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Androgen Induction of Male Sexual Behaviors in Female Goldfish

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The effectiveness of testosterone (T) and 11-ketotestosterone (K) in inducing male-typical sex behaviors in goldfish was examined by implanting intact adult females with one empty (blank) Silastic implant (B females), one implant containing T or K, or one T and one K implant (T + K females). Behavior of the four female groups was compared to that of untreated males and males containing a blank implant. Male-typical behaviors (coutship, spawning) and associated behavioral changes (increased activity, reduced spontaneous feeding) were assessed 3.5 and 4.5 months after implant in 30-min tests in which the test female or male was allowed to interact with a stimulus female in which sexual receptivity and attractivity had been induced by acute prostaglandin $F_{2\alpha}$ injection. Prostaglandin-induced female-typical spawning behavior in the test females and males was also assessed 4.5 months after implant in a 60-min test for female-typical behavior in which the test fish was injected with prostaglandin and placed immediately with a sexually active male. Blood samples 5 months postimplant showed that implants generated physiological levels of T and K. In both tests for male-typical behaviors, K and T + K females exhibited the full suite of behaviors shown by spawning males, e.g., male-typical courtship and spawning, increased swimming activity, and reduced spontaneous feeding. Although behaviors of K and T + K females did not differ, those of T + K females were more often equivalent to those of males and significantly different from those of B females. T females exhibited marginal male-typical behaviors which never differed significantly from those of B females. Androgen-treated females exhibited female-typical; spawning behaviors equivalent to that of males and B females. The results show that adult female goldfish can be behaviorally masculinzed without behavioral defeminization, and suggest that male-typical sex behaviors in goldfish are dependent on K, although other steroids also may be required. The inducible behavioral bisexuality of goldfish, a gonochoristic species, is discussed in terms of the prevalence of hermaphroditism in teleosts.

Although there is much evidence that male-typical reproductive behaviors in teleosts are influenced by gonadal androgens (reviews by Borg, 1994; Liley, 1969; Liley and Stacey, 1983), there is surprisingly little information as to the specific androgen(s) involved. Indirect evidence that androgens regulate male behavior comes from the temporal correlation between plasma androgen and reproductive activity in a variety of of teleosts (Borg, 1994), the general pattern being that putative androgens such as 11-ketotestosterone (K) and testosterone (T) reach peak plasma concentrations prior to or at the time of spawning (Borg, 1994), plasma concentrations of K being higher in males than in females, but levels of T often higher in females than in males. In sexually mature goldfish (*Carassius auratus*), plasma concentrations of T in males are equivalent to those in females, but plasma K is much higher in males than in females and plasma estradiol (E) is much higher in females than in males (Kobayashi, Aida, and Hanyu, 1986a; Rosenblum, Yamada, Callard, and Callard, 1985). Thus, whereas T is a gonadal steroid common to both sexes in goldfish, K is male-typical and E is female-typical. More direct evidence for androgen control of male behaviors comes from studies showing that male behaviors are reduced or abolished following castration, and can be restored in castrates (Borg, 1994), or induced in juveniles (Cardwell, Stacey, Tan McAdam, and Lang, 1995) and females (Landsman, David, and Drew, 1987), by androgen therapy.

In the two species where the behavioral effects of several androgens have been compared, 11-oxygenated androgens have been more effective than T. In the threespine stickleback (*Gasterosteus aculeatus*), where

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castration eliminated a variety of male reproductive behaviors (Borg, 1987), Silastic implants containing 11-ketoandrostenedione (KA; which in sticklebacks is converted to K extratesticularly; Mayer, Borg, and Schulz, 1990) were more effective in restoring behavior of castrates than were implants containing T, androstenedione, or 5α-dihydrotestosterone (DHT). In intact bluegill sunfish (*Lepomis macrochirus*) studied in the field, K implants were more effective than T implants in increasing several components of male reproductive activities (Kindler, Barr, and Philipp, 1991).

