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Development and Hormone Regulation of Androgen Receptor Levels in the Sexually Dimorphic Larynx of *Xenopus laevis*

DARCY KELLEY, DAVID SASSOON, NEIL SEGIL, AND MICHAEL SCUDDER

Department of Biological Sciences, Columbia University, New York, New York 10027

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Development of the sexually dimorphic larynx in African clawed frogs is controlled by secretion of androgenic steroids (D. Sassoon and D. Kelley, 1986, *Amer. J. Anat.* **177**, 457-472). Adult laryngeal muscle shows high levels of androgen binding relative to other skeletal muscles and binding activity in males is three times that in females (N. Segil, L. Silverman, and D. Kelley, 1987, *Gen. Comp. Endocrinol.* **66**, 95-101). To determine when androgen sensitivity and sex differences arise, we assayed [^{3}H]dihydrotestosterone (DHT) binding activity in larynges from metamorphic and postmetamorphic male and female frogs. Scatchard analyses indicate that DHT binds to a saturable component with high affinity. At metamorphosis, male and female juveniles have average binding levels of 262 and 269 fmoles/mg protein, respectively, approximately 7 to 20 times their adult values. At 3 months postmetamorphosis (PM), sexually dimorphic binding levels are observed. Binding activity declines gradually in females from metamorphosis to 9 months PM. In males, levels of binding activity remain high throughout the first 6 months PM and then decrease to near adult levels by 9 months PM. Administration of exogenous DHT to 3 months PM juveniles decreases average binding activity from 180 (male) or 74 fmoles/mg (female) to 33.5 fmoles/mg in both sexes. Testosterone has a less pronounced effect on binding activity in males than DHT and is ineffective in females. We conclude that sexually dimorphic adult levels of androgen binding in larynx arise by differential decrease from initially high, sexually monomorphic levels and that high titers of circulating androgens normally present by 6 months PM in males are responsible for the marked decrease in binding activity observed during laryngeal development.

INTRODUCTION

Sexual differentiation in vertebrates is largely under the control of steroid hormones secreted during development. Only a limited subset of cells responds to steroids. Responsive cells contain intracellular androgen receptors which, when complexed to steroid, act as DNA-binding proteins and can alter gene transcription (Eriksson and Gustafsson, 1983; Yamamoto, 1985). A central problem of sexual differentiation is identifying the cellular and molecular events controlled by hormone secretion during development. We have approached this question by studying the larynx, the sexually dimorphic vocal organ, of clawed frogs (*Xenopus laevis*).

The *X. laevis* larynx is markedly sexually dimorphic in adults. The male larynx has 32,000 muscle fibers as opposed to 4000 in females (Sassoon and Kelley, 1986). All muscle fibers in males are fast twitch, fatigue resistant, while the majority of female fibers are slow twitch (Sassoon *et al.*, 1987). Laryngeal muscle contains 10- to 20-fold more androgen receptor than nondimorphic muscle such as thigh and is thus an androgen target (Segil *et al.*, 1987). Androgen binding activity in laryngeal muscle is three or four times higher in adult males than in females (Segil *et al.*, 1987). Sex differences in muscle fiber number and twitch type reflect sex differences in androgen secretion during development (Sassoon *et al.*, 1986; Sassoon and Kelley, 1986; Lambdin and Kelley, 1986; Sassoon *et al.*, 1987). We wished to determine when the larynx acquires the ability to bind androgen and how adult, sexually dimorphic levels of binding develop. Androgen binding in adult muscle is not affected by castration or androgen treatment (Segil *et al.*, 1987). We thus also examined the regulation of binding by androgen in developing muscle.

MATERIALS AND METHODS

Animals

Postmetamorphic (PM) male and female *X. laevis* were purchased from Nasco (Ft. Atkinson, WI). Depending on the size of the animals, between 4 and 15 frogs were combined for each assay. Binding activity in whole male and female larynges was assayed at 0 months PM, 3 months PM, 6 months PM, 9 months PM, and adulthood (>24 months PM). The 0-month-PM group is at the final stage of tadpole metamorphic climax, stage 66, described in the normal table of *X. laevis* (Nieuwkoop and Faber, 1956). Each group of juvenile frogs in an assay was from the same ovulation group (i.e., all members of the group were the same chronological age). Postmetamorphic juveniles were gonadecto-

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mized at least 1 week prior to assay in order to clear endogenous steroid (Wetzel and Kelley, 1983). Sex was determined by inspection of the gonads at the time of removal. Metamorphic animals were not gonadectomized due to their small size but were sexed when assayed. Preliminary radioimmunoassay results indicate that androgen secretion (testosterone and dihydrotestosterone) at metamorphosis is low in both sexes (~25 pg/ml; Lambdin and Kelley, 1986).

