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From behavior to membranes: Testosterone-induced changes in action potential duration in electric organs

(sexual dimorphism/steroid hormones/electrocytes)

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ABSTRACT **The electric organ of mormyrid fishes consists of action potential-generating cells called electrocytes, which together produce a pulse-like electric organ discharge (EOD). The appearance of an EOD depends, in part, on the characteristic features of a single electrocyte's action potentials. In some species, gonadal steroid hormones induce increases in EOD duration, which mimic natural sex differences. We now show that testosterone-induced changes in EOD duration are associated with a 2- to 3-fold increase in the duration of action potentials generated by single electrocytes. Together with other anatomical and biochemical data, the results emphasize the exquisite interrelationship between steroid hormone action and the cellular machinery determining the electrical properties of single cells that underlie sexually dimorphic and seasonal behaviors.**

Among many vertebrates, the development and evolution of sex differences in social communication systems has been linked with the potent effects of gonadal steroid hormones on neurons and muscles (1–5). This includes the electrogenic system of the mormyrid fishes of Africa, which have an electric organ consisting of modified muscle cells called electrocytes (6) that generate an electric organ discharge (EOD) used in social communication and guidance systems (7, 8). The EOD is a unique behavior because it is also a discrete spike-like event whose principal features are determined by a single electrocyte (6). In several species, the EOD is sexually dimorphic and the male pulse may be 2–3 times the duration of the female's during the breeding season (8–12); gonadal steroid hormones can induce females to generate a male-like EOD (4, 12).

The mormyrid electric organ is located in the tail (Fig. 1*A*) and consists of four columns of electrocytes, which are wafer-shaped cells with distinct anterior and posterior faces (Fig. 1 *B* and *C*) (6, 13–15). Each electrocyte also has a "stalk" system that arises from the posterior face (Fig. 1*C*) and is innervated by spinal "electromotoneurons." A descending signal from the brain synchronously activates the motoneurons, which in turn synchronously activate all of the electrocytes (16). The sequential action potentials generated by the posterior and then the anterior faces of a single electrocyte determine the appearance of, respectively, the major head-positive and head-negative phases of an EOD, while the stalk determines an initial head negativity in species whose stalk penetrates through the electrocyte body (6, 15) (Fig. 1 *C* and *D*).

Using intracellular recording techniques, we have now found that the effects of gonadal steroids on EOD duration, a sexually dimorphic feature in mormyrid electric fish, is correlated with comparable changes in the duration of action potentials generated by single electrocytes. The results exemplify how steroid hormones can ultimately influence the induction of different behaviors by orchestrating changes in the fundamental events that determine the electrical and anatomical properties of individual cells, such as those used in communication systems during breeding periods.

MATERIALS AND METHODS

The study species was *Brienomyrus* sp. (total length, 70–90 mm), a species commercially available from American fish dealers. Using previously developed techniques (6), intracellular recordings were made from 138 cells in 24 animals: (*i*) 9 males and 3 females received no steroid treatments, (*ii*) 5 males and 4 females received a 2-mg pellet implant of 17a-methyl testosterone (Sigma) for periods ranging from 11 to 28 days (17), (*iii*) 2 males and 1 female received a 2-mg pellet implant of cholesterol [5(6)-cholestene-3-ol; Sigma] for 10–18 days (17). Cholesterol controlled for steroid effects that may not be specific to gonadal steroids (18). After anesthetization (tricaine methane sulfonate, 1:20,000), the upper spinal cord was exposed and severed, which both immobilized the trunk and "silenced" the electric organ since electromotoneurons, located at electrocyte levels (Fig. 1*B*), require excitation by a brain nucleus. The electrocytes were exposed and the entire tail was isolated in a Ringer's bath (19) whose temperature ranged from 22°C to 23°C. The animal was revived and respired on its own or had aerated water passed over its gills. Each electrocyte was impaled with two 3 M KCl-filled glass microelectrodes (10–20 Ω), each one used alternately for stimulating and recording so that we were able to record spikes from two different positions (Fig. 2 *A* and *B*). The capacitance of the recording electrodes was systematically compensated for (WPI M707, WPI M4A). All tests of significance were based on a two-tailed *t* test for a difference in means. Spike amplitude and spike duration (measured at half-amplitude values) were measured by an observer unaware of the treatment groups.

