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An *in vitro* model for evaluating peripheral regulation of growth in fish: Effects of 17β-estradiol and testosterone on the expression of growth hormone receptors, insulin-like growth factors, and insulin-like growth factor type 1 receptors in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

A central component of growth coordination in vertebrates is the growth hormone (GH)–insulin-like growth factor-1 (IGF-1) system. To date, most studies on the control of vertebrate growth have focused on regulation of pituitary GH production and release. In this study, we used liver, muscle, and gill tissue from sexually immature rainbow trout incubated *in vitro* to evaluate the extrapituitary effects of 17β-estradiol (E2) and testosterone (T) on mRNA and functional expression of growth hormone receptors (GHR), insulin-like growth factors 1 and 2 (IGF-1, IGF-2), and type 1 IGF receptors (IGFR1). E2 significantly decreased steady-state levels of GHR1, GHR2, and IGF-1 mRNAs in liver as well as of GHR1 and GHR2 mRNAs in muscle and of IGF-1 and IGF-2 mRNAs in gill in a time- and concentration-dependent manner. E2 had no effect on levels of IGFR1 mRNAs in muscle or on GHR and IGFR1 mRNAs in gill. Functional expression of GHRs as assessed by ^{125}I–GH binding capacity was reduced by E2 in liver and muscle; however, E2 did not affect ^{125}I–IGF-1 binding capacity in muscle or ^{125}I–GH and ^{125}I–IGF-1 binding capacity in gill. By contrast, T increased steady-state levels of GHR1, GHR2, IGF-1, and IGF-2 mRNAs in liver, of GHR1, GHR2, IGFR1A, and IGFR1B in muscle, and of GHR1, GHR2, IGF-1, IGF-2, IGFR1A, and IGFR1B mRNAs in gill in a time- and concentration-dependent manner. Binding capacity of ^{125}I–GH in liver and of ^{125}I–GH and ^{125}I–IGF-1 in both muscle and gill also was increased by T. These data indicate that E2 and T directly affect peripheral aspects of the GH–IGF system, and suggest, at least in immature rainbow trout, that E2 reduces hepatic sensitivity to GH as well as reduces peripheral production of IGFs and that T increases peripheral sensitivity to GH and IGF as well as increases peripheral production of IGFs.

1. Introduction

Animal growth is influenced by genetic, environmental, and nutritional factors. Extrinsic factors, such as temperature, photoperiod and food availability, are particularly important in the growth of fish, including the triggering of developmental processes such as hatching, metamorphosis, smoltification, sexual maturation, and spawning [3,28,36]. Although most species of fish have the capacity to grow throughout their life (indeterminate growth), their rate of growth typically slows over time, especially just prior to sexual maturity when energy is channeled to gonad growth and gamete production [9,31].

Integration of intrinsic and extrinsic factors results in modulation of the growth hormone (GH)–insulin-like growth factor (IGF) system. The GH–IGF system of fish is particularly complex given the existence of multiple receptors for GH (GHRs) and multiple type 1 IGF receptors (IGFR1s), IGF-2 production that, unlike the situation in mammals, is sensitive to GH in postembryonic animals, and in some species a gonad-specific IGF (IGF-3) that also is regulated by GH [2,34,37,47].

Accumulating evidence suggests that sex steroids may influence the growth of fish by modulating components of the GH–IGF system [6]. The observation that plasma levels of sex steroids increase during sexual maturation of fish, ranging from nondetectable to 60 ng/ml for E2 or to 160 ng/ml for T, and that these changes correlate with changes in plasma GH suggest that sex steroids regulate GH secretion [1,18,46]. This notion is supported by some studies that showed that 17β-estradiol (E2) increased plasma GH in goldfish, rainbow trout, and tilapia [17,29,45]; however, this response appears variable as E2 decreased plasma GH salmon in

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Atlantic salmon [24]. The effect of androgens also appears variable. Testosterone (T) increased plasma GH in goldfish, an effect that required aromatization of T–E2 [5], as well as in fasted but not fed rainbow trout [17]; however, T and 11-ketotestosterone (11KT) had no effect on plasma GH in coho salmon [23].

Data also indicate that sex steroids may act peripherally on aspects of the GH–IGF system. For example, in tilapia, elevated E2 levels did not correlate with high hepatic GHR mRNA expression [21]. Furthermore, E2 *in vivo* inhibited hepatic expression GHR mRNAs in black sea bream [19] as well as hepatic expression IGF-1 and IGF-2 mRNAs in gilthead sea bream [6]. Moreover, plasma levels of IGF-1 were reduced by E2 immersion of Atlantic salmon [24]. In tilapia, the expression of GHR and IGF-1 was reduced in the liver but not in the testes of E2-treated males [8]. Testosterone injection decreased the expression of GHR2 but not GHR1 mRNA in black sea bream [19]. In addition, T and 11KT implantation increased the expression of IGF-1 mRNA in coho salmon [23]. The variability of the response of GH–IGF system components to sex steroids may result from differences in the stage of sexual maturation, nutritional status, or other environmental influence as well as from tissue- and species-specific differences reflective of the animal's evolutionary/life history.

