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PLASTICITY OF THE ELECTRIC ORGAN DISCHARGE: IMPLICATIONS FOR THE REGULATION OF IONIC CURRENTS

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Summary

Weakly electric fish emit electric organ discharges (EODs) to locate objects around themselves and for communication. The EOD is generated by a simple hierarchically organized, neurophysiologically accessible circuit, the electromotor system. A number of forms of plasticity of the EOD waveform are initiated by social or environmental factors and mediated by hormones or neurotransmitters. Because the behavior itself is in the form of electric discharges, behavioral observations easily lead to testable hypotheses about the biophysical bases of these plasticities. This allows us to study ionic channels in their native cellular environments, where the regulation of various parameters of these currents have obvious functional consequences. In this review, we discuss three types of plasticity: a rapidly occurring, long-lasting, *N*-methyl-D-aspartate (NMDA)-receptor-dependent increase in baseline firing frequency of neurons in the pacemaker nucleus that underlies a readjustment of the baseline EOD frequency after long bouts of the jamming avoidance response; a rapidly occurring diurnal change in amplitude and duration of the EOD pulse that depends in part on modulation of the magnitude of the electrocyte Na^+ current by a protein kinase; and a slowly occurring, hormonally modulated tandem change in pacemaker firing frequency and in the duration of the EOD pulse in which changes in EOD pulse duration are mediated by coordinated shifts in the activation and inactivation kinetics of the electrocyte Na^+ and K^+ currents.

Key words: plasticity, electric organ, electric fish, pacemaker neuron, NMDA receptor, Na^+ channel, K^+ channel, androgen, estrogen, phosphorylation.

Introduction

Weakly electric fish are useful neuroethological subjects because their electrical signals are stereotyped and quantifiable, and the circuitry that generates and receives these signals is simple and accessible. Electric fish use their electric organ discharge (EOD) to convey information on the sender's species, gender and individual identity; therefore, the EOD waveform is species-specific, sexually dimorphic and individually distinct (Hopkins, 1972, 1974; McGregor and Westby, 1992). A fish's EOD waveform may also change in different behavioral contexts. To generate this diversity of EOD waveforms, the cells of the electromotor pathway must rely on the basic building blocks of the various ion channels used by all excitable cells. We believe that studying the mechanisms by which the ionic currents of the cells in the electromotor pathway generate this large diversity of signals and how these currents are altered during various forms of plasticity will illuminate the mechanisms of ion channel regulation in general. In this article, we illustrate various forms of plasticity of the EOD and the possible cellular mechanisms underlying them.

Plastic changes in the waveform of the EOD can occur rapidly (with a time course of hundreds of milliseconds to minutes) or slowly (with a time course of days to weeks). These changes are initiated by social interactions or stimuli that mimic them or by changes in environmental conditions, they are mediated by factors such as synaptic inputs or hormones, and they probably result from second-messenger-induced or transcriptionally controlled changes in ionic currents of cells in the electromotor pathway.

The EOD-generating circuitry

We will focus on the organization of the EOD-generating circuitry in the South American gymnotiforms, although there are striking parallels in the organization of this circuit in the independently evolved African mormyriforms. The EOD is driven by a small number of intrinsic pacemaking neurons located in a midline nucleus, called the pacemaker nucleus (PMN), in the ventral medulla (Fig. 1). These neurons then synapse on and drive a second group of neurons, called relay cells. The pacemaker of the Apteronotidae possesses, in addition, small, presumably glycinergic, interneurons of

