Distribution of Kv1-Like Potassium Channels in the Electromotor and Electrosensory Systems of the Weakly Electric Fish Apteronotus leptorhynchus

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ABSTRACT: The electromotor and electrosensory systems of the weakly electric fish Apteronotus leptorhynchus are model systems for studying mechanisms of high-frequency motor pattern generation and sensory processing. Voltage-dependent ionic currents, including low-threshold potassium currents, influence excitability of neurons in these circuits and thereby regulate motor output and sensory filtering. Although Kv1-like potassium channels are likely to carry low-threshold potassium currents in electromotor and electrosensory neurons, the distribution of Kv1α subunits in A. leptorhynchus is unknown. In this study, we used immunohistochemistry with six different antibodies raised against specific mammalian Kv1α subunits (Kv1.1–Kv1.6) to characterize the distribution of Kv1-like channels in electromotor and electrosensory structures. Each Kv1 antibody labeled a distinct subset of neurons, fibers, and/or dendrites in electromotor and electrosensory nuclei. Kv1-like immunoreactivity in the electrosensory lateral line lobe (ELL) and pacemaker nucleus are particularly relevant in light of previous studies suggesting that potassium currents carried by Kv1 channels regulate neuronal excitability in these regions. Immunoreactivity of pyramidal cells in the ELL with several Kv1 antibodies is consistent with Kv1 channels carrying low-threshold outward currents that regulate spike waveform in these cells (Fernandez et al., J Neurosci 2005;25:363–371). Similarly, Kv1-like immunoreactivity in the pacemaker nucleus is consistent with a role of Kv1 channels in spontaneous high-frequency firing in pacemaker neurons. Robust Kv1-like immunoreactivity in several other structures, including the dorsal torus semicircularis, tuberous electroreceptors, and the electric organ, indicates that Kv1 channels are broadly expressed and are likely to contribute significantly to generating the electric organ discharge and processing electrosensory inputs. © 2006 Wiley Periodicals, Inc. J Neurobiol 66:1011–1031, 2006

Keywords: pacemaker nucleus; electrosensory lateral line lobe; torus semicircularis; central pattern generation; ionic currents; teleost; gymnotiform

Weakly electric fish produce electric organ discharges (EODs) that function in electrolocation and communication. The discrete neural circuits that control EOD production and process electrosensory inputs serve as model systems for studying mechanisms of motor pattern generation and sensory integration (Szabo, 1975; Heiligenberg et al., 1996; Berman and Maler, 1999; Metzner, 1999; Zakon et al., 1999). The electromotor system is relatively simple, with only three types of neurons controlling EOD frequency. Furthermore, there is a straightforward relationship between the activity of neurons in this circuit and their behavioral output, the EOD (Meyer, 1984;
Studies of the electromotor system have examined the neural mechanisms of generating the remarkably precise and high-frequency command signal for the EOD (Dye, 1991; Moortgat et al., 1998; Smith and Zakon, 2000) and of modulating the EOD to produce complex electrical communication signals (Kawasaki et al., 1988; Keller et al., 1991; Heiligenberg et al., 1996; Zupanc and Maler, 1997; Metzner, 1999; Oestreich and Zakon, 2002; Stoddard et al., 2003). The electrical signals detected by the electrosensory system can be easily manipulated; their parameters (e.g., amplitude and phase) can be quantified; and the brain nuclei and cell types that encode electrosensory features have been well-characterized (Maler, 1979; Carr et al., 1981; Heiligenberg and Dye, 1982; Carr et al., 1986b; Berman and Maler, 1999). Previous studies have used the electrosensory system to investigate the cellular and network mechanisms underlying temporal filtering (Rose and Fortune, 1999; Fortune and Rose, 2003), adaptive filtering of sensory information based on sensory expectations (Bell, 1981; Bell et al., 1997; Bastian, 1999), and the production of dendritic burst discharges to encode stimulus features (Turner and Maler, 1999; Laing et al., 2003; Noonan et al., 2003).

Voltage-dependent potassium currents regulate excitability in most neurons, including those in the electromotor and electrosensory systems (Salkoff et al., 1992; Grissmer, 1997; Gan and Kaczmarek, 1998; Martina et al., 1998). Characterizing both the properties of these currents and the distribution of the channels that carry them is thus important for understanding the cellular mechanisms that contribute to pattern generation and sensory processing. In the brown ghost knifefish (*Apteronotus leptorhynchus*), for example, high-threshold potassium currents carried by AplKv3.3 potassium channels in the pyramidal cell layer of the electrosensory lateral line lobe (ELL) contribute to dendritic spikes and burst discharges, which encode amplitude modulations in the electric field (Gabbiani and Metzner, 1999; Turner and Maler, 1999; Rashid et al., 2001a,b). ELL pyramidal cells also express low-threshold potassium currents that influence subthreshold excitability and that are likely carried by Kv1 channels (Fernandez et al., 2005). Similarly, rapid, spontaneous firing of pacemaker and electromotor neurons, which control the EOD frequency, is regulated by potassium currents with pharmacological sensitivities similar to those mediated by Kv1 potassium channels (Dye, 1991; Smith and Zakon, 2000; Smith, 2006). Thus, both high-threshold potassium currents carried by Kv3-like channels and low-threshold potassium currents carried by Kv1-like channels are likely to regulate excitability in electrosensory and/or electromotor brain nuclei.

Although the distribution of a Kv3 potassium channel in *A. leptorhynchus* (AptKv3.3) has been described in electrosensory brain regions (Rashid et al., 2001a), the distribution of Kv1 channels in the brains of electric fish is not known. This study sought to examine the distribution of Kv1 channels in the electromotor and electrosensory systems of *A. leptorhynchus* by using immunohistochemistry with antibodies directed against different Kv1 α subunits. Our results demonstrate that Kv1 channels are broadly distributed in both the electromotor and electrosensory systems and are thus likely to contribute to EOD rhythm generation and electrosensory processing.

**METHODS**

**Subjects**

Sixteen *A. leptorhynchus* were obtained from commercial suppliers and were socially housed in 200 to 340-L aquaria at 25.5 to 26.5°C, pH 4.5 to 6.0, and conductivity of 100 to 500 μS/cm. All experiments complied with the NIH Guide for the Care and Use of Laboratory Animals and protocols approved by the Institutional Animal Care and Use Committee at Indiana University.