RESULTS

For *Brienomyrus* sp., captive males and females in nonreproductive condition have similar EODs that, in both sexes, increase 2–3 times in duration after testosterone treatments (4, 17, 20) (Fig. 1*D*). As determined by radioimmunoassays, the average plasma testosterone level in untreated individuals (n = 2 males and 2 females) was 3.9 ng/ml (SD = 1.43), while in testosterone-treated ones (n = 2 males and 3 females) it was 51.9 ng/ml (SD = 23.0). There were no significant differences between males and females within either sample group. The "untreated" and "treated" values compared with those in other teleost fishes in, respectively, nonreproductive and reproductive states (21). Long-duration EODs charac-

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Abbreviation: EOD, electric organ discharge.

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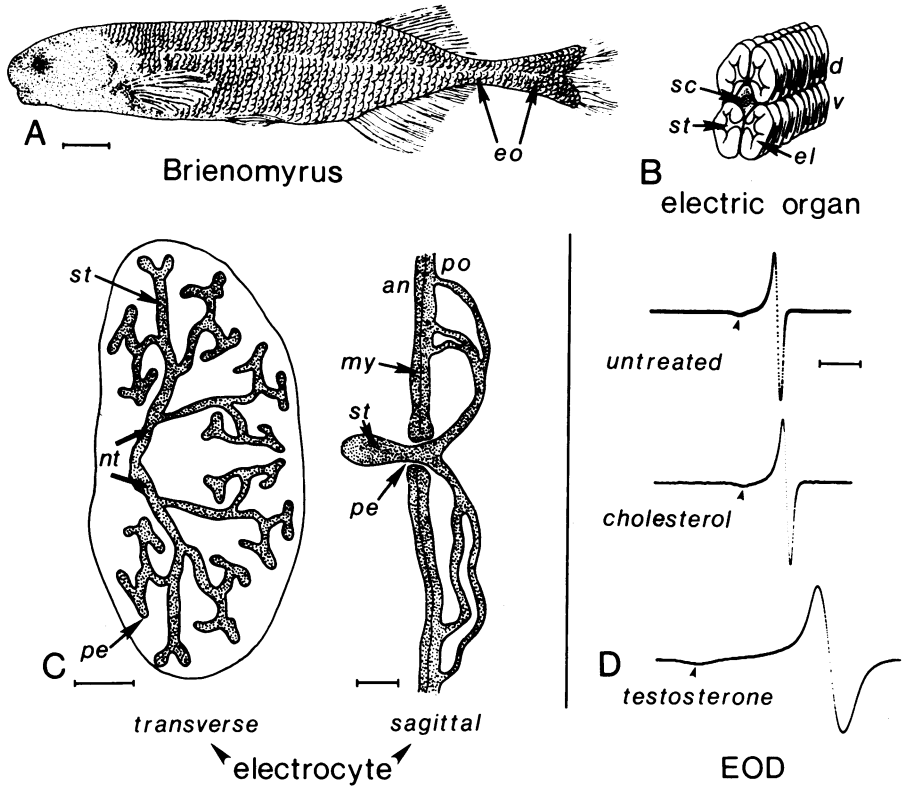

