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Effects of castration and androgen-treatment on the expression of FSH-β and LH-β in the three-spine stickleback, gasterosteus aculeatus—Feedback differences mediating the photoperiodic maturation response?☆

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ABSTRACT

In many animals, including the three-spine stickleback (*Gasterosteus aculeatus*), photoperiod strongly influences reproduction. The aim of this study was to investigate if feedback mechanisms on the brain–pituitary–gonadal axis play a role in mediating the photoperiodic response in the stickleback. To that end, stickleback males, exposed to either non-stimulatory short photoperiod (light/dark 8:16) or under stimulatory long photoperiod (LD 16:8), were subjected to either sham-operation, castration, castration combined with treatment with the androgens 11-ketoandrostenedione (11KA) and testosterone (T), and the effects on levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH)-β mRNA were analyzed. During breeding season the kidney of the stickleback male hypertrophies and produces a glue used for building nests. Kidney weight and expression of both LH-β and FSH-β were higher in sham-operated fish kept under long than under short photoperiod. Under both photoperiods, LH-β mRNA levels were lower in castrated males compared to sham-operated males and treatment with 11KA and T increased expression, indicating a positive feedback. A positive feedback was also found on FSH-β expression under long photoperiod, where castration decreased, and androgen replacement restored FSH-β mRNA expression. On the contrary, castration under short photoperiod instead increased FSH-β levels whereas treatment with 11KA and T decreased FSH-β expression, indicating a negative feedback on FSH-β under these conditions. The positive feedback on FSH-β expression under stimulatory photoperiod may accelerate maturation, whereas the negative feedback under inhibitory photoperiod may suppress maturation. This could be part of the mechanisms by which photoperiod controls maturation.

1. Introduction

The photoperiod is an important factor in the control of seasonal reproduction. Under stimulatory photoperiods, an increased secretion of gonadotropins (GTHs) from the pituitary activate vertebrate gonads. Previous studies have shown that photoperiod can alter the sensitivity of steroid feedback on the secretion of GTHs in the brain–pituitary–gonadal (BPG) axis in mammals (Turek, 1977; Rosa and Bryant, 2003). For instance, higher doses of testosterone (T) were needed to suppress LH and FSH plasma levels in castrated golden hamsters, *Mesocricetus auratus,* under a stimulatory long photoperiod than under a non-stimulatory short photoperiod (Turek, 1977). However, GTH secretion patterns may also change independently of changes in feedbacks on the BPG-axis. This has been observed in several studies on birds, e.g. in Japanese quail, *Coturnix japonica,* where both plasma LH and FSH levels were higher in castrated birds kept under stimulatory long photoperiods than under shorter photoperiods (Urbanski and Follett, 1982). These two ways in which photoperiods may control the BPG-axis are not mutually exclusive, both mechanisms were found to be present in tree sparrows, *Spizella arborea* (Wilson, 1985).

Feedback effects on the BPG-axis play an important role in controlling reproduction in teleost fishes. Both positive and negative feedback effects on the BPG-axis have been reported (Crim and Evans, 1979; Billard et al., 1977). Aromatization, the conversion of androgens to estrogens, has been found to be involved in both the positive and negative feedback effects in fishes (Crim et al., 1981; de Leeuw et al., 1986). It is, however, not known whether changes in feedback mechanisms play a role in the photoperiodic control of reproduction in fish.

The three-spine stickleback (*Gasterosteus aculeatus*) has a marked seasonal reproductive cycle in which spermatogenesis and the development of androgen-dependent secondary sexual characters in the male are separated in time (e.g. Borg, 1982).

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During the breeding season, the stickleback displays distinctive secondary sexual characters such as the red breeding colours and a hypertrophied kidney that secrets a glue, consisting of the androgen induced protein spiggin (Jakobsson et al., 1999; Jones et al., 2001), used for nest building. Sexual maturation in the stickleback is strongly dependent on photoperiod. In winter, long photoperiods induce maturation, while shorter photoperiods do not, even if temperatures are high (e.g. Baggerman, 1957; Borg et al., 1987).

The aim of the present study was to investigate whether feedback mechanisms play a role in mediating the photoperiodic response in the stickleback. To that end, males exposed to either short or long photoperiod were subjected to either sham-operation castration, castration combined with androgen replacement with the non-aromatisable androgen 11-ketoandrostenedione (11KA) or the aromatisable androgen testosterone (T), and the effects on expression of LH-β and FSH-β mRNA were analyzed.

2. Materials and methods

2.1. The animals and experimental protocol

The study was performed with the permission of the Stockholm Northern Animal Experiment Ethical Board (No. N184/00). Adult non-breeding sticklebacks were caught in the Öresund on the 15–16 December 2001. The fish were kept at 7 °C and under short day photoperiod (LD 8:16) until the onset of the experiment.

