The Journal of Neuroscience, May 1, 2000, *20*(9):3408–3414

Coregulation of Voltage-Dependent Kinetics of Na^+ and K^+ Currents in Electric Organ

M. Lynne McAnelly and Harold H. Zakon

Section of Neurobiology and Institute for Neuroscience, Patterson Laboratory, The University of Texas at Austin, Austin, Texas 78712

The electric organ cells of *Sternopygus* generate action potentials whose durations vary over a fourfold range. This variation in action potential duration is the basis for individual variation in a communication signal. Thus, action potential duration must be precisely regulated in these cells. We had observed previously that the inactivation kinetics of the electrocyte Na^+ current show systematic individual variation. In this study, using a two-electrode voltage clamp, we found that the voltage-dependent activation and deactivation kinetics of the delayed rectifying K^+ current in these cells covary in a graded and predictable manner across fish. Furthermore, when Na^+ and K^+ currents were recorded in the same cell, their voltage-dependent kinetics were highly correlated. This finding illustrates an unprecedented degree of coregulation of voltage-dependent properties in two molecularly distinct ionic channels. Such a coregulation of ionic channels is uniquely observable in a cell specialized to generate individual differences in electrical activity and in which the results of biophysical control mechanisms are evident in behaving animals. We propose that the precise coregulation of the voltage-dependent kinetics of multiple ionic currents may be a general mechanism for regulation of membrane excitability.

Key words: Na^+ current; K^+ current; electric organ; voltage-clamp; electric fish; Sternopygus*; regulation*

Electrical excitability is a fundamental property of neurons and muscle cells. Different cell types differ in their electrical behaviors, and each cell type must possess the correct complement of ionic currents with proper kinetics and magnitudes to generate its electrical "signature." Ionic currents must be carefully regulated to maintain a stable electrical phenotype or, conversely, to bring about adaptive changes in excitability during development, after changes in synaptic input or hormonal stimulation, or as a consequence of pathology (O'Dowd et al., 1988; Erulker et al., 1994; Turrigiano et al., 1995; Nerbonne, 1998; Xie and McCobb, 1998; Desai et al., 1999; Stemmler and Koch, 1999).

The electric organ cells (electrocytes) of weakly electric fish, which generate the electric organ discharge (EOD), are a good model cell to study how ionic currents are regulated. Because the EOD is used for social communication (Hopkins, 1972, 1974, 1999; Stoddard, 1999), EOD waveform varies across species, is sexually dimorphic, and is individually distinct; it is influenced by social factors and hormonal status and shows circadian variations in some species (Meyer, 1983; Mills and Zakon, 1987, 1991; Franchina and Stoddard, 1998). EOD waveform is primarily determined by the excitability properties of the electrocytes, and, because EOD waveform must be so finely tuned, the ionic currents of electrocytes are under exquisite regulatory control (Ferrari et al., 1995; Dunlap et al., 1997; Zakon et al., 1999). Furthermore, the EOD is easily recorded from freely swimming fish, providing an unmatched ability to unobtrusively assess biophysical events in behaving animals.

The EOD of the gold-lined black knifefish (*Sternopygus macrurus*) is a quasisinusoidal waveform that ranges from 50 to 200 Hz depending on age, sex, and individual identity (Hopkins, 1972; Mills and Zakon, 1987). To maintain the sinusoidal shape of the EOD, a fish discharging at a low frequency must produce long duration electrocyte action potentials (APs), whereas a fish discharging at a high frequency must generate short electrocyte APs; those discharging at intermediate frequencies make electrocyte APs of intermediate durations (Fig. 1). When compared across individuals, electrocyte AP duration is graded, varying from 3 to 12 msec, and is highly correlated with EOD frequency (Mills and Zakon, 1987, 1991).

We have shown previously that the rate of inactivation of the electrocyte Na^+ current shows systematic individual variation and is modifiable by hormone treatment (Ferrari et al., 1995; Dunlap et al., 1997). In this study, we show that the activation and deactivation kinetics of a voltage-dependent delayed rectifying K^+ current also vary in a graded manner and are systematically related to EOD frequency. Furthermore, when both currents are recorded from the same electrocytes, we find that the voltage-dependent kinetics of these two currents are highly correlated. This is the first report of such precise coordination of the kinetic properties of two ionic currents in the same cell, and this observation has general implications for the control of AP duration and membrane excitability.

MATERIALS AND METHODS

Animals. Gold-lined black knifefish (*Sternopygus macrurus)* were obtained commercially and maintained in aquaria in controlled temperature chambers. Immediately before electrocyte recording, recordings of the animal's EOD frequency were made in the home aquarium.

Tissue preparation. A 2.5–3.0 cm portion of the tail was removed and placed in saline containing (in mM): 114 NaCl, 2 KCl, 4 CaCl2, 2 MgCl2, 5 HEPES, and 3 glucose, pH 7.2, with curare (5 mg/l; Sigma, St. Louis, MO) to prevent contractions of the muscle fibers in the tail. The skin was removed to expose the electrocytes, and the tissue was pinned into a Sylgard recording chamber. We record from electrocytes *in situ* rather

Received Dec. 10, 1999; revised Feb. 8, 2000; accepted Feb. 15, 2000.

This work was supported by National Institutes of Health Grant R01 NS25513. We thank Kristina Schlegel for artwork, and Ying Lu and Rob Reinauer for fish care.

Correspondence should be sent to Dr. M. Lynne McAnelly at the above address. E-mail: l.mcanelly@mail.utexas.edu.

