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Individual Variation in and Androgen-Modulation of the Sodium Current in Electric Organ

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Electric fish of the genus *Sternopygus* produce a sinusoidal electric organ discharge (EOD) of low frequencies in males, high frequencies in females, and overlapping and intermediate frequencies in juveniles. Correspondingly, the cells of the electric organ, the electrocytes, generate action potentials which are of long duration in mature males, short duration in females, and intermediate duration in immatures. The androgen dihydrotestosterone (DHT) lowers EOD frequency and increases electrocyte action potential duration.

We examined the electrocytes under voltage clamp to determine whether variations in the kinetic properties of the Na^+ current might underlie these phenomena. We found that the fast inactivation time constants of the peak Na^+ current (0 mV) ranged from 0.5 to 4.7 msec and varied systematically with EOD frequency and action potential duration. Voltage dependence of steady-state inactivation also varied with EOD frequency with the midpoint of inactivation being more positive in fish with low EOD frequencies. There was no correlation between the voltage at which the Na^+ current activates, voltage at peak current, reversal potential, rate of recovery from inactivation, or TTX sensitivity and EOD frequency. We tested whether DHT influenced Na^+ current inactivation by recording from electrocytes before and after juvenile fish of both sexes were implanted with a DHT-containing or empty capsule. We found that inactivation time constants were significantly slower in DHT implanted, but not control, fish. This is the first observation of functionally relevant individual variation in the kinetics of a Na^+ current and the first demonstration that the kinetics of a Na^+ current may be modulated by an androgen.

[Key words: androgen, dihydrotestosterone, sodium current, inactivation, electric fish, electric organ, TTX]

Electric fish generate weak electric fields around themselves from an electric organ located along the tail and/or the body, and possess specialized receptor cells for detecting these fields. These nocturnally active fish rely on their electric fields to locate nearby objects and for social communication (Bullock and Heiligenberg, 1986).

The waveform characteristics of the electric organ discharge (EOD) of each species are species specific. For example, the species *Sternopygus macrurus*, the gold-lined black knifefish, generates a nearly sinusoidal EOD. The EOD frequency range of mature males is 50–90 Hz, that of females from 110–200 Hz, and that of juveniles is intermediate and overlaps the range of the adults (Hopkins, 1972, 1974; Meyer, 1983; Zakon et al., 1991). Within the appropriate range for its sex and age, each individual possess its own unique "signature" frequency which is stable over days (Bullock, 1969). Behavioral playback experiments have shown that EOD frequency is important in sex recognition: males respond to EODs in the female, but not the male, frequency range by giving electric courtship signals (Hopkins, 1974).

The firing pattern of the electric organ is controlled by a midline nucleus in the medulla, called the pacemaker nucleus (PMN). This nucleus is composed of two cell types. One type, the pacemaker cell, is entirely intrinsic to the nucleus and sets the rhythm of firing of the PMN. These cells are synaptically coupled with each other and to a second group of neurons whose axons descend in the spinal cord and innervate electromotor neurons. The electromotor neurons, in turn, activate the effector cells of the electric organ, termed electrocytes, via nicotinic cholinergic synapses (Bennett et al., 1967).

The sinusoidal form of the EOD in this species is determined both by the PMN and the electric organ (Mills and Zakon, 1987, 1991). The timing of the firing of the electric organ is determined by the PMN, while the actual wave shape of each discharge pulse is determined by the local properties of the electrocytes. The sinusoidal nature of the ongoing EOD is due to the fact that the pulses are rounded and the time interval between pulses is roughly the same as the duration of each pulse. The EOD pulse duration of each fish also varies individually and this is highly correlated with EOD frequency (Mills and Zakon, 1987, 1991). In this species, EOD pulse duration varies naturally by two- to threefold from 4 msec to over 10 msec and this range of durations is also evident in intracellular recordings from single electrocytes.

The sex difference in EOD frequency and pulse duration is under the control of gonadal steroids. EOD frequency varies with plasma androgen levels in field-caught males (Zakon et al., 1991). Furthermore, treating *Sternopygus* of either sex with the androgens testosterone or 5 α dihydrotestosterone (DHT) induces a downward shift in EOD frequency and a gradual increase of the average EO pulse width of about 1.3 msec (a 20% in-

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crease) over the course of 2 weeks (Meyer and Zakon, 1982; Meyer, 1983; Zakon and Meyer, 1983; Mills and Zakon, 1987). Intracellular recordings of electrocyte action potentials compared before and after androgen treatment showed that action potentials were significantly longer in duration after hormone treatment (Mills and Zakon, 1991). Thus, the properties of the pacemaker cells that determine firing frequency of the PMN and the electrocyte membrane properties that determine electrocyte spike duration must be correlated and must be coordinately altered during androgen treatment.

We have begun an analysis of this phenomenon by identifying the ion currents of the *Sternopygus* electrocytes. They have a Na^+ current, a delayed rectifier potassium current, and an inward rectifier potassium current as well as a chloride conductance, and a slow depolarizing conductance of the anterior membrane the ionic basis of which is not yet understood (Bennett, 1971; Ferrari and Zakon, 1993). In this study we examine the Na^+ currents of electrocytes from fish over a range of EOD frequencies to determine if there are any variations in their kinetic properties or sensitivity to TTX that correlate with EOD frequency, and whether the inactivation kinetics of the Na^+ current are influenced by androgens.

Materials and Methods

Animals. Juvenile *Sternopygus macrurus* of indeterminate sex were used in this study since their EOD frequencies and pulse durations cover the species range and since their EODs are androgen sensitive. Animal collection and maintenance have been previously described (Ferrari and Zakon, 1993). External recordings of EOD frequency were made in the home tank just before electrocyte recording. Individuals across the EOD frequency (and therefore APD) range were used to examine natural variations in the Na current. Those with mid- to high-frequency EODs were used in the androgen implant experiments.

Tissue preparation. The *Sternopygus* electric organ preparation has been previously described (Ferrari and Zakon, 1993; Mills and Zakon, 1991). Briefly, a small (1–1.5 cm) piece of the tail was removed and placed in standard Hickman's saline containing (mM) 111 NaCl, 2 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 $NaHCO_3$, 0.5 NaH_2PO_4, 5 HEPES, and 3 glucose (pH = 7.0). Curare (*d*-tubocurarine chloride, 10 mg/liter, Sigma) was added to prevent spontaneous contractions of the small muscle fibers located in this tissue. The tissue was pinned into a Sylgard recording chamber and the skin was peeled away to expose the electrocytes for intracellular recording.

