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Androgen binding in the brain and electric organ of a mormyrid fish

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Summary. The mormyrid fish of Africa produce a weak electric pulse called an Electric Organ Discharge (EOD) that functions in electrical guidance and communication. The *EOD waveform* describes the appearance of a single pulse which is produced by the electric organ's excitable cells, the electrocytes. For some species, there is a sex difference in the appearance and duration of the EOD waveform, which is under the control of gonadal steroid hormones. We now show, using biochemical techniques, that the steroid-sensitivity of the myogenic electric organ correlates with the presence of comparatively high levels of androgen-binding activity in the cytosol of electrocytes.

The *EOD rhythm* describes the rate at which the electric organ fires and is under the control of a central electromotor pathway. Sex differences have also been described for the EOD rhythm. Using steroid autoradiographic techniques, we found uptake of tritium-labelled dihydrotestosterone (^{3}H-DHT) by cells within the reticular formation that lie adjacent to the medullary 'relay nucleus' which innervates the spinal electromotoneurons that excite the electric organ. However, no DHT-binding was observed in the relay or electromotor nuclei.

Steroid-concentrating cells were also found in several other brainstem regions, the hypothalamus, and the thalamus. In particular, a group of DHT-concentrating, motoneuron-like cells were observed in the caudal medulla and were identified as a *swimbladder* or *sonic motor nucleus*.

The biochemical data suggest that the electric organ has evolved a sensitivity to gonadal steroid hormones that may underlie the development of known sex differences in the EOD waveform. The autoradiographic results suggest that if steroids do affect the development of sex differences in the EOD rhythm, it is at some level removed from known spinal and medullary electromotor nuclei.

Abbreviations: *ac* anterior commissure; *AD* area dorsalis telencephali; *AV* area ventralis telencephali; *CBL* cerebellum; *DT* dorsal thalamus; *E* electromotoneuron; *En* entopeduncular nucleus, *ef* lateral line efferent nucleus; *EG* eminentia granularis; *ELLL* electroreceptive lateral line lobe, *EO* electric organ, *FV* folded part of valvula of cerebellum; *H* hypothalamus; *M* mesencephalon; *MO* medulla oblongota; *OB* olfactory bulb; *OT* optic tectum; *PO* preoptic area; *R* medullary relay nucleus; *rf* reticular formation; *SC* spinal cord; *SMN* sonic motor nucleus; *T* telencephalon; *TP* posterior tuber of diencephalon; *TS* torus semicircularis; *UV* unfolded part of valvula of cerebellum, *v* ventricle; *VT* ventral thalamus

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Introduction

The mormyrid fish of Africa produce an electrical pulse, the Electric Organ Discharge (EOD), which functions in social communication and object detection or electrolocation (reviews: Heiligenberg 1977; Hopkins 1983). The EOD has two elements referred to as the EOD waveform and the EOD rhythm. The EOD waveform describes the shape of a single electric pulse which is produced by the specialized muscle-like cells (see Szabo 1960; Denizot et al. 1982) or electrocytes of the electric organ. The excitable membranes of a single electrocyte generate action potentials whose summed activity predicts the appearance of the entire EOD waveform (Bennett and Grundfest 1961). In some species, the EOD waveform is sexually dimorphic (Hopkins 1980; Moller 1980; Hopkins and Bass 1981; Westby and Kirschbaum 1982; Bass and Hopkins 1983, 1984, 1985), and androgens (testosterone or 5α-dihydrotestosterone) can induce females and juveniles to produce a signal that mimics the EOD waveform of sexually mature males (Bass 1986a; Bass and Hopkins 1983, 1984, 1985). Changes in the EOD waveform are correlated with