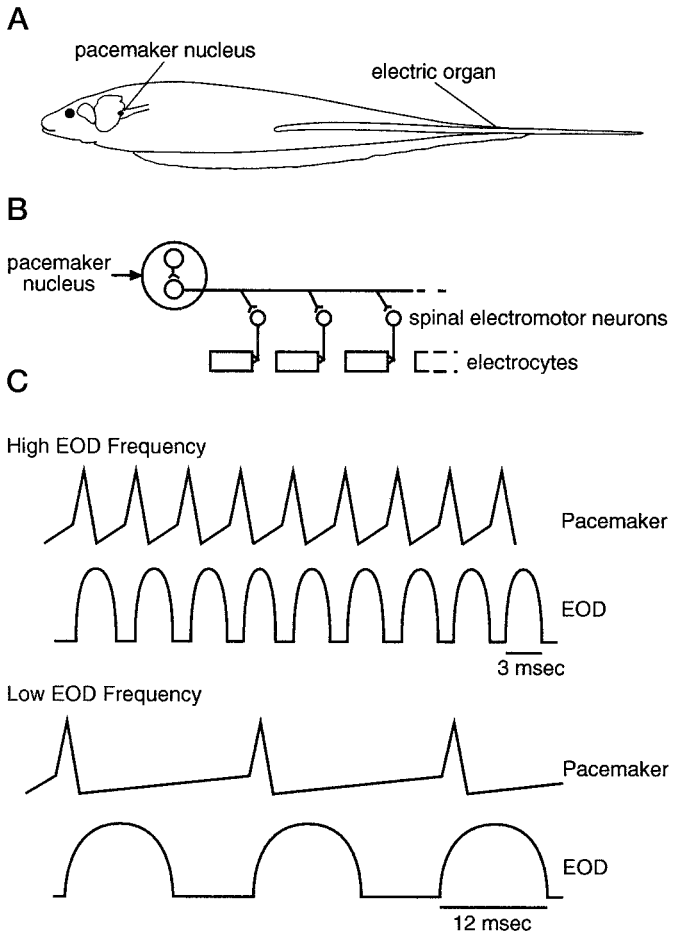

Figure 1. Schematic diagram of how the EOD is generated. *A*, The EOD is produced by the summation of the action potentials of the cells in the electric organ, called electrocytes. It is driven by cells in a midline medullary nucleus called the pacemaker nucleus. *B*, The output axons of the pacemaker nucleus synapse on a pool of spinal motoneurons, called electromotoneurons, which innervate the electrocytes. *C*, The firing frequency of the pacemaker neurons determine EOD frequency. For the EOD waveform to retain a sinusoidal shape, the duration of the electrocyte action potential must vary so that short action potentials are produced in electrocytes of fish with a high EOD frequency, long action potentials are produced in fish with a low EOD frequency, and action potentials of intermediate duration are generated in electrocytes of individuals with intermediate EOD frequencies.

than dissociate them to obviate possibly compromising channel function attributable to proteolysis (Hestrin and Korenbrot, 1987; Armstrong and Roberts, 1998).

Voltage clamp. A commercial two-microelectrode voltage-clamp amplifier [Axoclamp 2-A amplifier, TL-1 DMA interface, and pCLAMP software (Axon Instruments, Foster City, CA); Lab Master DMA boards (Scientific Solutions, Solon, OH); and Dell 486 computer (Dell Computers, Austin, TX)] was used. Two microelectrodes (with X1 head stage for voltage-recording and X10 head stage for current-passing electrodes) were placed in the posterior, active end of electrocytes with a grounded shield between them. The bath ground was a chlorided silver wire inserted into a plastic tube filled with 3% agar in 3 M KCl. Recordings were sampled at 20 kHz.

Holding potential was set near the resting potential of the cell (−90 to −75 mV). There was no systematic variation of electrocyte resting potential with EOD frequency so that this did not bias the results. Voltage-clamp steps of 75 msec duration were then given in 5 mV increments from −100 to + 45 mV. If a good recording was achieved, we switched to a saline in which the external Cl^- was replaced with the impermeant anion methylsulfate to decrease resting conductance and improve the space constant further and with 2 mM CsCl to block the inward rectifier. After current records were obtained in this saline, 1 μM TTX (Alomone Labs, Jerusalem, Israel) was added to block the Na^+ current and isolate the K^+ current.

The temperature of the preparation was not controlled, but room temperature was stable at 24–25°C. Recordings were made from fish of different EOD frequencies randomly over the course of this study so that slight seasonal variation in room temperature did not influence our results.

Under our recording conditions, the membrane potential settled in a few hundred microseconds at the start of the clamp step. We could record a rapidly activating inward rectifying K^+ current (data not shown) that was at its maximum magnitude by the time that the voltage clamp had settled. Thus, clamp speed was not a limiting factor in resolving the kinetics of the Na^+ and K^+ currents. Furthermore, there was no systematic relationship between electrocyte resting potential, apparent K^+ reversal potential, or resting resistance, and EOD frequency (data not shown). Finally, the variations in kinetics that we report below are evident, and even more exaggerated, at voltages close to the threshold for activation. At these voltages, activation and inactivation time constants are slow, which minimizes errors attributable to clamp speed, and current magnitudes are small, which minimizes series resistance errors.

Traces were leak subtracted, and analysis of currents was done with Clampfit (Axon Instruments). Graphs and *r* values were generated using Excel software (Microsoft, Seattle, WA). A more complete description of these procedures is available elsewhere (Ferrari et al., 1995).

RESULTS

Voltage-dependent activation and deactivation kinetics of K^+ current covary

The delayed rectifying K^+ current of the *Sternopygus* electrocyte is similar to that of the eel electrocyte in its general properties: that is, a noninactivating outward current that activates at approximately −40 mV (Shenkel and Sigworth, 1991). This current does not include any Ca^{2+}-activated K^+ currents because it is unaffected by removal of extracellular Ca^{2+} or addition of extracellular Co^+ or Cd^+ (Ferrari and Zakon, 1993).

Delayed rectifier K^+ current was recorded from electrocytes of fish across the range of EOD frequencies of the species. Typical currents from electrocytes of fish with EOD frequencies of 137, 89, and 43 Hz are illustrated in Figure 2. Outward currents from the fish with the highest EOD frequency activate more rapidly than those from the fish with a midrange EOD frequency, and these, in turn, activate more rapidly than those from the low EOD frequency fish (Fig. 2).

The rising phase, or activation, of a delayed rectifying K^+ current at each voltage step can be described quantitatively by fitting the current with a power function, namely $I_k(t) = I_{k,max}(1 - e^{-t/\tau})^n$, where $I_k(t)$ is the development of the current with time, $I_{k,max}$ is the maximum current attained, t is time, and τ is the time constant of activation (Hodgkin and Huxley, 1952). As sometimes occurs with the K^+ current, the value of n that gives the best fit varies with membrane potential or cell type (Campbell, 1992; Klemic et al., 1998). The best overall fit with our data were obtained with $n = 2$, which was the value we used in this study. Although the value of the exponent (2, 3, or 4) changed the value of the time constants, they were all shifted in the same direction, leaving the general conclusions of this study unchanged.