Voltage clamp. A commercial two-microelectrode set-up (Axoclamp 2-A amplifier, TL-1 DMA interface, and pCLAMP software, Axon Instruments; Lab Master DMA boards, Scientific Solutions; and Dell 325D computer) was used to voltage clamp the electrocytes. The *Sternopygus* electrocyte voltage clamp has been previously described in detail (Ferrari and Zakon, 1993). Microelectrodes were pulled from thin-wall filament glass (A-M Systems, Inc., #6160) on a Flaming-Brown model P-80/PC and had resistances of 2–3 MΩ when filled with 3 M KCl. The microelectrodes usually broke slightly upon penetration and often had final resistances of less than 1.5 MΩ. A grounded shield was placed between the electrodes and their holders and lowered as close to the bath surface as possible. The saline level was also adjusted so as to just cover the tissue surface and, in conjunction with the grounded shield, this served to effectively reduce coupling capacitance between the electrodes. Microelectrodes were placed in the posterior, active end of superficial electrocytes. The ground electrode was a plastic tube filled with 3% agar in 3 M KCl into which was inserted a chlorided silver wire. With this bridge, junction potential shifts were always less than 2 mV, even during complete chloride substitution.

In *Sternopygus*, the electrocytes are innervated at their posterior face and only this face is electrically active (Bennett and Grundfest, 1961; Bennett, 1971; Mills and Zakon, 1991; Ferrari and Zakon, 1993). Therefore, we clamped the active portion of the membrane and treated the rest of the cell as a large linear leakage in parallel. However, although the active membrane only extends 200–350 μm (273 ± 74 μm, N = 28 cells; based on the distribution of labeling with Na^+ channel antiserum Ab 2944; H. Zakon, S. Tinkle, and S. R. Levinson, unpublished observations) from the posterior end of the cell, the space constant for electrocytes in standard Hickman's saline is too low for adequate spatial voltage control (lambda = 540 ± 45 μm). Therefore, we used a recording saline that blocks the electrocyte's substantial chloride leakage current by replacement of NaCl with sodium methyl sulfate (NaMS). NaMS saline increases the space constant (lambda = 1208 ± 184 μm) without affecting APD, and we measured a voltage error of less than about 10% (at ±100 μm) and 15% (at ± 200 μm) deviation from isopotentiality by placing a third electrode at varying distances from the voltage-sensing electrode of the pair of electrodes used to voltage clamp the cell.

In order to further ensure a good space clamp and avoid any contamination from potassium currents, 40 mM tetraethylammonium (TEA) and 1 mM Cs^+ were added to the NaMS saline. With this saline (NaMS/Cs^+/TEA), if the Na^+ current is blocked by TTX, only linear leakage current remains. The Na^+ current was isolated for analysis by subtracting the linear leakage from the Na^+ current by either blocking the Na^+ current with TTX or using a depolarizing prepulse to inactivate the current. Under these conditions we recorded Na^+ currents that met all of the criteria for a well-clamped Na^+ current: the peak current was activated gradually with increasingly depolarizing voltage steps, the latency to peak decreased gradually, and there was a distinct reversal potential (Park and Ahmed, 1991; Kay et al., 1994). Additionally, no changes occurred in any of the voltage-dependent properties of the Na^+ current after partial blockage with TTX or partial inactivation with a depolarizing prepulse. The *I–V* curves for the Na^+ current recorded in this way are similar to averaged Na^+ currents recorded from a single to a few channels using patch electrodes from eel (*Electrophorus electricus*) electroplax (Shenkel and Sigworth, 1991).

Various conventional voltage pulse protocols were executed to determine the voltage dependence of characteristics such as current activation, fast inactivation, steady-state inactivation, reversal potential, peak current, and refractory behavior. Pulse protocols were generated by the pCLAMP software and delivered to the Axoclamp 2-A amplifier via the TL-1 DMA interface. During these acquisition episodes, the membrane current was sampled at 20 kHz with typical amplifier gain settings of 2500–9000 V/V. Because of the large amount of membrane capacitance and increased membrane resistance due to blocking the Cl^- current, clamp speed was generally between 100–500 μsec.

DHT implant experiments. Juveniles of undetermined sex were used for this study. Each fish's EOD frequency was measured for 2 weeks or longer to ensure that its EOD frequency was stable. Then, the terminal piece of the electric organ was rapidly removed and fish were returned to their aquaria. Na^+ currents were recorded from cells in the isolated piece of electric organ. The following day, fish were anesthetized in MS-222 (1:1000), implanted with DHT-filled or empty SILASTIC capsules in the abdominal cavity (Keller et al., 1986), and returned to their aquaria. Juvenile fish of both sexes have low levels of androgens (Zakon et al., 1991). Based on previous measurements, the SILASTIC capsules produced plasma concentrations of DHT in the range of 1–10 ng/ml, which is in the physiological range of plasma androgen in this species (Keller et al., 1986; Zakon et al., 1991). EOD frequency was tracked daily and 3–4 weeks later a more anterior section of the electric organ was removed and Na^+ currents were again recorded.

Results

General properties of the action potential and Na^+ current

Electrocyte action potentials were recorded in Hickman's saline before switching to the NaMS/Cs^+/TEA saline for voltage clamp. As shown previously (Mills and Zakon, 1991), APD and EOD frequency are highly negatively correlated ($r = -0.79$, $p < 0.0001$, $N = 50$ fish) (Fig. 1).

In the NaMS/Cs^+/TEA saline, the Na^+ current was well clamped (Fig. 2). The current activated gradually at about −45 mV, approached a maximum at 0 mV, decreased with increasing depolarization, and usually reversed near 55 mV. The Na^+ current also displayed the unusual feature of incomplete inactivation beyond the reversal potential in many cells (Ferrari and Zakon, 1993). Electrocytes are large cells and the average peak Na^+ current that they generated when the membrane was clamped at 0 mV was about 1000 nA. The numerical values of most of these voltage-sensitive properties are listed in Table 1.

