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Regulation of protogynous sex change by competition between corticosteroids and androgens: An experimental test using sandperch, *Parapercis cylindrica*

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Abstract

Cortisol, the dominant corticosteroid in fish, and 11-ketotestosterone (11KT), the most potent androgen in fish, are both synthesized and (or) deactivated by the same two enzymes, 11β-hydroxylase and 11β-hydroxysteroid dehydrogenase. Cortisol is synthesized in response to stress (such as that caused by interaction with a dominant conspecific), whereas 11KT is synthesized during protogynous sex change. It has been hypothesized that corticosteroids (such as cortisol) inhibit 11KT synthesis via substrate competition, thereby providing a mechanism for the regulation of socially mediated, protogynous sex change. We tested this hypothesis by administering cortisol (50 μg g^{-1} body weight) to female sandperch (*Parapercis cylindrica*) under social conditions that were permissive to sex change (i.e. in the absence of suppressive male dominance). Twenty-one days later, mean physiological cortisol concentration in cortisol-treated fish was 4.2-fold greater than that in 'socially stressed' female fish maintained in a semi-natural system. Although the dosage of cortisol was therefore considered to be favorable for engendering competitive inhibition of 11KT synthesis, all cortisol-treated fish changed sex, as did all sham-treated and control fish ($n=7$ fish per treatment). In addition, there was no effect of cortisol treatment on the rate of sex change or on the pattern of steroidogenesis. Thus, our results refute the hypothesis that protogynous sex change is regulated by substrate competition between corticosteroids and androgens.

Keywords: Social interaction; Stress; Cortisol; Endocrine regulation; Sex change; Protogyny; Coral reef fish

Introduction

Unlike almost all other vertebrates, a significant number of fishes mature first as female and later change sex to male (protogyny), or mature as male and later change sex to female (protandry) (Francis, 1992; Devlin and Nagahama, 2002). Often, initiation of sex change is coincident with a shift in the local social environment. For example, in group-forming, protogynous species, the death or removal of a male fish typically stimulates a female fish to undergo sex change, thereby restoring the operational sex ratio of the social unit (Robertson, 1972; Mackie, 2003).

The proximate cause of sex change appears to be a shift in the pattern of gonadal steroidogenesis (Frisch, 2004). In particular, sex change involves reciprocal changes in the relative concentrations of estradiol-17β (E_2), which tends to promote 'femaleness' (e.g. oogenesis), and 11-ketotestosterone (11KT), which tends to promote 'maleness' (e.g. aggressive behavior) (Nakamura et al., 1989; Cardwell and Liley, 1991; Cochran and Grier, 1991; Bhandari et al., 2003). Both E_2 and 11KT are synthesized from the same substrate, testosterone (T), which is typically found in all sexual stages of teleosts (Kime, 1993; Borg, 1994). The conversion of T to E_2 is catalyzed by the enzyme aromatase, while the conversion of T to 11KT proceeds via the intermediate 11β-hydroxytestosterone (11βOHT) and is catalyzed by the enzymes 11β-hydroxylase (11βH) and 11β-hydroxysteroid dehydrogenase (11βHSD) (Frisch, 2004). Not surprisingly, the relative activities of aromatase, 11βH and

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11βHSD in the gonad profoundly influence sexual status (Morrey and Nagahama, 2000; Bhandari et al., 2004; Sunobe et al., 2005).

Typically, sex change is limited to the highest ranking (or largest) individual within a social group (Munday et al., 2006). Thereafter, the dominant individual suppresses the transition of other (subordinate) fish in the social group, at least until that dominant individual dies or is removed (Ross, 1990). This suppressive effect is mediated via aggression and monopolization of resources, both of which cause stress to subordinates (Lutnesky, 1989; Fox et al., 1997; Perry and Grober, 2003). As in many other vertebrates, stress in fish manifests as, among other things, an increase in the synthesis of cortisol, the dominant corticosteroid in fish (Sumpter, 1997; Frisch and Anderson, 2000; Barton, 2002). This response presumably serves to increase the circulation of stored energy reserves in anticipation of sustained muscle activity (Barton, 2002).

Although the relationship between social interaction and sexuality has been well documented, little is known about the mechanisms by which social interactions influence gonadal steroidogenesis during sex change. This gap has been provisionally filled by a model that links stress-induced corticosteroid metabolism with inhibition of androgen synthesis – a prerequisite for protogynous sex change (Perry and Grober, 2003). Specifically, synthesis and deactivation of corticosteroids, such as cortisol, are catalyzed by 11βH and 11βHSD (respectively) – the same two enzymes responsible for the two-step synthesis of 11KT from T. The commonality of these enzymes to both steroid pathways creates the potential for competition between steroid substrates (Perry and Grober, 2003). It is hypothesized that female fish (under suppressive, male dominance) are unable to change sex because increased corticosteroid levels competitively inhibit the synthesis of 11KT. Conversely, the removal of suppressive dominance from a female fish is hypothesized to result in the clearance of corticosteroids and, ultimately, sex change via the synthesis of 11KT (and other androgens).