**Immunoblots**

To test the binding specificity of the Kv1 antibodies with brain tissue from *A. leptorhynchus*, immunoblots were performed on proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Six *A. leptorhynchus* were terminally anesthetized with 0.1% 2-phenoxyethanol. The brains were dissected in ice-cold homogenization buffer (HB: 50 mM Tris-HCl, 100 mM NaCl, 0.5 mM EDTA, 0.5 mM EGTA, 1 mM dithiothreitol, 0.6 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin A, 1 μM leupeptin, and 10 μg/L trypsin inhibitor; pH 7.5), were flash-frozen in liquid nitrogen, and were stored at −80°C until further processing. Tissues were pulverized with a mortar and pestle and homogenized over ice in HB. Cellular debris was removed by centrifugation (1000 g, 10 min, 4°C), and protein concentrations of the supernatants were determined by the method of Bradford (1976) with 2.0 mg/mL BSA as the standard. Supernatants were then diluted in sample buffer [60 mM Tris (pH 6.8), 14.4 mM 2-mercaptoethanol, 25% glycerol, 2% SDS, 0.1% bromophenol blue] to a final concentration of 0.5 μg/mL.

Proteins (7 μg/lane) were separated by SDS-PAGE on 4% to 15% Tris-HCl gels (Bio-Rad, Hercules, CA; 100 V, 120 min). Six replicate gels, one for each of the Kv1 antibodies, were prepared. Separated proteins were transferred from the gels to polyvinylidene fluoride (PVDF) membranes by using a Transblot SD semi-dry transfer cell (Bio...
Rad). Each of the membranes was then immunoblotted with one of the Kv1 antibodies by following the instructions in an Opti-4CN™ Substrate Kit (Bio-Rad). Membranes were briefly submersed in methanol, rinsed in Tween-PBS (PBST), blocked in 5% blocking media in PBST overnight, and incubated in one of the six Kv1 antibodies diluted in PBST (1:500; 2 h; room temperature). The membranes were rinsed in PBST and incubated in horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000; 1 h). Membranes were rinsed and processed for colorimetric detection by using the Western Blot Amplification Module Kits (Bio-Rad). Molecular weight standards (Kaleidoscope pre-stained, Bio-Rad) were run on the same gels. The relationship between the molecular weight of the standards versus the distance migrated on each gel was fit with a Boltzmann function. The molecular weights of the labeled proteins were estimated from the Boltzmann curves. To test for non-specific binding of the primary antibodies, membranes were also incubated with primary antibodies preadsorbed with the peptides used to generate the antibodies.

**Tissue Preparation for Immunohistochemistry**

*A. leptorhynchus* (*n* = 10) were deeply anesthetized by immersion in 0.1% 2-phenoxyethanol (Sigma, St. Louis, MO) and were perfused transcardially with heparinized Krebs-Henseleit buffer (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 2.0 CaCl2; 1 U/mL heparin; pH 7.4), followed by phosphate-buffered 4% paraformaldehyde (pH 7.4). Brains, tails, and skin from the head and muscle (63–70 kDa and 83–88 kDa; Yuan et al., 1998) were removed, postfixed in the same fixative (overnight, 4°C), and cryoprotected (24–48 h, 30% sucrose in 0.05M phosphate buffer, pH 7.2). Brains, tails, and skin from the head were removed, postfixed in the same fixative (overnight, 4°C), and cryoprotected (24–48 h, 30% sucrose in 0.05M phosphate buffer, pH 7.2). Tissues were sectioned at 10 μm on a cryostat and mounted directly onto microscope slides (SuperFrost Plus, Fisher, Pittsburgh, PA). Seven to twelve slides, each containing sections of tail, skin, and portions of the brain with electrosensory and electromotor nuclei, were prepared from each fish.

**Immunohistochemistry**

The tissue sections on the slides were surrounded with a hydrophobic barrier by using an ImmEdge pen (Vector Laboratories, Ingold, CA) to retain incubating solutions on the sections. Sections were postfixed on slides with 4% buffered paraformaldehyde (pH 7.4, 10 min, room temperature) and rinsed in 0.1M phosphate-buffered saline (PBS, pH 7.4). Sections were processed by immunohistochemistry with rabbit polyclonal antibodies affinity-purified for specific binding to Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, or Kv1.6 potassium channel α subunits (Alomone Laboratories, Jerusalem, Israel). The slides were incubated in 1% blocking reagent from a tyramide signal amplification kit (TSA kit, Molecular Probes, Eugene, OR) for 1 h at room temperature to block nonspecific binding sites. Each of the six Kv1 antibodies was diluted 1:50 in 1% blocking serum and was applied to separate slides for 15 to 20 h at room temperature. Negative control sections were incubated in 1% blocking serum without primary antibodies. Sections were rinsed in PBS and incubated in horseradish peroxidase-conjugated goat anti-rabbit IgG (provided in the TSA kit, diluted 1:100 in 1% blocking solution) for 1 h at room temperature.Slides were rinsed in PBS, and incubated in Alexa Fluor 488 tyramide working solution (5–10 min., diluted 1:100 in amplification buffer from the TSA kit and containing 0.0015% H2O2). Sections were again rinsed in PBS, and most sections were counterstained with propidium iodide (Molecular Probes, 1:1000, 1 min). Slides were coveredslipped with Vectashield Hard Set mounting media (Vector) or Gelmount (Biømeda, Foster City, CA).

**Microscopy and Imaging**

Slides were viewed with a Nikon E800 epifluorescence microscope, and images were collected with a CCD camera (Orca ER, Hamamatsu, Hamamatsu City, Japan) controlled by Metamorph imaging software (Universal Imaging, Downingtown, PA). Images were cropped and the brightness and contrast of images were uniformly adjusted by using Photoshop (v. 5.0LE, Adobe, San Jose, CA). The *A. leptorhynchus* brain atlas (Maler et al., 1991) was used to identify brain regions.

