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Endocrine control of sexual behavior in sneaker males of the peacock blenny *Salaria pavo*: Effects of castration, aromatase inhibition, testosterone and estradiol

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Abstract

The effects of castration and sex steroid manipulations on the expression of sexual behavior were investigated in a small fish, the peacock blenny, *Salaria pavo*. In this species, large males defend nests and attract females while small "sneaker" males reproduce by imitating the female morphology and courtship behavior in order to approach nests during spawning events and parasitically fertilize eggs. Sneakers switch into nest holders in their second breeding season, thus displaying both male and female-like sexual behavior during their lifetime. We tested the effects of castration and of an aromatase inhibitor (Fadrozole, F), testosterone (T) or 17β-estradiol (E_2) implants on the expression of male and female-like behavior in sneakers. Sneakers were either sham-operated, castrated or castrated and implanted with vehicle, F, T+F or E_2+F. Seven days after the treatment, sneakers were placed in a tank with a nesting male, two ripe females and an available nest. Castrated fish had lower levels of circulating T and increased the time spent displaying female typical nuptial coloration. T implants had the opposite effect, inhibiting the expression of female-like behavior and coloration. E_2 implants had no significant effect on the display of sexual behavior but the frequency of aggressive displays decreased. The results agree with previous findings in sneakers of *S. pavo* that demonstrated an inhibition of female-like behavior by 11-ketotestosterone (11-KT). The reported increase in T and 11-KT production when sneakers change into nest holders may thus contribute to behaviorally defeminize sneakers. Contrarily, both T and E_2 failed to promote male-like behavior, suggesting that behavioral masculization during tactic switching depends on other neuroendocrine mechanisms or that the time length of the experiment was insufficient to induce male-like behavioral changes in sneakers.

Keywords: Aromatase; Sex steroids; Reproductive behavior; *Salaria pavo*; Peacock blenny; Androgens; Estrogens; Implants; Alternative reproductive tactics; Fadrozole

Introduction

The influence of sex steroids on the control of sexual behavior has been investigated in detail in animal models from diverse taxa (e.g. rats, quails, lizards). From these studies, it has long been known that gonadal androgens administered to males promote male-typical sexual behavior. However, estrogen administration to males may also induce male typical sexual displays (e.g. Ball, 1937; Beach, 1942; Guhl, 1949). This paradoxical observation was the basis for the aromatase hypothesis (Naftolin et al., 1975), which suggests that the masculinizing effects of gonadal testosterone (T) on reproductive behavior are partially dependent on its local conversion to estradiol (E_2) by the enzyme aromatase in the brain. This hypothesis has been thoroughly corroborated by studies in mammals and birds where male sexual behavior was inhibited by blocking brain aromatase and reestablished by administering estrogens (e.g. Adkins et al., 1980; Vagell and McGinnis, 1997; for reviews see Baum, 2003; Ball and Balthazart, 2004).

Surprisingly, although fish have been extensively used to study endocrine mechanisms of sex determination and differentiation (review in Devlin and Nagahama, 2002), the role of the aromatization process on the regulation of fish sexual behavior has been poorly investigated. One reason for this is that the non-aromatisable androgen 11-ketotestosterone (11-

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KT) is thought to be the most potent androgen in fish (Borg, 1994). 11-KT is the most abundant circulating androgen in most male teleosts and was shown to restore male sexual behavior in castrated sticklebacks and white perches (Borg, 1987; Salek et al., 2001), to increase male sexual displays in intact bluegill sunfish (Kindler et al., 1991) and to induce male sexual displays in gynogenetic carps (Kobayashi and Nakanishi, 1999). However, in other species the most abundant circulating androgen during reproductive periods is T (e.g. Kime and Groves, 1986; Oliveira et al., 2001b) and in the only study conducted so far investigating the effects of blocking aromatase in fish sexual behavior, two of three investigated male sexual displays in guppies were reduced by aromatase inhibition (Hallgren et al., 2006). Thus, for some fish species, T aromatization into E2 may also mediate the expression of male sexual displays. The largely ignored role of brain aromatization of androgens on fish sexual behavior is also surprising as teleosts present by far the highest levels of brain aromatase activity in vertebrates (Callard et al., 1990) and, in fish, aromatase occurs in brain areas involved in the control of reproductive behavior (Forlano et al., 2001; Menuet et al., 2003, 2005) and peaks during the species reproductive seasons (e.g. Gelinas et al., 1998; Gonzalez and Piferrer, 2002, 2003; Forlano and Bass, 2005). On the other hand, exogenous estrogen administration to fish has been shown to decrease male sexual behavior (e.g. Bayley et al., 2003; Bjerselius et al., 2001; Doyle and Lim, 2005; Oshima et al., 2003) and there are no published studies suggesting that estrogens promote male sexual behavior in fish. Thus, the role of high aromatase levels in brain areas typically associated with the control of sexual behavior is unclear and, more generally, the mechanisms of neuroendocrine control of sexual behavior in fish are still poorly understood.

