyperpolarizing voltage step from −80 mV. Membrane capacitance was compensated using the amplifier controls and R_S was compensated by patch-clamp circuitry by 85–95%. The voltage-induced currents were filtered at 5 kHz (−3 dB cutoff) and digitized at 50 microseconds. Maximum peak conductance values (G) were obtained from the mean value of the peak leak-subtracted current (I) using Ohm's law ($G = I/(V_p - E_K)$) and a predicted Nernst K^+ equilibrium potential (E_K) of −83 mV. All voltages were corrected for a liquid junction potential of 8 mV. An unpaired t test was used to assess statistical differences of a control value compared with another one. A $p < 0.05$ was considered significant. C-Type inactivation was recorded by depolarizing from −80 mV holding to 20 mV for different times (0.1 to 10 seconds). Data were acquired at 10 kHz and low-passed filtered at 2 KHz. Leak current was estimated from membrane resistance using Ohm's law and subtracted off-line. Inactivated current amplitude was calculated by dividing the current at the end of the pulse by the initial peak current and shown as an inactivation ratio as a function of time.

Single-channel currents were recorded from transfected cells in the cell-attached configuration after forming giga-seal patches and a bath solution with high K^+ was used to zero the membrane potential. The general voltage-pulse protocol was to hold at −90 mV and step to −55 to 20 mV in 5 mV increments for 1.2 seconds with an interpulse duration of at least 2.0 seconds. Pipette solution (in mM) = NaCl 20, Kaspartate 5, $CaCl_2$ 2, $MgSO_4$ 2, HEPES 10, glucose 5, sucrose 130; bath solution (in mM): Kaspartate 120, KCl 25, EGTA 5, HEPES 10, glucose 5, sucrose 20, pH 7.3. Sylgarded pipette resistances were 5.5–7.5 Mohm.

Northern Blot Analysis

Poly A mRNAs (3 micrograms/lane) were run on a denaturing 1% agarose gel, transferred to a nylon membrane, and hybridized with radiolabeled probe to eel Kv1.1 untranslated 5′ end (−150–1 bp). The probe was labeled with ^{32}P-dCTP by the PCR method (Ausubel et al., 1990). The nylon filters were incubated in prehybridization solution one hour, and hybridized at high stringency (50% formamide/42°C/2 × SSPE) with the probe for 15 hours. The final high-stringency wash was in 0.2 × SSPE at 60°C. Blots were exposed to X-ray film (Kodak AR5) for 12–15 hours.

Results

Amino-Acid Sequence of Eel Kv1.1

A primary eel electric organ cDNA library was screened at low stringency with pore-cDNA probes from three K^+ channels subfamilies, *Shaker* (Kv1.1), *Shab* (Kv2.1), and *Shaw* (Kv3.4). The deduced amino-acid sequence of a 3.0 kbp cDNA clone isolated from an EEO cDNA library and its alignment to various Kv1 K^+ channels are shown in Fig. 1. The eel Kv1.1 protein was 478 amino acids in length and had a calculated molecular weight of 54,483 daltons. Eel Kv1.1 had the typical *Shaker*-type S1-S6 putative membrane-spanning regions as well as the pore region. The eel Kv1.l protein had the highest amino-

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eKv1.1  MTVVPGENLDETVALAALSQDVYDPE                    RADQECCERVVINISGLRFETQLK     50
xKv1.1  ---IA---M---SV-PGHP--S-H-D                    QD-H---------V----------     50
rKv1.2  ---AT-DPV--AA--PGHP--T----                     --H--------------------     49
rKv1.3  ------DH-L-PE-AGGGGG-PPQGGCVSGGGCDRYEPLPPALPAAGEQDC-G-----------------     70
rKv1.6  -RSEKSLT-AAPGEVRGPEGEQQDAG                EFQEAEGGGCCSS--L--------Y----R     57
rKv1.4  -E-AMVSAESSGCNSHMPYGYAAQAR    del:136     GGGYSSVRYSD--------V--------M-    193

eKv1.1  TLAQFPNTLLGDPRKRMRFFDPLRNEYFFDRNRPSFDAILYYYQSGGRLRRPVNVPVDVFMEEIKFYELG     120
xKv1.1  ------S----N-K----Y-------------------------------------L-M-S---------     120
rKv1.2  ------E------K----Y-------------------------------------L-I-S---R-----     119
rKv1.3  --C---E------KR---Y---------------L------------I-------I-I-S---R--Q--     140
rKv1.6  --SL--D------GR-V---------------------------------------L-I-----R--Q--DEA 130
rKv1.4  ------E------E--TQY-------------------------------K------F-I-TEE----Q--     263

                                                     _________S1___________
eKv1.1  EDVIENFKEDEGFIKEEERPLPENEFQRQVWLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFR     190