In this study, we used an *in vitro* rainbow trout system to examine the influence of sex steroids on the GH–IGF system in order to clarify their endogenous actions and to provide greater insight into the coordination of growth and reproduction. The specific hypothesis of the study was that E2 and T directly modulate the expression of GHRs, IGFs, and of IGFR1s. Our focus was on the primary source of peripheral IGF-1/2 (liver) and the major growth responsive tissues (muscle, gill) [12,33,35,47,48].

2. Materials and methods

2.1. Animals

Juvenile rainbow trout of both sexes (91.4 ± 5.3 g) were obtained from Dakota Trout Ranch near Carrington, ND, and transported to North Dakota State University where they were maintained in 800-l circular tanks supplied with recirculated (100% replacement volume per day), dechlorinated, municipal water at 14 °C under a 12L:12D photoperiod. Fish were fed to satiation twice daily with AquaMax Grower (PMI Nutrition International, Inc., Brentwood, MO), except 24–36 h before initiating experimental manipulations. Animals were acclimated to laboratory conditions for at least two weeks prior to experimentation.

2.2. Experimental conditions

At the time of sampling, animals were anesthetized in 0.05% (v/v) 2-phenoxyethanol, measured (body length and body weight), and bled from the severed caudal vessels. Gonads were removed and sex was determined by microscopic examination of fresh mounted gonadal tissue; all fish were sexually immature (gonad

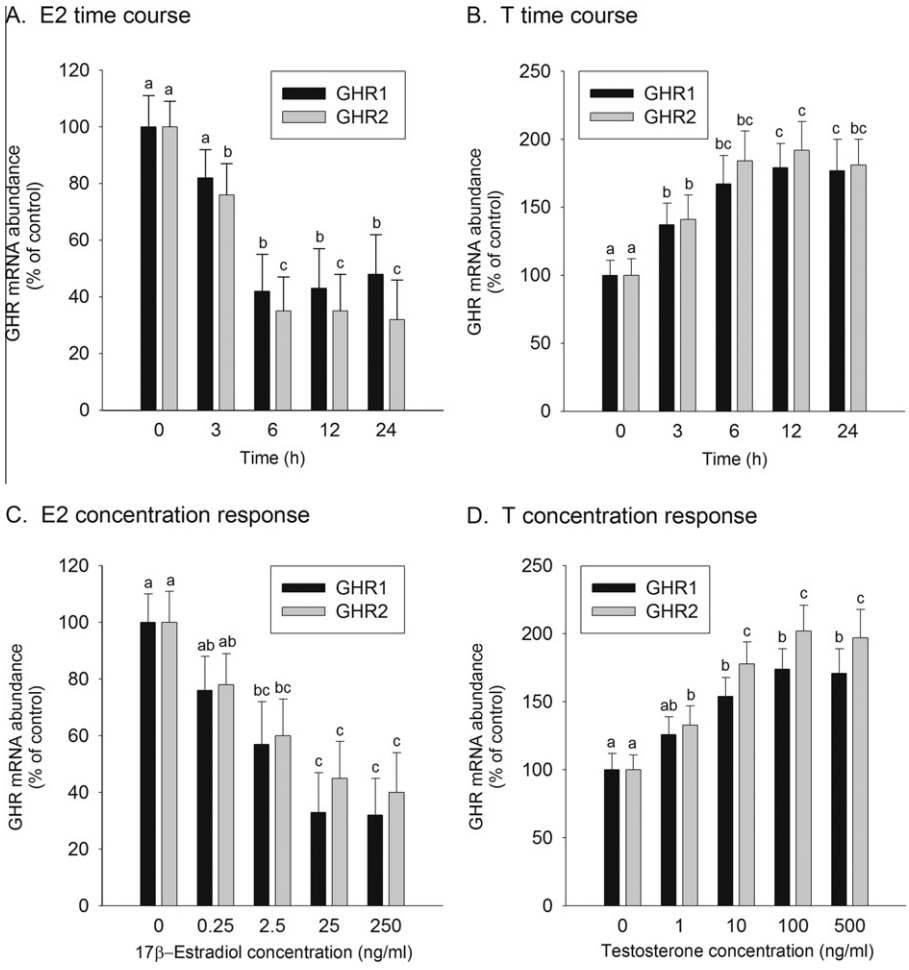

Fig. 1. Effects of 17β-estradiol (E2) and testosterone (T) on mRNA expression of growth hormone receptors in liver of rainbow trout incubated *in vitro*. Liver pieces were incubated for various times with 25 ng/ml E2 (A) or 100 ng/ml T (B) or with various concentrations of E2 (C) or T (D) for 6 h. Data are presented as mean ± SEM (n = 6–8). For a given GHR subtype, groups with different letters are significantly ($P < 0.05$) different from each other.