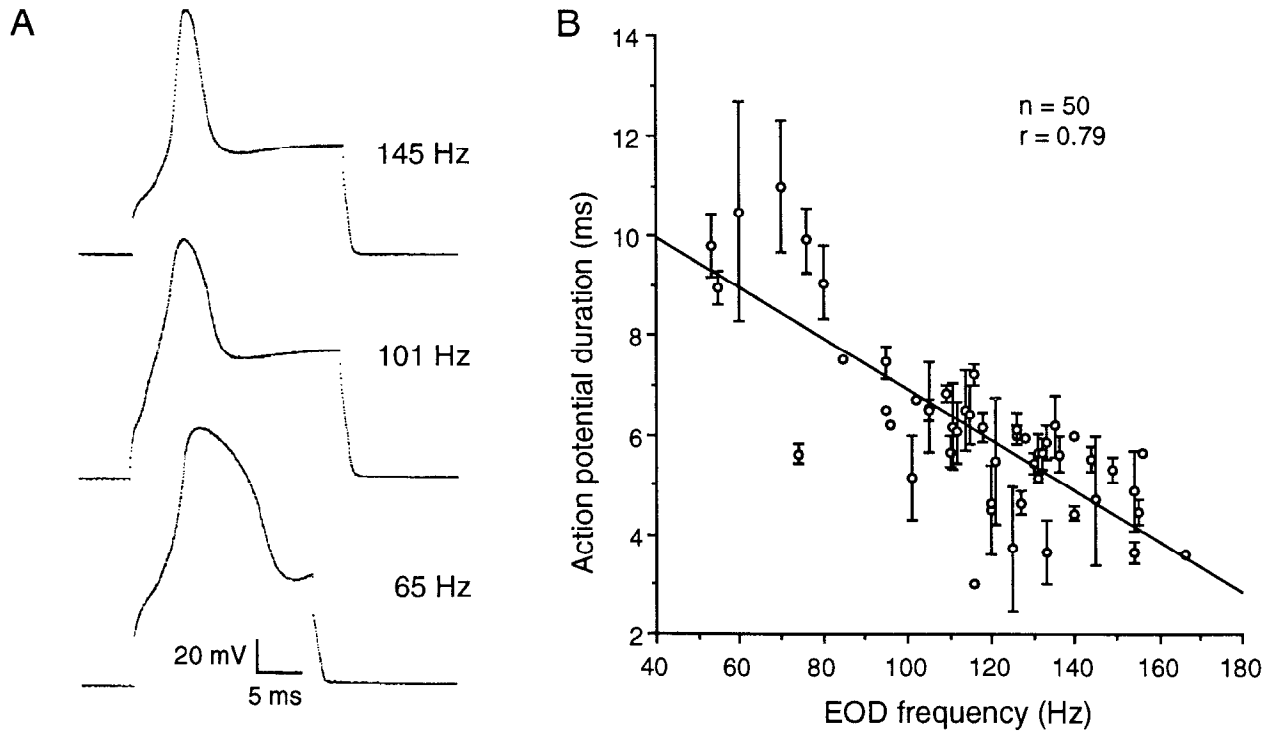

Figure 1. The relationship between electrocyte action potential duration and EOD frequency. *A*, Three action potentials evoked by current injection from electrocytes of fish with a high, mid-, and low-frequency EOD (indicated next to each trace). The current trace is not shown but the duration of current injection can be determined by the membrane charging curves in each recording. A long-duration current pulse was used to elicit action potentials since short-duration pulses often provided insufficient current to initiate action potentials due to the large size of the electrocytes. *B*, Mean electrocyte spike duration versus EOD frequency. Each point represents data from one fish, error bars are 1 SD.

Fast inactivation

Figure 2 shows a family of Na^+ currents from activation to peak current from fish with high-, mid-, and low-frequency EODs. The current exhibits both slower activation and inactivation kinetics as EOD frequency decreases. Due to the clamp speed (generally 100–500 μsec) and the possibility that part of the activation phase of the current might be obscured by the capacitative transient, no attempt was made to analyze the activation of the Na^+ current quantitatively.

The time constant of decay of the Na^+ current (τ_h) could usually be fit with a single exponential term, as is often the case for Na^+ currents (Pappone, 1980; Weiss and Horn, 1986; Tanguy and Yeh, 1991). However, at larger maintained depolarizations this Na^+ current showed a noninactivating or slowly inactivating component and, therefore, was best fit at these values with two exponentials. Similar behavior is seen in the eel electric organ Na^+ channel (Shenkel and Sigworth, 1991). In the voltage range of −50 to near 0 mV, the time constant τ_h declined; it then gradually increased with further depolarization in most cells (Fig. 3*A*). The peak current τ_h ranged from 0.5 to 4.7 msec and average values of τ_h for each fish were highly correlated with EOD frequency ($r = 0.70$, $p < 0.0001$, $N = 58$ fish) and average APD ($r = 0.67$, $p < 0.0001$, $N = 51$ fish) (Fig. 3*B*). An even better correlation was obtained with peak current τ_h versus EOD period (Fig. 3*C*).

Steady-state inactivation

The voltage dependence of the steady-state activation and inactivation of the Na^+ current was also examined. Activation parameters did not vary with APD or EOD, but the steady-state inactivation midpoint (h_∞) did. Figure 4 shows the steady-state inactivation curves for Na^+ currents from high, mid, and low frequency fish. Steady-state inactivation parameters were determined from a least-squares fit to a modified Boltzmann function $\%I_{total} = 1/1 + \exp([V_{pp} - V_{mid}]/k)$ where $\%I_{total}$ is the percentage of the Na^+ current elicited by a standard test pulse after a voltage prepulse, V_{pp} is voltage of the prepulse, V_{mid} is inactivation midpoint (50% reduction in current), and k is a constant. The midpoint of h_∞ ranged from −62.5 to −27.5 mV and was significantly correlated with EOD frequency ($r = 0.482$, $p < 0.017$, $N = 24$ fish), APD ($r = 0.666$, $p < 0.001$, $N = 21$ fish), and τ_h ($r = 0.458$, $p < 0.024$, $N = 24$ fish). The mean inactivation midpoint for the population was −48.6 ± 6.9 mV (SD; $N = 69$ cells).