xKv1.1  -EAM-K-R-----V--------DK-----------F--------I---I--M----------------LK     190
rKv1.2  -EAM-M-R----Y-----------------------------I------M-------S-------I--     189
rKv1.3  -EAM-K-R-----LR-------RRD---------------------------------------------     210
rKv1.6  LAAFREDEGCLPEGG-D-K---SQP----------------------------------------Q--     200
rKv1.4  -EALLK-R-----VR---DR------KK-I----------S---A-------------------------     334
                          N

                         •                          ____________S2__________      ______
eKv1.1  EDARMYEE      HYLVNGTMSAKKPN  PFTDPFFIVETLCIIWFSFELLVRFLACPSKPAFFKNIMNT     253
xKv1.1   -E-IFSR       RVN-S-VFY-SN   I------V-------------V---F------E-------F     250
rKv1.2  D-NED-HGGGVTF-TYS-S-IGYQQST  S----------------------F----F-----SC--T----I     257
rKv1.3  D-KDYPASPSQDVFEAA-NST-GASSGASS-S----V-----------------F-----AT-SR----L     280
rKv1.6  del:35 GSIPSGGLGTGGTS-FSTLGGSF----------V--V--T-------S-----A---R----I     299
rKv1.4  del:9  LSAGGHSRLL-D-SAPHNSGHTI-N--------V--V-----FV--CF----QAL-------I     407
                                    LE

        ______S3__________                  __________S4___________
eKv1.1  IDIVAIMPYFITLGLELAEHQSNGQ    QAMSLAILRVIRLVRVFRIFKLSRHSKGLQILGKTLQASMRE     320
xKv1.1  ------I-------T-I--  -EGN-KGE--T-----------------------------Q--K-----     319
rKv1.2  ----T-I-------T----KPEDA-QG  ----------------------------------Q--K-----     325
rKv1.3  ------I-------T----R-G---    ----------------------------------Q--K-----     347
rKv1.6  --L---F-------T--VQ  del:13  -----------------------------------------     376
rKv1.4  ----S-L-------TD--QQ-GG-GQQQ----F----I----------------------H--R-----     478
                            Q

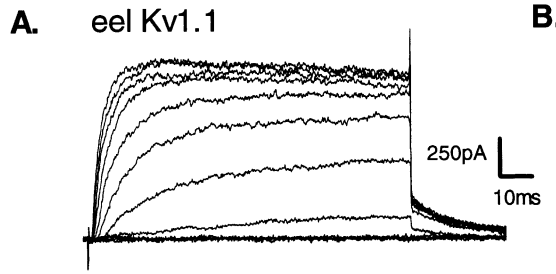
        ___________S5__________                __________H5___________       __________
eKv1.1  LGLLIFFLFIGVILFSSAVYFAETDDPDSGFSSIPEAFWWAVVSMTTVGYGDMCPVTIGGKIVGSLCAIA     390
xKv1.1  -----------------------AEEDE-H-T---D-----------------Y----------------     389
rKv1.2  -----------------------A-ER--Q-P---D-----------------V-T--------------     396
rKv1.3  -----------------------A---S---N---D-------T---------H----------------     417
rKv1.6  -----------------------A--V--L-P---D-------T---------Y-M-V------------     447
rKv1.4  -----------------------A-E-TTH-Q---D-------T---------K-I-V------------     548

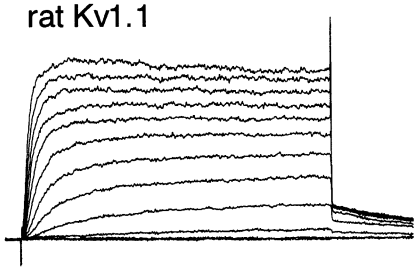
        __S6____________
eKv1.1  GVLTIALPVPVIVSNFNYFYHRETEHEEQLQYTHVTCGQQQPSFG ELKKSDSKPSLSKSDYLDSEDA     457
xKv1.1  -------------------------G---A-LL--SSPNLASNS  D- SRR-SSAM---E-MEI-EDLN     456
rKv1.2  -------------------------G---A--LQ--SCPKI--SP D----R-ASTI-----MEIQEGVN     465
rKv1.3  -------------------------G---A--M--GSC-HLS-SAE--R-AR-NST----E-MVI-EGGM     487
rKv1.6  -------------------------Q---G---------PT-DLKATDNGLG --DFAEASRERRSSYLP     515
rKv1.4  -------------------------N---T-L-QNAVSCPYLPSN L---FR-ST-S---E--EM-EGKE     618
                                                               LGD

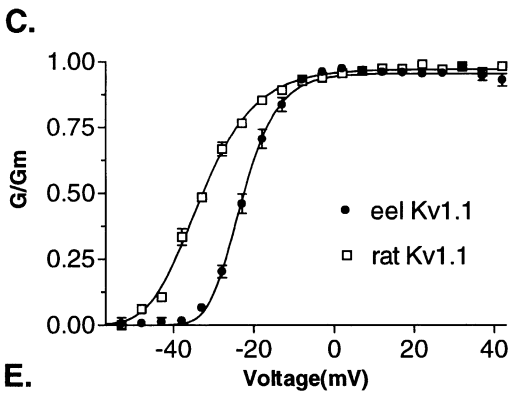
eKv1.1              DSIKYTNCSPHKA      YTGKLTDV                                   478
xKv1.1  NSIDNF    REAN-RTG--TI      NCVNKSKL----                                  489
rKv1.2  NSNEDF    REENL-TA--TLANTNY  VNI-KM----                                   498
rKv1.3  NHSAFPQTPFKTGNS TAT-TTNNNPNSCVNIKKIF---                                   525
rKv1.6                      T--R-     YAEKRM--E-                                  530
rKv1.4     EKCQGKGDDSETDKN---NA--          VE---                                  654
           GVKESLC
```

Fig. 1. Eel Kv1.1 amino-acid sequence is most similar to Kv1 K^+ channels. Amino-acid sequences were aligned with gaps to maximize long sequence alignment. Deletion in the Kv1.4 and Kv1.5 sequences have been introduced as noted. The *Xenopus* Kv1 channel (Ribera, 1991; Ribera and Nguyen, 1993) was denoted by an *x* and the rat Kv1 channels (Christie et al., 1989; Stuhmer et al., 1989; Grupe et al., 1990; Swanson et al., 1990) are denoted by an *r*. Dashes represent amino-acid identities to eel Kv1.1. The S1–S6 putative transmembrane domains and the H5 pore region are overlined. The dot symbol denotes a conserved putative extracellular N-linked glycosylation site (NXS/T) on Kv1 channels, except for Kv1.6.