As is true of the delayed rectifying K^+ current in other cells, the rate of activation varied with membrane potential, being faster at more positive voltages (Fig. 3*A*). We plotted the time constant of activation at 25 mV above threshold (threshold is defined as the voltage step with the first indication of an outward current using voltage steps of 5 mV intervals) for the complete sample of cells to determine whether the rate constant of activation varied systematically with EOD frequency (Fig. 3*B*). We used this value as our standard for comparison because this point is in

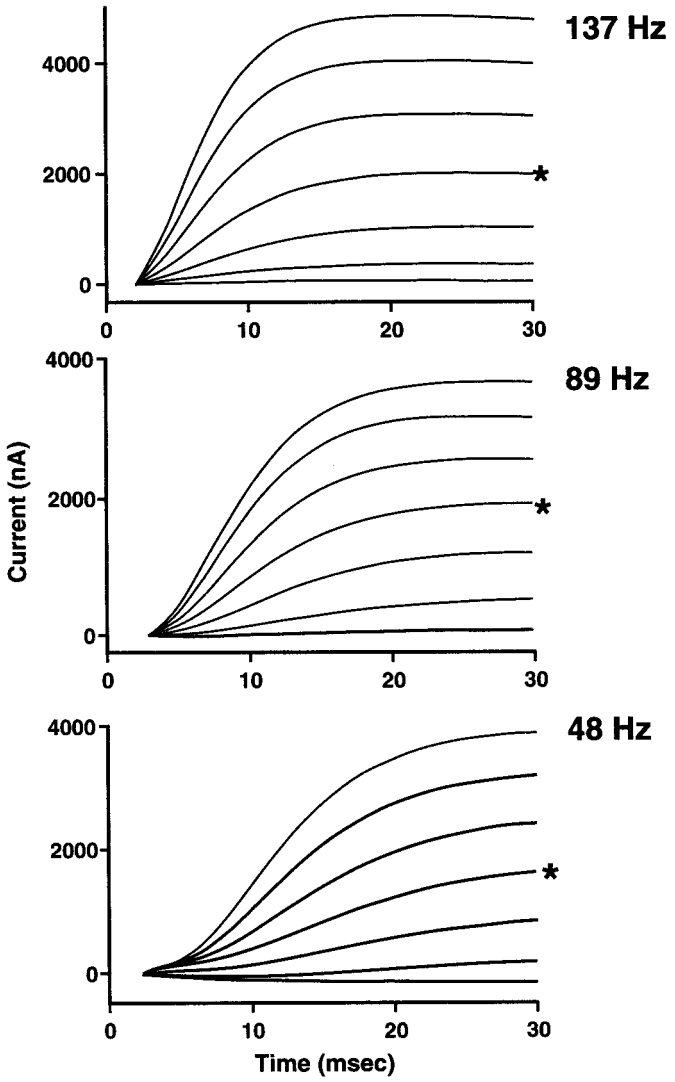

Figure 2. Delayed rectifying K^+ currents from electrocytes of fish over a range of EOD frequencies. Each fish's EOD frequency is indicated to the *right* of the currents. The activation time constants for the currents at 25 mV above activation threshold (*traces* indicated by *asterisks*) are 4.65 msec (137 Hz), 5.81 (89 Hz), and 8.40 (48 Hz). The illustrated currents are from voltage steps in 10 mV increments with the first trace at 5 mV below activation threshold.

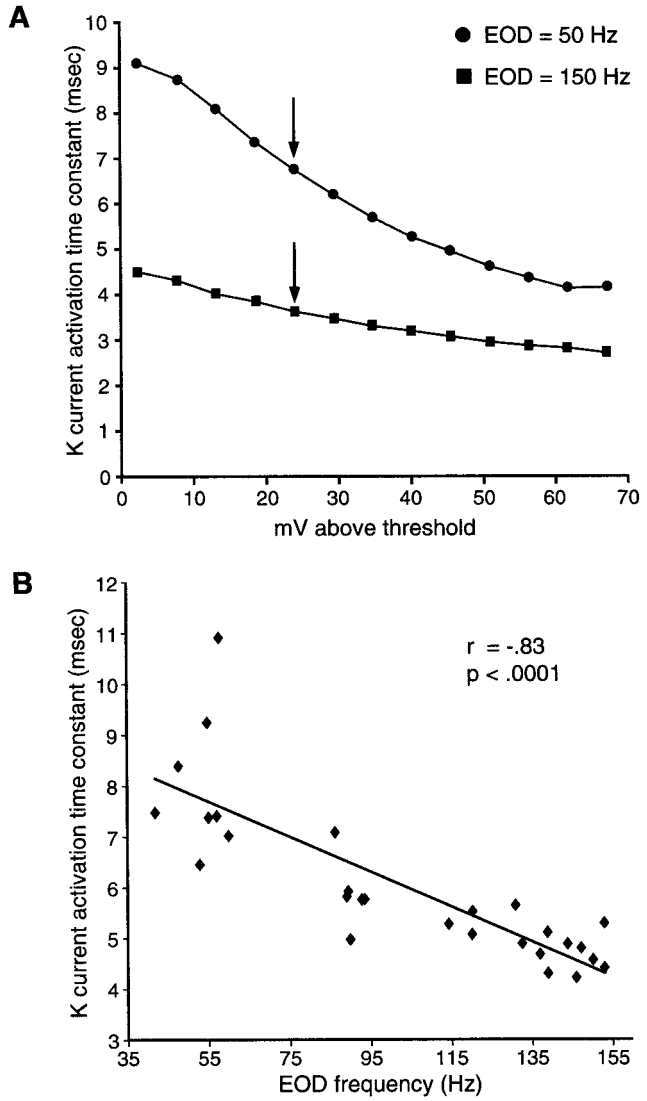

Figure 3. Activation time constant as a function of voltage and EOD frequency. *A*, Activation time constant varies with voltage within a cell, becoming faster at more depolarized voltages. *Arrows* denotes the value taken at 25 mV above activation threshold that was used for comparison across fish. *B*, Time constant of activation (at 25 mV above activation threshold) varies with EOD frequency.

the linear portion of the current–voltage curve. Rates of activation varied from 4.19 to 10.93 msec and were highly correlated with EOD frequency (-0.83; $p < 0.0001$; $n = 28$ cells, each from a different fish).