Sodium current recovery

The Na^+ current recovery from fast inactivation was examined using a twin-pulse protocol. It showed a typical time-dependent recovery from inactivation which was best fit with a single exponential, and, at a holding potential of −90 mV, it recovered to 90% of its initial magnitude in 2.3 ± 0.1 msec ($N = 51$ cells). The refractory behavior of the Na^+ current was also examined as a function of the holding and interpulse potentials. The time course of the recovery was highly voltage dependent, where more depolarized holding and interpulse potentials substantially slowed the recovery (Fig. 5). There was no correlation between the 90% recovery value, and either fast or steady state inactivation or EOD frequency.

TTX sensitivity

The *Sternopygus* electrocyte Na^+ current was blocked by nanomolar concentrations of TTX, usually being completely

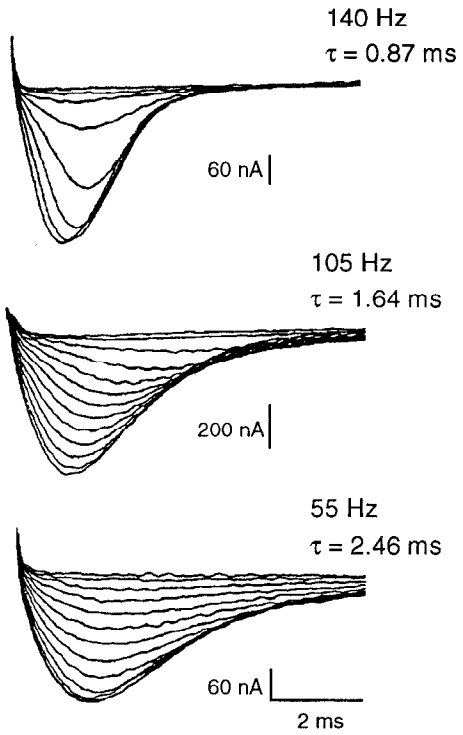

Figure 2. Sodium currents from fish with high- (*top*), mid- (*middle*), and low- (*bottom*) frequency EODs. Each family of curves represents sodium currents elicited by a 25 msec voltage-clamp step in 5 mV (*middle* and *bottom traces*) or 10 mV (*top trace*) increments from just below activation threshold (−60 mV) to peak current (0 mV) (holding potential = −90 mV). The inactivation time constant of the peak current (τ_h) was determined from a least squares fit to the declining phase of the current and is given for each cell.

blocked in 100–250 nM TTX (Fig. 6). There was no change in the inactivation kinetics of the Na^+ current after TTX treatment; currents before and during TTX treatment were superimposable. In different experiments the K_d ranged from 4 to 28 nM and the average K_d was 16.8 nM (N = 12 cells, one cell per fish). There was no relationship between EOD frequency and TTX sensitivity.

DHT effects on Na^+ current

As in previous studies, the EOD frequency of fish implanted with DHT-containing capsules was lowered from baseline values (average change = −43.5 ± 13.2 Hz) whereas those of the fish implanted with control capsules showed virtually no change (average change = −2.5 ± 3.4 Hz). The change from baseline EOD frequency between these two groups was significantly different (Mann-Whitney U test, $p < 0.008$) (Fig. 7).

After taking baseline measurements of electrocyte Na^+ currents, each fish was implanted with either a DHT-filled or empty capsule. Figure 8*A* illustrates representative traces for the peak Na^+ currents from electrocytes of a fish before and four weeks after implantation of an empty SILASTIC capsule. After the second recording period this fish was subsequently implanted with a DHT capsule and recordings were made from its electrocytes four weeks later. The Na^+ current activation and inactivation were visibly slower (Fig. 8*B*).

The Na^+ current inactivation time constants of all the cells recorded from an individual fish in one session were averaged. Pre- and postimplant mean peak current τ_h values were subtracted for each fish to give a difference score, and average difference scores for all fish in the DHT-implanted and control groups were calculated (Fig. 7*B*). Pre- and postimplant peak current τ_h were not significantly different in the control group but differed significantly in the DHT-treated group ($p < 0.008$, Mann-Whitney U test). The difference between the control and DHT-treated groups was similarly significantly different. On the other hand, there were no statistically significant differences among the magnitudes of the Na^+ currents in any of these conditions.

Table 1. Na^+ current parameters in *Sternopygus* electrocytes

	Mean	SD	SE	*N* Fish	*N* Cells
Peak Mag (nA)	928.2	581.7	77.0	57	186
Peak V_m (mV)	0.05	13.8	1.8	57	183
Activ V_m (mV)	−45.5	7.4	1.0	56	178
E_{rev} (mV)	56.1	16.3	2.3	51	150
Peak τ_h (mS)	1.47	0.82	0.11	58	186
h_∞ (mV)	−49.1	6.3	1.3	24	78
Recov (mS)	2.35	0.95	0.13	16	57

Means shown are calculated from individual fish averages and are similar to means calculated for individual cells (N = 1–9 cells/fish). Peak Mag, peak current magnitude in nanoamps; Peak V_m, membrane potential at peak current; Activ V_m, membrane potential of current activation; E_{rev}, reversal potential; Peak τ_h, current decay time constant in milliseconds; h_∞, steady-state inactivation midpoint; Recov, time to 90% recovery of current magnitude in twin pulse protocol.

Discussion

The main findings of this study are that the fast and steady-state inactivation kinetics of the Na^+ current of the *Sternopygus* electric organ vary with EOD frequency and APD, that this variation in kinetics does not correlate with other voltage-dependent properties of the current nor with TTX sensitivity, and that the Na^+ current fast inactivation time constant is lengthened by treatment with androgens.

Inactivation kinetics

The fast inactivation (τ_h) of the Na^+ current is strongly correlated with EOD frequency and APD. The systematic shift in τ_h with EOD frequency cannot be ascribed to a voltage-clamp artifact. Factors like electrocyte size and the extent of the excitable membrane could contribute to an apparent relationship between kinetic parameters and EOD frequency if these properties varied with EOD frequency and if spatial voltage control were inadequate. However, while the extent of the excitable membrane does correlate with electrocyte size (H. Zakon, S. Tinkle, and S. R. Levinson, unpublished observations), each fish possesses electrocytes of all sizes in its electric organ (Mills et al., 1992) so there would be no systematic error. More directly, measurements of membrane potential along the length of the electrocyte during voltage clamp showed good spatial control, and no relationship was found between membrane resistance and τ_h (M. Ferrari, personal observation). The midpoint of steady-state inactivation also correlated with EOD frequency although somewhat less strongly than did τ_h.