acid identity to Kv1.1, Kv1.2, and Kv1.3 channels and the highest overall similarity to the *Xenopus* homolog of Kv1.1. The eel Kv1.1 S5-S6 region (321–404), which contained the putative pore region, had the highest amino-acid identity to rat Kv1.3 (85%) and rat or *Xenopus* Kv1.1 or Kv1.2 (80%). The N-







D.

	n	V1/2(mV)	Slope(mV)
eel Kv1.1	12	-22.8 ± 0.7**	-3.9 ± 0.2**
rat Kv1.1	12	-33.5 ± 0.9	-6.1 ± 0.1

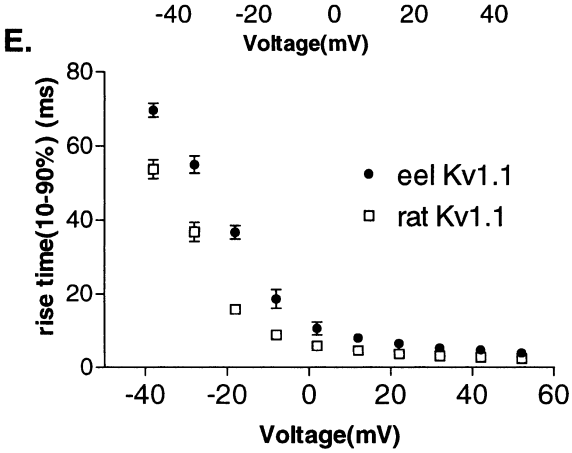


Fig. 2. Functional analysis of activation parameters of eel Kv1.1 and rat Kv1.1 in mammalian cells. (*A*) A family of outward K^+ currents for eel Kv1.1 in response to depolarizing voltage steps from −80 mV to depolarized voltages in 10 mV increments from −60 mV to 50 mV. (*B*) A family of outward K^+ currents for rat Kv1.1 as in *A*. (*C*) Normalized conductance (G/G_m) versus voltage plots for eel Kv1.1 and rat Kv1.1 from depolarizations in 5 mV increments. Data points were fitted using a Boltzmann equation as described in Methods. (*D*) Group data for the $V_{1/2}$ and the slope (*a*) of the G/V curve for both eel Kv1.1 and rat Kv1.1.(*E*) Group data for activation rise time, which is the time required for the current to rise from 10% to 90% of its final value, plotted as a function of voltage for eel Kv1.1 and rat Kv1.1.

terminus of eel Kv1.1 (1–166) was highly conserved with the Kv1.1/Kv1.2 channels, while the C-terminus (405–478) showed the greatest degree of sequence divergence from these channels. Eel Kv1.1 had one putative extracellular N-linked glycosylation site (NXT/S) at position N203 and a number of putative intracellular protein kinase phosphorylation sites: Protein Kinase A (PKA) sites; T47, S85, and T414, and Protein Kinase C (PKC) sites; S317, S459, and T472. However, eel Kv1.1 does not have a canonical PKA site, RRXS, at the C-terminus that is conserved in many mammalian K^+ channels and a site that has been shown to be phosphorylated on rat Kv1.1 (Ivanina et al., 1994) and Kv3.2 channels (Moreno et al., 1995).