We examined tail currents to observe whether rates of deactivation also vary with EOD frequency and whether the rates of activation and deactivation of the current from a single cell were correlated. Electrocytes were clamped to 0 mV for 45 msec to activate the delayed rectifying K^+ current fully and then clamped for 45 msec to voltages ranging from -105 to -30 mV in 5 mV steps. Figure 4*A* illustrates tail currents from electrocytes of two fish with EOD frequencies of 42 and 154 Hz. Tail currents were well fit with a single exponential time constant whose value was voltage-dependent (Fig. 4*B*).

We used the deactivation time constant at -40 mV as our standard of comparison across cells because this value is positive enough to avoid contamination from any unblocked inward rectifier current. Deactivation time constants (at -40 mV) for the complete sample of cells ranged from 4.80 to 8.77 msec and were highly correlated with EOD frequency ($r = -0.90$; $p < 0.001$; $n = 15$) and the activation time constants of each current ($r = 0.84$; $p < 0.001$; $n = 15$) (Fig. 5).

No other attributes of the K^+ current were systematically correlated with EOD frequency or K^+ current activation time constant, including activation voltage (mean, -38.4 mV; SD, 5.96 mV; $n = 27$) or current magnitude (taken at 25 mV above threshold; mean, 1524 nA; SD, 492 nA).

Voltage-dependent properties of K^+ and Na^+ currents recorded in the same cell are correlated

A previous study found that the inactivation kinetics of the electrocyte Na^+ current vary with EOD frequency (Ferrari et al., 1995). To examine how well correlated the voltage-dependent kinetics of K^+ and Na^+ currents are on a cell-by-cell basis, both

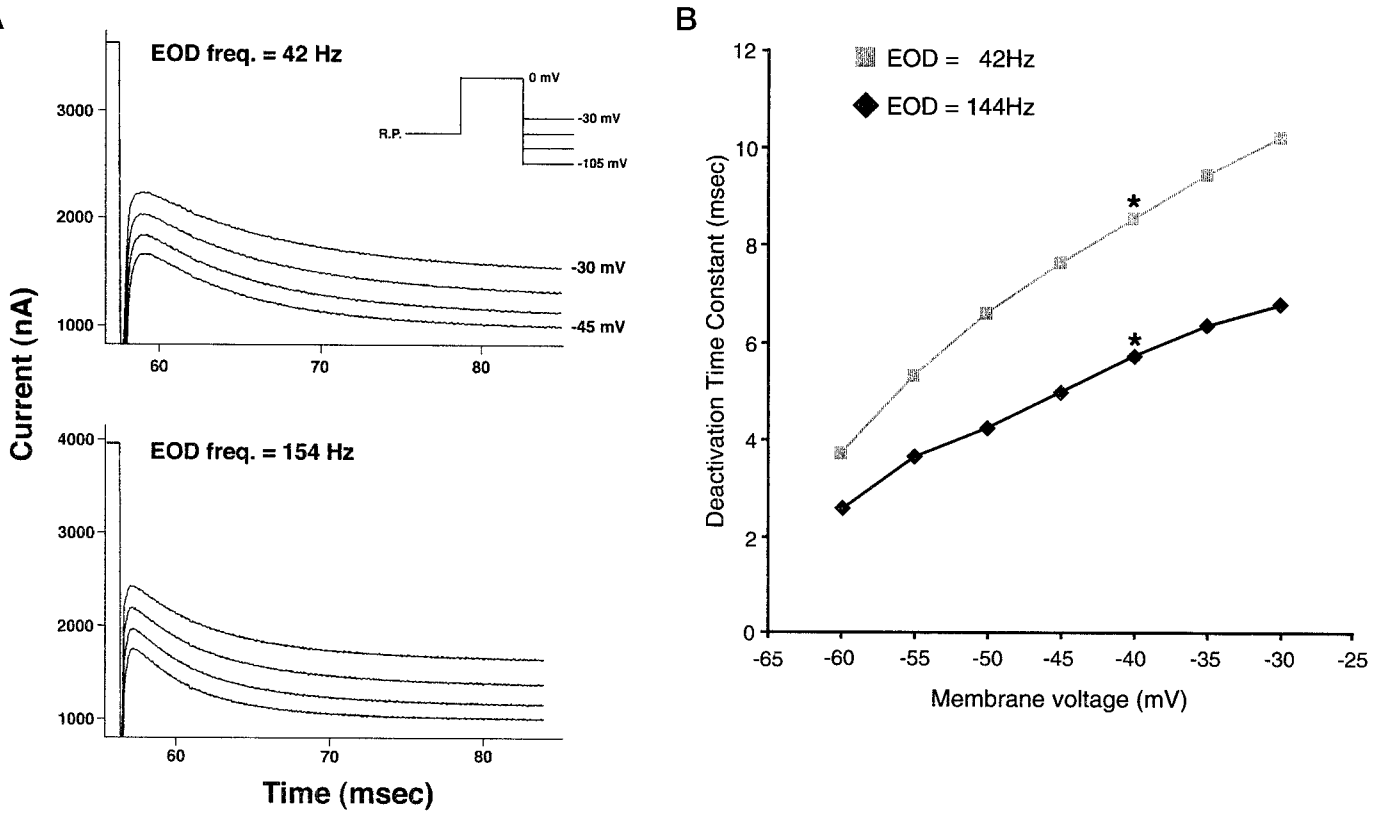

Figure 4. Potassium current deactivation kinetics vary with EOD frequency. *A*, Tail currents were recorded at voltages from −105 to −30 mV in 5 mV steps (see *inset* of voltage protocol). *Traces* from −30 to −45 mV are shown. Time constant of decay of the current at −40 mV is 8.47 msec for the fish with an EOD frequency of 42 Hz and 5.66 msec for the fish with an EOD frequency of 154 Hz. *B*, Deactivation time constant changes with membrane potential. Values at −40 mV are indicated by *asterisks*.

currents were measured in a subset of cells. This was accomplished by measuring the total current under voltage clamp, measuring K^+ currents after eliminating the Na^+ current with 1 μM TTX, and then retrieving the Na^+ current by digital subtraction of these two traces at each voltage step. The Na^+ currents measured in this way are identical to those recorded by pharmacological isolation after complete blockade the K^+ current with tetraethylammonium (Ferrari et al., 1995).