**RESULTS**

**Immunoblots**

Immunoblots of brain tissue from *A. leptorhynchus* revealed that all of the Kv1 antibodies except for the Kv1.4 antibody labeled proteins similar in size to corresponding mammalian Kv1 proteins (Fig. 1). The Kv1.1 antibody labeled proteins of 78 and 86 kDa. The 78-kDa protein, which was more intensely labeled by the Kv1.1 antibody, had a similar molecular weight as Kv1.1 in cultured Schwann cells from mice (75 kDa; Sobko et al., 1998). The Kv1.2 antibody strongly labeled an 87-kDa protein, and weakly labeled a 50-kDa protein. The more robustly labeled 87-kDa protein is similar in size to Kv1.2 expressed in rat brain, oligodendrocytes, Schwann cells, and PC12 cells (75–90 kDa; Sheng et al., 1994; Attali et al., 1997; Sobko et al., 1998; Xu et al., 1999; Conforti et al., 2000). As in the present study, several other studies have reported a smaller protein that was also weakly labeled with the Kv1.2 antibody (Sheng et al., 1994; Attali et al., 1997; Sobko et al., 1998; Nashmi et al., 2000). The Kv1.3 antibody labeled 74- and 84-kDa proteins; these molecular weights are similar to that of Kv1.3 in rat brain and arterial smooth muscle (63–70 kDa and 83–88 kDa; Yuan et al., 1998; Xu et al., 1999). A prominent band corresponding to a molecular weight of 128 kDa was robustly
labeled with the Kv1.4 antibody, with fainter bands at 104 and 97 kDa. The most prominent, 128 kDa, band is somewhat larger than Kv1.4 expressed in mammalian glial cells (115 kDa; Attali et al., 1997; Sobko et al., 1998). This discrepancy could be caused either by differences in the molecular weight of a Kv1 channel in *A. leptorhynchus* that bound to the Kv1.4 antibody (due to sequence differences and/or differences in post-translational processing) or by the Kv1.4 antibody binding to a protein other than a Kv1/C11 subunit in *A. leptorhynchus*. The Kv1.5 antibody labeled 81-kDa and 123-kDa proteins. The smaller of these proteins is similar in size to Kv1.5 in cultured rat and mouse neural tissues (75–90 kDa; Attali et al., 1997; Sobko et al., 1998; Xu et al., 1999). Proteins of 92 and 108 kDa were labeled by the Kv1.6 antibody. The smaller and more intensely labeled of these proteins is similar in size to Kv1.6 in cultured rat oligodendrocytes (88 kDa; Attali et al., 1997).

The fact that all of the Kv1 antibodies except Kv1.4 most strongly labeled proteins whose molecular weights were consistent with those of the specifically targeted Kv1 α subunits suggests that the Kv1 antibodies bound primarily to Kv1 proteins in *A. leptorhynchus*. In some cases, however, the Kv1 antibodies also labeled additional, fainter bands on the immunoblots. For example, the Kv1.2 antibody labeled a 50-kDa protein as well as the expected 87-kDa protein, and the Kv1.6 antibody labeled a 108-kDa protein as well as the expected 92-kDa protein. These additional faint bands are similar to those found in immunoblots with Kv1 antibodies against mammalian neural and vascular tissues and have been postulated to result from the antibodies binding to alternative isoforms or degradation products of the targeted Kv1 proteins (Wang et al., 1993; Sheng et al., 1994; Barry et al., 1995; Attali et al., 1997; Sobko et al., 1998; Yuan et al., 1998; Xu et al., 1999; Nashmi et al., 2000). Additional bands on the Western blots could have also resulted from the mammalian Kv1 antibodies binding to duplicate homologous proteins in *A. leptorhynchus*. As a result of whole-genome duplication in the evolution of ray-finned fishes, many mammalian genes have duplicate homologs in teleost fishes (Amores et al., 1998; Christoffels et al., 2004). Indeed, two homologs of both Kv1.1 (Kv1.1a and Kv1.1b) and Kv1.2 (Kv1.2a and Kv1.2b) are expressed in the weakly electric fish *Sternopygus macrurus* (Few and Zakon, 2003), and it is thus possible that the mammalian antibodies cross-reacted with both of the duplicate fish Kv1 homologs. Another possibility is that the additional faint bands resulted from a low level of cross-reactivity of some of the Kv1 antibodies with unrelated proteins expressed in the *A. leptorhynchus* brain.

**Kv1-Like Immunoreactivity in Electrosensory Structures**

Immunoreactivity with each of the Kv1 antibodies was widespread in both electrosensory and electromotor structures in the CNS and the periphery. Below we describe the distribution of Kv1-like immunoreactivity in both the ascending electrosensory pathway from the tuberous electroreceptors in the skin to the torus semicircularis in the midbrain and the descending electromotor pathway from the thalamic prepace-maker nucleus to the electric organ (Fig. 2; Tables 1 and 2).

**Tuberous Electroreceptors.** The receptor cells of tuberous electroreceptor organs and their afferents were labeled to varying degrees by all six of the Kv1 antibodies (Fig. 3; Table 1). Kv1.1 and Kv1.4 immunoreactivity was moderately strong in the electroreceptor cells and intense in the afferent axons of the electroreceptor organs [Fig. 3(A,D)]. In contrast, the Kv1.2 antibody intensely labeled the electroreceptor cells, but only lightly labeled the afferents [Fig.
Kv1.5 immunoreactivity was robust in both the electroreceptor cells and afferent axons [Fig. 3(E)]. Kv1.3 and Kv1.6 antibodies also labeled both electroreceptor cells and afferents, but the staining was less intense than that obtained with the other Kv1 antibodies [Fig. 3(C,F)].

**Electrosensory Lateral Line Lobe (ELL).** The ELL is the first-order electrosensory nucleus in the brain and contains four functionally and neuroanatomically distinct somatotopic maps, each of which has a laminar organization (Maler, 1979; Shumway, 1989). The layers of the ELL (from ventral to dorsal) include the following: (1) the deep fiber layer (DFL), through which electrosensory afferents traverse; (2) the deep neuropil layer (DNL), which contains phase-encoding spherical cells; (3) an interneuron-rich granular cell layer (GCL); (4) a plexiform layer that contains efferent axons; (5) a polymorphic layer containing pyramidal cells, one of two types of projection neurons; and (6) an extensive molecular layer, which contains the apical dendrites of the pyramidal cells and is the site of termination of major electrosensory feedback pathways [Fig. 2(B); Maler, 1979; Berman and Maler, 1999].

All of the Kv1 antibodies labeled the ELL, although different neuron types and structures were immunoreactive with each of the different Kv1 antibodies (Fig. 4; Table 1). The Kv1.1 antibody strongly labeled electroreceptor afferents in the DFL and DNL. The cell bodies of pyramidal cells in the polymorphic layer as well as their dendrites in the molecular layer and the GCL were also immunoreactive for Kv1.1 [Fig. 4(A)]. The Kv1.2 antibody labeled fine fibers throughout the ELL, although these fibers were most dense in the DFL and polymorphic layer [Fig. 4(B)]. The only immunoreactivity in the ELL for Kv1.3 was found in the electroreceptor afferents in the DFL and DNL, and GCL. The Kv1.4 antibody also faintly labeled spherical cells in the DNL, granular cells in the GCL, and pyramidal cells in the polymorphic layer and their dendrites in the molecular layer and GCL [Fig. 4(E,F)]. The electroreceptor afferents in the DFL were strongly immunoreactive for Kv1.5, and there was also faint Kv1.5-like immunoreactivity in the granular cells in the GCL [Fig. 4(D)]. Afferents in the DFL and DNL and thick fibers in the GCL were immunoreactive with the Kv1.6 antibody. ELL pyramidal cells in the polymorphic layer and their dendrites in the molecular layer and GCL were also labeled by the Kv1.6 antibody [Fig. 4(G,H)].