Analysis of the Functional Properties of Eel Kv1.1 Expressed in Mammalian CHO Cells

Whole-cell outward currents of 1–2 nA were elicited upon depolarizations in cells transfected with eel Kv1.1 or rat Kv1.1 as shown in Fig. 2*A* and 2*B*. The conductance versus voltage curves are shown in Fig. 2*C*. The activation parameters for eel Kv1.1 were compared with rat Kv1.1 (Stuhmer et al., 1989), which was the most closely related channel at the amino-acid level, and are listed in Fig. 2*D*. The voltage required for half maximal activation ($V_{1/2}$) of eel Kv1.1 (−22.8 ± 0.7 mV) was more positive than that for rat Kv1.1 (−33.5 ± 0.9 mV). The slope (*a*) of the conductance versus voltage curve was also steeper

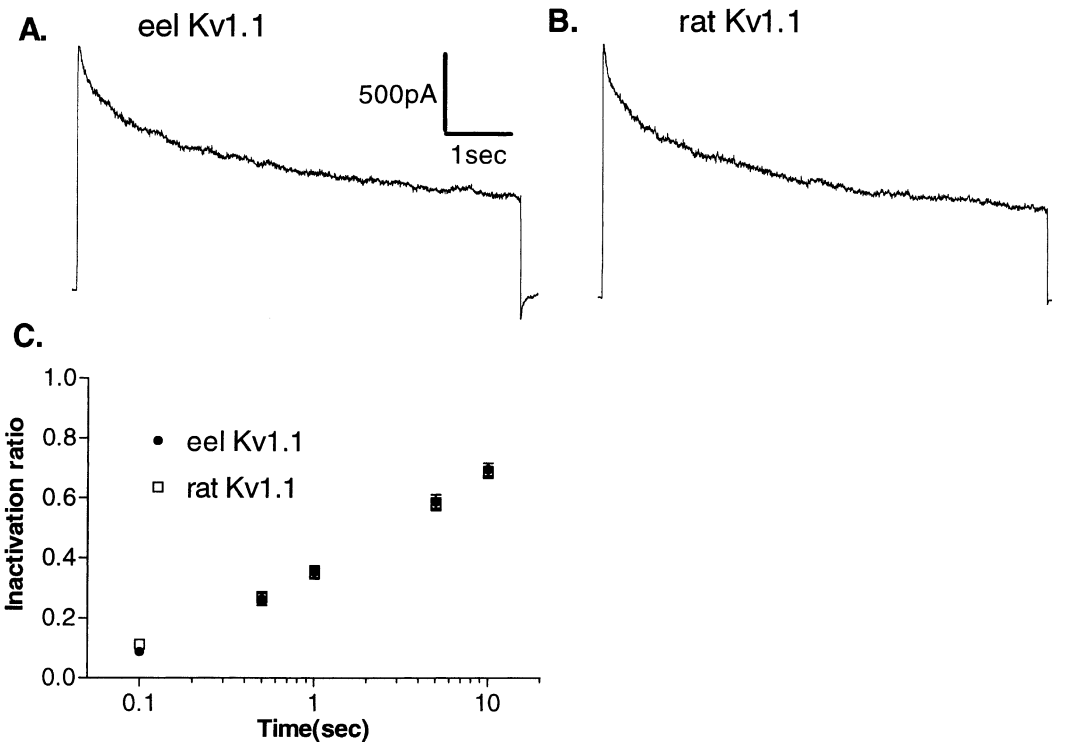


Fig. 3. Functional analysis of C-type inactivation parameters of eel Kv1.1 and rat Kv1.1 in mammalian cells. (*A* and *B*) C-Type inactivation of eel Kv1.1 (*A*) and rat Kv1.1 (*B*) in CHO cells. Cells were held at −80 mV and depolarized to 20 mV. (*C*) Plot of the inactivation ratio (inactivated current at end of pulse divided by the initial peak current) as a function of pulse duration.

for eel Kv1.1 (−3.9 ± 0.2 mV) versus rat Kv1.1 (−6.1 ± 0.1 mV). Activation kinetics were quantified by estimating rise times, i.e., the time required for the current to rise from 10% to 90% of its final value following various depolarizations, and plotted as a function of voltage. Eel Kv1.1 had significantly slower activation rise times versus rat Kv1.1 (Fig. 2*E*). Given that the cytoplasmic S4-S5 linker has been shown to be important in gating parameters (McCormack et al., 1991; Isacoff et al., 1991), the relatively slower rise time for eel Kv1.1 may be related to the amino-acid sequence of its S4-S5 linker, which was identical to Kv1.5 and Kv1.6, which both have slower rise times than rat Kv1.1 (Grupe et al., 1990; Swanson et al., 1990). It should also be noted that the $V_{1/2}$ values for Kv1.5 and Kv1.6 were relatively positive compared to the other members of the Kv1 subfamily, and that these values are closer to the eel Kv1.1, which may also be related to the identical amino-acid sequence of the S4 to S5 region on these molecules. C-type inactivation parameters appeared to be similar for eel Kv1.1 and rat Kv1.1 (Fig. 3). Single-channel current traces for eel Kv1.1 were elicited from cell-attached patches (Fig. 4*A*). The single-channel conductance of eel Kv1.1 (16.3 ± 0.7 pS, $n = 7$) (Fig. 4*B*) was somewhat higher than the single-channel conductance for rat Kv1.1 (12.6 ± 0.8 pS, $n = 4$).