Hormone Treatment

Age-matched, 3-month-PM males and females were used for studies of androgen effects on dihydrotestosterone (DHT) binding activity and laryngeal weight (Figs. 4 and 5). Androgen was administered by implanting a compressed pellet of crystalline hormone (~3 mg) into the dorsal lymph sac (see Kelley and Pfaff, 1976; Wetzel and Kelley, 1983). Such a pellet releases a supraphysiological dose of androgen for this stage in development (Lambdin and Kelley, 1986). For each assay, sibling animals were treated for 3 weeks with either testosterone (4-androsten-17β-ol-3-one) propionate or dihydrotestosterone (5α-androstan-17β-ol-3-one). An additional group of females was treated for 5 weeks with testosterone propionate. Pellets were removed 1 week prior to assay. For some experiments (pre-gonx), animals were gonadectomized immediately prior to pellet implantation. In others (intact), males and females were gonadectomized at the same time as pellet removal, i.e., 1 week prior to assay. Control (non-hormone-treated) juveniles were gonadectomized 1 week prior to assay and were sham operated by making a small incision into the dorsal lymph sac at the time experimental animals received pellets. All surgical procedures were performed under cold narcosis.

Tissue Preparation

Whole larynges were dissected free of surrounding tissue and stored in TEGM (pH = 7.4, 8 m*M* Tris, 1.5 m*M* EDTA, 10% glycerol, 2 m*M* sodium molybdate) at −70°C for no more than 2 weeks prior to assay. Duplicate assays of fresh and frozen larynges revealed no degradation of binding over this 2 week period. Thigh muscle (*m. gluteus* and *m. semimembranosus*)—which is not sexually dimorphic—was assayed at 0 and 6 months PM and in adulthood. All subsequent procedures were carried out at 4°C. At the time of assay, larynges were thawed on ice, homogenized in TEGMD buffer (TEGM plus 1.0 m*M* dithiothreitol; Sigma) in a glass-Teflon homogenizer (Kontes), and centrifuged (Beckman) in 0.8 ml Ultraclear (Beckman) ultracentrifuge tubes in a Beckman SW50.1 rotor at 40,000 rpm (140,000*g*) for 45 min. Supernatant minus lipid layer was assayed for DHT binding activity (cf. Jung and Baulieu, 1972; Max *et al.*, 1981; Gustafsson *et al.*, 1984). Muscle nuclei are fragile and are disrupted by freezing and homogenization. Androgen binding activity was thus measured in the supernatant and represents total extractable activity under these conditions. The protein concentration of the supernatant, determined according to the method of Bradford (Bradford, 1976), ranged from 0.2–1.5 mg/ml. Preliminary experiments indicated that binding was not affected by protein concentrations over this range.

Determination of B_{max} and K_d

Supernatant was incubated at 4°C for 4 hr with concentrations of [^{3}H]DHT (New England Nuclear, Cambridge, MA; sp act, approx 170 Ci/m*M*). In order to carry out the incubations to saturation, [^{3}H]DHT concentrations used ranged from 0.1–10 n*M*. Under these conditions, equilibrium binding is reached by 1 hr of incubation and does not change over the next 5 hr (Segil *et al.*, 1987). The ligand [^{3}H]DHT was chosen because it is a major circulating androgen in *X. laevis* and can be obtained at a specific activity higher than that of [^{3}H]-R1881 (a synthetic androgen) used in our previous studies (Segil *et al.*, 1987). To correct for nonspecific binding, duplicate incubates containing a 100-fold excess of nonradioactive DHT were also assayed. [^{3}H]-Dihydrotestosterone bound to the macromolecular fraction was separated from unbound steroid by gel filtration on Sephadex LH-20 (Sigma) minicolumns (bed volume = 0.4 ml) as described by Ginsburg and colleagues (Ginsburg *et al.*, 1974) with modifications (Sheets *et al.*, 1985). The macromolecular fraction was eluted into scintillation vials with TEGMD and counted with liquid scintillant (Histofluor, National Diagnostics, Somerville, NH). Aliquots of the original incubates were also prepared to determine the concentration of [^{3}H]DHT. All samples were counted in a Beckman liquid scintillation counter. Counting efficiency was determined at the time of each assay with [^{3}H]toluene (New England Nuclear).