**Dorsal Torus Semicircularis (TSd).** The torus semicircularis (TS) is a prominent midbrain region that is a major site for integration of mechanosensory and electrosensory information. The dorsal subdivision of the TS (TSd) comprises most of the TS and is a multilaminar structure specialized for electrosensory processing (Carr et al., 1981). Pyramidal cells in the
ELL convey electroreceptive probability/amplitude information via projections to layers 3, 5, 7, 8, and 9 of the TSd. Neurons in the deep layers of the TSd (7, 8, and 9) give rise to an ascending projection to nucleus electrosensorius in the diencephalon, and neurons in all layers except layers 1, 6, and 8b project to nucleus preeminentialis, which conveys descending feedback to the ELL [Fig. 2(C); Carr et al., 1981]. Layer 6 of the TSd is specialized for precisely processing phase information (Carr et al., 1986b), and Kv1-like immunoreactivity in this layer will be presented separately (see below).

As in the ELL, Kv1-like immunoreactivity was widespread in the TSd (Figs. 5 and 6; Table 1).

### Table 1  Summary of Immunoreactivity of Electrosensory Structures with Kv1 Antibodies

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<tr>
<th>Nucleus/Structure</th>
<th>Kv1.1</th>
<th>Kv1.2</th>
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<th>Kv1.4</th>
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<td>Tuberous electroreceptors</td>
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<td>Afferent axons</td>
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<td>Electroreceptive lateral line lobe (ELL)</td>
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<td>Pyramidal cell bodies</td>
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<td>Pyramidal apical dendrites</td>
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<tr>
<td>Neuropil</td>
<td>++</td>
<td>+</td>
<td>—</td>
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</tr>
<tr>
<td>Medium, round neurons</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Small, round neurons</td>
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<td>+++</td>
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<td>Fusiform neurons</td>
<td>++++</td>
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<tr>
<td>Layer 9</td>
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<tr>
<td>Neuropil</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Small, ovoid neurons</td>
<td>—</td>
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</table>

+, ++++, faint, moderate, intense immunoreactivity; — no specific immunoreactivity; (p), punctate staining pattern.
Immunoreactivity for each of the Kv1 antibodies, except Kv1.3, was present in the TSd, but different cell types and structures were labeled by each antibody. Light-to-moderate punctate immunoreactivity for Kv1.1 was present in the neuropil in layers 3, 4, 5, 7, 8, and 9. Kv1.1-like immunoreactivity was also present in small (5–6 μm diameter) neurons in layer 3, 10 to 15 μm diameter round neurons in layers 5 and 8, and fusiform neurons (5–7 μm × 10–12 μm) in the ventral part of layer 8 [Fig. 5(A,B)]. Immuno-
reactivity for Kv1.2 was much less abundant, and was limited to sparse punctate labeling in the neuropil of layers 8 and 9 and faint staining of medium-sized round neurons (similar to those labeled for Kv1.1) in layer 8 [Fig. 5(C)]. No Kv1.3-like immunoreactivity was present in the TSd. The Kv1.4 antibody lightly labeled the neuropil of layers 3, 4, and 5, and also labeled five different classes of toral neurons: small ovoid cells in layer 4; medium-sized round neurons in layer 5 (similar to those labeled for Kv1.1); small, round neurons (5–7 μm diameter) in layer 8; fusiform neurons in the ventral part of layer 8 (similar to those labeled for Kv1.1); and small (6–8 μm) ovoid cells in layer 9 [Fig. 5(D,E)]. The neuropil of layers 3, 4, and 5 and 5 was also immunoreactive with the Kv1.6 antibody; and this antibody also labeled neurons similar in size and distribution to those labeled for Kv1.1 and/or Kv1.4 in layers 4, 5, and 8, as well as ovoid cells in layer 7 [Fig. 5(F,G)].

Layer 6 of the TSd is specialized for precisely processing electrosonre phase information (Carr et al., 1986a,b). Two cell types of layer 6, giant cells and medium cells, receive afferent input from spherical cells in the ELL and form a circuit that compares relative phase information from electroreceptors located in different areas of the skin [Fig. 2(C)]. Structures in layer 6 were immunoreactive with all of the Kv1 antibodies except Kv1.3 (Fig. 6; Table 1). Fine fibers and terminals in layer 6 were strongly immunoreactive for Kv1.2, Kv1.4, and Kv1.6. These fibers formed dense networks around the somata of giant cells, although the giant cells themselves were not specifically labeled with any of the Kv1 antibodies. The Kv1.5 antibody produced lighter, more sparse and punctate labeling of fibers in layer 6. Medium cells and their processes were lightly to moderately immunoreactive with all of the Kv1 antibodies except Kv1.3.

### Table 2: Summary of Immunoreactivity of Electromotor Structures with Kv1 Antibodies

<table>
<thead>
<tr>
<th>Nucleus/Structure</th>
<th>Kv1.1</th>
<th>Kv1.2</th>
<th>Kv1.3</th>
<th>Kv1.4</th>
<th>Kv1.5</th>
<th>Kv1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central posterior/prepacemaker nucleus (CP/PPn)</td>
<td></td>
<td></td>
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<tr>
<td>Circumventricular cells</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dorsomedial fibers/neuropil</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>(+p)</td>
<td>—</td>
</tr>
<tr>
<td>Dorsomedial (CP) neurons</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ventrolateral neuropil</td>
<td>+/+(+p)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>++(p)</td>
<td>—</td>
</tr>
<tr>
<td>Ventrolateral neurons</td>
<td>+</td>
<td>—</td>
<td>+</td>
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<tr>
<td>PPN-C projection neurons</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+++</td>
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<tr>
<td>Pacemaker nucleus (Ph)</td>
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<tr>
<td>Pacemaker cells</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+++</td>
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<tr>
<td>Relay cells</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Relay axons</td>
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<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>+++</td>
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<tr>
<td>Parvocells</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>+++</td>
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<tr>
<td>Afferent axons</td>
<td>+++</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Fine fibers</td>
<td>—</td>
<td>+++</td>
<td>++</td>
<td>+/+(+p)</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Spinal cord</td>
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<tr>
<td>Relay axons</td>
<td>++</td>
<td>—</td>
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<td>+++</td>
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<tr>
<td>Electromotor neurons</td>
<td>—</td>
<td>—</td>
<td>+(p)</td>
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<td>Fine fibers</td>
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<td>+++</td>
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<tr>
<td>Electric organ</td>
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<tr>
<td>Axons</td>
<td>—</td>
<td>++(p)</td>
<td>++</td>
<td>—</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Schwann cells/myelin</td>
<td>+/++</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

+, ++, ++++, faint, moderate, intense immunoreactivity; — no specific immunoreactivity; (p), punctate staining pattern.