Fast and steady-state inactivation of the *Sternopygus* electrocyte Na^+ current are correlated such that the voltage curve of steady-state inactivation is shifted to more hyperpolarized values as fast inactivation time constant becomes more rapid (and with higher EOD frequencies). This relationship has been observed

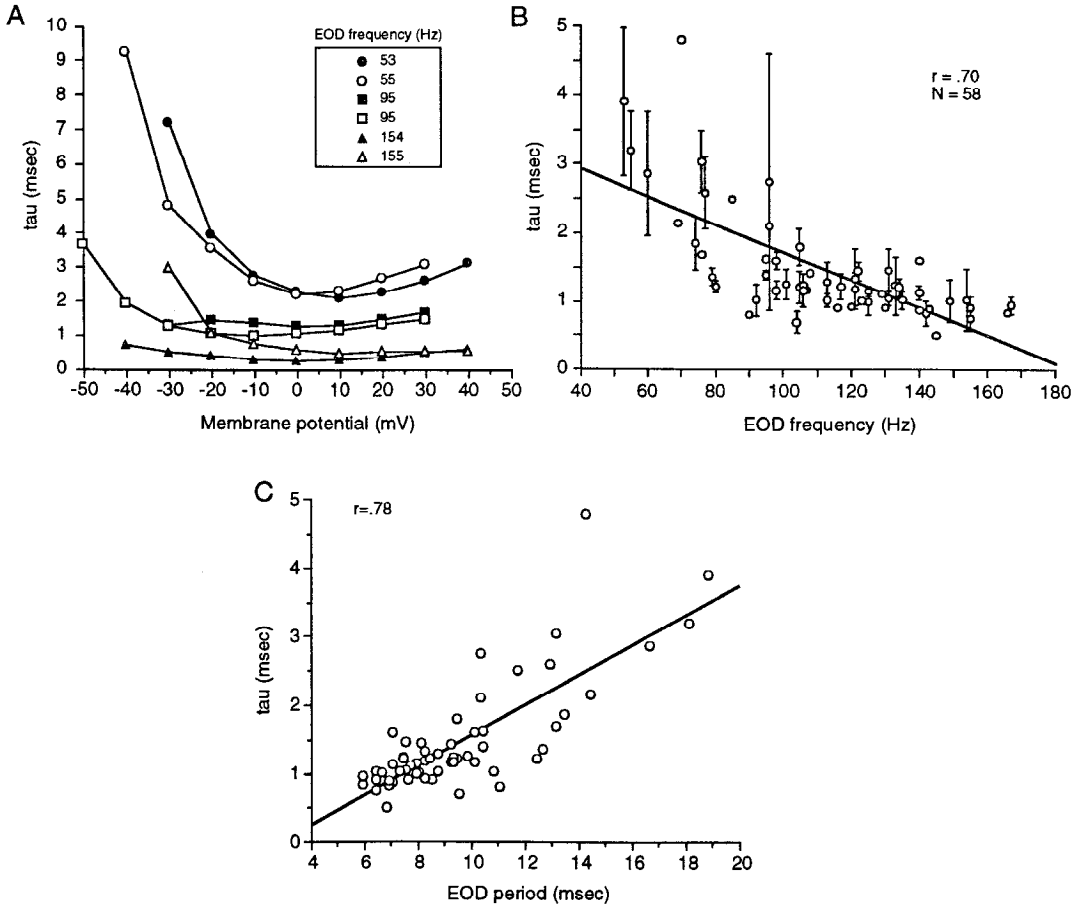

Figure 3. τ_h varies with voltage and EOD frequency. *A*, τ_h as a function of membrane voltage for electrocytes from fish with high-, mid-, and low-frequency EOD. *B*, The relationship between peak current τ_h and EOD frequency. Each data point is the average (and SD) τ_h value for a single fish. *C*, The relationship between mean peak current τ_h and EOD period.

in a number of cell types studied to date (Krafte et al., 1988; Schwartz et al., 1990; Isom et al., 1992; Roy and Narahashi, 1992; Zhang et al., 1992). It is important to bear in mind that while these two parameters are often linked, they have been shown to vary independently under some circumstances (Pappone, 1980; West et al., 1991).

Sodium current kinetics may vary during development in a given tissue. For example, in ventricular myocytes Na^+ current activation and inactivation becomes faster over the whole volt-

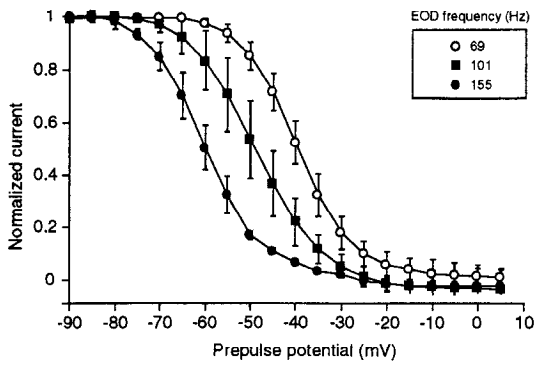

Figure 4. Relationship between steady state inactivation and EOD frequency. *A*, Averaged steady-state inactivation curves for multiple cells from fish of high, mid, and low EOD frequency. Inactivation curves determined with a conditioning pulse of −115 mV for 200 msec, a prepulse which varied from −90 to 40 mV for 25 msec, and a test pulse of 0 mV. The mean inactivation midpoints (h_∞) are −38.5 mV, −48 mV, and −60 mV respectively. Peak current is normalized to current elicited by a single pulse and plotted against prepulse potential. Error bars are 1 SD, and curves are line fits (Boltzmann function) to the data.