FIG. 1. (*A*) Line drawing of *Brienomyrus* indicating position of electric organ (eo). (Bar = 1 cm.) (*B*) Oblique anterior view of electric organ, which consists of two dorsal (d) and two ventral (v) columns of serially stacked cells called electrocytes (el). Each electrocyte is separated within a gelatinous compartment from its neighbor. The columns surround the spinal cord (sc), which contains the electromotoneurons that synchronously activate all of the electrocytes. (*C*) Line drawings of electrocytes. To the left is a transverse view looking at the anterior face of the electrocyte labeled el in *B*. The stalk system (st, also in *B*) has several branches but is innervated by electromotoneurons in a restricted zone (nt). To the right is a sagittal view showing the anterior (an) and posterior (po) faces of the electrocyte. A stalk branch penetrates through the electrocyte body at a particular site (pe, also see transverse view) and has small branches that are continuous with the posterior face. A central zone of myofilaments (my) denotes the electrocytes' origin from striated muscle. [Bars = 500 (transverse) μm and 30 (sagittal) μm.] (*D*) Differential recordings (positive electrode near the head) of the EOD of *Brienomyrus* sp. scaled to the same peak-to-peak amplitude. Shown are representative EODs of untreated, cholesterol-treated (18 days), and testosterone-treated (18 days) individuals. Arrowheads indicate small initial negative phase of the EOD. (Bar = 0.4 msec.) Average EOD durations (msec) were as follows: untreated, 0.6 (SD = 0.05; n = 12); cholesterol-treated, 0.7 (SD = 0.05; n = 3); testosterone-treated, 1.9 (SD = 0.44; n = 9).

terize males during the breeding season in species whose EOD duration is known to undergo reversible changes in the field (4).

As in other mormyrids (6), there were three major classes of intracellularly recorded potentials. The largest class consisted of single spikes (Fig. 2 *A–F*). In a second response class, an initial stimulus elicited a single spike, while succeeding stimuli presented at variable delays during the cell's relative refractory period evoked double spikes (Fig. 3 *A* and *B*). The two spikes represent the separate activity of the two faces (6); their degree of separation varied with stimulus frequency, intensity, and duration. A third class of "stalk" spikes (6) differed from all others in having fast rise times, slow decays, and the longest durations (Fig. 3 *C* and *D*).

The average duration of single spikes was >2 times greater in testosterone-treated, compared to untreated or cholesterol-treated, animals (Fig. 2 *A–G*). There was a small increase in spike (Fig. 2*F*) and EOD (Fig. 1*D*) duration in the cholesterol-treated animals. For double spikes, the total duration of the response and the duration of each of the individual spikes were 2 times greater in the testosterone-treated group compared to either cholesterol or untreated groups, which were not significantly different from each other (Fig. 3 *A* and *B*). Comparable results were found for stalk spikes (Fig. 3 *C* and *D*). There were no significant differences in average resting potentials or current thresholds for spike generation between any groups (Fig. 2 legend).

The average amplitude of single spikes was significantly greater in testosterone-treated animals (Fig. 2*H*). However, there was appreciable overlap in the amplitude data collected from the experimental and control groups (Fig. 2*H*).

DISCUSSION

In this study, physiological levels of gonadal steroid hormones were found to induce dramatic increases in the duration of action potentials (or spikes) generated by the electrocytes of a mormyrid fish. The effect was consistently robust and was found in all cells tested. For recordings of single spikes, the largest data group, the distribution of pulse durations was virtually nonoverlapping between the testosterone and control groups. In contrast, the effects of steroids on spike amplitude were variable with appreciable overlap between experimental and control groups, although there were some differences in average amplitudes.

In a previous study on a gymnotid electric fish with a pulsatile EOD like that of mormyrids, steroid treatment induced a dramatic shift in some electrocytes in the relative amplitudes of spikes recorded differentially across the two electrocyte faces, reflecting a change in the relative amplitudes of the two phases of the EOD (22). Steroids did not affect the amplitude (or duration) of *intracellularly* recorded spikes. These authors presented data for one electrocyte suggestive that steroids induced changes in the duration of the action potential as recorded differentially across the