Kv1-Like Immunoreactivity in Electromotor Structures

**Central Posterior/Prepacemaker Nucleus (CP/PPn).** The CP/PPn is a thalamic nucleus that controls modulations of EOD frequency that are used as communication signals (Heiligenberg et al., 1991; Zupanc and Maler, 1997). Several Kv1 antibodies labeled struc-
tures in the CP/PPn (Fig. 7; Table 2). The Kv1.1 antibody faintly labeled scattered, medium-sized (7–10 μm diameter) neurons throughout the CP/PPn [Fig. 7(A)]. Sparse punctate Kv1.1-like immunoreactivity was also present in the neuropil of the lateral portion of the CP/PPn, including the gradual rise (PPn-G) and chirp (PPn-C) portions of the PPn. The only structures in the CP/PPn that were immunoreactive for Kv1.2 were small, intensely labeled cells adjacent to the ventricle [Fig. 7(B)]. Numerous small (5–7 μm) round cells throughout the CP/PPn were immu-

noreactive with the Kv1.3 antibody [Fig. 7(C)]. The Kv1.4 antibody intensely labeled small cells adjacent to the ventricle and fibers that extended from this region into the more lateral portions of the CP/PPn [Fig. 7(D)]. Moderately intense, punctate Kv1.5-like immunoreactivity was present throughout the neuropil of the CP/PPn; and the relatively large (9–18 μm) ovoid projection neurons of the PPn-C were intensely immunoreactive for Kv1.5 [Fig. 7(E,F)]. The Kv1.6 antibody did not specifically label any structures in the CP/PPn.

Figure 3 Kv1-like immunofluorescence (green) in tuberous electroreceptor organs. Cell nuclei counterstained with propidium iodide (red). Primary afferent axons are indicated with arrowheads. Scale bars = 50 μm.
Figure 4  Kv1-like immunofluorescence (green) in the electrosensory lateral line lobe (ELL). Cell nuclei are counterstained with propidium iodide (red). Abbreviations: DFL, deep fiber layer; DNL, deep neuropil layer; GCL, granular cell layer; Poly, polymorphic layer; Str. fib., stratum fibrosum; Mol, molecular layer. Dorsal is up. (A) Kv1.1 immunoreactivity in electroreceptor afferents in the DFL and DNL and in pyramidal cell bodies (arrows) in the polymorphic layer and their dendrites in the molecular layer and GCL. (B) Kv1.2-immunoreactive fibers throughout the ELL. Note the higher density of these fibers in the DFL and polymorphic layer. (C) Kv1.3 immunoreactivity in afferent fibers in the DFL. (D) Kv1.5 immunoreactivity in afferent fibers in the DFL. Note the faint Kv1.5 immunoreactivity in granular cells in the GCL (arrowheads). (E, F) Faintly Kv1.4-immunoreactive fine fibers in the DFL, DNL, and GCL. Granular cells (arrowheads in E) and spherical cells (open triangles in F) were also labeled by the Kv1.4 antibody. Pyramidal cells (arrows in E) and their dendrites in the molecular layer were faintly labeled. Image in (F) is from the same section, but ventral to the image in (E). (G, H) Robust Kv1.6 immunoreactivity in electroreceptor afferents in the DFL, thick processes in the GCL, and pyramidal cell bodies (arrows in G) in polymorphic layer and their dendrites in the molecular layer and GCL. Image in (H) is from the same section, but ventral to the image in (G). Scale bars = 100 μm in (A, B); 50 μm in (C–H).
Pacemaker Nucleus. EOD frequency is controlled by the pacemaker nucleus (Pn). Extensively electrotonically coupled pacemaker cells and relay cells in the Pn fire synchronously at high frequencies (600–1100 Hz) to generate the command signal for the EOD. Kv1 immunoreactivity was widespread in the Pn (Fig. 8; Table 2). The Kv1.1 antibody robustly labeled axons that entered the Pn dorsolaterally, ramified within the Pn, and appeared to terminate on relay cells [Fig. 8(A)]. These Kv1.1-positive axons may be those of afferents from the prepacemaker nucleus, which form glutamatergic synapses on relay cells and depolarize them to produce chirps (Heiligenberg et al., 1996). The cell bodies of relay and pacemaker

Figure 5 Kv1-like immunofluorescence (green) in the dorsal torus semicircularis (TSd) layers 3 to 5 and 7 to 9. Cell nuclei are counterstained with propidium iodide (red). Numbers on each figure indicate layers. Dorsal is up. Labeled neurons are indicated by arrowheads or arrows. (A, B) Kv1.1 immunoreactivity in neuropil of all layers, and in neurons in layers 3, 5, and 8. A Kv1.1-immunoreactive fusiform neuron in the ventral portion of layer 8 is indicated by an arrow in (B). (C) Punctate Kv1.2 immunoreactivity in the neuropil of layers 8 and 9. Neurons in layer 8 also labeled with the Kv1.2 antibody. (D, E) Kv1.4 immunoreactivity in the neuropil of layers 3 to 5 and in neurons in layers 4, 5, 8, and 9. A Kv1.4-positive fusiform neuron in the ventral part of layer 8 is indicated by the arrow in (E). (F, G) Kv1.6 immunoreactivity in neuropil of layers 3 to 5 and in neurons in layers 4, 5, 7, and 8. Scale bars = 50 μm.
cells were also faintly Kv1.1-positive. Numerous fine fibers throughout the pacemaker nucleus were intensely immunoreactive with the Kv1.2 antibody and were particularly dense around the edges of pacemaker and relay cell bodies [Fig. 8(B)]. These fine fibers may be astrocytic processes because they have a similar distribution in the pacemaker nucleus as fibers immunoreactive for glial fibrillary acidic protein (GFAP; Smith et al., 2000). Double-label immunohistochemistry with Kv1 and GFAP antibodies is needed to confirm this identification. Fine fibers with a similar distribution, as well as the cell bodies of pacemaker and relay cells, were faintly stained by the Kv1.3 antibody [Fig. 8(C)]. The Kv1.4 antibody labeled all of the structures labeled by the Kv1.3 antibody, but also labeled the large-diameter axons of the relay cells [Fig. 8(D)]. The Kv1.5 strongly labeled the cell bodies and processes of parvocells, small interneurons that form chemical synapses on relay and pacemaker cells and are also coupled by gap junctions to these cells [Smith et al., 2000; Fig. 8(E)]. Kv1.5-like immunoreactivity was also present in relay, but not pacemaker, cells. The Kv1.5 staining in relay cells was more intense around the edges of these cells, and it is possible that some of this immunoreactivity was in terminals of parvocells. Both the cell bodies and the axons of relay and pacemaker cells were strongly immunoreactive with the Kv1.6 antibody [Fig. 8(F)].