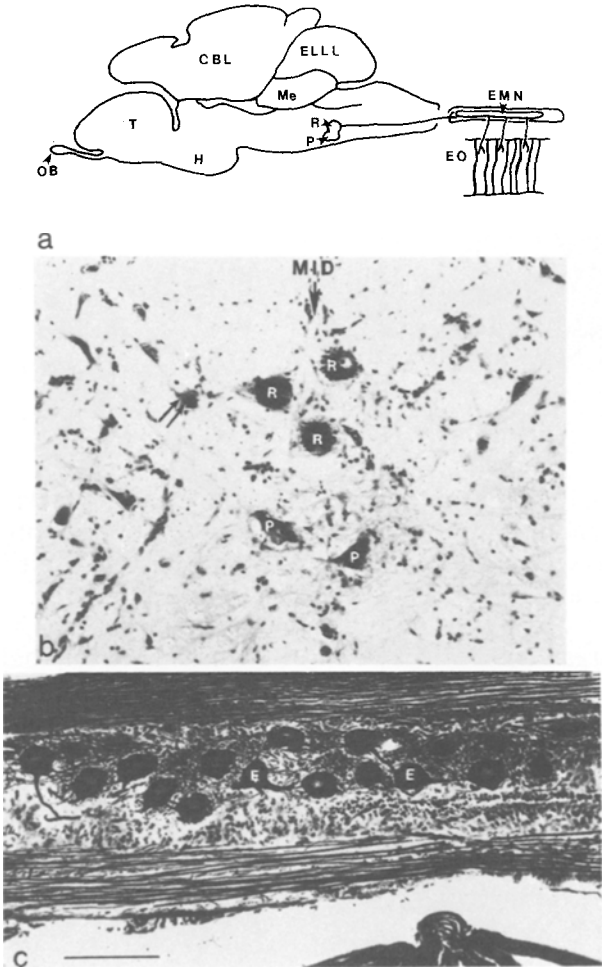

Fig. 1. a Line drawing of a sagittal view of a mormyrid brain illustrating the electromotor pathway. Shown are the spinal electromotor nucleus (*EMN*) which is directly innervated by a medullary relay nucleus (*R*). The relay nucleus is innervated in turn by a ventrally adjacent pacemaker nucleus (*P*). Both R and P have additional connections as described in Bell et al. (1983). See text for details. **b** Photomicrograph of Nissl-stained, transverse section through the medulla of *Brienomyrus brachyistius* (long biphasic) at the level of the relay (*R*) and pacemaker (*P*) cells. Double arrows point to 'other' cells within the medial reticular formation that lie near the relay neurons. Midline is indicated (*MID*). **c** Photomicrograph of a Bodian-stained, sagittal section through the spinal cord of *B. brachyistius* (long biphasic) showing the electromotor neurons. Bar scale for **b** and **c** 100 μm

morphological (Bass et al. in press) and physiological (Bass and Volman 1985) changes in individual electrocytes.

The EOD rhythm is the rate at which the electric organ produces an EOD waveform, and is under the control of a central electromotor pathway. Although sex differences have been reported for the EOD rhythm of mormyrids (Lucker and Kramer 1981), there are no reports of the possible effects of steroid hormones on the EOD rhythm or its associated motor pathway (but see Meyer 1983 for a gymnotiform electric fish). The central motor pathway that innervates the electric organ of mormyrids has been described in detail elsewhere (Bennett et al. 1967; Bell et al. 1983). The electric organ is innervated by the electromotor nucleus (EMN) which is found in the spinal cord at the level of the electric organ (Fig. 1a, c). The EMN receives direct input from a 'relay' nucleus in the medulla (Fig. 1a, b). The relay nucleus (Szabo 1957; Bennett et al. 1967) lies in the midline reticular formation and contains 25–30 (40–50 μm diameter) cells (R, Fig. 1a, b). Recent anatomical and physiological studies (Bell et al. 1983; Elekes et al. 1985) have identified 15–20 smaller (12–25 μm) cells that lie directly ventral to the relay nucleus and are identified as a 'pacemaker nucleus' (P, Fig. 1a, b). The pacemaker nucleus directly innervates the more dorsal relay nucleus and is considered to make all 'final' decisions as to the excitation rate of the electromotor nucleus, and its target, the electrocytes (Grant et al. 1986).

We have now used biochemical techniques and steroid autoradiography to characterize androgen-binding in the electric organ and the brain of mormyrids. Biochemical methods suggested the presence of an androgen receptor protein in the electric organ. Autoradiography revealed steroid uptake in several brain regions including a swimbladder motor nucleus. Steroid-concentrating cells were *not* found in the known medullary relay or spinal electromotor nuclei. However, one candidate group of labelled cells in the medial reticular formation might affect the activity of the central electromotor pathway. Portions of the results have appeared elsewhere (Bass et al. 1984).

Material and methods

Steroid autoradiography. Autoradiographic studies were carried out with a species from Gabon, West Africa – *Brienomyrus brachyistius* (long biphasic), which has a steroid-sensitive EOD waveform (Bass and Hopkins 1983; Bass 1986a). Three females and two males were castrated at least one week prior to injection of 50 μCi of 1, 2, 4, 5, 6, 7, 16, 17-^{3}H-dihydrotestosterone (DHT) (New England Nuclear, specific activity: 200 Ci/mM). Just prior to injection, the DHT was evaporated with a rotary evaporator from a benzene-ethanol mixture and redissolved in a mixture of 25 μl of 100% ethanol and 25 μl of 0.65% saline. Injections were made intraperitoneally with a Hamilton syringe. Two hours after injection, the fish were anesthetized by cold narcosis and then decapitated. The brain and tail (which contains the electric organ and the spinal electromotoneurons (see Bass 1986b) were removed and quickly frozen with powdered dry ice on a cryostat chuck. All material was sectioned on a Bright cryostat (−16 °C) at 10 μm in a darkroom equipped with a Thomas Duplex Safelight. Three brains (two female, one male) were cut in the horizontal plane and two (one female,

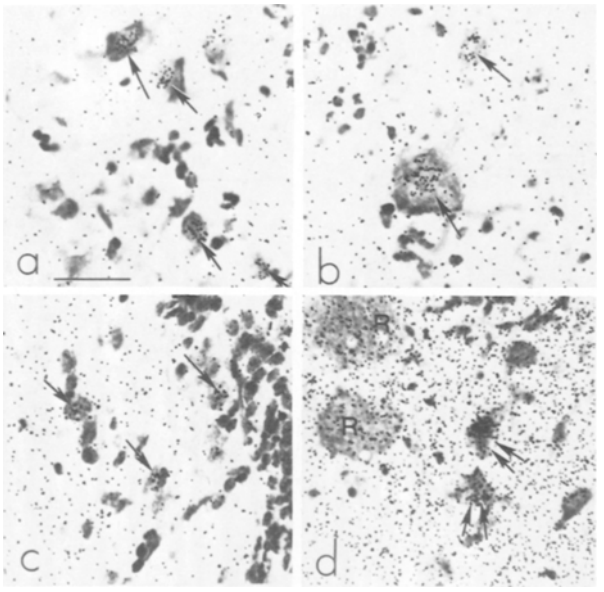

Fig. 2a–d. Photomicrographs of Nissl-stained, horizontal sections from *Brienomyrus brachyistius* (long biphasic) illustrating examples of DHT-concentrating cells (arrows). **a** Sonic motor nucleus, small-celled division. **b** Sonic motor nucleus, large-celled division. **c** Ventral thalamus. **d** Labelled cells (double arrows) in the medial reticular formation that lie adjacent to unlabelled relay (*R*) cells. Bar scale is 40 μm for **a–d**