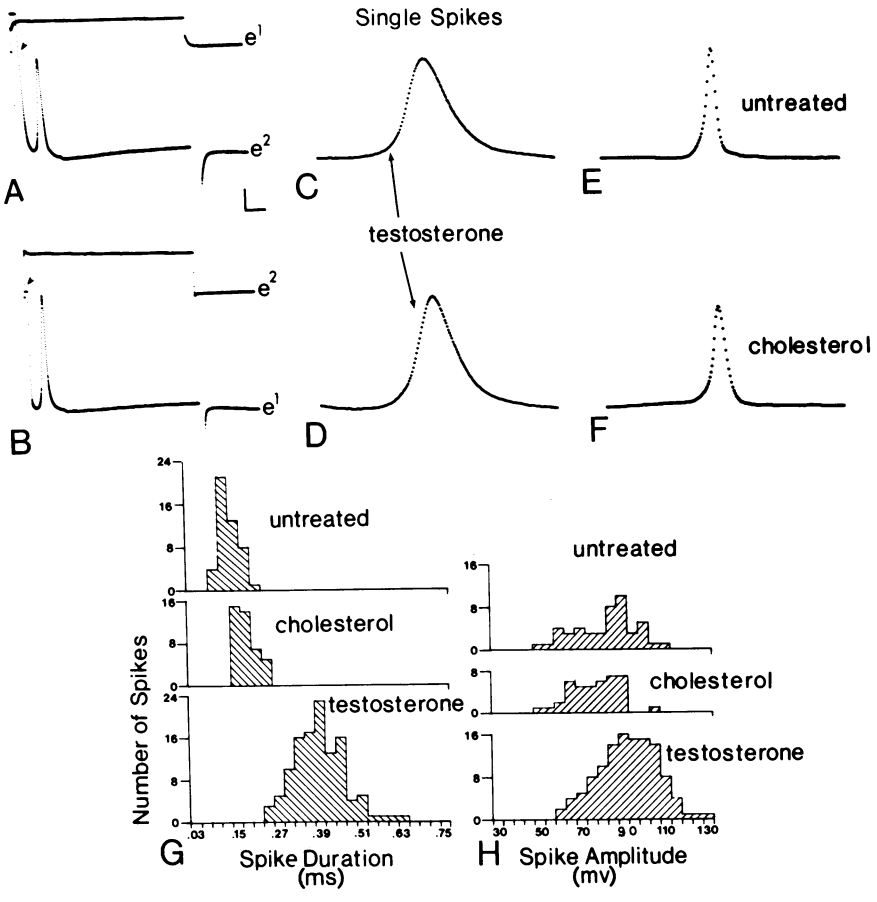

FIG. 2. (*A–F*) Intracellularly recorded action potentials from single electrocytes. Vertical bar scale in *A* is 20 mV for all spike records and 1 μA for current pulses in top trace of *A* and *B*. Horizontal scale is 2.0 msec for *A* and *B* and 0.2 msec for *C–F*. Arrowheads indicate stimulus artifact. (*A* and *B*) Each of two electrodes (e^1, e^2) were used alternately for stimulating (square pulses, top trace) and recording so that spikes (bottom traces) were recorded at two positions in a cell. (*C–F*) The largest class of testosterone-sensitive spikes (as shown on expanded time scale without stimulus pulse) were single spikes. Except where indicated, spike duration or spike amplitude was significantly greater ($P < 0.0001$) in the testosterone-treated group compared to cholesterol-treated or untreated groups, and these two groups were not significantly different from each other ($P > 0.05$). Spike duration was 2–3 times greater in testosterone (*C* and *D*, which are, respectively, *A* and *B* on an expanded scale), compared to untreated (*E*) or cholesterol-treated (*F*) groups. (*G*) Histogram of single spike durations (measured at half-amplitude values). Averages (msec) were as follows: untreated, 0.14 (SD = 0.03; $n = 47$); cholesterol, 0.18 (SD = 0.03; $n = 41$); testosterone, 0.39 (SD = 0.07; $n = 113$). Cholesterol group is significantly greater than untreated one ($P < 0.0001$). (*H*) Histogram of total amplitude of single spikes as measured from baseline to peak. Averages (mV) were as follows: untreated, 82.0 (SD = 14.2; $n = 47$); cholesterol, 76.8 (SD = 11.2; $n = 41$); testosterone, 93.4 (SD = 13.7; $n = 113$). Average resting potentials (RPs) or spike thresholds (STs) were not significantly different between any groups: RPs (mV) were as follows: untreated, −92.7 (SD = 6.9; $n = 11$); cholesterol, −95.8 (SD = 9.5; $n = 38$); testosterone, −97.1 (SD = 7.6; $n = 83$). STs (μA) were as follows: untreated, 0.9 (SD = 0.5; $n = 45$); cholesterol, 1.0 (SD = 0.4; $n = 50$); testosterone, 1.0 (SD = 0.5; $n = 122$).