**Spinal Cord.** The axons of relay cells project from the pacemaker nucleus and fasciculate in a distinct tract in the dorsolateral spinal cord. These axons were strongly immunoreactive for both Kv1.1 and Kv1.6 [Fig. 9(A,D)]. The terminals of the relay axons form electrotonic synapses on the cell bodies of electromotor neurons (Bennett et al., 1978). The cell bodies of the electromotor neurons

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**Figure 6** Kv1-like immunofluorescence in TSD layer 6 in sections from the rostral part of the TSD, where layer 6 occupies most of the medial portion of the TSD. G, giant cells. Numbers indicate layers. (A) Kv1.1 immunoreactivity in medium cells (arrowheads) and their processes (arrows). Labeling in giant cells was faint and not clearly above nonspecific staining levels observed in negative control sections. (B) Intense Kv1.2 immunoreactivity in networks of fine fibers throughout layer 6 as well as fainter Kv1.2 staining in medium cells (arrowheads). (C) Labeling of more sparsely distributed fibers (arrows) as well as medium cells (arrowheads) by the Kv1.5 antibody. A column of vertical neuropil (vn) passes through the center of the image. (D, E) Robust immunoreactivity for Kv1.4 (D) and Kv1.6 (E) in fine fibers and medium cells (arrowheads). Scale bars = 25 μm in (A), 50 μm in (B–E).
EMNs) contained faint, punctate immunoreactivity for Kv1.3 [Fig. 9(C)], but did not label with any of the other Kv1 antibodies. The axons of the EMNs in the electric organ, however, were strongly labeled by several Kv1 antibodies (see below). Fine fibers in the central grey matter of the spinal cord, including around the cell bodies of EMNs, were immunoreactive for Kv1.2; and the Kv1.6 antibody labeled fine fibers throughout the spinal cord [Fig. 9(B,D)].
Electric Organ. The electric organ of apteronotid fish, unlike that of other gymnotiform fish, is neurogenic and comprised of the specialized, hypertrophied, and targetless axons of spinal electromotor neurons (Bennett, 1971). All six Kv1 antibodies labeled the electric organ, although different patterns of labeling were produced with each antibody (Fig. 10). The intensity of staining with all six Kv1 antibodies varied along the length of the axons, and in most cases showed a banded pattern of staining that corre-
sponded to the distribution of nodes and internodes. This pattern of staining was apparent as alternating light and dark bands of staining in parasagittal sections of the electric organ or as variation in the intensity of staining of the axonal profiles in transverse sections. Propidium iodide counterstaining, which labeled the nuclei of Schwann cells, was used to determine whether the immunoreactivity with each antibody was localized to axonal or Schwann cell membranes. Both Kv1.1-like and Kv1.4-like immunoreactivity was localized in the myelinating membranes of Schwann cells [Fig. 10(A,B,G,H)]. The pattern of Kv1.4-like immunoreactivity was distinctive and included circumferential bands of intense staining around each axon coupled with shorter, intense bands radiating perpendicularly from these bands [Fig. 10(G)]. This pattern of staining resembled that around the “peculiar nodes” described by Bennett (1971, p. 420). In contrast with Kv1.1 and Kv1.4 antibodies, immunoreactivity with the Kv1.2, Kv1.3, Kv1.5, and Kv1.6 antibodies was localized primarily in the axons of the electric organ with little or no immunoreactivity in Schwann cells. Furthermore, the Kv1.2 antibody produced a distinct punctate pattern of staining in the axonal membranes [Fig. 10(C,D)].

**DISCUSSION**

The primary goal of this study was to characterize the distribution of Kv1 potassium channels in the electro-sensory and electromotor systems of *A. leptorhynchus*. We found widespread immunoreactivity for different Kv1 α subunits in several electromotor and electro-sensory structures. Below we compare the distribution of Kv1-like immunoreactivity in the brain of *A. leptorhynchus* with that reported in other vertebrates and discuss how the distribution of Kv1 channels might relate to their functions in electro-sensory processing and electromotor pattern generation.
Distribution of Kv1-Like Immunoreactivity

Although the expression of Kv1 channels has been well-characterized in the brains of many mammalian species, relatively few studies have examined the distribution of these channels in the brains of nonmammalian vertebrates. Kv1-like immunoreactivity was distributed throughout the brain of A. leptorhynchus.
This broad distribution of Kv1-like immunoreactivity is similar to widespread expression of Kv1 channels in the brains of other vertebrates (Nguyen et al., 2000; Trimmer and Rhodes, 2004). The distribution of immunoreactivity with each of the specific Kv1 antibodies used in this study was also consistent with the expression of specific Kv1 α subunits in other species. In the mammalian brain, Kv1.1, Kv1.2, and Kv1.4 are the most broadly expressed Kv1 subunits in the CNS, whereas the expression of Kv1.3 and Kv1.5 is more restricted (Trimmer and Rhodes, 2004). Similarly, we found immunoreactivity for Kv1.1, Kv1.2, and Kv1.4 in numerous electromotor and electrosensory structures in the brain of A. leptorhynchus, whereas fewer structures were labeled with the Kv1.3 and Kv1.5 antibodies (Tables 1 and 2).

The subcellular distribution of Kv1-like immunoreactivity in A. leptorhynchus was also consistent with previous reports of Kv1 expression in other systems. In the mammalian brain, Kv1 channels are commonly expressed in the axons and terminals, and one major role of potassium currents carried by Kv1 channels is to influence the shape of action potentials and regulate the depolarization and repolarization of presynaptic terminals (Sheng et al., 1994; Trimmer and Rhodes, 2004). Kv1.2, for example, is rare in neuronal cell bodies, but abundant in axons and terminals in the mouse brain (Wang et al., 1994). Consistent with this distribution, Kv1.2-like immunoreactivity in the A. leptorhynchus brain was primarily present in fine fibers; the only somatic staining with the Kv1.2 antibody was faint labeling of neurons in layers 6 and 8 in the TsD. Other Kv1 channels, including Kv1.1, Kv1.5, and Kv1.6, however, have broader subcellular distributions that include neuronal somata and dendrites as well as axons and terminals in the mouse brain (Wang et al., 1994; Chung et al., 2001). Similarly, Kv1.1, Kv1.5, and Kv1.6 antibodies labeled cell bodies and dendrites as well as axons, terminals, and neuropil in A. leptorhynchus (e.g., Kv1.1 and Kv1.6 immunoreactivity in the somata and dendrites of ELL pyramidal cells and Kv1.6 immunoreactivity in relay and pacemaker cell bodies). The similar subcellular distribution of Kv1-like immunoreactivity in fish and mammals suggests that specific Kv1 channel types may similarly regulate neuronal excitability across taxa.