The castration/implant experiment was started on the 4–6 January 2002. The fish were anaesthetized with 0.1% 2-phenoxyethanol, ca. 1.5 mm long incisions were made into the abdominal cavity on each side and the testes were excised using fine forceps. This type of surgery drastically reduces circulating levels of T and 11-ketotestosterone (11KT) in breeding sticklebacks (Mayer et al., 1990). Sham-operated fish were treated similarly except that the testes were mot removed. The castrated control and sham-operated fish were implanted with empty Silastic capsules (length 5 mm, 1.2 mm outer diameter) sealed with silicone glue. The other two castrated groups were implanted with capsules, described as above, containing either crystalline T (KEBO) or 11KA (Sigma), which is converted to 11KT in blood cells of sticklebacks (Mayer et al., 1990). The fish were divided between two 700 l aquaria kept under long photoperiod (16:8 light/dark) or under short photoperiod (8:16 LD). In the castration/replacement experiment, 16 sham-operated, 18 control castrated, 17 11KA treated and 17 T treated fish were placed under each regime.

Temperature was raised gradually to 20 °C over the first week to allow adaptation. All aquaria contained brackish water (0.5% salinity), which was constantly filtered and aerated, and the bottoms were covered with sand. The fish were fed daily with frozen red midge larvae. The fish in the castration/replacement experiment were sampled on the 14–15 February 2002. The fish were anaesthetized and weighed. Pituitaries were collected and frozen in liquid nitrogen, and kidneys were excised and weighed (±1 mg). Visual inspection under a dissecting microscope confirmed that the presence of capsules and the completeness of castration (absence of testes remnants) in all fish.

2.2. Dot-blot analysis of LH-β and FSH-β mRNA expression

Total RNA was extracted from single pituitaries using Trizol reagent (Invitrogen) following the manufacture's instructions, and was then denatured and blotted on Hybond-N+, Nylon membrane (Amersham Pharmacia Biotech) through a Hybri-blot manifold (Life Technologies). The membranes were allowed to dry and were then UV-crosslinked.

The membranes were hybridized using a cRNA probe. The ca. 300 bp long antisense probes, based on stickleback LH-β and FSH-β (Hellqvist et al., 2004, Accession Nos. AJ534969 and AJ534871, respectively) were synthesized by *in vitro* transcription using SP6 or T7 RNA polymerase, respectively, and labelled with (α-^{32}P) UTP according to the ^{TM}Strip-EZ™ RNA kit (Ambion). The blots were prehybridized for 1 h at 65 °C, with ULTRAhyb hybridization solution (Ambion) and then incubated over night with the radioactive probe (1×10^{6} cpm/ml hybridisation solution). The blots were washed twice in 1× SSC, 0.1% SDS at 65 °C for 15 min each and then once for 15 min at 65 °C and once for 30 min at 68 °C in 0.1× SSC, 0.1% SDS. The hybridization signals of the blots were counted and analyzed using a biophosphoimager (BioRad). The membranes were stripped according to the ^{TM}Strip-EZ™ protocols and subsequently hybridized with a ^{32}P-labeled stickleback 18S rRNA probe to correct for unequal RNA loadings.

2.3. Statistics

The data were analyzed with STATISTICA '99 edition (StatSoft Inc.), using two-way ANOVA for multiple comparisons and Tukey honest significant difference (post hoc) test for comparisons between pair of groups. Prior to analysis, the data were log transformed.

3. Results

3.1. Kidney somatic index

Sham-operated males displayed significantly higher KSI under long photoperiod than under short photoperiod ($p < 0.001$, Table 1). Under long photoperiod, castrated males had significantly lower kidney somatic index (KSI) compared to sham-operated males ($p < 0.001$), whereas under short photoperiod, where KSI was low in both sham-operated and castrated males, there was no significant difference in KSI. Treatment with 11KA significantly increased KSI in castrated males under both photoperiods ($p < 0.001$ in each comparison), whereas treatment with T did not. There was no effect of photoperiod on KSI in the groups treated with 11KA, T or in castrated controls.

3.2. FSH-β and LH-β mRNA expression

In both experiments, expressions of both FSH-β and LH-β in sham-operated males were significantly higher under long photoperiod than under short photoperiod ($p < 0.001$, Figs. 1 and 2).

There was no difference in LH-β levels between the two photoperiods in castrated control males and in castrated males treated with either 11KA or T (Fig. 2). Under long photoperiod, LH-β mRNA levels were significantly reduced in castrated males compared to

Table 1
Kidneysomatic indices (KSI) in sham-operated males, castrated controls and castrated males treated with 11-ketoandrostenedion (11KA) or testosterone (T) implants and kept under different photoperiods (16 h light/8 h dark, 8 h light/16 h dark)*

Treatment	KSI
16:8 sham	2.67 ± 0.17[b]
16:8 castrated	0.78 ± 0.06
16:8 11KA	2.72 ± 0.25[b]
16:8 T	1.21 ± 0.15
8:16 sham	0.94 ± 0.17[a]
8:16 castrated	0.74 ± 0.09
8:16 11KA	2.65 ± 0.18[b]
8:16 T	1.17 ± 0.19

* Values are mean ± SEM, $n = 10–13$ fish per group.
[a] $p < 0.001$ compared to sham-operated fish under LD 16:8.
[b] $p < 0.001$ compared to castrated to castrated controls under the same photoperiod.