Data Reporting

Results are plotted as femtomoles per milligram protein bound against the concentration of bound/free ligand. The apparent K_d and B_{max} were obtained by Scatchard analysis (Scatchard, 1949). Each value for androgen binding represents the mean of two to four assays (4 to 15 frogs per assay). In some cases, assays were repeated to ensure contemporaneous controls (i.e., 3-month-PM control frogs in the hormone treatment experiment, also female frogs treated with testosterone for 5 weeks). Data are reported as means and ranges of values. The statistical significances of differences in

binding and in laryngeal weights after hormone treatment were evaluated using the Mann-Whitney *U* test (Siegel, 1956).

Competition Assays

The specificity of [^{3}H]DHT binding to components of the macromolecular fraction of laryngeal supernatant was assayed by a series of competition experiments performed with testosterone (T), DHT, estradiol (E), progesterone (P), corticosterone (C), and triamcinolone acetonide (TA) (Sigma). The supernatant was incubated with 10 n*M* [^{3}H]DHT as in the saturation assays. T, DHT, E, P, and C were included in parallel incubations at concentrations 10, 100, and 1000 times that of [^{3}H]-DHT. All data concerning specificity of binding are expressed as the percentages of competition with bound [^{3}H]DHT.

RESULTS AND SPECIFIC DISCUSSION

Receptor Levels during Postmetamorphic Development

We have determined the amounts of androgen binding activity in the larynx at four stages during the postmetamorphic development of *X. laevis* (Fig. 1). At 0 months PM, male and female DHT binding activities are the same (male, 262 fmole/mg protein; female, 269 fmole/mg). By 3 months PM, laryngeal DHT binding is lower in both sexes and is sexually dimorphic (male, 189 fmoles/mg protein; female, 106 fmoles/mg, $P < 0.01$). These reduced levels are maintained until 9 months PM. In males, binding increases to 170% of metamorphic levels between 3 and 6 months PM and thereafter declines, reaching adult levels (52 fmoles/mg) by 9 months PM. Values for both males and females are more variable at 6 months PM than at any other stage. Nonetheless, the range of binding values for males (555–325 fmoles/mg protein) is the highest observed at any age and does not overlap with the range of values in females (185–40 fmoles/mg protein) which is considerably lower ($P < 0.01$). At 9 months PM, ranges of DHT binding values in males and females overlap. Between 9 months PM and adulthood, levels of binding activity in females continue to decline, while levels in males do not change, so that by adulthood (>24 months PM), binding is again dimorphic (males, 40 fmoles/mg protein; females, 18 fmoles/mg protein, $P < 0.01$). Androgen binding activity in thigh muscle was assayed at 0 and 6 months PM and in adults and found to be uniformly low (0–6 fmoles/mg protein) and similar for both sexes at these stages.

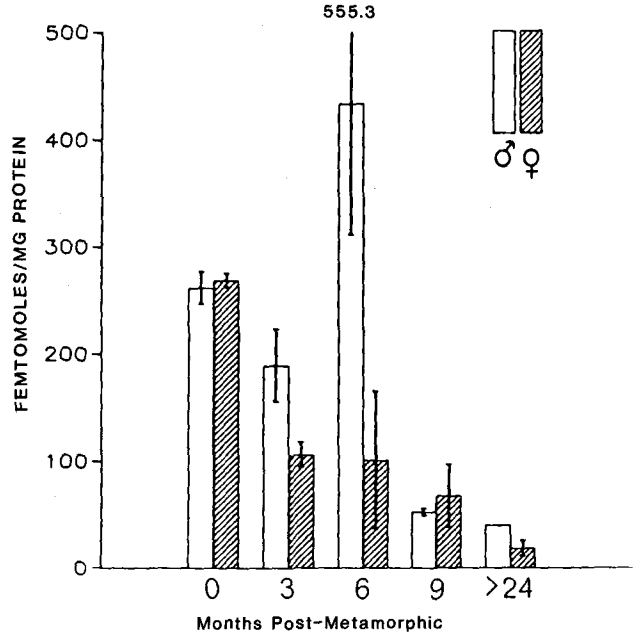

FIG. 1. Androgen receptor levels in larynx of developing and adult *X. laevis*. The mean and ranges of two to four assays (4 to 15 larynges per assay) are indicated. Values for receptor levels were obtained from Scatchard plots (see Fig. 2). At metamorphosis, receptor values are high and sexually monomorphic. Male values remain elevated for the first 6 months; female values decline by half during this period.