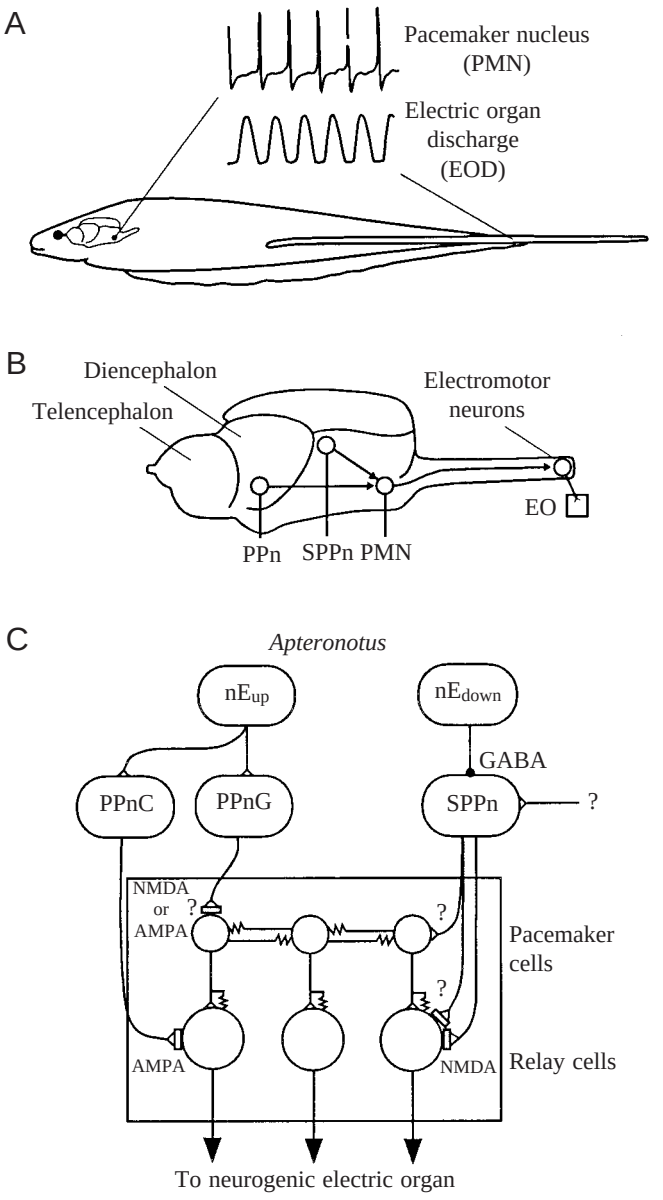

Fig. 1. Schematic illustration of the electromotor circuit. (A) Extracellular field recordings from the pacemaker nucleus (PMN) and their relationship to the electric organ discharge (EOD) in *Sternopygus*. Each action potential in the PMN is followed by an EOD pulse. (B) The PMN is a midline medullary nucleus whose firing rate determines basal EOD frequency. The PMN drives spinal electromotor neurons (EMNs), which in turn drive the myogenically derived electrocytes in all families but the Apteronotidae, whose electric organ is composed of axons of the EMNs. Modulations of the basal EOD frequency are accomplished by glutamatergic inputs from the prepacemaker nucleus (PPn) and the sublemniscal prepacemaker nucleus (SPPn). (C) Pacemaker neurons, which are intrinsic to the nucleus, are electrotonically coupled to each other and drive relay neurons. The axons of the relay neurons run down the spinal cord and innervate the EMNs. The inputs from the PPn and SPPn activate pacemaker or relay cells *via* alpha-amino-3-hydroxy-5-methyl-4 isoxazole proprionic acid (AMPA)- and *N*-methyl-D-aspartate (NMDA)-type glutamate receptors. There are species differences in these inputs and in the extent of electrotonic *versus* chemical coupling between pacemaker and relay cells. This figure shows the synaptology of the PMN of the genus *Apteronotus*. nE_{up}, nucleus electrosensorius, increasing EOD frequency; nE_{down}, nucleus electrosensorius, decreasing EOD frequency; GABA, γ-aminobutyric acid; PPnC, part of the PPn controlling 'chirps'; PPnG, part of the PPn controlling gradual increases in EOD frequency. A and B are taken from Zakon et al. (1991); C is taken from Juranek and Metzner (1997).

unknown function that make chemical synapses on both pacemaker and relay cells (Turner and Moroz, 1995; G. T. Smith, Y. Lu and H. Zakon, unpublished observations).

Pacemaker neurons are endogenously active oscillatory neurons that are electrotonically coupled to each other and to the relay cells (Elekes and Szabo, 1981; Dye, 1988; Moortgat et al., 1998a). The relay neurons send their axons out of the nucleus and down the spinal cord to innervate electromotor neurons (EMNs) (Bennett et al., 1967). In most species, the EMNs then innervate the electrocytes, the cells of the electric organ (in the Apteronotidae, the axons of the EMNs themselves constitute the electric organ).

The resting frequency of the EOD is determined by the firing frequency of the pacemaker neurons, while the shape of each EOD pulse is dictated by the membrane properties of the electric organ. Those species with a highly regular PMN firing frequency and whose pulses are usually monophasic and approximately of the same duration as the interpulse intervals generate a periodic sine-wave-like EOD; these are called 'wave fish'. Those species in which the EOD pulse is usually multiphasic and is generated more irregularly with much longer interpulse intervals are referred to as 'pulse' fish.

The regular rhythm of the EOD can be modified by inputs to the pacemaker. The PMN has only two known inputs, the prepacemaker nucleus (PPn) and the sublemniscal prepacemaker nucleus (SPPn), which control the rapid modulations of the EOD that occur during social signaling and the slower modulations that occur during the jamming avoidance response (Bennett et al., 1967; Kawasaki and Heiligenberg, 1988, 1989, 1990; Juranek and Metzner, 1997, 1998).