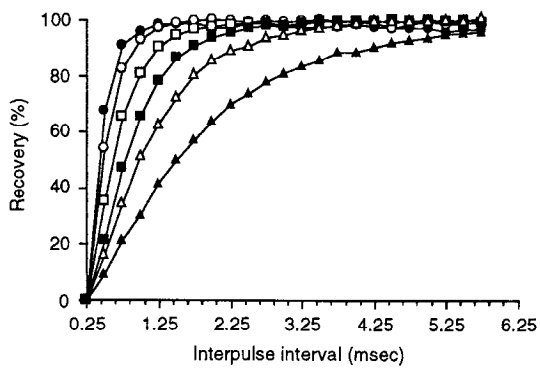

Figure 5. Recovery of Na^+ current from inactivation with different holding potentials. Holding potentials were −120 mV (*solid circles*), −110 mV (*open circles*), −100 mV (*open squares*), −90 mV (*solid squares*), −80 mV (*open triangles*), −70 mV (*solid triangles*) in 10 mV steps. The cell was held at the indicated holding potentials for 2 min and recovery was examined using a two pulse protocol (each pulse was + 10 mV for 10 msec) in which the interpulse interval was varied. Interpulse potential was the same as the holding potential.

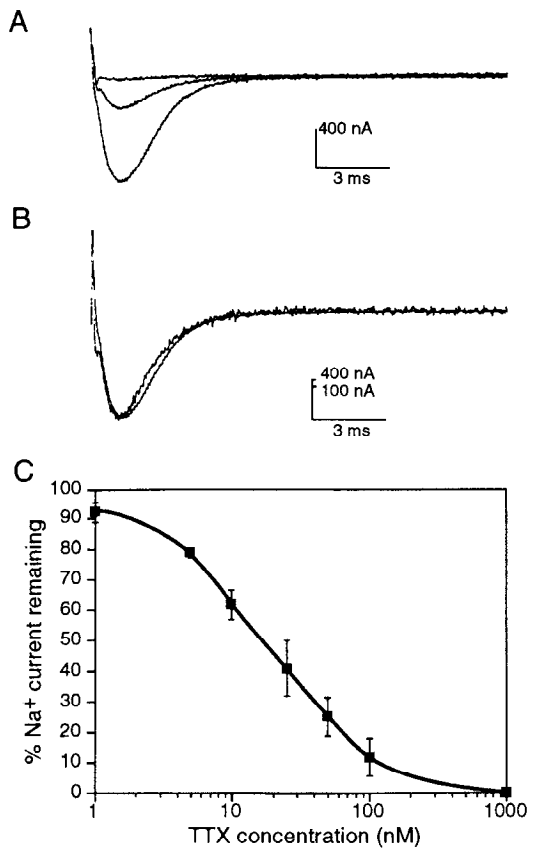

Figure 6. TTX blocks electrocyte Na^+ current. *A*, Peak Na^+ current in 0, 25, and 100 nM TTX (EOD frequency = 122 Hz). *B*, Superposition of the scaled Na^+ currents in 0 and 25 nM TTX. *C*, Averaged dose–response curves of Na^+ current in increasing concentrations of TTX. Note that the Na^+ current is about 90% blocked in 100 nM TTX.

age range and the steady state inactivation curve shifts 15–20 mV more negative during development. This shift from slow to fast channel kinetics contributes to the developmental increase in the rate of rise and conduction velocity of the cardiac action potential (Rosen et al., 1978; Fuji et al., 1988; Zhang et al., 1992). Other studies have shown similar shifts in channel populations with development in skeletal muscle and dorsal root ganglion cells (Kallan et al., 1990; Schwartz et al., 1990; Trimmer et al., 1990; Roy and Narahashi, 1992), although the functional importance of the shift is not clear.

To our knowledge, this study is the first to report systematic individual variations in the kinetics of a Na^+ current in a mature cell. In the *Sternopygus* electrocyte, the variation in sodium current kinetics has a clear functional significance: variations in current kinetics determine the duration of the electric organ pulse which is the fundamental unit of the EOD, a social signal.

Recovery from fast inactivation

The rate of sodium current recovery from inactivation in a two-pulse protocol was highly dependent on the holding potential. Negative holding potentials led to much faster recovery than positive holding potentials; above −80 mV, recovery was substantially slowed. In this regard, it is interesting to note that the average resting potential for electrocytes is −85 mV and each action potential terminates with a quick return to this value (Ferrari and Zakon, 1993). This quick and complete return to the

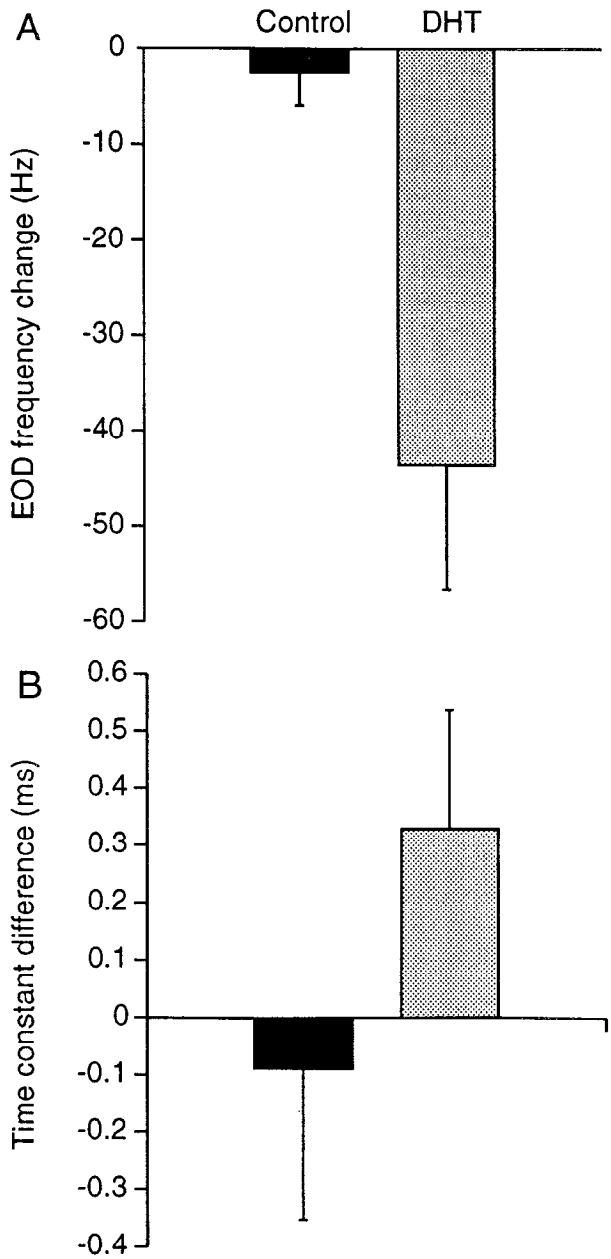

Figure 7. The effects of DHT on EOD frequency (*A*) and inactivation time constants (*B*) (τ_h) of the peak Na^+ current. Baseline measures of inactivation time constants were calculated from one to five electrocytes per fish and averaged. These parameters were measured again 3–4 weeks after androgen or control implant. Pre- and postimplant mean values were subtracted for each fish and the group difference scores were plotted.