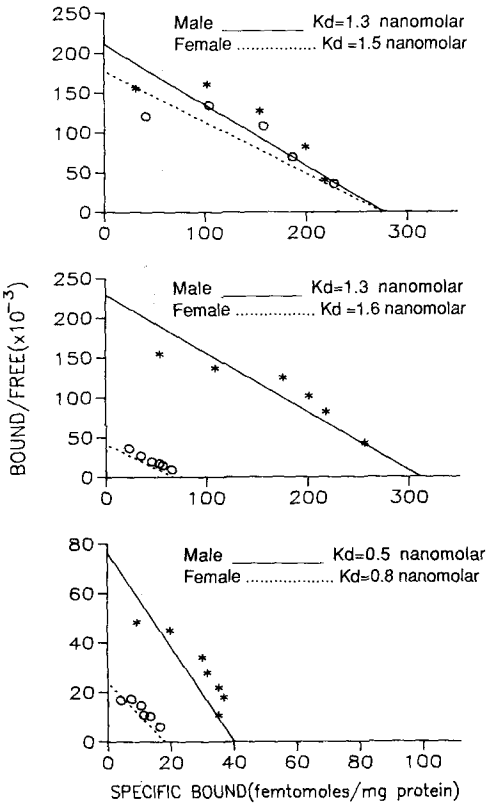

FIG. 2. Representative Scatchard plots of [^{3}H]DHT binding to supernatant preparations of 0- and 6-month postmetamorphic and adult larynges. Levels (inferred from the B_{max}) are monomorphic at 0 months, but sexually dimorphic at 6 months and in adulthood. Affinities (inferred from the K_d) are similar for males and females at all ages. Top, 0 months; middle, 6 months; bottom, adult.

Scatchard analysis reveals that DHT binds saturably and with high affinity to a macromolecular component in the larynx. Sample Scatchard plots are shown in Fig. 2. The apparent affinity (K_d) at each stage varies between 0.3 and 1.5 n*M*. We observe no consistent sex differences in affinity at any age. We consider that [^{3}H]DHT binding observed is not due to serum proteins because (1) affinities obtained are 2 orders of magnitude greater than those of steroid-binding serum proteins for steroids (Bonne and Raynaud, 1976), (2) the binding affinity for [^{3}H]DHT is equal to or greater than that of [^{3}H]R1881, a steroid analog that does not bind to serum proteins in rats (Bonne and Raynaud, 1976), and (3) thigh muscle has very low levels of [^{3}H]DHT binding.

Our data indicate that in the juvenile, as in the adult (Segil *et al.*, 1987), the larynx is an androgen target tissue. Most, if not all, skeletal muscle contains some androgen receptor (Gustafsson *et al.*, 1984). Binding of [^{3}H]DHT in larynx, however, was between 10 and 50 times binding in thigh. Androgen binding activity in thigh does not change with age; binding in larynx decreases between metamorphosis and 9 months PM. The affinity of [^{3}H]DHT and of [^{3}H]R1881 (Segil *et al.*, 1987) binding in larynx is similar to values reported for binding to androgen receptors in other sexually dimorphic muscles (e.g., Jung and Baulieu, 1972; Lieberburg and Nottebohm, 1979). Immunoprecipitation assays, using a human antiserum that recognizes the androgen receptor, yield receptor levels from *X. laevis* larynx equivalent to binding levels from [^{3}H]DHT or [^{3}H]R1881 studies (D. Tindall, personal communication). The most likely explanation for [^{3}H]DHT binding in juvenile larynx, therefore, is the presence of an androgen receptor protein such as that found in other androgen targets (Janne and Bardin, 1984).

The specificity of DHT binding was tested in 0- and 6-month-PM male and female frogs by competition with other steroid hormones at 10, 100, and 1000 times the concentration of DHT. As expected, unlabeled DHT displaces greater than 90% of [^{3}H]DHT binding at all concentrations (Fig. 3). At 10 times the [^{3}H]DHT concentration, none of the other steroids (T, E, C, P, or TA) competes better than 60% for DHT binding. At this concentration, C, T, and TA compete especially poorly (10, 15, and 30%, respectively), while P and E compete more effectively than T (see below). At 100 times the [^{3}H]DHT concentration, P, E, and T compete almost as well as DHT and at the 1000 times concentration, there is no difference between DHT, E, T, and P competition. Corticosterone and TA compete poorly at all concentrations. Competition results were similar for males and females at the two stages examined.