one male) in the transverse plane. The electric organs and spinal cord were cut in the horizontal plane. Every third section was picked up on a slide that had previously been coated with Kodak NTB-3 nuclear track emulsion. Sections were allowed to dry at room temperature for two hours and then placed in light tight boxes containing dessicant and stored at 4 °C. After periods ranging from 2 months to 1 year, the slides were developed in Kodak D-19 developer at 16 °C, fixed, and counterstained with cresyl violet or methylene blue (procedure courtesy of Dr. Joan Morrell of Rockefeller University).

All autoradiograms were scanned at 400×. The number of grains over a nucleus (or the entire soma in those cases where a nucleus was not readily discernable, e.g. (Fig. 2c) was counted at 1,000×. Grain counts were also made over ten adjacent neuropil zones of similar area and an average background grain count was determined. A cell was considered labelled if the grain count over the nucleus or soma exceeded or equaled the 99% confidence interval derived from a Poisson distribution based on the mean background grain density (Arnold 1980, 1981).

One male of a closely related species, *Brienomyrus niger* (which also has a steroid-sensitive EOD waveform; Bass 1986a), was castrated and injected with an ethanol-saline mixture. Following sacrifice as above, brain sections were mounted on pre-exposed, emulsion-coated slides or unexposed emulsion-coated slides and then processed for steroid autoradiography as above. This material controlled for fading of the latent image and artifactual grain reduction respectively (Rogers 1973). Additional reference brain series for *Brienomyrus brachyistius* (long biphasic) were prepared from paraffin embedded material cut in the transverse or horizontal plane at 15 μm and counterstained with cresyl violet.

Androgen binding activity. Studies of androgen-binding activity in the electric organ were carried out with *Brienomyrus* sp. (also referred to as *B.* sp. (2) in Bass 1986a and Bass and Hopkins 1984), a species available commercially from American fish dealers. *Brienomyrus* sp. has a steroid-sensitive EOD waveform (Bass 1986a; Bass and Hopkins 1984) and is the focus of our studies of the effects of steroid hormones on the anatomy and physiology of electric organs (Bass et al. 1984, in press; Bass and Volman 1985).

The amount of androgen-binding was estimated from the binding of a radioactive steroid to muscle cytosol in vitro (method of Ginsburg et al. 1974 with modifications of Sheets et al. 1985). Each assay was based on a pooled sample of electric organs from 6–10 male or female *Brienomyrus* sp. gonadectomized 6–7 days prior to the experiments. Animals were gonadectomized to clear endogenous steroid from the circulation which would otherwise interfere with our assay. Animals were sacrificed by cold narcosis and kept on crushed ice during the dissection. The electric organ consists of four columns, two on either side of the midline, of wafer-shaped cells called electrocytes (see Bass 1986b, c). Scales, overlying connective tissues, and tendons were removed to reveal the electrocytes. Each column was then dissected free with a razor blade and placed in TEGMD buffer (pH = 7.4, 0.01 *M* Tris, 1.5 m*M* EDTA, 10% glycerol, 0.02 *M* sodium molybdate, 1.0 *M* dithiothreitol, Sigma) at 4° C. The tissue was minced and then homogenized in the TEGMD buffer. The homogenate was centrifuged at 40,000 RPM for 45 min in an SW 50.1 rotor (Beckman) and the supernatant was removed for preparation of the incubates. The protein concentration of the cytosol fraction was determined according to the method of Bradford (1976).

Cytosol was incubated with a radioactive synthetic androgen, methyltrienolone (^{3}H-R1881, 87Ci/m*M*, New England Nuclear). R1881 was used because of, as Max (1981) points out, its selective binding to androgen receptors (Bonne and Raynaud 1976), but not serum proteins (Bonne and Raynaud 1975), and because it remains unmetabolized under conditions employed in our assays (Tremblay et al. 1977; Snochowski et al. 1980). Cytosol was incubated at 4 °C for 4 h. Following incubation, labelled R1881 bound to receptor protein was separated from unbound steroid by gel filtration through sephadex LH-20 (Sigma) columns.

The amount of binding and binding affinity were determined by saturation analysis (Scatchard 1949). Cytosol fractions were incubated with concentrations of (^{3}H)-R1881 ranging from 0.1–10 m*M*. To control for nonspecific binding, parallel incubates contained a 100 fold excess of unlabelled R1881 (New England Nuclear). Results were analyzed by the method of Scatchard (1949). Values for B_{max} and K_d were obtained by linear regression of the data from the Scatchard analysis.

In an additional set of experiments, binding activity was assayed by incubating the cytosolic fraction with one concentration (at least 5.0 m*M*) of ^{3}H-R1881. Cytosol was incubated with and without a 100 fold excess of unlabelled R1881 and specific binding was determined as above.