The S5-S6 region of K^+ channels contains the pore that appears to be the site of channel block by various pharmacological agents (Jan & Jan, 1990; Perney and Kaczmareck, 1991; Rudy et al., 1991; Pongs, 1992; Salkoff et al., 1992; Vegas-Saenz de Miera et al., 1994). Tetraethylammonium (TEA) ions block K^+ channels from both the external and internal side of the membrane, apparently interacting with amino acids in the S5-S6 region (MacKinnon & Yellen, 1990; Kavanaugh et al., 1991; Taglialatela et al., 1991; Kirsch et al., 1991). Different *Shaker*-type channels are blocked by extracellular TEA (TEA_{ex}) at low (0.1–0.3 mM), intermediate (4–11 mM), and high (50–500 mM) IC_{50} values. Eel Kv1.1 currents elicited at 20 mV were blocked by 10 mM TEA_{ex} by 73% ±3%, $n = 2$, so its IC_{50} appeared to be < 10 mM. A critical eel Kv1.1 S5-S6 amino acid that confers this intermediate TEA_{ex} sensitivity may be cysteine 373 near the C-terminus of the pore (Fig. 5). This residue was identical in the *Drosophila Shab* channel, which had an IC_{50} of about 10 mM. Although the amino acid at this position has been reported to be involved in TEA_{ex} block (MacKinnon & Yellen, 1990; Kavanaugh et al., 1991; Taglialatela et al., 1991; Kirsch et al., 1991), inspection of the amino-acid sequences and TEA_{ex} IC_{50} values for the different K^+ channels suggested that additional residues were involved (Fig. 5).

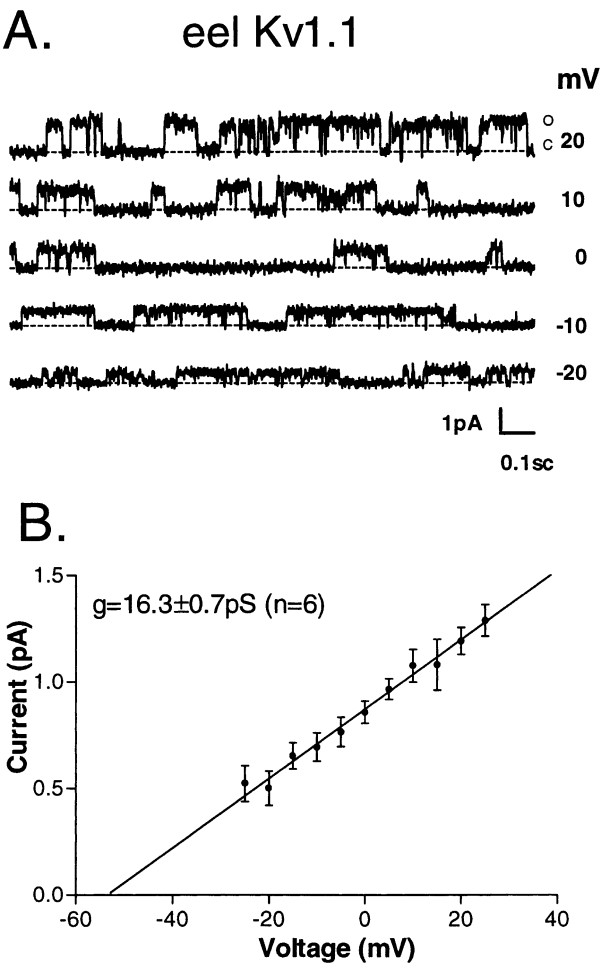


Fig. 4. Single-channel conductance of eel Kv1.1 in mammalian cells. (*A*) Representative single-channel current traces of eel Kv1.1 channels expressed in CHO cells. Cells were held at −80 mV and depolarized to different voltages. (*B*) Single-channel current amplitude plotted as a function of voltage gave a single-channel conductance of 16.3 ± 0.7 pS, $n = 6$.

Analysis of Eel Kv1.1 mRNA Expression

The distribution of the eel Kv1.1 mRNA in eel electric main organ, brain, skeletal muscle, and cardiac muscle was determined by Northern blot analysis by using a 5′ untranslated cDNA ^{32}P-labeled probe to eel Kv1.1. The probe hybridized to a prominent band at ~4.3 kp, but it also recognized less prominent bands of larger and smaller sizes, from the main EEO, but no signal was detected from eel brain, skeletal muscle, or heart muscle (Fig. 6). Stripping and rehybridizing the same Northern blot with a labeled actin probe revealed that the actin mRNA was intact in all mRNA preparations and expressed to similar levels in EEO and brain, and to higher levels in skeletal and cardiac tissue (*data not shown*).

