ANNUAL CHANGES IN PLASMA LEVELS OF CORTISOL AND SEX STEROID HORMONES IN MALE RAINBOW TROUT, ONCORHYNCHUS MYKISS *

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Abstract The profiles of cortisol, testosterone, 11-ketotestosterone and 17α, 20β-dihydroxy-4-pregnene-3-one in male rainbow trout reared under constant water temperature and natural photoperiod were determined by radioimmunoassay. Gonads of male rainbow trout reached maturity when the fish were two years old. Changes in the plasma levels of both sex steroid hormones and cortisol were closely related to the GSI. Plasma levels of testosterone, 11-ketotestosterone and 17α, 20β-dihydroxy 4-pregnene-3-one showed a clear peak in the annual breeding season, when the GSI reached their maxima. Plasma cortisol levels also showed clearly seasonal changes in both two- and three-year-old fish. The results suggest that the elevated plasma levels of cortisol may not just be due to stresses during the breeding season but have certain physiological functions in the reproduction of rainbow trout.

Key words: rainbow trout, sex steroid hormones, cortisol

INTRODUCTION

There are a lot of published papers describing the annual or seasonal endocrine changes associated with reproductive activity in teleost fishes (Scott et al., 1980; Lou et al., 1984, 1986; Suzuki et al., 1997; Hou 1998; Yaron et al., 1986), and evidence that the gonadal maturation of salmonid fish was accompanied by adrenocortical hypertrophy (Schreck, 1996). Plasma concentrations of cortisol were observed to elevate during the spawning season in goldfish (Delahunty et al., 1979) and in Pacific salmon (Maule et al., 1996). But the changes in cortisol levels are usually attributed to stress (Pickering et al., 1981). Moreover, many of these studies showed changes in plasma levels of either cortisol or sex steroid hormones during gonadal maturation (Carragher et al., 1989; Foo and Lam, 1993). The correlation between circulating cortisol and sex steroid hormones in annual changes cannot be clearly understood. The present study was thus conducted to clarify the annual changes in plasma levels of cortisol and sex steroid hormones (testosterone, 11-ketotestosterone and 17α, 20β-dihydroxy-4-pregnene-3-one) in male rainbow trout reared under constant water temperature and natural photoperiod.

MATERIALS AND METHODS

Experimental fish

Two-year-old to three-year-old male rainbow trout used in this study were reared in a concrete
tank with running spring water at 10°C, under natural day light, at the Oizumi Fisheries Research Laboratory of Tokyo University of Fisheries, Japan. Average body weights of the fish were from 352 ± 96 g at the beginning of the experiment to 1263 ± 214 g at the end of experiment.

Fish were sampled at about monthly intervals. Six fish were netted each time, and anesthetized immediately with 1000 × 10⁻⁶ of 2-phenoxyethanol. Within 5 minutes after netting, blood was taken from the caudal vasculature using a heparinized syringe. The blood samples were centrifuged at 1000 × g at 4°C for 10 minutes and blood plasma was separated, and plasma samples were stored at -25°C until use. Fish and gonad were weighed and gonado-somatic indices (GSI: gonad weight × 100/body weight) were calculated.

Experimental methods

Steroid hormone radioimmunoassay (RIA) Testosterone and 11-ketotestosterone were measured by radioimmunoassay (RIA) as described previously (Lou et al., 1984, 1986; Hou, 1998).

Plasma cortisol levels were determined as described previously (Hou et al., 1999). Antiserum (Cosmo-Bio Co.) and labeled cortisol (NEN research products) were used. The antiserum cross-reacts with cortisol, deoxycorticosterone, corticosterone, and testosterone at 100, 12, 8, and 0.1% levels, respectively. The percentage recovery of tritiated cortisol from plasma was 96%. The intra-assay and inter-assay coefficient of variations of the RIA were 6% and 12%, respectively.

The assay for 17α, 20β-dihydroxy-4-pregnene-3-one (17α, 20β-DP) were newly developed for this study. Labeled (2, 4, 6, 7-3H) 17α, 20β-DP were purchased from New England Nuclear (USA). Unlabeled 17α, 20β-DP used for standards were purchased from Sigma Chemical Company (USA). The rabbit anti-17α, 20β-dihydroxy-4-pregnene-3-oxime-BSA serum was purchased from Teikokozoki Pharmaceutical Company (Japan). The cross reactivity of 17α, 20β-dihydroxy-4-pregnene-3-oxime-BSA with 17α, 20β-dihydroxy-4-pregnene-3-one, 5β-pregnan-3β, 17α, 20β-triol, 20β-hydroxyprogesterone and 17α-hydroxyprogesterone were 100, 2.54, 1.55, 0.82, 0.028 and < 0.01%, respectively. The intra-assay and inter-assay coefficients of variation were 6.0% (n = 6) and 7.1% (n = 7). Recovery was 95.9%. The lowest limit of detection was 45 pg/ml.

Statistical analysis

All data are expressed as mean ± SEM. Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS

Changes in GSI

The two-year-old fish’s GSI rapidly increased in September, peaked in October (5.4 ± 0.8), then declined in November; that of the three-year-old rapidly increased in August, peaked in September, then declined in October. Spermiogenesis began in October, and lasted until January (Fig.1). Solid symbols in Fig.1 indicate the time points where the hormone concentrations were significantly different from those at other time points not marked by solid symbols (P < 0.05).

Changes in steroid hormone levels

The two-year-old fish’s plasma cortisol levels in the period from August to November were higher than those in other months, and started to decline from December; those of the three-year-old fish rose sharply in October, when spermiogenesis began (Fig.2).

Plasma testosterone level began to rise in August, maximized in September (35.86 ± 4.92 ng/