The specificity of the (^{3}H)-R1881 binding was further evaluated in a set of competition experiments with 17α-methyltestosterone (MT), dihydrotestosterone (DHT), 17-β-estradiol (E) and β-corticosterone (C) (Sigma Chemical). Either MT, DHT, E, or C was substituted for non-radioactive R1881, at concentrations in 10-, 100-, and 1000-fold excess of (^{3}H)-R1881.

Steroid injections. We tested the effectiveness of R1881 in inducing elongation of the EOD. Four specimens of *Brienomyrus* sp. (two males, 78 and 85 mm standard length; two females, 80 and 85 mm) received two injections, separated by 24 h, of 10 μl of cold R1881 (10^{-2} *M* in absolute ethanol). Injections were made into the dorsal epaxial musculature. Four other specimens (three males, 65–100 mm; one female, 78 mm) served

as vehicle controls and received two injections of 10 µl of absolute ethanol into epaxial muscle.

Results

Steroid autoradiography. Figure 3a is a line drawing of a sagittal view of a *Brienomyrus* brain, showing the approximate horizontal levels illustrated in Figs. 3–5. The position of DHT-labelled cells in a male brain are illustrated in line drawings of horizontal brain sections of *Brienomyrus brachyistius* (long biphasic). We detected no reliable differences in labelling between males and females. Within the forebrain, labelled cells (7.5 µm diameter) were found in the posterior tuber region of the diencephalon, just lateral to the periventricular cells of the third ventricle (Fig. 3b). A few, scattered DHT-concentrating cells (5–7 µm) were also seen in the thalamus (Figs. 2c, 4b, 5a, b). The largest populations of labelled cells, however, were located in several regions of the medulla. These cells will be the focus of our report as they are most relevant to our studies of the electromotor (and sonic motor; Bass 1986d, e) pathway and represent new data on gonadal steroid-binding in a teleostean brain.

The medullary relay nucleus in *B. brachyistius* (long biphasic) has a rostral-caudal extent of about 500 µm. We did not find any steroid-concentrating cells in the electromotor or relay nuclei. However, there was a large population of small (12 µm), labelled cells in the medial reticular formation dorsal, lateral and caudal to the relay nucleus (rf, Figs. 4a, b, 5a). A pair of larger, fusiform shaped (12 × 25 µm), DHT-concentrating cells were found about 30–40 µm lateral to the relay neurons (Figs. 2d, 4a). Morphologically, these cells resemble those of the pacemaker nucleus although they lie dorsal and lateral to that nucleus. These cells have been identified in one male brain.

A major area containing labelled cells was found at the caudal end of the vagal motor column. Two size classes of cells were labelled. One group of large, oval-shaped (25–30 µm) cells was found near the midline. Smaller (12–15 µm) cells lie rostral and lateral to the large cells, scattered throughout the vagal motor region. We designated these cells as belonging to a swimbladder or sonic motor nucleus (SMN, Figs. 2a, b, 5b) (see Discussion).

Androgen binding. Analysis by linear regression of a representative Scatchard plot presented in Fig. 6 indicates an apparent affinity of R-1881 for its binding component in the cytosol of $K_d = 4.2$ n*M*.

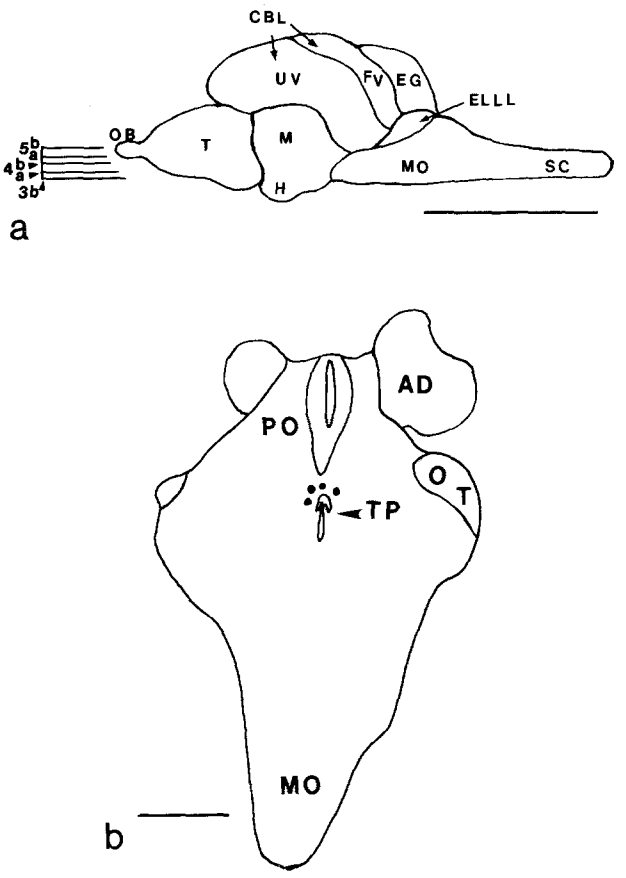

Fig. 3. a Line drawing of sagittal view of *Brienomyrus* brain showing approximate levels of line drawings of horizontal sections in Figs. **b**, 4a, b, 5a and b. Bar scale 5 mm. **b** In this, and Figs. 4 and 5, position of actual numbers of DHT-labelled cells in single horizontal sections is shown by dots. Diameters of dots have no relation to a cell's actual dimensions. Shown here are labelled cells in the area of the posterior tuber (*TP*) zone of the diencephalon. Bar scale 1 mm

The average from three separate assays (6–10 animals per assay) was 3.5 n*M*, (±0.7). The extrapolated maximum binding from one assay is presented in Fig. 6 and is about 20 f*M*/mg cytosolic protein. The average of the same three assays was 20.8 f*M*/mg (±5.3) cytosolic protein.