Discussion

We report the cloning and functional expression of a Kv1.1-type K^+ channel from the main organ of the electric eel. The eel Kv1.1 channel was most similar in amino-acid sequence and function to the Kv1 *Shaker* subfamily of K^+ channels when expressed in tissue culture cells. Overall the amino-acid homology of eel Kv1.1 was most similar to the *Xenopus* Kv1.1 homologue. However, there are regions on the eel Kv1.1 protein, such as the S4-S5 region and the pore, that have greater sequence identity to Kv1.6 and Kv1.3 proteins, respectively. Additionally, two amino acids in the pore region, which have been proposed to be determinants for TEA sensitivity, were identical to dShab. These findings suggest that the eel Kv1.1 amino-acid sequence shares similarities with numerous members of the mammalian Kv1 subfamily, and the Kv family, and implies that this subfamily may not be fully differentiated in eel.

Electrophysiology of EEO Electrocytes

Eel electrocytes from the main organ and Sachs organ have been extensively analyzed by the two-microelectrode voltage clamp (Keynes & Martins-Ferreira, 1953; Nakamura et al., 1964, 1965; Ruiz-Manresa et al., 1970). Under conditions in which voltage-gated sodium channels were blocked, electrocyte depolarization from rest resulted in an increase in membrane resistance due to the closing of a robust inward rectifying K^+ current, and there was no evidence of voltage-gated outward K^+ currents (Nakamura et al., 1964, 1965; Ruiz-Manresa et al., 1970). Although the action potential in an electrocyte was somewhat broad compared to a neuronal action potential, it has been proposed that the relatively rapid membrane repolarization following sodium channel inactivation in this cell was due to a fast membrane time constant of ~95 microseconds ($\tau_m = R_m C_m$) resulting from a large resting leak conductance (Keynes & Martins-Ferreira, 1953; Nakamura et al., 1965). Thus, it has been argued that electrocytes do not express, nor would they require, outward K^+ currents for the repolarization of an action potential (Nakamura et al., 1964, 1965; Ruiz-Manresa et al., 1970). Additionally, since the EEO basically functions as a battery used to generate large currents to stun prey or predators, and plays a role in electrolocation, a rapid neuronal-type action potential would not be required (Nakamura et al., 1964, 1965; Ruiz-Manresa et al., 1970). However, a more recent patch-clamp study of the innervated membrane of eel electrocytes was undertaken to characterize sodium currents in this tissue in greater detail (Skenkel & Sigworth, 1991). Unexpectedly, this work revealed the presence of small voltage-gated outward currents characteristic of K^+ channels. The outward current

			IC50 TEA_{ex}(mM)	References
		H5		
eel Kv1.1		FAETDDPDSGFSSIP**E**AFWWAVVSMTTVGYGDM**C**PVTIGGK	<10	
rKv1.3	(85%)	---A---S---N---D-------T---------H--------	50	Stuhmer et al., 1989
rKv1.1	(80%)	---AEEAE-H-----D-----------------Y--------	0.3	Stuhmer et al., 1989
rKv1.2	(80%)	---A-ER--Q-P---D-----------------V-T-----	>100	Stuhmer et al., 1989
rKv1.6	(78%)	---A--V--L-P---D-------T---------Y-M-V---	4	Swanson et al., 1990
rKv1.4	(73%)	---A-E-TTH-Q---D-------T---------K-I-V---	>100	Stuhmer et al., 1989
dShab	(68%)	---K-EK-TK-V---------GIT--------I--T-AL--	10	Wei et al., 1990
rKv2.1	(61%)	---K-ED-TK-K---AS----TIT--------IY-K-LL--	10	Frech et al., 1989

Fig. 5. Eel Kv1.1 S5-S6 linker region is most similar to Kv1 K^+ channels. The S5-S6 linker regions of seven K^+ channels are aligned to EEO Kv1.1 (*d* denotes *Drosophila* and *r*, rat). Dashes represent amino-acid identities to the eel Kv1.1 sequence. The % amino-acid identity is shown on the left and the first sequence, rKv1.3, has the highest amino-acid identity of 85% although its TEA sensitivity was not similar to eel Kv1.1. The IC_{50} values for TEA_{ex} are also listed for other channels. The H5 pore region is overlined and the glutamic acid (E) and cysteine (C) in eel Kv1.1 are in bold. Rat Kv1.1 and Kv1.2 channels have an H5 amino-acid sequence that is identical to *Xenopus* Kv1.1 and Kv1.2 homologues (Ribera, 1991; Ribera & Nguyen, 1993).

was detected in only 3 of 28 patches in the study and the three patches had only 17%, 2.4% and 0.9% of the peak inward sodium current further attesting to their very low density. These K^+ currents were not detected by previous investigators using the two-microelectrode voltage-clamp, possibly due to their low functional density.

Eel Kv1.1 Expression in Electrocytes and Its Possible Role

Northern blot analysis indicated that eel Kv1.1 mRNA was expressed in electrocytes at appreciable levels for an ion channel. However, we have no data on whether the mRNA was efficiently translated by electrocytes, if the protein was targeted to the innervated membrane of the electrocyte, or whether it was clustered or uniformly distributed on the innervated cell surface. Nevertheless, we have shown that EEO Kv1.1 exhibited characteristics of a delayed-rectifier K^+ channel when expressed in tissue culture cells and displayed peak currents similar to other K^+ channels that have been expressed here.