Long-term frequency elevation of pacemaker neurons and the EOD

In the wave fish *Apteronotus leptorhynchus*, the electric organ discharges very stably at high frequencies because of the extreme regularity of firing of the pacemaker and relay neurons and the electrotonic coupling between them (Bullock, 1970; Meyer, 1984; Moortgat et al., 1998a,b). Dye (1988) developed a pacemaker slice preparation that included the presynaptic axons from the prepacemaker nuclei. He showed that a brief tetanic stimulation of these axons resulted in the expected rapid increase in PMN firing frequency during synaptic activation and, surprisingly, a slight elevation of a few hertz in the baseline firing frequency for 10 s following this stimulation. He called this a long-term frequency elevation (LTFE).

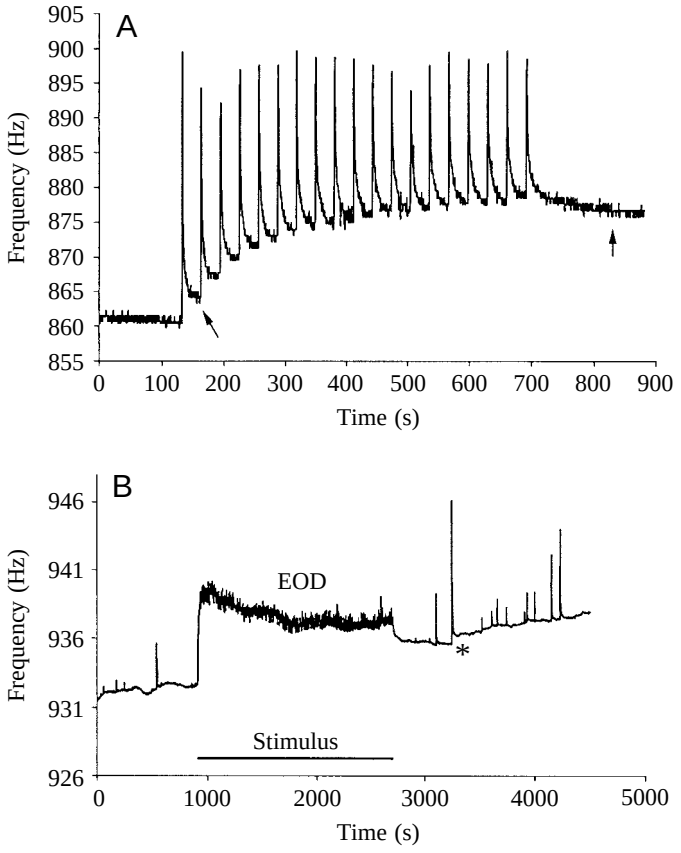

Fig. 2. Long-term frequency elevation and plasticity of the jamming avoidance response in the brown ghost fish (*Apteronotus leptorhynchus*). (A) Plot of firing frequency of a pacemaker neuron with a baseline discharge rate of 862 Hz. A single high-frequency (350 ms train at 1000 Hz of 100 μs 10 V pulses) burst of stimuli to the presynaptic input to the pacemaker nucleus results in a slight increase in baseline pacemaker firing frequency (first arrow). A series of such tetanic stimuli causes baseline pacemaker firing frequency to increase and to remain at that new value for minutes (second arrow). (B) A likely behavioral correlate of this effect is a long-term elevation in the electric organ discharge (EOD) frequency after a 30 min exposure to a jamming stimulus 5 Hz below the fish's EOD frequency. The activity superimposed on the EOD during the stimulus is made up of chirps given by the male in response to the stimulus. The larger spikes that occur mainly after the stimulus presentation are yodels (indicated by the asterisk), which are longer-duration communication signals (J. Oestreich and H. H. Zakon, unpublished data).

Two recent studies have extended these observations. First, *in vivo* stimulation of the SPPn causes a rapid increase in EOD frequency followed by an evident LTFE of a few hertz in EOD frequency. This shows that the LTFE is not an artifact of the slice preparation and that it is actually sufficient to cause changes in EOD frequency (Heiligenberg et al., 1996). Second, it has been shown in the slice preparation that repeated bouts of tetanic stimulation elicit an LTFE after each bout until the firing frequency of the PMN neurons is raised by as much as 25–30 Hz, at which point it cannot be increased with further stimulation (Oestreich and Zakon, 1998) (Fig. 2A).