anterior face. It is difficult to compare these results with the present study because the effect and the number of electrocytes were not quantified. However, it is intriguing to consider that the effects of gonadal steroids on electrocyte action potentials differ between these two distantly related groups of teleost fishes: the principal effect in gymnotiforms may be on the action potentials' amplitude, while in mormyrids it is on their duration. This difference may further relate to a number of fundamental features distinguishing the electric organs and EODs of these two groups (23). Among mormyrids, EOD duration is the most prominent feature distinguishing the EODs of different species and sexes. Species differences have over a 30-fold range from 250 μsec to 8 msec, while sex differences may range from 0.8 msec to 3.0 msec. In gymnotiforms that generate pulse-like EODs (some species produce quasi-sinusoidal wave-like EODs), EOD durations range only from 1 to 5 msec (there are no detailed data on sex differences, but see ref. 22). From an anatomical perspective, the evolution of species differences in mormyrids focuses on the diversity in architecture of individual electrocytes. Among gymnotiforms, the evolution of species diversity in EODs relates more to the development of multiple sets of electric organs that fire asynchronously.

Anatomical studies have shown that an increased EOD duration in testosterone-treated mormyrids is correlated with an increase in the surface area of the electrocyte's membranes (13). If the conductive properties of the membranes remain unchanged, then changes in surface area may directly affect total membrane capacitance (24, 25). Unfortunately, we were not able to measure the capacitance of electrocytes accurately, owing to a large stimulus artifact unavoidably caused by our electrode configuration (see Fig. 2 *A* and *B*). Capacitative changes could potentially account for the observed increases in action potential duration (ref. 26, A.H.B. and B. Land, unpublished data). In any case, increases in membrane surface area appear to be confined to the anterior face of the electrocyte (13), while the data from double spikes, presumably generated separately by the two faces (6), indicate that both anterior and posterior face spikes are of longer duration. Changes in the surface area of the anterior face may effectively increase its capacitance so that a larger amount of the initial current flow across this face is capacitative, thus increasing the delay of spike initiation in the anterior face relative to that of the posterior face. An increase in delay time could ensure that the two spikes, which have undergone an increase in duration, do not have any appre-