negative resting potential between spikes would allow for almost complete recovery of the Na^+ current within 2–3 msec. *Sternopygus* produces a constant wave-like EOD that may reach frequencies of 200 Hz (Hopkins, 1972; Hopkins, 1974; Zakon et al., 1991). At these high frequencies the interval between successive pulses is only about 2–3 msec. The Na^+ current should be recovered from inactivation within this time and should be capable of maximum current generation.

TTX sensitivity

Muscle fibers of adult vertebrates express a TTX-sensitive sodium current. During early development and after denervation

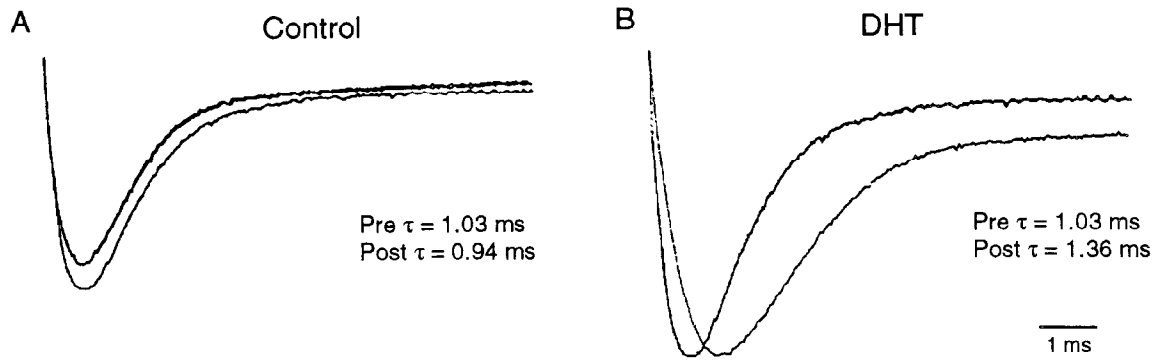

Figure 8. Effects of DHT on peak Na+ currents. *A*, Comparison of a baseline Na^+ current and that recorded 4 weeks after implanting a fish with a control SILASTIC capsule. *B*, Comparison of the same baseline Na^+ current and one recorded after the same fish was implanted with a DHT-filled capsule for 4 weeks. Traces were normalized for ease of comparison. The traces in *A* are slightly offset for clarity. The inactivation time constant of the Na^+ current is slower following androgen treatment. Although we did not quantitatively analyze the activation phase of the Na^+ current, it appears to be slower as well.

of adult muscle a second, TTX-insensitive (K_d of approximately 1 μM) current appears (Pappone, 1980; Gonoi et al., 1989). The TTX-insensitive channels in muscle have slower kinetics than the TTX-sensitive channels. Thus, we reasoned that the variation in Na^+ current kinetics in the *Sternopygus* electrocyte might be due to differential levels of expression of TTX-sensitive and TTX-insensitive currents with different inactivation kinetics. We found, however, that electrocyte Na^+ currents were sensitive to TTX in the same range (average K_d = 16.8 nM) as the TTX-sensitive Na^+ current of mature vertebrate skeletal muscle (Narahashi, 1988), and that the K_d did not correlate with EOD frequency.

The K_d values that we determined for different electrocytes varied from 4 nM to 28 nM. This range is comparable to but slightly higher than that reported for electrocytes from eel based on binding kinetics of channels in membrane homogenates (1–10 nM: Agnew et al., 1978; Yanagawa et al., 1987).

While it appears that mature electrocytes do not express a TTX-insensitive Na^+ current with slow inactivation kinetics, it is possible that the TTX-insensitive current might be present in electrocytes during early development and/or after denervation, as is observed in skeletal muscle (Weiss and Horn, 1986; Gonoi et al., 1989; Kallan et al., 1990). This possibility must be investigated in light of the long-duration action potentials (~15–25 msec) observed in regenerating electrocytes (M. L. McAnelly, unpublished observations).

Modulation by androgen treatment

Long-term androgen treatment slows the rate of fast inactivation of the *Sternopygus* electrocyte Na^+ current. The electrocytes also possess both inward rectifying and outward delayed rectifying K^+ currents (Ferrari and Zakon, 1993). We have not yet subjected these two other currents to an analysis of their kinetics before and after androgen treatment so we do not know if they are affected as well. Nevertheless, we can conclude from this study that the androgen-induced broadening of the action potential is in part or entirely due to the slowing of the inactivation kinetics of the Na^+ current. No variations in or androgen modulation of the kinetics of the Na^+ current have been reported for the eel electrocyte. However, it is not clear whether they do not exist in the eel or whether they would be observed in reproductively mature or androgen-treated animals.

Chronic treatment with steroids (estrogen, corticosterone) or thyroxine is known to alter K^+ and Ca^{2+} currents in a variety of neural, muscle, and glandular tissues (Binah et al., 1987; Boyle et al., 1987; Pragnell et al., 1990; Inoue and Sperelakis, 1991; Levitan et al., 1991; Joëls and de Kloet, 1992; Karst et al., 1993; A. Russo, personal communication). Long-term actions of androgens on membrane excitability have been reported in a few other studies. Androgens broaden the electric organ action potential in other species of electric fish (the South American genus *Hypopomus* and a number of the African mormyrids: Hagedorn and Carr, 1985; Bass and Volman, 1987) and increase the excitability of motoneurons innervating a sexually dimorphic forearm muscle in *Xenopus* (Erulker et al., 1981). However, the ionic basis of these effects is not understood. It would be interesting to determine whether androgens also alter Na^+ current kinetics in a similar way in the electric organs of the independently evolved African mormyrids.