Results of single concentration (one-point) assays are presented in Table 1 and are in general agreement with the value obtained for B_{max} by the Scatchard analysis. As with all other experiments, each assay was done on pooled tissue from 6–10 animals. The average value from 4 separate assays of male electric organ was 16.1 (±3.4) femtomoles (f*M*)/mg cytosolic protein, and for 2 assays of female electric organ was 15.1 (±2.5) f*M*/mg. The free ligand concentration used in these assays ranged from 6.4 to 31.4 n*M*/mg cytosolic protein. In spite of this large range of ligand concentration, variation in binding activity is low, con-

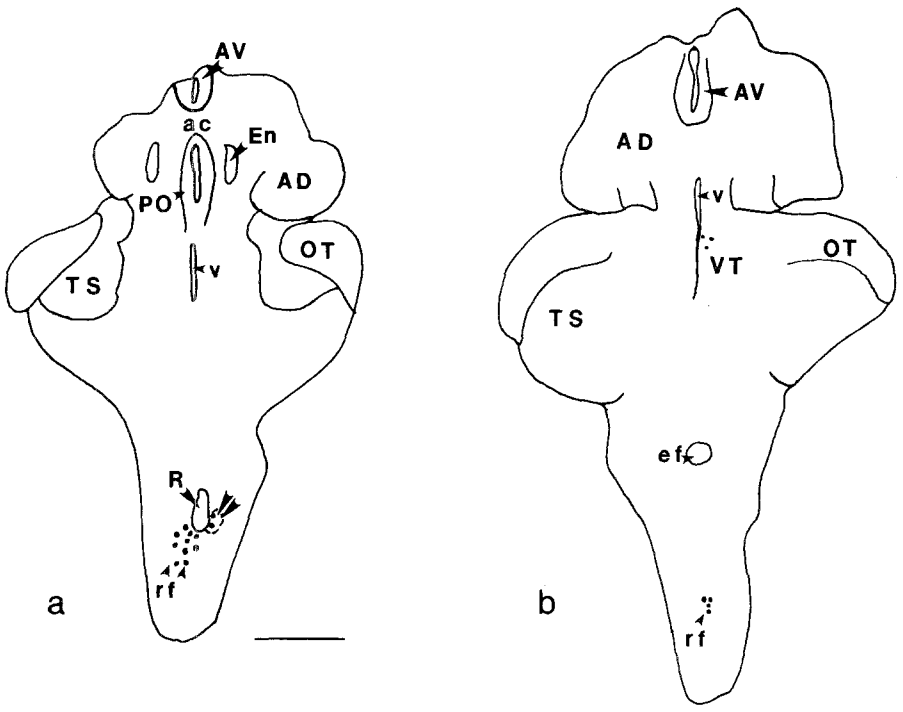

Fig. 4. a Line drawings at level of the medullary relay nucleus (*R*). The two labelled cells in Fig. 2d are indicated with a double arrowhead. Labelled cells are also found in the medial reticular formation (*rf*) that lies caudal and lateral to the relay and pacemaker cells. **b** A section at the level of the efferent nucleus of the lateral line nerve (ef) showing labelled cells in the ventral thalamus (*VT*) and again in the medial reticular formation (*rf*). Bar scale 1 mm for **a** and **b**