Figure 6 illustrates K^+ and Na^+ currents from electrocytes of two fish with EOD frequencies at 131 and 55 Hz. The activation of the K^+ current and the inactivation of the Na^+ current are more rapid in the electrocyte from the fish with the higher EOD frequency. Currents were sampled from electrocytes of fish across the range of EOD frequencies and their voltage-dependent kinetics were compared. As reported previously, inactivation of the Na^+ current was faster in individuals with higher EOD frequencies (Ferrari et al., 1995). The inactivation kinetics of the peak Na^+ current were each also correlated with the activation kinetics of the K^+ current ($r = 0.88$; $p < 0.0001$; $n = 17$) (Fig. 7).

We did not measure the rate of activation of the Na^+ current because its onset was sometimes obscured by the capacitative artifact of the voltage clamp. However, visual inspection suggests that the activation phase is slower in electrocytes from fish with low EOD frequencies.

Additionally, the magnitudes of the Na^+ (at peak current) and K^+ currents (at 25 mV above activation threshold) were correlated ($r = 0.68$; $p < 0.002$; $n = 17$) as in a previous study (Ferrari and Zakon, 1993). This likely reflects variation in electrocyte size (Mills et al., 1992) with larger electrocytes possessing more active membrane and, therefore, larger currents. This point is suggested by the correlation between the length of membrane labeled with a sodium channel antibody and the length of the electrocyte ($r = 0.52$; $p < 0.05$) (H. Zakon, S. R. Levinson, unpublished observations).

DISCUSSION

We have shown in the *Sternopygus* electrocyte that the voltage-dependent kinetic properties of a delayed rectifying K^+ current systematically covary and that these also covary with those of a voltage-dependent Na^+ current. This finding demonstrates the precision with which the voltage-dependent kinetics of a delayed rectifying K^+ channel may be regulated and illustrates the extent to which a high degree of coregulation of voltage-dependent properties of two independent ion channels can occur. These observations have implications for studies (Desai et al., 1999) and models (Stemmler and Koch, 1999) of how excitable cells may regulate their active conductances.

Variation in the voltage-dependent kinetics of delayed rectifying K^+ currents

The voltage-dependent kinetics of the K^+ current differ in different cell types, may change in a single cell type during development or under different hormonal conditions, or show regional variations in its expression (Barish, 1986; Harris et al., 1988; Campbell, 1992; Erulker et al., 1994; Goodman and Art 1996; Kros et al., 1998; Nerbonne, 1998). Our observation of systematic

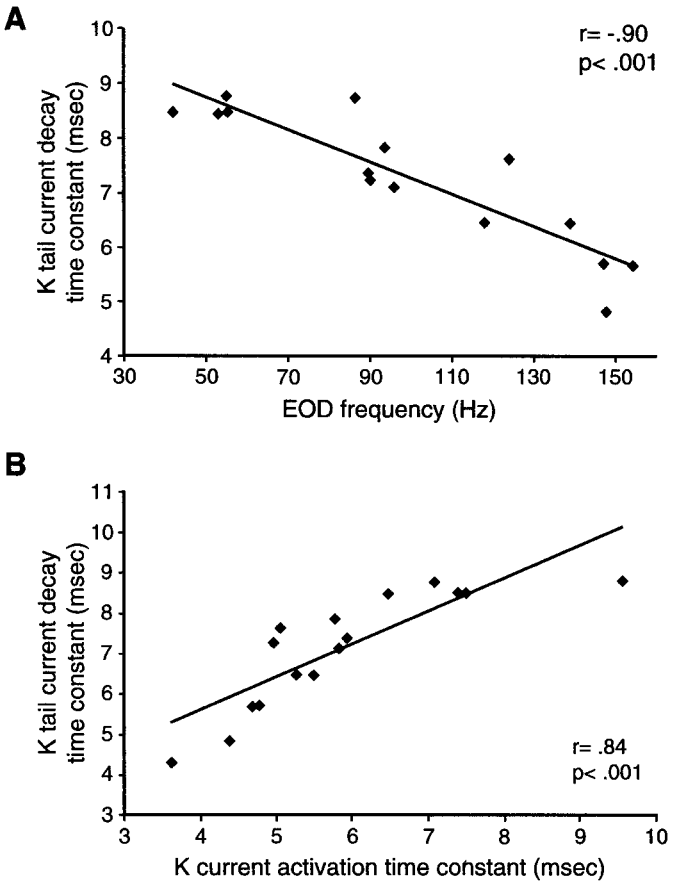

Figure 5. Potassium current deactivation is correlated with EOD frequency (*A*) and the activation time constant of the current from the same cell (*B*).

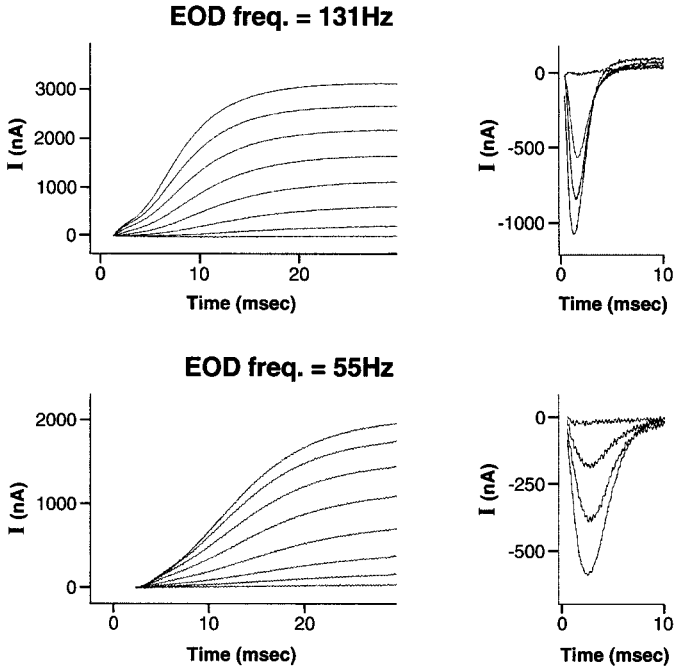

Figure 6. The voltage-dependent kinetics of the K^+ (*left*) and Na^+ (*right*) currents in an electrocyte from a fish with a high EOD frequency (131 Hz) are more rapid than those of an electrocyte from a fish with a low EOD frequency (55 Hz). *Traces* of K^+ currents as in Figure 2; Na^+ currents illustrate the peak Na^+ current for each cell and currents at two other values of membrane potential arbitrarily chosen for each cell. By visual inspection, the activation of the Na^+ current is also more rapid in fish with a high EOD frequency. However, we did not fit time constants to its activation phase because we could not be certain there was no influence of the capacitative transient caused by charging the membrane on the early part of the current record.