The objective of the present study was to test the above-mentioned model. To do this, we first assessed 'natural' levels of cortisol in females of protogynous sandperch (*Parapercis cylindrica*) that were assumed to be under chronic stress from suppressive (male) dominance (Experiment 1). We then artificially elevated cortisol levels in high-ranking female fish and, at the same time, relieved them of suppressive dominance, thus creating social conditions permissive to sex change (Experiment 2). Predictions based on the model suggest that cortisol-treated fish would not synthesize 11KT, but would instead continue to synthesize E_2, thereby failing to undergo sex change.

Materials and methods

Experimental animals

P. cylindrica is a group-forming, protogynous hermaphrodite that inhabits shallow coral reefs of the western Pacific Ocean (Randall et al., 1990). Social units typically contain one male and two or more females, and males suppress sex change in females via dominance interactions. Social rank is strictly size-based, and maturation occurs at ~70 mm total length (TL) (Walker and McCormick, 2004). Importantly, *P. cylindrica* is well suited to manipulative experiments in captivity: individuals acclimate rapidly to tank conditions; males and females have sexually dimorphic coloration; and sex change occurs rapidly (~21 days) (Walker and McCormick, 2004).

Experiment 1: cortisol levels in a semi-natural system

To simulate the natural habitat of *P. cylindrica*, a generous quantity of coral rubble and algae-encrusted rock was added to seven 100-l plastic tanks, each supplied with high-quality circulating seawater (~2 l min^{-1}), supplemental aeration, and ambient light. Thirty-seven adult *P. cylindrica* (7 males, size range: 107–115 mm TL; 30 females, size range: 70–94 mm TL) were collected from the Great Barrier Reef, Australia using a dip-net and anesthetic (10% clove oil in ethanol). 1 male and ≥2 females were added to each of the seven tanks, after which all fish were left undisturbed, except for regular feeding with shrimp and fish flakes. Just as in the wild, a size-based social hierarchy was established within each group of fish (Walker and McCormick, 2004). This enabled social rank to be assigned based on TL (rank 1=largest fish [male], rank 2=second largest fish [dominant female], rank 3=third largest fish [subordinate female], etc.). After 30 days, all fish were captured with a dip-net and subsequently euthanized with an overdose of anesthetic (~1 min in 30 ppt clove oil). Each fish was captured and anesthetized as quickly as possible, thereby minimizing handling stress (Strange and Schreck, 1978; Morgan and Iwama, 1997; Wagner et al., 2003). To confirm each fish's sex, the gonad was removed by dissection and subsequently stored in 10% buffered formaldehyde (pH 7.4) until histological sectioning (see below). The remaining body was frozen (−80 °C) until steroid extraction (see below).

Experiment 2: exogenous cortisol administration

Sixty-three adult *P. cylindrica* (21 males, size range: 106–123 mm TL; 42 females, size range: 73–106 mm TL) were captured (as described above) and placed into 21 tanks, with one male and two females per tank. Tank conditions were the same as in Experiment 1, except that algae-encrusted bricks and pots were used to simulate the natural habitat, and minced fish and squid were used as food. Daily observations confirmed that a size-based social hierarchy was established among each group of fish (Walker and McCormick, 2004). After 2 days, a dip-net was used to permanently remove all male fish. At the same time, dominant female (rank 2) fish were individually captured, briefly anesthetized (~1 min in 20 ppm clove oil), and restrained in a wet foam cradle. Dominant female fish were then randomly assigned to one of three treatment groups: 'cortisol', 'sham' or 'control' (7 fish per treatment). Fish in the cortisol group received an implant containing cortisol, while fish in the sham group received an implant without cortisol. Control fish did not receive an implant, but were restrained in the cradle for an equivalent amount of time as fish in the other two groups.

Previous studies have demonstrated that silicone implants are a reliable technique for chronic administration of exogenous steroids (Pankhurst et al., 1986; Gamperl et al., 1994; Shelton and Mims, 2003). In particular, cortisol implants are known to maintain elevated cortisol concentrations in the plasma of experimental fish for at least 20–30 days (Pickering and Duston, 1983; Pickering and Pottinger, 1985). In studies where the objective was to simulate chronic stress, the appropriate dose of cortisol was found to be ~18–60 μg g^{-1} body weight (Pickering and Duston, 1983; Pickering and Pottinger, 1985; Carragher et al., 1989). In the present study, we chose to use cortisol at doses of 50 μg g^{-1} body weight, which is in the upper part of this range.

To prepare the cortisol implants, 9.9 mg of cortisol (No. H4001, Sigma, Castle Hill, Australia) was added to 0.6 ml of medical grade silicone (No. Q7-4850, Dow Corning, Midland, USA) in a petri dish. After thorough mixing with a spatula, the cortisol–silicone composite was loaded into a 0.5-ml syringe and refrigerated (4 °C) overnight. A small, semi-solid implant (size range: 25–32 mm^3) was then inserted into the ventral, posterior region of the peritoneal cavity of relevant fish using a short, blunt, 19-gauge hypodermic needle. The size of each implant was adjusted to ensure that each fish received the correct dose of cortisol. Sham implants were prepared in the same way but without the cortisol. The insertion of each implant was completed in ~1 min, after which each fish was returned to its tank. Within 24 h, dominant female fish (all three treatment groups) resumed normal behavior (e.g. feeding), and subsequent survival of these