We suspected that a possible behavioral correlate of LTFE is a gradual readjustment of the baseline EOD frequency induced by long periods of a positive-going jamming avoidance response (JAR). The JAR in *Apteronotus* occurs when a fish is presented with a sine-wave stimulus a few hertz below its own EOD frequency that mimics the EOD of a neighboring fish. The beating of the fish's own EOD with the EOD mimic interferes with its electrolocation (Bastian, 1987). The JAR, which is a transient increase in the fish's EOD frequency of 5–10 Hz in the presence of the jamming stimulus, is believed to be a reflex for minimizing this beating. According to most studies of the JAR, which present the jamming stimulus for seconds to minutes, once the stimulus is over, the EOD returns to its former value. The JAR is believed to be mediated by input from the SPPn that depolarizes the relay, and perhaps pacemaker, neurons *via N*-methyl-D-aspartate (NMDA) receptors (Dye et al., 1989; Heiligenberg et al., 1996; Juranek and Metzner, 1997, 1998; Bottai et al., 1997).

We tested our hypothesis about the function of the LTFE and observed that, following the presentation of a sine-wave a few hertz below a fish's EOD frequency which elicits a JAR for 30 min, the fish's EOD frequency was increased by 5 Hz for at least tens of minutes and perhaps for hours (Oestreich and Zakon, 1998) (Fig. 2B). EOD frequency was also increased after fish spontaneously 'yodeled.' A yodel, which is a signal of unclear communication function, is an increase in EOD frequency of tens of hertz and lasting for hundreds of milliseconds. It is believed to occur by simultaneous activity of AMPA and NMDA receptors (defined in Fig. 1) from inputs from both the PPn and SPPn (Dye, 1988). The stimulation of the afferents in the slice preparation, which results in the co-activation of AMPA and NMDA receptors, resembles most closely a neural analog of yodeling.

LTFE does not involve plasticity in synaptic transmission, such as in long-term potentiation, but depends on a long-term change in the ionic currents of the postsynaptic cells since it is an elevation of the endogenous firing frequency of the PMN neurons. The cellular basis of this phenomenon is not known. LTFE in the slice preparation is blocked by an NMDA, but not an AMPA, receptor blocker (Dye et al., 1989). Thus, presumably through the entrance of Ca^{2+} *via* the NMDA receptor, a second-messenger pathway is engaged, leading to a change in an ion conductance of pacemaker and/or relay cells. These cells possess a number of likely Ca^{2+}-sensitive second-messenger candidates, the foremost being nitric oxide synthase and ryanodine and inositol trisphosphate receptors (Zupanc et al., 1992; Turner and Moroz, 1995; Berman et al., 1995) and at least three candidate ionic currents, a voltage-dependent K^+ current, a T- or R-type Ca^{2+} current and a Na^+ current with a persistent component (Dye, 1991; G. T. Smith and H. H. Zakon, unpublished). The fact that an increase in EOD frequency is observable after a 100 ms stimulation requires that any putative cellular mechanism work with this rapidity.

We propose that the adaptive value of LTFE is that, during the prolonged activation of the NMDA receptor which occurs during long-lasting bouts of jamming, the control of the EOD

frequency is gradually shifted away from an NMDA-receptor-dependent synaptic mechanism that would probably cause large continuous influxes of Ca^{2+} to one that is determined by a postsynaptic mechanism.

Rapid changes in EOD pulse properties

Hagedorn and Heiligenberg (1985), studying the behavior of freely swimming electric fish (*Eigenmannia*) in a group tank, noted that in the evening the EOD of the dominant male became so loud that it drowned out the EODs of all other fish. This observation was anecdotal in that they were unable to make accurate measurements of EOD amplitude under these circumstances. However, there are now well-documented examples of changes in EOD amplitude and waveshape in *Hypopomus*, a fish with a pulse-type discharge (Hagedorn and Zelick, 1989; Hagedorn, 1995). Male *Hypopomus* have a longer-duration EOD pulse than females, and it is of greater amplitude. When two male *Hypopomus* with comparable EODs fight, the EOD of the loser decreases in pulse duration and amplitude within a few hours (Hagedorn and Zelick, 1989). A comprehensive study of similar changes was made recently in the hypopomid *Brachyhypopomus pinnicaudatus* (Franchina, 1998). In that study, the EODs of fish were recorded non-invasively as they rested in or swam through a narrow central compartment of a larger tank. The diphasic EOD waveform of this species is sexually dimorphic, with males having a longer second head-negative phase (Franchina, 1998; Hopkins et al., 1990). *B. pinnicaudatus* males, but not females, show increases in EOD amplitude and in the duration of the second phase during the night (Fig. 3A,B). Interestingly, these changes may occur prior to the onset of darkness and they continue in a circadian fashion when fish are kept in constant darkness. They change over tens of minutes, but may show significant differences from baseline measurements within 6 min (P. Stoddard, personal communication).