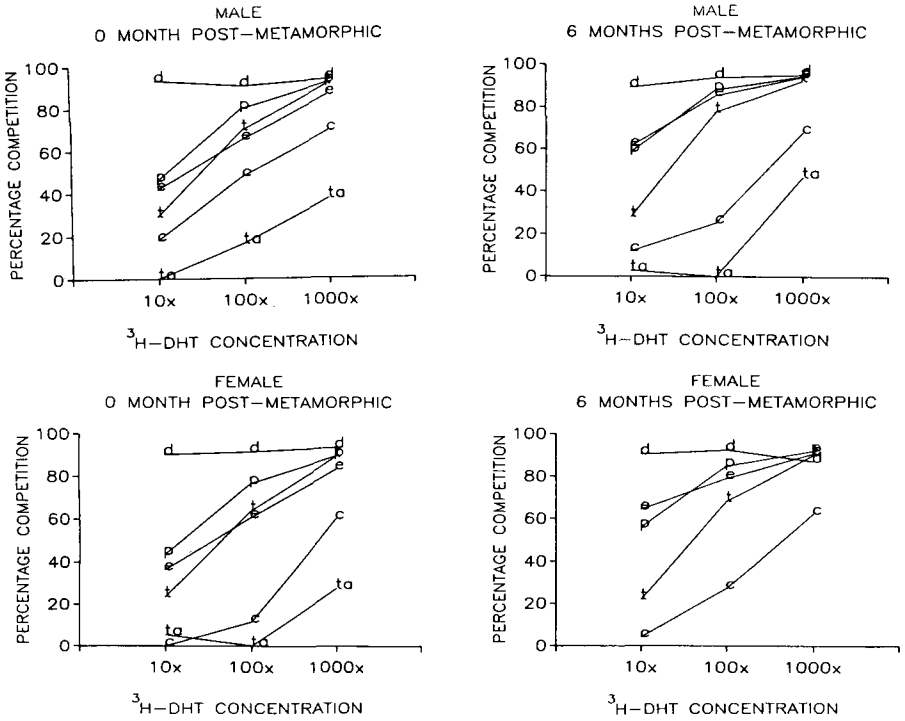

FIG. 3. Competition of unlabeled steroids for [^{3}H]DHT binding to the macromolecular fraction of laryngeal cytosol in 0- and 6-month postmetamorphic juveniles. Incubates included 10, 100, and 1000 times the [^{3}H]DHT concentration of dihydrotestosterone (D), estradiol (E), progesterone (P), testosterone (T), triamcinolone acetonide (TA), and corticosterone (C). Unlabeled DHT was a very effective competitor. Estradiol, progesterone, and testosterone were good competitors (especially at 100 and 1000×). Corticosterone and TA were poor competitors.

Testosterone and dihydrotestosterone are the two major circulating androgens in *X. laevis* (Kelley, 1980; Wetzel and Kelley, 1983). Binding results are consistent with the presence of a receptor with high affinity for DHT rather than testosterone in *X. laevis* larynx (Segil *et al.*, 1987). In human androgen receptor transcribed from cloned cDNAs, DHT is more effective than T in competing for R1881 binding (Chang *et al.*, 1988; Lubahn *et al.*, 1988). Because of the effectiveness of DHT as a competitor in binding assays, its high circulating levels, and its biological efficacy in masculinization (see below), DHT is likely to be an active androgen in *X. laevis* larynx.

Estradiol and progesterone compete as or more effectively than testosterone for [^{3}H]DHT binding. It is possible that [^{3}H]DHT binds to progesterone or estradiol receptors present in the supernatant incubate. The presence of a specific progesterone receptor in larynx is, however, unlikely because TA, a potent progesterone analog in mammals (Zava *et al.*, 1979), is a poor competitor. The inferred amino acid sequence of the cloned androgen receptor is highly similar to that of the progesterone receptor (94% homologous over the putative DNA-binding domain; Lubahn *et al.*, 1988). Thus progesterone could be binding to the androgen receptor in our assays. The presence of estrogen receptors in larynx is also unlikely, since preliminary studies (O'Dougherty, Sassoon, and Kelley, unpublished) suggest that there is no specific estradiol binding in larynx. Effective competition by estrogens has been reported for androgen receptors in other species (Fox, 1975; Weill, 1986). Do progesterone or estradiol interfere with DHT binding under physiological conditions in *X. laevis?* In *X. laevis* both estradiol and progesterone circulate at levels at least 1 order of magnitude below testosterone (Ozon, 1972a,b; Fortune and Tsang, 1981). Levels of estrogen and progesterone used in competitions here are thus at least 10^3 to 10^5 times physiological values and are unlikely to compete with DHT-binding under physiological conditions.