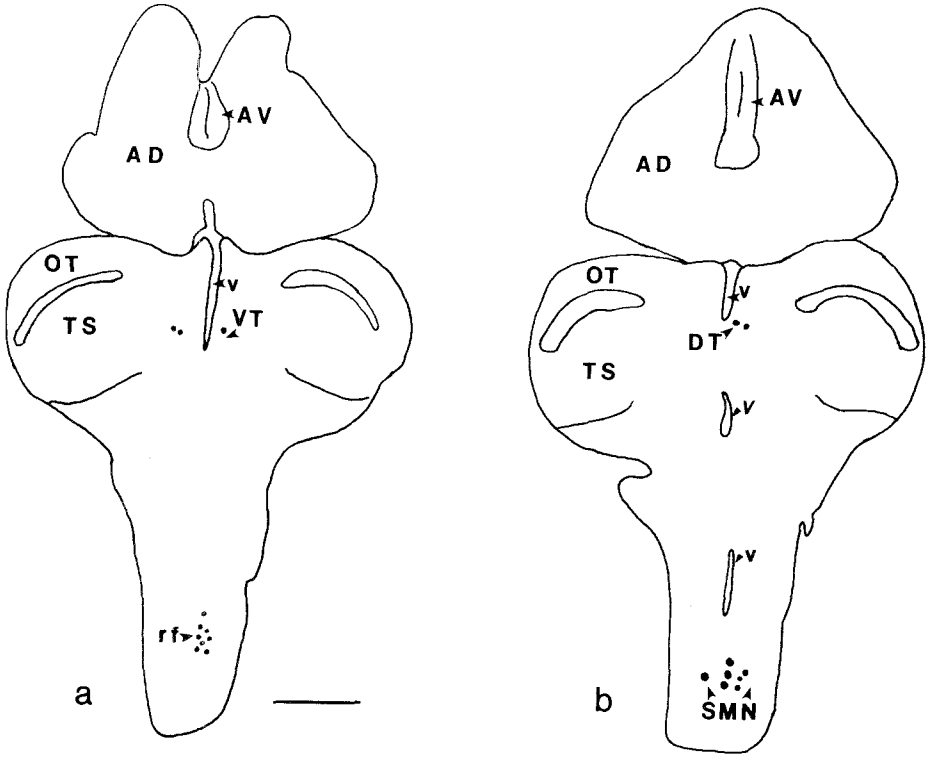

Fig. 5. a Labelled cells are scattered throughout the ventral thalamus (*VT*) and reticular formation (*rf*). **b** Labelled cells are also found more dorsal in the thalamus (*DT*) and in a nucleus at the caudal end of the vagal motor column that is identified as a sonic motor nucleus (*SMN*). The SMN spans the midline and consists of two size classes (see Fig. 2). Bar scale 1 mm for **a** and **b**

firming that our putative androgen receptor was saturated under these conditions (Table 1). Although our preliminary data suggested a sex difference in binding activity (Bass et al. 1984); additional assays did not confirm this result.

Due to the limited amount of tissue available, only three competitor steroids were used; these results are presented in Fig. 7. Percentage competition was adjusted for non-specific binding of R1881. DHT competes with 100% efficiency rela-

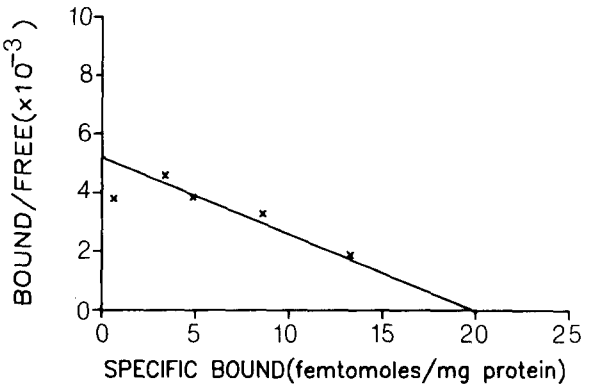

Fig. 6. Scatchard plot of ^{3}H-R1881 binding to cytosol from male electric organ. The B_{max} is 19 femtomol/mg cytosolic protein, while the Kd is 4.2 n*M*

Table 1. Single concentration assays for androgen binding activity in the electric organ of *Brienomyrus* sp.

Electric organ		Epaxial muscle	
Binding Activity (f*M*/mg)[a]	R-1881 (n*M*/mg)[b]	Binding Activity (f*M*/mg)[a]	R-1881 (n*M*/mg)[b]
Males			
13.1	7.5		
20.1	6.4		
13.5	9.1	1.7	5.8
17.9	31.4	0.6	6.2
Females			
13.3	11.5		
16.8	28.6	0.0	5.4

[a] Binding activity expressed in femtomol (f*M*)/mg cytosolic protein

[b] Concentrations of free ligand (R-1881) in nanomol (n*M*)/mg protein

tive to R1881 at the 10× concentration of (^{3}H)-R1881, the lowest concentration used in the competition studies. At this same concentration, 17α-methyl testosterone displaced approximately 70%, and 17-β estradiol about 50%, of specifically bound R1881. At 100× excess, methyl-testosterone still competes poorly, while estradiol competes as well as DHT. In one experiment, enough tissue was available to carry out a competition with a 100 fold excess of β-corticosterone (Sigma) (Fig. 7). The result was a very low level of competition (19%) compared to DHT and estradiol, which again competed equally well. The greater effectiveness of DHT than estradiol as a competitor at 10× concentrations suggests that (^{3}H)-R1881 binding is androgen specific. Although DHT competes very efficiently with R1881, we do not know if DHT is an active steroid in this system. Further studies

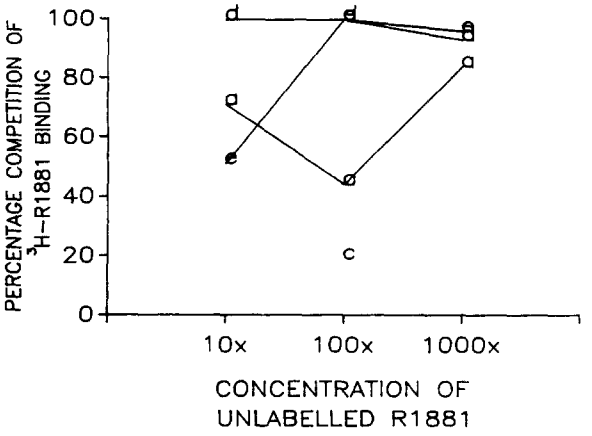

Fig. 7. Receptor specificity. Competition for specific binding in male electric organ. Cytosol was incubated with 6 m*M* (^{3}H)-R1881 for 4 h at 4 °C with 10 fold, 100 fold and 1000 fold excess of DHT (*d*), 17β-estradiol (*e*) or 17 α-methyl-testosterone (*a*) and a 100 fold excess of β-corticosterone (*c*). Competitions are expressed as percentage competition of specifically bound ^{3}H-R1881 as adjusted for non-specific binding of R1881