**Potential Roles of Kv1 Channels in Electrosensory Processing in ELL Pyramidal Cells**

The ELL of A. leptorhynchus is an established model system for studying cellular and synaptic mechanisms of sensory processing (Mathieson and Maler, 1988; Turner et al., 1994, 1996; Bastian, 1999; Turner and Maler, 1999). The contribution of voltage-dependent ionic conductances in ELL pyramidal cells to fast oscillatory burst discharges has been particularly well-studied (Turner and Maler, 1999). Oscillatory burst discharges are common in neurons and are thought to contribute to feature detection, frequency tuning, and correlated activity across networks (Wehr and Laurent, 1996; Ritz and Sejnowski, 1997). In ELL pyramidal cells, these discharges encode amplitude modulations in the electric field and are produced by the interplay of fast somatic and slower dendritic action potentials (Turner et al., 1994; Gabbiani and Metzner, 1999; Turner and Maler, 1999). High-threshold potassium currents repolarize dendritic spikes and thereby regulate burst threshold and structure (Rashid et al., 2001b; Noonan et al., 2003). These high-threshold currents also permit high-frequency firing in ELL pyramidal cells via frequency-dependent interactions with sodium currents and low-threshold potassium currents (Fernandez et al., 2005). The most likely carriers of these high-threshold potassium currents are channels composed of AptKv3.3 subunits, which are abundantly expressed in the somata and dendrites of ELL pyramidal cells (Rashid et al., 2001a,b).

In addition to high-threshold potassium currents carried by Kv3 channels, ELL pyramidal cells also express low-threshold potassium currents that regulate action potential waveform and firing frequency, particularly at low firing rates (Fernandez et al., 2005). The molecular basis of the low-threshold potassium currents in these neurons is unknown, but Kv1 channels are likely candidates. Kv1 channels produce low-threshold potassium currents; and we found immunoreactivity for three Kv1 α subunits, Kv1.1, Kv1.4, and Kv1.6, in the somata and dendrites of ELL pyramidal cells. Although the Kv1.1 and Kv1.6 immunoreactivity in ELL pyramidal cells was much stronger than the Kv1.4 immunoreactivity, the effects of channel blockers are not entirely consistent with Kv1.1- or Kv1.6-like channels influencing spike waveform in these cells. α-dendrotoxin, which blocks mammalian Kv1.1 and Kv1.6, but not Kv1.4, channels (Coetzee et al., 1999), did not affect spike waveform in ELL pyramidal cells (Rashid et al., 2001b; Fernandez et al., 2005). It is possible that potassium currents carried by Kv1.4 channels influence spike waveform and that the Kv1.1- and Kv1.6-like channels expressed in ELL pyramidal cells influence other aspects of excitability (e.g., subthreshold integration of synaptic inputs). Alternatively, the potassium channels recognized by the Kv1.1 and/or Kv1.6 antibodies in A. leptorhynchus might differ from mam-
malian Kv1.1 and Kv1.6 in their sensitivity to α-dendrotoxin. Further molecular, pharmacological, and biophysical characterization of the Kv1 channels in the ELL is needed to test these hypotheses.

Potential Roles of Kv1 Channels in the Electromotor Pattern Generation

Neurons in the electromotor system fire spontaneously at high frequencies to control the EOD. EOD frequency in *A. leptorhynchus* ranges from 600 to 1100 Hz. Pacemaker and relay cells in the pacemaker nucleus and electromotor neurons in the spinal cord fire at these frequencies to generate the command signal for the EOD, and are among the fastest and most precisely-firing neurons in any animal (Bennett et al., 1967; Bullock, 1970; Meyer, 1984; Schaefer and Zakon, 1996; Moortgat et al., 1998). The direct correspondence of the activity of neurons in the electromotor system with the behavior they control combined with the relative simplicity of the electromotor circuit make it a useful model for studying mechanisms of high-frequency motor pattern generation (Smith, 1999).

The effects of channel blocking drugs on the firing rates of pacemaker cells and EMNs indicate that potassium currents carried by Kv1-like channels are essential for high-frequency firing (Dye, 1991; Smith and Zakon, 2000; Smith, 2006). In both of pacemaker neurons and EMNs, high-frequency firing is disrupted by submillimolar concentrations of 4-aminopyridine (4-AP), but is unaffected by high concentrations of tetraethylammonium (TEA). Sensitivity to low concentrations of 4-AP coupled with resistance to TEA is characteristic of several types of Kv1 channels. The firing rates of EMNs, but not pacemaker cells, are also strongly affected by α-dendrotoxin, which blocks mammalian Kv1.1, Kv1.2, and Kv1.6 channels (Coetzee et al., 1999; Smith and Zakon, 2000; Smith, 2006). This suggests that different Kv1 channels may regulate high-frequency activity in these two cell types. One of the goals of this study was to determine whether Kv1 channels were expressed in pacemaker and electromotor neurons.

Consistent with the hypothesis that Kv1-like channels contribute to high-frequency pattern generation in the electromotor system, immunoreactivity with Kv1 antibodies was abundant in the pacemaker nucleus. The cell bodies of pacemaker and relay cells were strongly labeled with the Kv1.6 antibody and more weakly labeled with antibodies against Kv1.1, Kv1.2, Kv1.3, and Kv1.4. Relay cells, but not pacemaker cells, were also lightly immunoreactive for Kv1.5. This suggests that Kv1-like channels are expressed in pacemaker and relay cells and might carry the potassium currents underlying spontaneous, high-frequency activity in these cells. The observation that the most robust Kv1-like immunoreactivity in these cells occurred with the Kv1.6 antibody, however, is less consistent with previous pharmacological results. Mammalian Kv1.6 channels are blocked by α-dendrotoxin, which had no effect on spontaneous firing in pacemaker neurons (Smith et al., 2000; Smith, 2006). One possible explanation for this inconsistency is that the Kv1.6 antibody might have bound to a Kv1 channel in *A. leptorhynchus* that is not sensitive to α-dendrotoxin. Only some Kv1 channels are blocked by α-dendrotoxin, and it is not known how well the sensitivity of particular Kv1 channel subunits (e.g., Kv1.6) to α-dendrotoxin is conserved across taxa. The epitope used to raise the Kv1.6 antibody was at the C-terminus of the protein, whereas α-dendrotoxin binds in the loop between the S5 and S6 domains (Tytgat et al., 1995), and it is thus possible that the Kv1.6 antibody bound to a Kv1 channel that lacked the α-dendrotoxin binding domain. An alternative possibility is that pacemaker and relay cells express α-dendrotoxin–sensitive Kv1.6 channels that do not contribute to high-frequency firing. In this case, other Kv1 channels that are α-dendrotoxin-resistant and are also expressed in pacemaker and/or relay cells (e.g., Kv1.4 or Kv1.5) might regulate high-frequency firing.