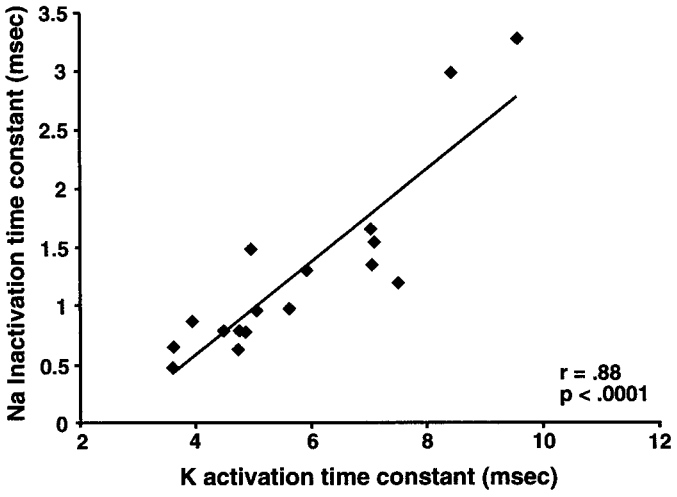

Figure 7. Activation time constant of the K^+ current versus inactivation time constant of the Na^+ current for 17 cells in which both currents were measured.

graded variations in the voltage-dependent kinetics of a delayed rectifying K^+ current in electrocytes demonstrates the precision with which this current may be controlled. The only observation of comparable precision in the control of the kinetics of this current is in hair cells in which the voltage-dependent kinetics of the current vary with position along the cochlea (Goodman and Art, 1996).

Activation and deactivation kinetics of electrocyte K^+ current covary, and this is observed in other cell types with spatial or developmental variation in K^+ current kinetics (Barish, 1986; Goodman and Art, 1996). On one hand, covariation in rates of activation and deactivation of the K^+ current is supported by kinetic models and mutational analysis of channel function (Monks et al., 1999). On the other hand, these properties need not always covary; K^+ channels with rapid activation kinetics may have either rapid (Kv3.1) or slow (Kv1.5) rates of deactivation (Grissmer et al., 1994), and rates of activation and deactivation may be uncoupled in a variety of experimental circumstances (Augustine and Bezanilla, 1990; Patton et al., 1997; Jerng et al., 1999). Thus, some active regulation may be required for these parameters to covary so precisely.

The covariation of these two kinetic parameters likely has important functional consequences; the activation phase of the K^+ current occurs during the falling phase of the EO pulse, whereas deactivation phase occurs during the period between EO pulses. Thus, a fish with a low-frequency EOD must have a K^+ current that develops slowly to prolong the AP and slow deactivation kinetics to ensure that the K^+ current contributes to electrocyte hyperpolarization between EOD pulses. A fish with a higher frequency EOD must have a K^+ current that activates faster to repolarize the AP faster and a deactivation phase that is more rapid so that the K^+ current is terminated before the next EOD pulse. The voltage-dependent kinetics of the K^+ current

appear to be graded to accommodate the individual variation in EOD frequency and pulse duration in this species.

Covariation in voltage-dependent kinetics of the Na^+ and K^+ currents

The most novel finding of this study is that the kinetics of a K^+ and a Na^+ current are tightly coregulated. There are numerous examples of covariation in the magnitudes of two currents (O'Dowd et al., 1988; Erulker et al., 1994; Desai et al., 1999), and a few cases reporting covariation in the magnitude of one current and the kinetics and/or magnitude of another, such as the calcium current and the calcium-activated K^+ current in cochlea hair cells (Wu and Fettiplace, 1996). However, this is the first report of covariation of the voltage-dependent kinetics of two ionic currents.

The cellular mechanisms by which this occurs are unknown. Variations in the kinetics of a single current could arise by molecular control, such as differential expression of different channel genes, or splice products or edited mRNAs of a single gene, each of which produces channels with different kinetic properties (Schaller et al., 1992; Gurantz et al., 1996; Patton et al., 1997; Liu and Kaczmarek, 1998; Martina et al., 1998; Nerbonne, 1998; Hanrahan et al., 1999). There is evidence for all of these mechanisms controlling voltage-dependent Na^+ and K^+ channel variation in the literature, although control by alternative splicing is rare in vertebrates, and RNA editing is only known for invertebrate Na^+ and K^+ channel genes.

One might imagine that coregulation of two currents occurs through complementary processes in the expression of both Na^+ and K^+ channel genes. This scenario is potentially complicated by the fact that Na^+ channels are a single protein, whereas K^+ channels are tetramers of smaller subunits (Wei et al., 1990). Additionally, some K^+ channel subunits (i.e., Kv2.3), although incapable of forming functional channels themselves, influence the activation and deactivation kinetics of the K^+ channels with which they coassemble (Castellano et al., 1997).

We know that at least two muscle Na^+ channel genes (*SKM1* and *SKM2*) (Lopreato et al., 1999) and a number of K^+ channels from different families (Kv1, Kv2, Kv3, and Kv4) (P. Few, unpublished observations) are expressed in the electrocytes of *Sternopygus*. However, we do not yet know their expression patterns in the electrocytes of fish over the range of EOD frequencies.

Regulation of kinetics could also occur by association of the channel with β subunits (Isom et al., 1992; Rettig et al., 1994; Ramanathan et al., 1999). However, the known β subunits that associate with Na^+ and K^+ channels are of molecularly distinct gene families. Regulation of two different currents via this mechanism, again, requires coordinated expression of different β subunit genes and comparable actions of each β subunit on its particular channel type.