The significance of these changes is believed to be that, during the day, when fish are inactive, the male can emit a low-amplitude symmetrical waveform that is less conspicuous to electroreceptive predators. At night, when most social communication occurs, males increase the amplitude of their EODs and the duration of the second phase so that the EOD is 'loud' and more sexually dimorphic. In some instances, EOD duration and amplitude change independently (P. Stoddard, personal communication), suggesting that they are independently controlled at the cellular level.

Such an increase in EOD amplitude would result if each electrocyte generated more current. In fact, the magnitude of the electrocyte Na^+ current of the wave gymnotiform *Sternopygus* increases within minutes after activation of protein kinase A by 8-bromo cyclic AMP, a membrane-permeable cyclic AMP analog (McAnelly and Zakon, 1996) (Fig. 3C). Further support for this idea comes from the observation that injections of 8-bromo cyclic AMP, but not saline, into the electric organ of lightly restrained fish result in an increase in the magnitude of their EODs (A. Gulledge and

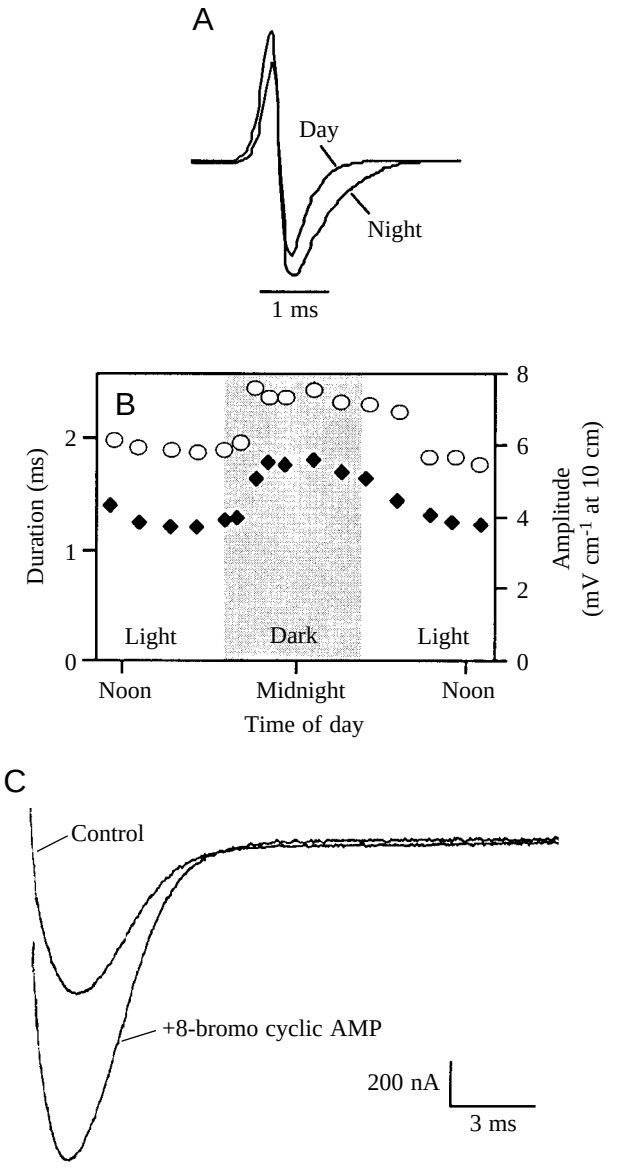

Fig. 3. Diurnal changes in the electric organ discharge (EOD) of a pulse fish (*Brachyhypopomus pinnicaudatus*) and its possible biophysical basis. (A) Traces of EOD waveform during the day and night illustrating the nocturnal increases in amplitude of the positive and negative phases of the EOD and in the duration of the negative phase of the EOD. (B) Measurements of peak-to-peak amplitude (open symbols) and the duration (filled symbols) of the second phase of the EOD pulse of a male over 24 h. (C) Voltage-clamp recordings of the peak Na^+ current from an electrocyte of a wave fish (*Sternopygus*) before and after treatment with 8-bromo cyclic AMP. Note that the amplitude of the Na^+ current is enhanced. Such an increase in the Na^+ current is likely to increase the current output of each electrocyte, thereby increasing the amplitude of the EOD. A and B are taken from Franchina and Stoddard (1998); C is taken from McAnelly and Zakon (1996).