We speculate that if EEO Kv1.1 channel proteins are appreciably expressed in the innervated membrane they may be chronically inhibited and unresponsive to depolarization. Some possibilities that may lead to inhibition include modulation of the EEO Kv1.1 phosphorylation state (Kaczmareck & Levitan, 1987; Levitan, 1988) or modulation by an uncharacterized EEO Kv1 beta subunit and/or other unknown modulators. If an outward K^+ current was not required for repolarization of action potentials in electrocytes then what physiological role might eel Kv1.1 play? In response to environmental conditions that may require faster electrical discharges from the electric organ, such as stunning of prey or electrolocation, the eel Kv1.1 channel may be de-inhibited and thus responsive to membrane depolarization. The consequence would then be a shorter-duration action potential due to faster membrane repolarization. Evidence suggested that action potentials in pituitary nerve terminals may be influenced by both protein phosphatase and protein kinase activity, indicating that enzymatic modulation of action potentials may occur under physiological conditions (Bielefeldt & Jackson, 1994). Further studies are required to determine whether EEO Kv1.1 channel proteins are expressed and whether they are chronically inhibited in the native tissue.

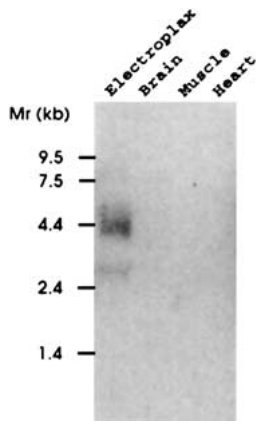


Fig. 6. Eel Kv1.1 mRNA is expressed predominately in EEO. A 5′ untranslated ^{32}P-radiolabeled cDNA probe to eel Kv1.1 was used for Northern blot analysis of poly A mRNA (3 micrograms) from different eel tissues. From left to right: main electric organ; brain; skeletal muscle; and heart. The film was exposed to the nylon membrane for 15 hours. The positions of the RNA markers in kilobases are shown on the left of the blot.

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References

Ausubel, F.M., Brent, R., Kingston, R.E., More, D.D., Seidman, J.G., Smith, J.A., Struhl, eds 1990. *Current Protocols in Molecular Biology*: vol 1 and 2. John Wiley & Sons, New York, NY

Chomczyznski, P., Sacchi, N. 1987. Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. *Analyt. Biochem.* **162:**156–159

Bielefeldt, K., Jackson, M.B. 1994. Phosphorylation and dephosphorylation modulate a Ca-activated K channel in rat peptidergic nerve terminals. *J. Physiol.* **475:**241–254

Christie, M.J., Adelman, J.P., Douglass, J., North, R.A. 1989. Expression of a cloned rat brain potassium channel in *Xenopus* oocytes. *Science* **244:**221–224

Frech, G.C., VanDongen, A.M.J., Schuster, G., Brown, A.M., Joho, R.H. 1989. A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning. *Nature* **340:**642–645

Grupe, A., Schroter, K.H., Ruppersberg, J.P., Stocker, M., Drewes, T., Beckh, S., Pongs, O. 1990. Cloning and expression of a human voltage-gated potassium channel. A novel member of the RCK potassium channel family. *EMBO J.* **9:**1749–1756

Hille, B. 2001. Ionic channels of excitable membranes, 3rd ed. Sinauer: Sunderland, Massachusetts

Isacoff, E.Y., Jan, Y.N., Jan, L.Y. 1991. Putative receptor for the cytoplasmic inactivation gate in the Shaker K^+ channel. *Nature* **353:**86–89

Ivanina, T., Perez, T., Thornhill, W.B., Levin, G., Dascal, N., Lotan, I. 1994. Phosphorylation of RCK1 K^+ channels expressed in *Xenopus* oocytes by protein kinase A. *Biochemistry* **33:**8786–8792

Jan, L.Y., Jan, Y.N. 1990. How might the diversity of potassium channels be generated? *Trends Neurosci.* **13:**415–419

Kaczmareck, L.K., Levitan, I.B. 1987. Neuromodulation: The biochemical control of neuronal excitability. Oxford University Press: N. Y.