Numerous studies show that phosphorylation by a variety of kinases affects activation or inactivation rates of either Na^+ or K^+ currents (Augustine and Bezanilla, 1990; Desarmenien and Spitzer, 1991; Numann et al., 1991; Li et al., 1993; Jonas and Kaczmarek, 1996; Roeper et al., 1997). Coordinate control by this mechanism would have the virtue that a single kinase could regulate both channel types. However, because most studies typically focus on the effects of phosphorylation on a single current, it is not known whether kinases can coordinately regulate the kinetics of two currents with the precision that we observe here. To date, only the actions of protein kinase A on the Na^+ current in the *Sternopygus* electric organ have been studied; activation of protein kinase A increases the magnitude of the electrocyte Na^+ current but does not affect its voltage-dependent kinetics (McAnelly and Zakon, 1996).

Other possibilities include regulation by variations in the lipid microenvironment of the surrounding membrane (Burnashev et al., 1991; Kang and Leaf, 1996) or the association with the cytoskeleton (Undrovinas et al., 1995), both of which are known to influence the voltage-dependent parameters of the cardiac Na^+ current. Each of these could conceivably also influence the gating of K^+ channels and thus account for the coordination between them.

General functional consequences

The coregulation of Na^+ and K^+ currents and their role in shaping AP duration is easily observed in the electrocyte in which AP wave shape must be precisely regulated over a fourfold range of duration (3–12 msec) in different individuals and in which the result of these biophysical control mechanisms is evident in recordings from behaving animals. Such coregulation of ionic channels would be much more difficult to observe in the CNS. However, even subtle manifestations of this phenomenon in CNS neurons could have profound implications for a variety of processes. Coregulation of Na^+ and K^+ channels underlying the action potential in synaptic terminals could strongly influence Ca^{2+} influx and neurotransmitter release (Jackson et al., 1991; Quattrocki et al., 1994; Whim and Kaczmarek, 1998). Similarly, the coregulation of these channels in dendrites could affect the gain and frequency response of the dendritic membrane to subthreshold synaptic events, as well as the characteristics of backpropagated action potentials. Thus, we propose coregulation of channel kinetics as a more general mechanism for regulating action potentials and membrane excitability.

REFERENCES

Armstrong C, Roberts W (1998) Electrical properties of frog saccular hair cells: distortion by enzymatic dissociation. J Neurosci 18:2962–2973.

Augustine C, Bezanilla F (1990) Phosphorylation modulates potassium conductance and gating current of perfused giant axons of squid. J Gen Physiol 95:245–271.

Barish ME (1986) Differentiation of voltage-gated potassium current and modulation of excitability in cultured amphibian spinal neurons. J Physiol (Lond) 375:229–250.

Burnashev N, Undrovinas A, Fleidervish I, Makielski J, Rosenstraukh L (1991) Modulation of cardiac sodium channel gating by Lysophosphatidylcholine. J Mol Cell Cardiol [Suppl] 23:23–30.

Campbell DT (1992) Large and small vertebrate sensory neurons express different Na and K channel subtypes. Proc Natl Acad Sci USA 89:9569–9573.

Castellano A, Chiara M, Mellström B, Molina A, Monje F, Naranjo J, López-Barneo J (1997) Identification and functional characterization of a K^+ channel α-subunit with regulatory properties specific to brain. J Neurosci 17:4652–4661.

Desai NS, Rutherford LC, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. Nat Neurosci 2:515–520.

Desarmenien M, Spitzer N (1991) Role of calcium and protein kinase C in development of the delayed rectifier potassium current in *Xenopus* spinal neurons. Neuron 7:797–805.

Dunlap K, McAnelly M, Zakon H (1997) Estrogen modifies an electrocommunication signal by altering the electrocyte sodium current in an electric fish, *Sternopygus*. J Neurosci 17:2869–2875.

Erulker S, Rendt J, Nori R, Ger B (1994) The influence of 17β oestradiol on K^+ currents in smooth muscle cells isolated from immature rat uterus. Proc R Soc Lond B Biol Sci 256:59–64.

Ferrari MB, Zakon HH (1993) Conductances contributing to the action potential of *Sternopygus* electrocytes. J Comp Physiol [A] 173:281–292.

Ferrari MB, McAnelly ML, Zakon HH (1995) Individual variation in and androgen-modulation of the sodium current in electric organ. J Neurosci 15:4023–4032.

Franchina CR, Stoddard PK (1998) Plasticity of the electric organ discharge waveform of the electric fish *Brachyhypopomus pinnicaudatus*. I. Quantification of day–night changes. J Comp Physiol [A] 183:759–768.

Goodman MB, Art JJ (1996) Variations in the ensemble of potassium currents underlying resonance in turtle hair cells. J Physiol (Lond) 497.2:395–412.

Grissmer S, Nguyen A, Aiyar J, Hanson D, Mather R, Gutman G, Karmilowicz M, Auperin D, Chandy K (1994) Pharmacological characterization of five cloned voltage-gated K^+ channels, types Kv1.1, 1.2, 1.3, 1.5 and 3.1, stably expressed in mammalian cell lines. Mol Pharmacol 45:1227–1234.

Gurantz D, Ribera A, Spitzer N (1996) Temporal regulation of Shaker- and Shab-like potassium channel gene expression in single embryonic spinal neurons during K^+ current development. J Neurosci 16:3287–3295.

Hanrahan C, Palladino M, Bonneau L, Reenan R (1999) RNA editing of a *Drosophila* sodium channel gene. Ann NY Acad Sci 868:51–66.

Harris G, Henderson L, Spitzer N (1988) Changes in densities and kinetics of delayed rectifier potassium channels during neuronal differentiation. Neuron 1:739–750.

Hestrin S, Korenbrot JI (1987) Voltage-activated potassium channels in the plasma membrane of rod outer segments: a possible effect of enzymatic cell dissociation. J Neurosci 7:3072–3080.

Hodgkin A, Huxley A (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond) 117:500–544.

Hopkins CD (1972) Sex differences in electric signaling in an electric fish. Science 176:1035–1037.

Hopkins CD (1974) Electric communication in the reproductive behavior of *Sternopygus macrurus* (Gymnotoidei). Z Tierpsychol 35:518–535.

Hopkins CD (1999) Design features for electric communication. J Exp Biol 202:1217–1228.