A. Ridgel-Wonsettler, personal communication). The kinetics of the Na^+ current remain unchanged after treatment with protein kinase A (PKA), suggesting that PKA controls EOD magnitude but not its duration. It is not yet known whether

other ionic currents in the electrocyte are influenced by PKA activation and, if so, how their amplitudes change in conjunction with the Na^+ current.

Comparable changes in the amplitude of the second phase of the diphasic EOD occur in the mormyrid *Campylomormyrus* sp. with changes in water conductance (Kramer and Kuhn, 1993). When *Campylomormyrus* are moved from low-resistance to high-resistance water, as might occur more gradually during the transition from the dry to the rainy seasons in the wild, the first phase of the EOD, which is produced by neural activation of the posterior face of the electrocytes, increases proportionately. However, the second phase of the EOD, which is generated by current from the neurally innervated face of the electrocytes flowing across and depolarizing the anterior face, decreases in amplitude. Over the course of a few days, the amplitude of the second phase of the EOD is partially restored. When fish are moved back to low-conductance water, the amplitude of the second phase 'overshoots' and eventually returns to normal. While it seems likely that changes in the amplitude of ionic currents might underlie this phenomenon, nothing is known about the currents of these electrocytes.

In none of these cases of EOD pulse plasticity are the upstream physiological mechanisms known. It is likely that these changes are the result of rapid changes in hormone levels. For example, circadian changes induced by melatonin, changes in social status induced by corticosteroids or sex hormones, and changes in water conductivity induced by prolactin or other osmoregulatory hormones. Whatever the mediating factors in these examples, it seems that the ultimate targets of these putative hormonal signals are the ion channels of the electroyctes.

Long-term changes in EOD: effects of steroid hormones

Because EODs are used as communication signals to convey information on species, gender and individual identity, there is great diversity in the waveforms. This variation emphasizes the specialization of the cells in the electromotor circuitry for regulation of excitability. The most intensely studied aspects of this variation are the sexual dimorphism and hormonal regulation of the EOD waveform. The sexual dimorphism in EOD waveforms occurs in wave- and pulse-type fish in both orders and has been shown to be causally linked to naturally occurring or experimentally manipulated levels of sex steroid hormones (Hopkins, 1972, 1974; Meyer and Zakon, 1982; Bass and Hopkins, 1983; Hagedorn and Carr, 1985; Bass, 1986; Bass et al., 1986; Bass and Volman, 1987; Mills and Zakon, 1987, 1991; Landsman and Moller, 1988; Freedman et al., 1989; Zakon et al., 1991a,b; Zakon, 1996; Schaefer and Zakon, 1996; Dunlap et al., 1997; Dunlap and Zakon, 1998; Carlson and Hopkins, 1998).

In pulse fish of both groups, androgens increase electrocyte size and total membrane surface area. This is believed to influence their passive membrane properties (Bass and Volman, 1987; Hagedorn and Carr, 1985). Active ionic conductances are also likely to be affected by hormones in the electrocytes of pulse fish but, with the exception of one recent study on the genus *Gymnotus*, a group in which no sexual dimorphism has been noted (F. Sierra and O. Macadar, personal communication), ionic currents have not yet been isolated and studied in the electrocytes of any pulse fish.

The most comprehensive study of sex steroid action at the cellular level in the electromotor system is on the wave fish *Sternopygus macrurus*. In this species, mature males discharge from 50 to 90 Hz, mature females from 110 to 200 Hz and juveniles at intermediate and overlapping frequencies. Since the pacemaker firing frequency determines the EOD frequency, the pacemaker neurons fire at different frequencies in each individual. However, the waveform of the *Sternopygus* EOD also depends on the duration of the electrocyte action potential: a fish with a low EOD frequency has a long-duration action potential; a fish with a high EOD frequency has a shorter-duration action potential. Thus, EOD frequency and action potential duration vary from individual to individual as well as between the sexes, and these parameters are tightly correlated over the whole range of EOD frequencies of this species (Mills and Zakon, 1987) (Fig. 4A).

When fish are treated with the androgen dihydrotestosterone (DHT), EOD frequency is lowered, EOD pulse duration is lengthened (Fig. 4C) and electric organ action potentials become broadened. Conversely, when fish are treated with estrogen, EOD frequency is raised and EOD pulse duration is shortened ((Dunlap et al., 1997; Meyer, 1983; Meyer and Zakon, 1982; Mills and Zakon, 1987, 1991; Zakon et al., 1991a,b). In contrast to pulse fish, these changes in EOD waveform are not accompanied by any changes in electrocyte morphology (Mills et al., 1992).