Effects of Hormone Treatment on Binding Activity

As expected, DHT binding is already sexually dimorphic in 3-month-PM (Fig. 4, Normal) juveniles. When 3-month-PM male frogs are treated with testosterone for 3 weeks, levels of [^{3}H]DHT binding are reduced to 39 fmoles/mg protein (Fig. 4, intact). [^{3}H]Dihydrotestosterone binding in control males at these ages ranges from 150 to 220 fmoles/mg protein. Testosterone treatment thus results in a decrease of [^{3}H]DHT binding to approximately 20% of control values. If males are, however, gonadectomized at the time of T pellet insertion (pre-gonx group) instead of at the time the

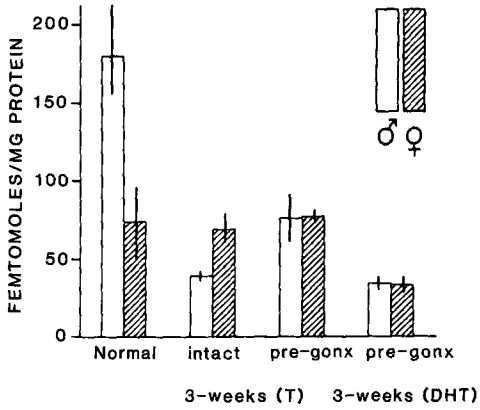

FIG. 4. Androgen regulation of [^{3}H]DHT binding activity in 3-month-PM male and female *X. laevis*. The means and ranges of receptor levels (inferred from Scatchard analyses; (see Fig. 5)) for control (Normal) and hormone-treated frogs are shown. Frogs were treated with androgen for 3 weeks. Some experimental frogs were gonadectomized immediately prior to pellet implantation. Alternatively, frogs were gonadectomized at the same time as pellet removal, 1 week prior to assays. The numbers of assays (three to seven frogs for each assay) were four for controls, three for DHT-treated frogs, and two for all other groups. Two control assays were performed at the time androgen pellets were implanted into experimental animals. The remaining control assays were performed 3 weeks later.

pellet is removed 3 weeks later (intact group), much less reduction in DHT binding is observed. Binding in juvenile males with intact testes and T pellets is 40% of untreated values (76 fmoles/mg protein), a value similar to the binding activity in intact (74 fmoles/mg pro-

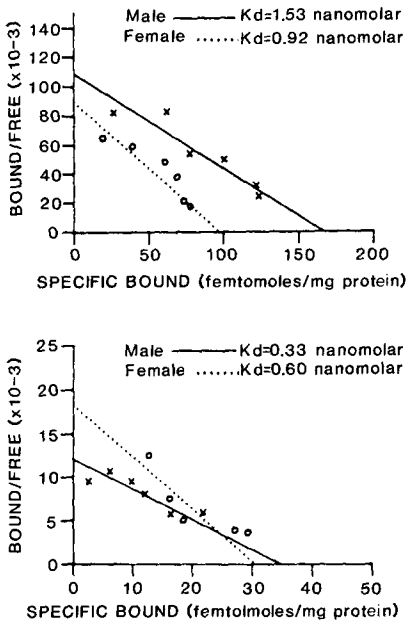

FIG. 5. Representative Scatchard plots of [^{3}H]DHT binding to supernatant preparations of 3-month-PM control (top) and DHT-treated (bottom) larynges.

tein) and gonadectomized, T-treated females (72 fmoles/mg protein). Binding of DHT in females was not affected by testosterone treatment (intact or pre-gonx versus Normal) even when these frogs were treated for an additional 2 weeks (5 weeks total, data not shown). These results suggested that differential metabolism of T by intact males, females, and castrated males might influence the results obtained. In particular, testosterone can be metabolized by 5α-reductase to dihydrotestosterone. If DHT is the active androgen, differences in testosterone metabolism between the sexes and/or by the gonads could account for our results. We thus treated gonadectomized 3-month-old juveniles of both sexes with DHT, and observed marked decreases in [^{3}H]DHT binding in both sexes (Fig. 4, pre-gonx). Values for binding activity in DHT-treated males and females are similar to those of T-treated intact males.