Isom LL, De Jonhg KS, Patton DE, Reber BFX, Offord J, Charbonneau H, Walsh K, Goldin AL, Catterall WA (1992) Primary structure and functional expression of the β1 subunit of the rat brain sodium channel. Science 256:839–842.

Jackson M, Konnerth A, Augustine G (1991) Action potential broadening and frequency-dependent facilitation of calcium signals in pituitary nerve terminals. Proc Natl Acad Sci USA 88:380–384.

Jerng H, Shahidullah M, Covarrubias M (1999) Inactivation gating of Kv4 potassium channels: molecular interactions involving the inner vestibule of the pore. J Gen Physiol 113:641–659.

Jonas E, Kaczmarek L (1996) Regulation of potassium channels by protein kinases. Curr Opin Neurobiol 6:318–323.

Kang J, Leaf A (1996) Evidence that free polyunsaturated fatty acids modify Na^+ channels by directly binding to the channel proteins. Proc Natl Acad Sci USA 93:3542–3546.

Klemic K, Durand DM, Jones S (1998) Activation kinetics of the delayed rectifier potassium current of bullfrog sympathetic neurons. J Neurophysiol 79:2345–2357.

Kros C, Ruppersberg J, Rusch A (1998) Expression of a potassium current in inner hair cells during development of hearing in mice. Nature 394:281–284.

Li M, West JW, Numann R, Murphy BJ, Scheuer T, Catterall WA (1993) Convergent regulation of sodium channels by protein kinase C and cAMP-dependent protein kinase. Science 261:1439–1442.

Liu S, Kaczmarek L (1998) The expression of two splice variants of the Kv3.1 potassium channel gene is regulated by different signaling pathways. J Neurosci 18:2881–2890.

Lopreato G, Lu Y, Atkinson N, Zakon H (1999) Two Na^+ channel genes are expressed in the electric organ of *Sternopygus*. Soc Neurosci Abstr 25:1733.

Martina M, Schultz J, Ehmke H, Monyer H, Jonas P (1998) Functional and molecular differences between voltage-gated K^+ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. J Neurosci 18:8111–8125.

McAnelly ML, Zakon HH (1996) Protein kinase A activation increases sodium current magnitude in the electric organ of *Sternopygus*. J Neurosci 16:4383–4388.

Meyer JH (1983) Steroid influences upon the discharge frequencies of a weakly electric fish. J Comp Physiol [A] 153:29–37.

Mills A, Zakon HH (1991) Chronic androgen treatment increases action potential duration in the electric organ of *Sternopygus*. J Neurosci 11:2349–2361.

Mills A, Zakon HH, Marchaterre MA, Bass AH (1992) Electric organ morphology of *Sternopygus macrurus*, a wave-type, weakly electric fish with a sexually dimorphic EOD. J Neurobiol 23:920–932.

Mills AC, Zakon HH (1987) Coordination of EOD frequency and pulse duration in a weakly electric wave fish: the influence of androgens. J Comp Physiol [A] 161:417–430.

Monks S, Needleman D, Miller C (1999) Helical structure and packing orientation of the S2 segment in the shaker K^+ channel. J Gen Physiol 113:415–423.

Nerbonne JM (1998) Regulation of voltage-gated K^+ channel expression in the developing mammalian myocardium. J Neurobiology 37:37–59.

Numann R, Catterall WA, Scheuer T (1991) Functional modulation of brain sodium channels by protein kinase C phosphorylation. Science 254:115–118.

O'Dowd D, Ribera A, Spitzer N (1988) Development of voltage-dependent calcium, sodium, and potassium currents in *Xenopus* spinal neurons. J Neurosci 8:792–805.

Patton D, Silva T, Bezanilla F (1997) RNA editing generates a diverse array of transcripts encoding squid Kv2 K^+ channels with altered functional properties. Neuron 19:711–722.

Quattrocki E, Marshall J, Kaczmarek L (1994) A Shab potassium channel contributes to action potential broadening in peptidergic neurons. Neuron 12:73–86.

Ramanathan K, Michael T, Jiang G, Hiel H, Fuchs P (1999) A molecular mechanism for electrical tuning of cochlear hair cells. Science 283:215–217.

Rettig J, Heinemann S, Wunder F, Lorra C, Parcej D, Jo D, Pongs O (1994) Inactivation properties of voltage-gated K^+ channels altered by presence of β-subunit. Nature 369:289–294.

Roeper J, Lorra C, Pongs O (1997) Frequency-dependent inactivation of mammalian A-type K^+ channel Kv1.4 regulated by Ca^{2+}/calmodulin-dependent protein kinase. J Neurosci 17:3379–3391.

Schaller KL, Krzemien DM, McKenna NM, Caldwell JH (1992) Alternatively spliced sodium channel transcripts in brain and muscle. J Neurosci 12:1370–1381.

Shenkel S, Sigworth FJ (1991) Patch recordings from the electrocytes of *Electrophorus electricus*. J Gen Physiol 97:1013–1041.

Stemmler M, Koch C (1999) How voltage-dependent conductances can adapt to maximize the information encoded by neuronal firing rate. Nat Neurosci 2:521–527.

Stoddard P (1999) Predation enhances complexity in the evolution of electric fish signals. Nature 400:254–256.

Turrigiano G, LeMasson G, Marder E (1995) Selective regulation of current densities underlies spontaneous changes in the activity of cultured neurons. J Neurosci 15:3640–3652.

Undrovinas A, Shandler G, Makielski J (1995) Cytoskeleton modulates gating of voltage-dependent sodium channel in heart. Am J Physiol 269:H203—H214.

Wei A, Covarrubias M, Butler 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.

Whim M, Kaczmarek L (1998) Heterologous expression of the Kv3.1 potassium channel eliminates spike broadening and the induction of a depolarizing afterpotential in the peptidergic bag cell neurons. J Neurosci 18:9171–9180.

Wu Y, Fettiplace R (1996) A developmental model for generating frequency maps in the reptilian and avian cochleas. Biophys J 70:2557–2570.

Xie J, McCobb D (1998) Control of alternative splicing of potassium channels by stress hormones. Science 280:443–446.

Zakon HH, McAnelly L, Smith GT, Dunlap K, Lopreato G, Oestreich J, Few WP (1999) Plasticity of the electric organ discharge: implications for the regulation of ionic currents. J Exp Biol 202:1409–1416.