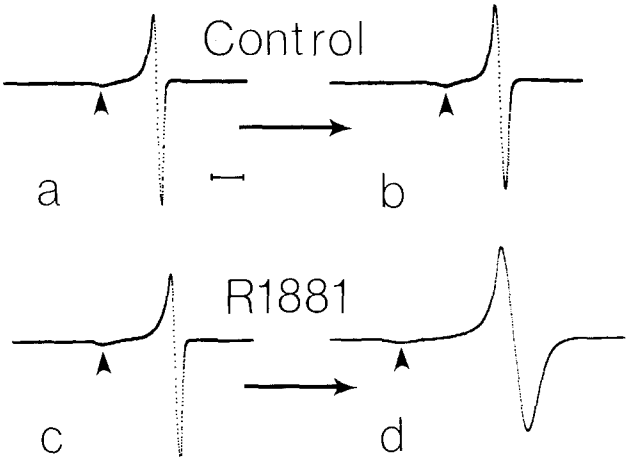

Fig. 8 a–d. Steroid injections. Shown are oscilloscope records of the EOD of *Brienomyrus* sp. scaled to the same peak-to-peak amplitude. EODs were measured differentially with Ag/AgCl electrodes, positive polarity near the head. **a, b** Following two injections of absolute ethanol, there was no significant change in the EOD duration of a male (65 mm, standard length) five days after (**b**) the first injection (**a**). **c, d** In contrast, two injections of R1881 dissolved in ethanol induced a two fold increase in the EOD duration of a female (80 mm) five days after (**d**) the first injection (**c**). The EOD has a small initial negative phase as indicated by the arrowheads in **a–d**. Bar scale in **a** equals 0.2 ms

are underway testing other steroids, in particular 11-keto-testosterone, which has been shown to be an active steroid in other fishes (see Pottinger and Pickering 1985) and also induces elongation of the EOD waveform (A.H. Bass, unpublished observations).

In order to compare androgen binding levels in electric organ with another myogenic derivative,

we assayed dorsal epaxial trunk muscle from the same animals used for the electric organ assays. Assays of epaxial muscle from either males or females indicated little or no binding activity. In those cases where activity was found, it ranged from 0.6 to 1.7 f*M*/mg protein for (^{3}H)-R1881 concentrations ranging from 5.4 to 6.2 n*M*/mg protein (Table 1). Since epaxial assays were done at the same time as those on electric organs from the same animals, it is clear that higher levels of androgen binding are present in the electric organ.

Steroid injections. The average EOD duration doubled in the four specimens treated with R1881 (e.g., Fig. 8c, d), increasing from an average of 0.6 ms (SD = 0.0) to 1.2 ms (SD = 0.05). The four vehicle controls (e.g., Fig. 8a, b) showed no change in their average EOD duration of 0.6 ms (SD = 0.05).

Discussion

Steroid autoradiography

Electromotor pathway. To our knowledge, this report for mormyrids is the first study identifying DHT-binding sites in the central nervous system of any fish (also see below). One intriguing result was the discovery of DHT-labelled cells in the medial reticular formation adjacent to the medullary relay nucleus which innervates electromotoneurons. We do not know if the labelled reticular cells contact relay or pacemaker cell processes. However, other studies show that the dendritic processes of pacemaker (Bell et al. 1983; Elekes and Szabo 1985; Grant et al. 1986) and relay (Elekes et al. 1985; Grant et al. 1986) cells extend laterally into the reticular formation or neighboring fiber bundles. Steroid-binding cells in the reticular formation could affect the activity of either pacemaker or relay cells if they in fact establish functional contacts with those cells. If shown, this would be strong evidence suggesting that steroid hormones may influence the activity of the central electromotor pathway and so affect the development of known sex differences in the EOD rhythm (Lucker and Kramer 1981). Only more detailed studies can resolve these questions.

Steroid hormones are known to affect the development of sex differences in the EOD rhythm of gymnotiform electric fish (Meyer 1983). In this case, steroids have been shown to affect the firing rate of a medullary pacemaker nucleus which determines the EOD rhythm (Meyer 1984; Meyer et al. 1984). To date, autoradiographic studies have not identified steroid-concentrating cells in the pacemaker nucleus of gymnotiforms (H.J. Meyer, A.H. Bass, D.B. Kelley unpublished studies).

Sonic motor pathway. We also found binding in two size classes of cells at the caudal end of the vagal motor column. Based on their location in the brainstem, these cells are possible candidates for swimbladder or sonic motoneurons (a midline, caudal brainstem nucleus in other teleosts; Pappas and Bennett 1966; Fine et al. 1982; Bass 1986d, e). The tentative identification of a sonic motor nucleus in mormyrids is reinforced by other studies in which a similar group of cells have been retrogradely labelled with horseradish peroxidase (HRP), following HRP injections into the putative sonic musculature of another mormyrid, *Brienomyrus* sp. (Bass 1986e). Sound production has been recently reported for mormyrids in reproductive condition (Crawford et al. 1985), suggesting a possible role of gonadal steroids in this behavior.

Comparisons with other studies. Several reviews discuss the presence of steroid binding cells in the brains of different classes of vertebrates (Morrell et al. 1975; Kelley and Pfaff 1978; Kim et al. 1978a; Demski 1984). For brevity we will discuss only pertinent aspects of those studies on fish.