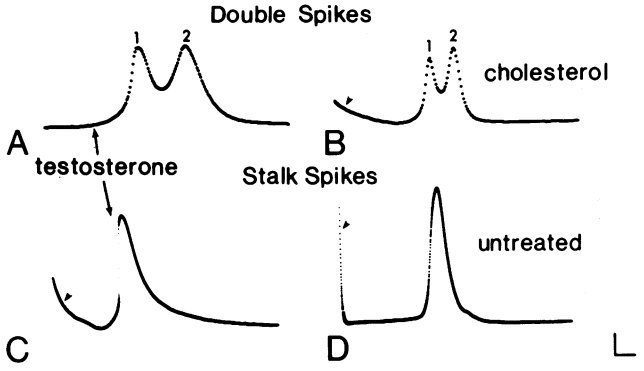

FIG. 3. (*A–D*) Additional classes of testosterone-sensitive spikes: double and stalk spikes. Action potentials recorded intracellularly from single electrocytes as in Fig. 2. Vertical bar scale in *A* is 20 mV for all records. Horizontal scale is 0.2 msec for *A* and *B* and 0.5 msec for *C* and *D*. Duration measurements as in Fig. 2. Double spikes: total (spikes 1 and 2 combined) duration in testosterone-treated specimens (*A*) was 2–3 times greater than cholesterol-treated (*B*) or untreated ones. Averages (msec) were as follows: testosterone, 0.78 (SD = 0.22; $n = 12$); cholesterol, 0.32 (SD = 0.09; $n = 6$); untreated, 0.28 (SD = 0.07; $n = 4$). Average duration (msec) of spike 1: testosterone, 0.27 (SD = 0.06; $n = 7$); cholesterol, 0.13 (SD = 0.01; $n = 2$); untreated, 0.10 (SD = 0.06; $n = 2$); spike 2: testosterone, 0.36 (SD = 0.11; $n = 9$); cholesterol, 0.15 (SD = 0.04; $n = 2$); untreated, 0.15 (SD = 0.03; $n = 2$). There were no significant differences ($P > 0.1$) between spikes 1 and 2 within any experimental group. Some spikes could not be measured because their falling or rising phase did not extend below half-amplitude values because of overlap of the two spikes. Stalk spikes: spike duration in the testosterone treatment group (*C*) was significantly greater than cholesterol ($P < 0.005$) or untreated (*D*) ($P < 0.01$) ones. Averages (msec) were as follows: testosterone, 0.62 (SD = 0.2; $n = 8$); cholesterol, 0.38 (SD = 0.05; $n = 4$); untreated, 0.33 (SD = 0.07; $n = 4$). The small number of cases in which we recorded stalk spikes most likely results from the lower probability of impaling a stalk branch, which is a thin cable-like structure (see Fig. 1*C*). In 9 of 13 cases, recordings of double spikes on one electrode coincided with recording a stalk spike on a second electrode. This is expected when the stalk electrode is used for stimulation, which might initiate a sequential activation of the two faces and so double spikes (6).

ciable temporal overlap so as to maximize the amplitude of an external potential. A delay between the spikes generated by the two faces of an electrocyte is essential to the formation of a biphasic external potential (6, 22, 27). An external pulse would not be generated if both faces produced spikes simultaneously, since they would cancel each other. Finally, the increases we found in spike duration in both electrocyte faces may be caused by changes in the distribution, number, or behavior of one or more types of ion channels.

A number of other studies have shown that steroid hormones can affect the morphology of cells that are implicated in the generation of communication signals (1–3, 5). In only a few cases have the neurophysiological properties of such cells been examined (22, 28–30); however, in many of these studies, the direct effects of steroids may be confounded by preferential use, innervation by steroid-concentrating motoneurons, or survival of distinct cell types. The increase in action potential duration that we have recorded is unlikely to be use dependent, since both treated and untreated fish produce EODs that are used in continuously ongoing orientation and communication behaviors (7, 8). It still remains possible that there are changes in the rate or frequency at which an EOD is generated (i.e., the EOD rhythm), which would be under central control (16). However, several lines of evidence, together with the present study, indicate that steroids exert their effects on the EOD pulse waveform by directly changing the anatomy and physiology of the electrocytes rather than components of the central electromotor pathway. First, autoradiographic studies do not indicate that steroids exert a direct effect on the central electromotor pathway either at the level of spinal electromotoneurons or medullary nuclei that control the excitation rate of the electric organ (20). Second, neurophysiological recordings show that steroids do not affect the temporal properties of the spinal command signal, which underlies the excitation of an EOD (4, 12). Third, steroids exert a dramatic effect on the anatomical properties of the entire complement of adult electrocytes (13). Lastly, biochemical data indicate that mormyrid electrocytes have high levels of androgen-binding activity in comparison to trunk muscles (20). It is the development and evolution of this steroid sensitivity that underlies the sexual dimorphisms in EODs and in the surface membranes and action potentials of mormyrid electrocytes.

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