Do sex steroids alter the electrical properties of the electric organ directly or indirectly *via* the changed activity of the pacemaker neurons? The former view is supported by two observations. First, small implants of androgens in the electric organ, which raise androgen concentration locally without exposing the pacemaker nucleus to elevated levels of androgens, broaden the electric organ pulse. In this experiment, the pulse broadens despite the electric organ being driven at a constant frequency (Few and Zakon, 1998). Second, nuclear androgen and estrogen receptors have been identified immunocytochemically in the electrocytes (Dunlap et al., 1997; Gustavson et al., 1994). Although it is clear that androgens influence electrocyte action potential directly, reciprocal experiments must be performed to test whether altering the pacemaker firing frequency results in changes in electric organ pulse duration.

Using the voltage-clamp technique, three voltage-dependent ionic currents have been identified in the *Sternopygus* electrocyte: a Na^+ current, an inwardly rectifying K^+ current and an outwardly rectifying K^+ current (Ferrari and Zakon, 1993). The inwardly rectifying K^+ current, which sets the electrocyte resting potential, shows little variation in any of its properties among individuals. The Na^+ current and outwardly rectifying K^+ current, in contrast, differ among individuals in

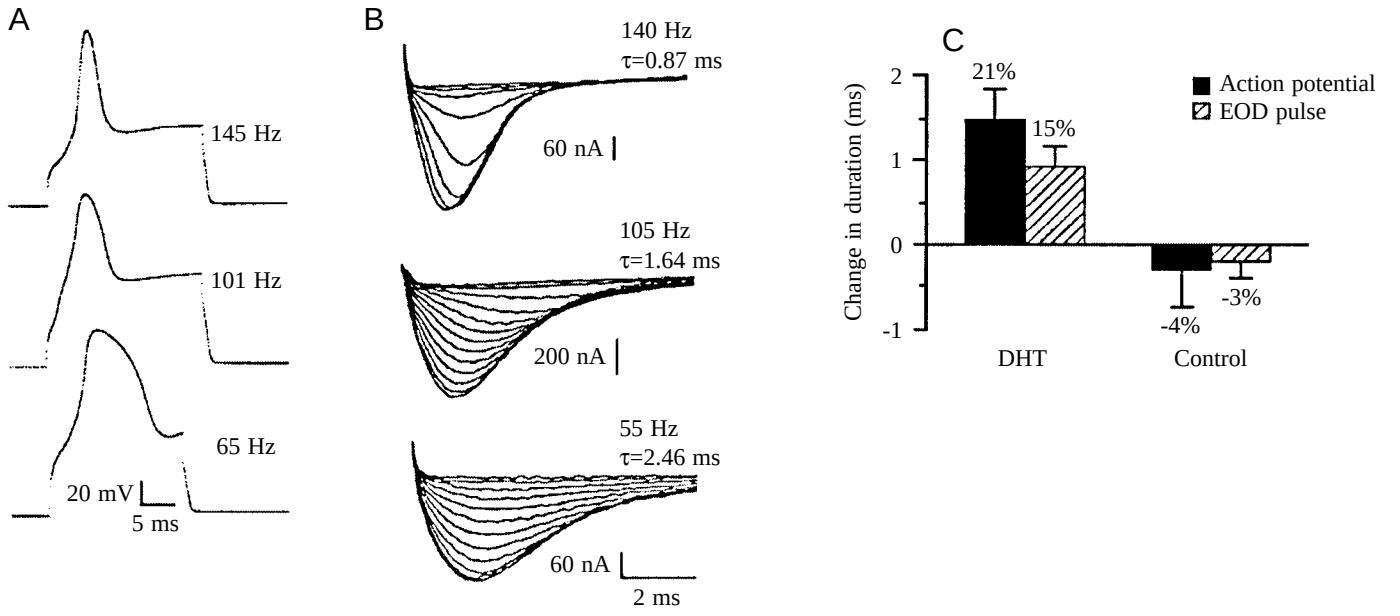

Fig. 4. Action potential and Na^+ current kinetics vary with electric organ discharge (EOD) frequency and are shifted by androgen treatment in *Sternopygus*. (A) Action potentials from electrocytes evoked by intracellular current injection. On the right is the EOD frequency of the fish from which each recording was made. Note that action potentials are longer in duration as EOD frequency becomes lower. (B) Na^+ currents recorded from electrocytes of fish with comparable EOD frequencies to those in A. To the right of each family of Na^+ currents is the fish's EOD frequency and the time constant of inactivation (τ) of the peak Na^+ current. Note that Na^+ currents inactivate faster as EOD frequency becomes higher. (C) Change in the duration of the EOD pulse and intracellularly evoked action potential. Baseline measurements were made of each fish's EOD frequency and in the duration of its electrocyte action potentials, and fish were implanted with a dihydrotestosterone (DHT)-containing or an empty capsule. Recordings of both variables were made 2–3 weeks later. Note that control fish showed no significant change in EOD pulse or action potential, whereas both were prolonged (P=0.005; one-tailed t-test) in DHT-treated fish. Values are means + S.E.M., N=14 for DHT group, N=8 for controls. A and B are taken from Ferrari et al. (1995); C is taken from Mills and Zakon (1991).