Steroid autoradiographic studies have been done in six species of fish: male and female platyfish (*Xiphophorus maculatus*) (estradiol-binding; Kim et al. 1979); male sunfish (*Lepomis cyanellus*) (testosterone-binding; Morrell et al. 1975); male and female adult lampreys (*Ichthyomyzon unicuspis*) (estradiol-binding; Kim et al. 1980); male paradise fish (*Macropodus opercularis*) (testosterone and estradiol-binding; Davis et al. 1977), male and female goldfish (*Carassius auratus*) (estradiol-binding; Kim et al. 1978b), and male and female toadfish (*Opsanus tau*) (testosterone-binding; Fine et al. 1982).

In general, estradiol and testosterone binding is reported in the ventral hypothalamus and telencephalon (area ventralis telencephali), the preoptic area, and the thalamus. We have seen DHT-binding in all of these areas in mormyrids excepting the preoptic area and the telencephalon proper. Only Davis et al. (1977) and Fine et al. (1982) examined the brainstem and spinal cord for binding sites. Davis et al. report no uptake of labelled testosterone or estradiol in the medulla or spinal cord of paradise fish. Fine et al. report four testosterone-binding sites in the brainstem of toadfish: the midbrains' torus semicircularis (homologue of the mammalian inferior colliculus) and optic tectum

(homologue of the mammalian superior colliculus), and two medullary nuclei identified as nucleus ventrolateralis medullae and nucleus periventricularis medullae. Steroid-binding to motor neurons was not reported in any of the above studies.

Androgen receptor biochemistry

We attempted to localize steroid-binding sites in the electric organ using steroid autoradiography. However, a high level of background, which is commonly seen in muscle steroid autoradiography (Darcy Kelley, personal communication), prevented us from distinguishing any labelled nuclei. As an alternative strategy, we initiated a series of biochemical assays to determine if the myogenic electric organ binds androgens. Our biochemical studies suggested the presence of a cytosolic receptor protein in the electric organ of male and female *Brienomyrus* sp. For comparison, we examined dorsal epaxial muscle, another myogenic tissue. In contrast to the electric organ, we found little or no evidence of androgen-binding activity in epaxial muscle.

It is important to note that the male and female *Brienomyrus* sp. used in this study had similar EODs; a characteristic of this species when maintained in captivity in non-reproductive condition. The EOD of male *Brienomyrus* sp. probably undergoes seasonal changes in duration, as in other mormyrids (Bass 1986a). In addition to the similarity in their EODs, the electrocytes of non-reproductive males and females have similar morphological (Bass et al., in press) and physiological (Bass and Volman 1985) properties. For such individuals, testosterone induces a 2–3 fold increase in the duration of the EOD waveform (Bass and Hopkins 1984; Bass and Volman 1985) which is paralleled by changes in electrocyte morphology (Bass et al. in press) and a 2–3 fold increase in the duration of action potentials generated by individual electrocytes (Bass and Volman 1985; see Bass 1986c and Bass et al., in press, for more detailed discussions). The discovery of high androgen-binding levels in the electric organ reinforces our suggestion that gonadal steroid hormones directly affect the properties of electrocytes.

Our assays were limited by the small number of animals of our study species that were commercially available and the limited amount of tissue available from each animal (each electric organ weighs about 50 mg) for preparing cytosol fractions. While we can characterize the electric organ as an androgen-target tissue, further study is needed to rigorously define absolute quantities of binding activity and the specificity of binding. The small number of steroids tested could not adequately test the specificity of the R1881 binding activity. However, we feel that R1881 was the ligand of choice since it exerted a strong behavioral effect, i.e., induced elongation of the EOD waveform. When we know the endogenous active steroid in mormyrids, further receptor specificity analyses will be performed.

When estradiol was present in concentrations of 100 fold excess that of the R1881, it displaced about 50% of R1881 binding. Fox (1975) has suggested the presence of a steroid receptor in brain extracts that interacts with both androgens and estrogens (also see Sheridan 1983). Fox studied androgen (testosterone and DHT bind to 'the same macromolecular entities') and estrogen binding in extracts of the hypothalamus and preoptic area of mice. A 500-fold excess of estradiol blocked 83% of ^{3}H-testosterone binding when incubated below saturation levels of testosterone. A 500 fold excess of testosterone also blocked, though less efficiently (42%), ^{3}H-estradiol binding below saturation levels. Fox suggested a mechanism whereby brain receptors detect 'the relative concentrations of androgens and estrogens', rather than independently assessing the concentration of each steroid. Both 17-β-estradiol and androgens can induce changes in the EOD waveform (Bass 1986a), although the relative potencies of each steroid in effecting a response have not yet been determined. If mormyrids have significant circulating plasma levels of both androgens and estrogen, the electric organ may also have a receptor that interacts with both major classes of steroids.

For comparison, we note that several other myogenic structures involved in the motor control of reproductive behavior also have high levels of androgen-binding and are characterized as androgen-target tissues. These include the larynx of the frog, *Xenopus laevis* (Segil et al. 1983), the syrinx of zebra finches (Lieberburg and Nottebohm 1979), the penile muscles of rats (Dube et al. 1976) and the swimbladder drum muscles of toadfish (A.H. Bass and N. Segil, unpublished observations). We can now also characterize the electric organ of mormyrids as an androgen-target tissue.

To summarize, the sexual dimorphism in EOD waveforms is due in part or in whole to their sensitivity to androgens. Androgen-binding in the electric organ can allow for changes in the EOD waveform. While sex differences have been reported for the EOD rhythm of mormyrids (Lucker and Kramer 1981), we have not discovered androgen-binding in any known portion of the central elec-

tromotor pathway, excepting of course the electric organ itself.

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