Despite pharmacological evidence indicating that Kv1-like channels regulate spontaneous, high-frequency activity in electromotor neurons (Smith, 2006), the cell bodies of electromotor neurons were not immunoreactive with most of the Kv1 antibodies, and showed only weak immunoreactivity with the Kv1.3 antibody. Several possibilities might explain the relative lack of Kv1 immunoreactivity in EMNs despite the sensitivity of these neurons to toxins that target Kv1 channels. One possibility is that potassium currents that support high-frequency firing in EMNs are carried through Kv1.3-like channels that generated the weak Kv1.3 immunoreactivity in the EMN somata. This possibility is not likely both because Kv1.3 immunoreactivity was relatively weak in EMNs and because mammalian Kv1.3 is resistant to α-dendrotoxin, which disrupts high-frequency firing in EMNs (Smith, 2006). A second possibility is that the potassium currents that support spontaneous firing in EMNs are carried by channels composed of Kv1 subunits that did not bind to any of the Kv1 antibodies used in this study. PCR amplification of voltage-gated potassium channel genes from *A. leptorhynchus* genomic DNA identified 10 unique Kv1 gene fragments (Rashid and Dunn, 1998); and it is possible
that the protein products of some Kv1 genes in A. leptorhynchus were not recognized by any of the specific Kv1 antibodies used in this study. A third possibility is that potassium currents that regulate high-frequency firing of EMNs arise in the axons rather than the somata of these neurons. The axons of the EMNs in the electric organ were immunoreactive with several Kv1 antibodies (see below).

Extensive Kv1-like immunoreactivity in the electric organ, which is composed of the large axons of EMNs, is consistent with previous reports that Kv1 channel proteins are commonly targeted to axons and terminals (Sheng et al., 1994; Trimmer and Rhodes, 2004). Furthermore, the distribution Kv1-like immunoreactivity in the electric organ was similar to that in mammalian peripheral nerves. Mammalian Schwann cells, for example, express Kv1.1, Kv1.2, Kv1.4, and Kv1.5 subunits, although the expression of Kv1.2 and Kv1.5 is downregulated in adults (Sobko et al., 1998). Consistent with this finding, immunoreactivity of the electric organ with the Kv1.1 and Kv1.4 antibodies was primarily localized to Schwann cell membranes. Immunoreactivity with the other Kv1 antibodies was associated with electromotor axons in the electric organ. In myelinated axons in mice, voltage-gated potassium channels are typically concentrated in the juxtaparanodal region of the axon (Wang et al., 1993; Rasband, 2004). Although the propidium iodide counterstain used in this study labeled only cell nuclei and therefore did not allow us to confirm the location of nodal, paranodal, and juxtaparanodal regions of the axons in the electric organ, the banded pattern of Kv1-like immunoreactivity in the axonal membranes in the electric organ is consistent with the juxtaparanodal distribution found in other myelinated axons. Future studies comparing the co-distribution of Kv1-like channels and voltage-gated sodium channels in the electric organ may contribute to an understanding of the function of neurogenic electric organs. The nodes of Ranvier vary dramatically in structure and excitability along the length of the axons in the electric organ (Bennett, 1971), and the distribution of voltage-gated potassium and sodium channels is likely to underlie some of this variation.

Possible Functions of Kv1 Channels in Other Electrosonory and Electromotor Structures

Kv1-like immunoreactivity was also present in many electrosonory and electromotor structures in which the function of voltage-dependent potassium currents has not yet been investigated. For example, Kv1-like immunoreactivity was widespread in neurons and neuropil in the TSd. Many neurons in the TSd have strong temporal filtering properties, and voltage-dependent conductances contribute to these sensory filters by amplifying inputs in particular frequency ranges (Rose and Fortune, 1999). Voltage-gated sodium currents allow regenerative postsynaptic potentials that amplify high-frequency sensory input (Fortune and Rose, 2003), but the role of voltage-dependent potassium conductances in temporal filtering in TSd neurons is not known. The expression of Kv1 channels in the TSd raises the possibility that potassium currents carried by these channels might shape sensory filters in TSd neurons.

Similarly, the robust immunoreactivity for Kv1 in tuberous electroreceptors suggests that Kv1 channels might influence the excitability and/or tuning of electroreceptors. Tuberous electroreceptors are tuned with best frequencies near the fishes’ own EOD frequency; and in species with sexually dimorphic EOD frequencies, steroid-induced plasticity in EOD frequency is accompanied by parallel changes in electroreceptor tuning (Viancour, 1979; Meyer and Zakon, 1982; Meyer et al., 1987). Calcium currents are the principal inward currents in electroreceptor cells and amplify receptor potentials produced in response to small changes in the electric field (Bennett and Clusin, 1979); but the role of voltage-dependent potassium currents in tuberous electroreceptors has not been studied. One possibility is that Kv1 channels carry outward currents that shape the tuning curves of receptor cells. Because the potassium currents carried by Kv1 channels are activated at relatively negative membrane potentials, they could have a strong influence over subthreshold membrane resonance and tuning. In central auditory neurons, for example, both low-threshold potassium currents carried by Kv1 channels and high-threshold potassium currents carried by Kv3 channels can influence tuning by narrowing temporal integration windows and increasing high-frequency fidelity (Brew and Forsythe, 1995; Rathouz and Trussell, 1998; Svirsksis et al., 2003). Additional electrophysiological studies of tuberous electroreceptors are needed to determine the biophysical properties of the voltage-dependent potassium currents in these cells and to test the hypothesis that currents carried by Kv1 channels contribute to tuning.

Overall, the distribution of Kv1-like immunoreactivity in A. leptorhynchus indicates specific expression of several different Kv1 α subunits in electromotor and electrosonory neurons. Future studies characterizing the molecular and biophysical properties of
native Kv1 channels in Apteronotus and examining how the potassium currents carried by these channels influence neuronal excitability are needed to elucidate the roles of these channels in generating and modulating high-frequency motor rhythms and in encoding electrosensory features.

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