Long-term androgen treatment diminishes the Na^+ current in a muscle cell line (C2) and this effect is blocked by the androgen receptor antagonist flutamide (Tabb et al., 1994). However, only the magnitude of the current, and not its kinetics are altered in these cells.

At least two cases are known of other steroids influencing Na^+ currents: chronic treatment of PC12 cells with the corticosteroid analog dexamethasone and of uterine myometrium with estrogen increases the number of cells expressing Na^+ currents (Garber et al., 1989; Inoue and Sperelakis, 1991). Additionally, the peptide thyrotropin release hormone (TRH) has been shown to reduce the magnitude of the Na^+ current in dissociated septal cells (Lopez-Barneo et al., 1990). In these cases, as well as in the cases cited above, it is the magnitude of the current or the number of cells expressing it that is affected rather than a change in kinetics. The effect that we report here is also novel in that the kinetics and not the magnitude of the Na^+ current are altered by a hormone.

Androgens influence sexual and aggressive behaviors in many vertebrates, including primates (Becker et al., 1992). Yet little is known about how androgens modulate the electrical activity of the neurons in the circuits underlying these behaviors. If a similar androgen-dependent spike broadening were to occur in certain neurons, their transmitter release might be prolonged, thus strengthening their inputs relative to others in the relevant circuits.

Site of action of androgens

DHT lowers EOD frequency (determined by the PMN) and broadens pulse duration (determined by electrocyte membrane properties, presumably by Na^+ current) with the same time course. Thus, DHT either acts directly and independently in the brain and in the EO or else directly in the brain and indirectly in the EO through an activity dependent mechanism sensitive to changes in EOD frequency. The likeliest scenario is that DHT acts directly on the electrocytes. First, the electrocytes possess androgen receptors (Gustavson et al., 1994). Second, EOD frequency can be lowered by lightly anesthetizing the fish. When this is done the duration of the EOD pulse remains constant for at least an hour (as long as tested) (Mills and Zakon, 1987). In addition, in our *in vitro* experiments electrocytes which are unstimulated for up to 10 hr still generate action potentials whose durations approximate that of the fish's EOD pulse. Nevertheless, while we can exclude short-term stimulation at EOD frequency as a determinant of EO pulse duration, we cannot presently exclude long-term stimulation.

Possible sources of variation of inactivation kinetics

The individual variation in and androgen-dependent modulation of *Sternopygus* electrocyte Na^+ current kinetics may arise from one or more known mechanisms. One source of the kinetic variation could be kinetically distinct channels derived from separate genes or transcripts since voltage-dependent Na^+ channels comprise a multigene family with at least six distinct genes (Noda et al., 1986; Rogart et al., 1989; Trimmer et al., 1989; Kallan et al., 1990; George et al., 1992). In skeletal muscle, different types of Na^+ channels derive from different genes and these channels have kinetically distinct inactivation properties (Kallan et al., 1990). Multiple transcripts for rat skeletal muscle channels have also been reported although it is unknown whether these isoforms generate channels with kinetically distinct currents (Schaller et al., 1992). Interestingly, the gene that encodes the Na^+ channel isoform with slow inactivation kinetics (skm2), originally cloned from fetal skeletal muscle, is expressed in the uterus (George et al., 1992). It would be interesting to determine whether the kinetics of this channel are modulated by steroid hormones.

We do not know how many Na^+ channel genes or transcripts are present in the *Sternopygus* electric organ, but there is only a single gene reported so far in the electric organ of the eel, a phylogenetically close relative (Noda et al., 1984). If more than one Na^+ channel type is expressed in the *Sternopygus* electrocyte, they are not distinguishable by TTX sensitivity as in muscle.

While the α-subunit alone is sufficient to produce a Na^+ channel when expressed in an oocyte or lipid membrane, one or two β-subunits are associated with it to form the complete channel in some mammalian tissues, and this association influences the inactivation kinetics of the Na^+ channel (Isom et al., 1992). Beta subunit coexpression accelerates the macroscopic current decay and shifts steady-state inactivation to more negative potentials (Krafte et al., 1988; Isom et al., 1992). Thus, in *Sternopygus*, the level of expression of the β-subunit might be correlated with EOD frequency and regulated by androgens. However, in the eel, attempts to identify β-subunits by Northern blot analysis with probes from mammalian tissue failed (Isom et al., 1992). Whether a β-subunit exists in *Sternopygus* electric organ and could explain the differences in inactivation kinetics remains to be determined.

Last, several recent studies have shown that Na^+ channel kinetics can be modulated by phosphorylation *in vitro* (Dascal and Lotan, 1991; Numann et al., 1991; Schreibmayer et al., 1991; West et al., 1991). For example, protein kinase C (PKC)-mediated phosphorylation of a specific serine in the "inactivation loop" (domain III-IV linker) of a brain-derived sodium channel dramatically decreases the rate of decay of the sodium current (Numann et al., 1991; West et al., 1991; Li et al., 1993). Eel sodium channel and rat skeletal muscle channel can be heavily phosphorylated by PKA, but not by PKC (Emerick and Agnew, 1989; Yang and Barchi, 1990). However, it is unknown if these PKA-mediated changes in phosphorylation result in changes in kinetics.

Kinases typically act on a time course of minutes while the time course of the androgen-dependent change in the electric organ is days to weeks. While phosphorylation would seem an unlikely mechanism to underlie such slow changes, it is possible that a gradual increase in the level of expression of a kinase or phosphatase could result in a gradual increase in the mean number of phosphorylated channels and a shift in whole current inactivation. Indeed, PKC activity in the pituitary has been shown to be gradually enhanced over the course of at least 5 d following estrogen treatment (Drouva et al., 1990).

Conclusions

We found that the Na^+ current of the *Sternopygus* electrocytes shows individual variations in inactivation kinetics that correlate with EOD frequency and underlie individual variations in APD TTX sensitivity does not vary with EOD frequency or APD suggesting that, if a heterogeneity of Na^+ channel types forms the basis for this variation in inactivation kinetics, they are not differentially sensitive to TTX. Last, in keeping androgen's ability to increase action potential duration, we found that long-term treatment of fish with androgens causes a slowing of the fast inactivation of the Na^+ current. This is the first observation of individual differences in and androgen sensitivity of the inactivation kinetics of the Na^+ current. How androgens cause these changes will be studied in future experiments.

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