Here, the effects of castration and sex steroid manipulation on male and female-like displays were tested in the peacock blenny *Salaria pavo*. In this species, males are larger than females and have a pronounced head crest and an anal gland in the first two rays of the anal fin (Fishelson, 1963; Papaconstantinou, 1979; Patzner et al., 1986). Males defend nests in crevices or holes and, in the studied population, a shortage of nest sites promotes a strong male-male competition for nests (Almada et al., 1994, 1995). Females compete for the access to nesting males and take the initiative in courtship. Females court males by quickly beating the pectoral fins and open-and-closing the mouth in synchrony while displaying a typical nuptial coloration, which consists of an alternated pattern of vertical dark and light bars in the head and anterior portion of the body (Almada et al., 1995). Nesting males assume a passive role during courtship but occasionally will also court females from the nest with intense but low-frequency jerking movements of the anterior portion of the body (Patzner et al., 1986). Small "sneaker" males are unable to acquire nests and reproduce by imitating the female's morphology, courtship displays and nuptial coloration in order to approach and deceive nesting males and parasitically fertilize eggs (Gonçalves et al., 1996). When compared with size-matched females, small sneakers are equally courted and attacked by nesting males suggesting that their female-mimicry is efficient (Gonçalves et al., 2005). Sneaker males develop secondary sexual characters and may reproduce as nesting males in their second breeding season (T. Fagundes, D. Gonçalves and R.F. Oliveira, unpublished data). Thus, at least some males perform female-like displays in their first breeding season and male-like displays in the following reproductive seasons. Sneakers have lower circulating and gonadal levels of both T and 11-KT than nesting males, and T is the most abundant androgen in the two morphs both in the plasma (D. Gonçalves and R.F. Oliveira, unpublished data) and in the gonads (Oliveira et al., 2001a). In sneakers, 11-KT implants inhibit female-like displays and promote the development of male secondary sexual characters but it does not induce male-like sexual behavior (Oliveira et al., 2001d).

In this experiment, the effects of castration and of administration to castrated sneakers of an aromatase blocker, T or E_2 on male and female-like sexual displays were tested.

Methods

Animals

During the breeding season (June and July 2005), males, females and sneakers were collected at Culatra Island (36°59′N, 7°51′W, Algarve, Southern Portugal) during morning low tide and transported to a field station located in the island. In the field, sneakers were classified as such if they lacked or had vestigial male typical secondary sexual characters (head crest, anal gland and male-like coloration) and sperm could be easily extruded from their *vas deferens* by gently pressing the abdomen (Gonçalves et al., 1996). In relation to nesting males, sneakers have proportionally larger gonads that lack a testicular gland (Gonçalves et al., 1996) and this criterion was also used to exclude potential non-sneakers from the experiment during the surgical procedure. Animals were kept in 20 l tanks under natural photoperiod (14L; 10E) and temperature (24±2 °C) and fed with frozen cockles until testing. Nesting males were kept together with females and provided with abundant nest sites so that they were defending eggs before the experiment. Sneakers and females used in the experiment were kept in separate tanks.

Reagents

The aromatase inhibitor Fadrozole (F) was gently donated by Novartis Pharma AG (Basel, Switzerland). T and E_2 were purchased from Steraloids (Newport, USA) and the tritiated water and androstenedione used in the radiometric procedure purchased from PerkinElmer (Oporto, Portugal). All other reagents were purchased from Sigma-Aldrich (Madrid, Spain).

Castration and implant procedure

After being transported to the field station, sneakers were anesthetized with a light dosage of MS222 (tricaine methanesulfonate, dilution 1:10,000), measured and weighed. To assess treatment effects on male-like typical characters, the head and body height were measured to provide an indicator of head crest development (head height/body height) and the genital papilla and the anal gland development were classified in an ordinal scale from 0 (not developed) to 9 (fully developed). Sneakers were recovered in a small tank with full aeration and transferred to a provisional container provided with shelters in abundance until the surgical procedure. Silastic implants (internal diameter = 1.47 mm, external diameter = 1.96 mm) were then prepared and their length adjusted to the sneaker's weight in order to keep the implant concentration equal for all fish. The implant concentration was based on previous studies in fish (Kobayashi et al., 1991; Oliveira et al., 2001c; Modesto and Canário, 2003; Ros et al., 2004) and was of 100 μg per gram of fish for T and for the aromatase blocker Fadrozole (F) and of 1 μg per gram of fish for E_2. Steroids and F were thoroughly dissolved in castor oil. A total of 54 sneakers were randomly assigned to one of six treatments: (1) Sham; (2) castrated (Cast); (3) castrated controls (C; castor oil