These results provide additional evidence that DHT is the active androgen metabolite responsible for decreases in androgen binding in the juvenile larynx. Dihydrotestosterone is more effective than testosterone in all experimental groups. Why is testosterone more effective in reducing binding in intact males than in pregonadectomized males? One possibility is that the male gonad is a major metabolizer of T and converts T to DHT very efficiently; gonadectomized males would thus lack sufficient DHT for a complete response. A similar mechanism may account for the failure of T to reduce binding in females. Females may lack sufficient 5α-reductase activity (even in gonads) and thus show no reduction in androgen binding unless DHT itself is supplied.

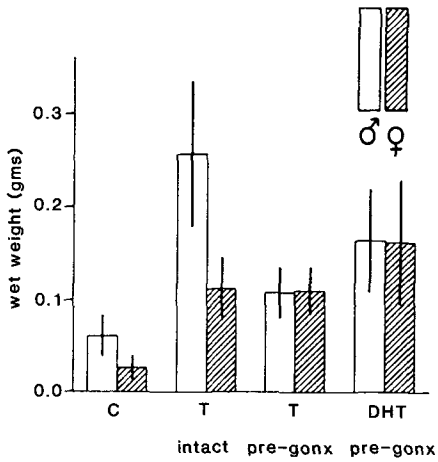

FIG. 6. Effects of T and DHT treatment on laryngeal weights of male and female, 3-month-PM *X. laevis*. Means and ranges of values are shown. The numbers of larynges weighed were control, 6 male, 13 female; T intact, 3 male, 8 female; T pre-gonx, 8 male, 9 female; DHT pre-gonx, 7 male, 8 female. Laryngeal weights are already dimorphic at 3 months and are increased in both sexes by androgen (T or DHT) treatment.

Effects of Hormone Treatment on Laryngeal Weights

By 3 months PM, male larynges weigh twice as much as those of females ($P < 0.001$). Androgen (T or DHT) increases laryngeal weights in both sexes ($P < 0.001$); gonadectomized juveniles treated with DHT have somewhat larger larynges than those treated with T (Fig. 6). The weights of these T- and DHT-treated juveniles are the same for both sexes. Testosterone increases laryngeal weight significantly more in intact males than in pregonadectomized males ($P < 0.05$). In females, the effects of androgen on laryngeal weights are the same regardless of gonadal state.

As was the case for regulation of DHT binding, testosterone is more effective in increasing male laryngeal weight when the testes are present. Again, we suggest that this result could reflect the contribution of testicular 5α-reductase activity, a contribution that may be missing in females. Unlike the observed reduction in DHT binding, DHT by itself does not exert a maximal effect on laryngeal weight, suggesting that both T and DHT contribute to laryngeal growth.

GENERAL DISCUSSION

The development of secondary sexual characteristics is dependent on both the presence of circulating androgens and the intracellular expression of functional steroid receptor proteins in target tissues (Wilson *et al.*, 1981). In adults, sexually differentiated targets continue to be responsive to steroids and exhibit high levels of specific steroid receptors relative to other tissues. Given the androgen sensitivity of juvenile larynx (Sassoon *et al.*, 1986; Sassoon and Kelley, 1986) and the presence of androgen receptor in adult laryngeal muscle (Segil *et al.*, 1987), we expected to find some androgen binding in juvenile larynx. The surprising finding, however, was the very high levels of receptor present in juvenile larynx.

At metamorphosis, androgen binding activity is approximately an order of magnitude greater than that in the adult larynx. In juvenile males, levels of binding are high for the first 6 months of postmetamorphic development and then decline rapidly to adult values. In juvenile females, however, the level of receptor has declined to half of its initial value by 3 months after metamorphosis and then continues to decrease until adult values are attained. During the period between metamorphosis and adulthood, levels of androgen binding in thigh remain at the same and comparatively low values found in adult thigh. In adults, levels of androgen binding in both laryngeal muscle (Segil *et al.*, 1987) and whole larynx (this report) are sexually dimorphic—

males have approximately three times the female level. The present results demonstrate that, at metamorphosis, binding levels are *not* sexually dimorphic. The adult dimorphic levels of receptor result from a differential decrease in binding activity in both sexes.