Kavanaugh, M.P., Varnum, M.D., Osborne, P.B., Christie, P.B., Busch, M.J., Adelman, J.P., North, R.A. 1991. Interaction between tetraethylammonium and amino acid residues in the pore of cloned voltage-dependent potassium channels. *J. Biol. Chem.* **263:**7584–7587

Keynes, R.D., Martins-Ferreira, H. 1953. Membrane potentials in the electroplates of the electric eel. *J. Physiol.* **119:**315

Kirsch, G.E., Taglialatela, M. Brown A.M. 1991. Internal and external TEA block in a single cloned K^+ channel. *Am. J. Physiol.* **261:**C583–C590

Latorre, R., Aberhauser, A., Labarca, P., Alvarez, O. 1989. Varieties of calcium-activated potassium channels. *Annu. Rev. Physiol.* **51:**385–399

Levinson, S.R., Duch, D., Thornhill, W.B. 1986. Unusual molecular properties of the sodium channel isolated from the electric organ of *Electrophorus electricus*. *Neurol. Neurobiol.* **20:**375–393

Levitan, I.B. 1988. Modulation of ion channels in neurons and other cells. *Annu. Rev. Neurosci.* **11:**119–136

MacKinnon, R., Yellen, C. 1990. Mutations affecting TEA blockade and ion permeation in voltage-activated K channels. *Science* **250:**271–279

McCormack, K., Tanouye, M.A., Iverson, L.E., Line, J.W., Ramaswami, M., McCormack, T., Campanelli, J.T., Mathew, M.K., Rudy, B. 1991. A role for hydrophobic residues in the voltage-dependent gating of Shaker K channels. *Proc. Natl Acad. Sci.* **88:**2931–2935

Moreno, H., Kentros, C., Bueno, E., Weiser, M., Herdandez, A., Vega-Saenz de Miera, E., Thornhill, W.B., Rudy, B. 1995. Thalamocortical projections have a K channel that is phosphorylated and modulated by cAMP-dependent protein kinase. *J. Neurosci.* **15:**5486–5501

Nakamura, Y., Nakajima, S., Grundfest, H. 1964. Eel electroplaques: spike electrogenesis without potassium activation. *Science* **146:**266

Nakamura, Y., Nakajima, S., Grundfest, H. 1965. Analysis of spike electrogenesis and depolarizing K inactivation in electroplaques of *Electrophorus electricus*. *J. Gen. Physiol.* **49:**321–348

Perney, T.M., Kaczmarek, L.K. 1991. The molecular biology of K channels. *Curr. Opin. Cell Bio.* **3:**663–670

Pongs, O. 1992. Molecular biology of voltage-dependent potassium channels. *Physiol. Rev.* (Supplement on Forty Years of Membrane Current in Nerve) **72:**S79–S88

Ribera, A.B. 1991. A K channel gene is expressed at neural induction. *Neuron* **5:**691–701

Ribera, A.B., Nguyen, D.A. 1993. Primary sensory neurons express a Shaker-like K channel gene. *J. Neurosci.* **13:**4988–4996

Rudy, B. 1988. Diversity and ubiquity of K channels. *Neuroscience* **25:**729–750

Rudy, B., Kentros, C., Vega-Saenz de Miera, E. 1991. Families of K channel genes in mammals: Toward an understanding of the molecular basis of K channel diversity. *Mol. Cell. Neurosci.* **2:**89–102

Ruiz-Manresa, F., Ruarte, A.C., Schwartz, T.L., Grundfest, H. 1970. Potassium inactivation and impedance changes during spike electrogenesis in eel electroplaques. *J. Gen. Physiol.* **55:**33–47

Salkoff, L., Baker, K., Butler, A., Covarrubias, M., Pak, M.D.A., Wei, A. 1992. An essential "set" of K^+ channels conserved in flies, mice and humans. *Trends Neurosci.* **15:**161–166

Skenkel, S., Sigworth, F.J. 1991. Patch recordings from the electrocytes of *Electrophorus electricus*. *J. Gen Physiol.* **97:**1013–1041

Stuhmer, W., Ruppersberg, J.P., Schruer, K.H., Sakmann, B., Stocker, M., Giese, K.P., Perschke, A., Baumann, A., Pongs, O. 1989. Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. *EMBO J.* **8:**3235–3244

Swanson, R., Marshall, J., Smith, J.S., Williams, J.B., Boyle, M.B., Folander, K., Luneau, C.J., Antanavage, J., Oliva, C., Buhrow, S.A., Vennett, C., Stein, R.B., Kaczmarek, L.K. 1990. Cloning and expression of cDNA and genomic clones encoding three delayed rectifier potassium channels in rat brain. *Neuron* **4:**929–939

Taglialatela, M., VanDongen, A.M.J., Drewe, J.A., Joho, R.H., Brown, A.M., Kirsch, G.R. 1991. Patterns of internal and external tetraethylammonium block in four hololog ous K channels. *Mol. Pharmacol.* **40:**299–307

Thormhill, W.B. Wu, M.B., Jiang X., Wu, X., Morgan, P., Margiotta, J.F. 1996. Expression of Kv1.1 delayed-rectifier potassium channels in Lec Mutant CHO cell lines reveals a role for sialidation in channel function. *J. Biol. Chem.* **271:**19093–19098

Vega-Saenz de Miera, E., Weiser, M.E., Kentros, C., Lau, D., Moreno, H., Serodio, P., Rudy, B. 1994. Shaw-related K channels in mammals. *In:* Handbook of Membrane Channels, pp. 41–78, Academic Press,

Wei, A., Covarrubias, M., Bulter, A., Baker, K., Pak, M., Salkoff, L. 1990. K current diversity is produced by an extended gene family conserved in Drosophila and mouse. *Science* **248:**599–603