Despite the evidence that K is a behavioral androgen in fish, and has been shown to be the androgen most potent in inducing male secondary sex structures in a number of fish species (Borg, 1994), there is no evidence in fish for classical nuclear receptors that preferentially bind K. Indeed, nuclear extracts of trout skin (Pottinger, 1987) and goldfish brain (Pasmanik and Callard, 1988), bind K with considerably less affinity than T and DHT. The relative binding of K and T in trout skin is particularly problematic because K is more potent than T in stimulating epidermal changes (Pottinger and Pickering, 1985).

In this study, we examined the ability of T and K implants, alone and in combination, to induce male-typical sex behaviors in intact adult female goldfish. The goldfish is appropriate for such a study because there is considerable information on steroid levels of both sexes (Kobayashi *et al.*, 1986a; Rosenblum *et al.*, 1985), and because the hormonal control of female-typical behaviors already have been investigated (Kobayashi and Stacey, 1993). Because female-typical behavior in goldfish is regulated by prostaglandin, and is not affected by steroid treatment (Kobayashi and Stacey, 1993), we felt it unlikely that steroid treatment effects on male-typical behaviors would be confounded by alterations in female-typical behavior systems. We initially attempted this work in castrated males, but were unsuccessful due to high rates of testicular regeneration, a problem with many fish species (Liley, 1969; Liley and Stacey, 1983; Borg, 1994).

MATERIALS AND METHODS

Animals

Goldfish were purchased in spring from Ozark Fisheries Co. (Stoutland, MI), and held in the Biological Sciences Department (University of Alberta, Edmonton) in 1000-liter flow-through fiberglass stock aquaria (15–20°C; 16L:8D—lights on at 0800 hr) for several months prior to commencing experiments. Prior to and during experiments, fish received daily *ad libitum* feedings of a commercial trout chow. On June 28, 88 sexually regressed females (mean body wt = 63 g; range, 30–130 g) and 30 sexually mature, spermiated males (mean body wt = 53 g; range, 31–84 g) were selected from the stock aquaria, implanted with steroid-filled or empty Silastic capsules, and placed separately by sex in two 250-liter flow-through fiberglass holding aquaria (20°C) held under the same photoperiod as the stock aquaria. On September 18, 85 females and 22 males were transferred from the fiberglass holding aquaria and placed in 70-liter flow-through glass holding aquaria (20°C; 16L:8D) provided with gravel substrate and artificial vegetation (clumps of floating green acrylic yarn). Each glass holding aquarium received 5 or 6 fish, each from a different treatment group. At this time, groups of untreated fish (to serve either as stimulus females in the tests for male-typical behaviors, or as male spawning partners in the tests for female-typical behaviors) were removed from stock aquaria and placed in additional glass holding aquaria.

Experimental Design

At 3.5 and 4.5 months postimplant, all experimental fish were tested for their tendency to perform male-typical courtship and spawning behaviors with a non-ovulated, prostaglandin $F_{2\alpha}$ (PGF)-injected stimulus female. These two tests are not strictly comparable, because a video-recorder malfunction resulted in the loss of data from 14 fish in the first test for male-typical behaviors (2 or 3 fish from each treatment group). In addition, 3 fish (from different treatment groups), which had been healthy and displaying behavior typical of their treatment group during the first test for male-typical behaviors, were removed from the experiment for health reasons before the second test for male-typical behaviors. One week after the second test for male-typical behaviors, all fish were injected with PGF and tested for their tendency to perform female-typical oviposition behavior with an intact, spermiated male. At 5 months postimplant, fish were anesthetized and a blood sample taken from the caudal vasculature to determine steroids levels (Kobayashi, Aida, and Hanyu, 1986b). At 6 months postimplant, all fish were euthanized by overanesthesia, and the gonads removed to determine gonadosomatic index (GSI; gonad weight expressed as a percentage of total body weight).

Steroid Implants

Dow Corning medical grade Silastic tubing (1.98 mm i.d.; 3.18 mm o.d.) was used to prepare empty (30 mm