A paradoxical aspect of the decrease in androgen binding during laryngeal development is that it coincides with a period of marked growth and differentiation (Sassoon and Kelley, 1986). The DNA content of the entire male larynx increases from 51 to 473 μg between metamorphosis and adulthood. Female laryngeal DNA values show a net increase from 50 μg/larynx at metamorphosis to 343 μg/larynx at adulthood. The male larynx adds 28,000 muscle fibers during postmetamorphic development. In females there is no net addition of laryngeal muscle fibers after metamorphosis. At metamorphosis, muscle fiber type in the larynx is heterogeneous in both sexes and resembles the adult female pattern of fiber type expression (mostly slow twitch fibers, Sassoon *et al.*, 1987). By 3 months PM, however, male laryngeal muscle has begun to switch to the fast twitch type, a process complete by 6 months PM (Tobias *et al.*, 1988).

Masculinization of the larynx appears to be controlled by androgen secretion from the developing testes (Kelley, 1986). At metamorphosis, androgen levels are low in both sexes but then proceed to rise rapidly in males; adult values are present at 6 months PM (Lambdin and Kelley, 1986). Androgen induces myogenesis and switching of muscle twitch fiber types (Sassoon *et al.*, 1987). Blockade of the androgen receptor with flutamide prevents fiber addition in males (Sassoon and Kelley, 1986). Androgen treatment also, as we show here, decreases DHT binding in developing larynx.

Are the effects of androgen on myogenesis and on receptor levels related? If myoblasts require androgen for survival, females may lose myoblasts because of insufficient hormone levels. Cell loss could contribute to the decrease in androgen binding seen in developing female larynx. Such a model would suggest that laryngeal myoblasts contain high levels of androgen receptor. Between metamorphosis and 3 months PM the female larynx does show a 50% decrease in DNA content suggesting that cell loss may be occurring at early postmetamorphic stages (Sassoon and Kelley, 1986). Rising titers of androgen in developing males may maintain proliferating myoblasts at high enough levels to keep androgen receptor levels elevated. As myoblasts withdraw from the cell cycle and fuse, receptor levels would fall. In this scenario, the decrease in androgen binding in developing male larynx is due to terminal cell differentiation of relatively receptor-rich myoblasts into relatively receptor-poor myotubes. In female larynx, decrease in receptor binding would be due to loss of the receptor-rich cell type perhaps due to cell death.

Our results in *X. laevis* larynx can be compared to the development of other sexually dimorphic muscles and to muscle development in general. All skeletal muscle has some androgen receptor; this characteristic is thought to account for the anabolic effects of male sex hormones on muscle (Gustafsson *et al.*, 1984). Krieg (1976) has reported that skeletal muscle from prepubescent rats has higher levels of androgen receptor than adult skeletal muscle. Our results in larynx of *X. laevis* agree with this earlier finding. Max and colleagues (Max *et al.*, 1981) compared androgen receptor values in intact and regenerating sexually dimorphic muscles (*m. levator ani*) of adult rats. Since receptor levels were lower in regenerating muscle, these authors suggest that the proliferating myoblasts responsible for regeneration do not express high levels of receptor until myotube formation and maturation. It is not clear, however, whether these results in adults are indicative of how androgen receptor is regulated in developing *levator ani* muscle in which androgen receptor expression is essential to muscle survival (Venable, 1966). There is increasing evidence that different populations of myoblasts are involved in muscle formation at different stages of development (Seed and Hauschka, 1984; Crow and Stockdale, 1986a,b). The satellite (myoblastic) cells of adult muscle could represent a lineage distinct from primary and secondary myoblasts responsible for initial muscle formation. Myoblasts present in *levator ani* at earlier stages in development may be regulated quite differently than satellite cells.

Masculine development of *levator ani* in rats and laryngeal muscle in *X. laevis* appears to require androgenic steroids. *Levator ani* involutes in females or castrated males during the neonatal period unless androgen is supplied (Venable, 1966). In larynx, myogenesis, fusion, and muscle fiber type expression are androgen-regulated between metamorphosis and 9 months PM (Sassoon and Kelley, 1986; Sassoon *et al.*, 1987). Androgen sensitivity of the developmental program in these muscles could be due to the presence of specialized, highly androgen-sensitive myoblasts absent from conventional skeletal muscle. Such myoblasts could belong to a distinct tertiary lineage or to a secondary lineage in which androgen receptor expression has been induced. In either case, the program of differentiation is controlled by androgen. Such control may prove useful in understanding the cellular and molecular mechanisms responsible for development not only of sexually dimorphic muscles but of all skeletal muscles.

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