their kinetic properties (Fig. 4B). Specifically, the Na^+ current activates and inactivates rapidly in fish with higher EOD frequencies and slowly in fish with lower EOD frequencies. The K^+ current also activates more rapidly in fish with higher EOD frequencies (Ferrari et al., 1995; McAnelly and Zakon, 1998). The kinetics of these two currents co-vary significantly over the range of EOD frequency, illustrating how tightly co-regulated they are. Long-term treatment of fish with DHT slows down and long-term treatment of fish with estrogen speeds up the kinetics of the Na^+ current (Dunlap et al., 1997; Ferrari et al., 1995). It is not yet known whether the kinetics of the K^+ current are affected by hormones, but this seems likely.

We are now studying the hormonal regulation of the Na^+ and K^+ currents at the molecular level, since the long time course of the hormone-dependent changes and the presence of nuclear steroid receptors suggest that they are subserved by changes in transcription. It is unclear whether the regulated molecules are the ion channels themselves, modulatory channel-associated proteins, such as beta subunits, other proteins that link the channels to each other or to the cytoskeleton, or enzymes that modify the channels post-translationally.

The first step is to determine whether there are different isoforms of the channels (either from different genes or from alternatively spliced variants of the same gene) whose abundance is regulated by sex steroids and whether these channels display the predicted differences in their kinetics in an expression system such as a *Xenopus* oocyte. Two Na^+ channel genes have been partially cloned from the electric organ, SKM1 and SKM2, which are homologs of mammalian muscle Na^+ channel genes (Lopreato et al., 1998). While studies of these channels are still in their infancy, two observations are of particular interest. First, since SKM2, which is the predominant Na^+ channel in the mammalian heart, had only been cloned and sequenced in mammals, it was not certain how widespread this Na^+ channel was among vertebrates. The cloning of this channel in a teleost fish indicates that it may be expressed in many, if not all, vertebrate groups. Second, while SKM1 is only expressed in muscle in mammals, it seems also to be expressed in the brain in *Sternopygus*. This suggests that transcriptional regulation of this channel differs among groups. It is not yet known whether there are any variants of these genes, but identification of these genes in fish is an important first step to an eventual molecular analysis of the regulation of the Na^+ current.

It will also be interesting to compare the mechanisms of steroid-dependent plasticity of ionic currents in the electrocytes of *Sternopygus*, whose EOD is essentially a monophasic discharge, with those of gymnotiform pulse fish, such as *Brachyhypopomus*. In this species, the EOD is diphasic

with a sexually dimorphic second phase; the posterior face of the most caudal electrocytes produces an action potential that is similar in both sexes, while activation of the anterior face generates an action potential of longer duration in males than in females (Hagedorn and Carr, 1985; B. Rasnow and P. Stoddard, personal communication). It will be intriguing to discover whether similar changes in Na^+ and K^+ currents underlie variation in action potential duration in this species and also whether these changes are limited to one membrane. This may have implications for our understanding of selective targeting of channels in excitable cells. This comparative analysis should also be extended to the independently evolved mormyriform fish, which include a wave-type and numerous pulse-type species.

While most of the biophysical analysis has been of the ionic currents in the electrocytes, it is important to study the pacemaker neurons as well. It will be interesting to know how the ionic currents expressed in these neurons generate oscillatory potentials that range from tens of hertz in some species to over a kilohertz in others. Are the same currents used in the pacemaker neurons in all species, or do those species with higher EOD frequencies express different types of channels? How is the firing frequency of the pacemaker regulated by steroid hormones, and does the rapid NMDA-dependent LTFE of the EOD act *via* the same ionic currents that are regulated more slowly by steroids.

Conclusions

In this review, we illustrate the utility of using the electromotor system for studying functionally relevant regulation of ion channels. Questions for the future include understanding how rapid socially or environmentally mediated and longer-term transcriptionally mediated events interact at the cellular level, how separate cellular signals independently control the kinetics and amplitudes of ionic currents, how variations in the properties of ionic currents underlie a tremendous variation of almost two orders of magnitude in pacemaking frequencies, and the molecular identities of the channels that show plasticity and how they relate to their previously cloned mammalian counterparts.

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