Effects of acute 17\( \alpha \)-methyltestosterone, acute 17\( \beta \)-estradiol, and chronic 17\( \alpha \)-methyltestosterone on dopamine, norepinephrine and serotonin levels in the pituitary, hypothalamus and telencephalon of rainbow trout (Oncorhynchus mykiss)

Abstract This study investigated: (a) the effects of acute 17\( \alpha \)-methyltestosterone (MT) or 17\( \beta \)-estradiol (E\(_2\)) administration on norepinephrine (NE), dopamine (DA), serotonin (5-HT), 3,4, dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) contents in the hypothalamus, telencephalon and pituitary of previtellogenic female rainbow trout Oncorhynchus mykiss, and (b) the effects of chronic MT administration on the levels of these neurotransmitters in these brain regions in immature male rainbow trout. The acute administration of MT induced a significant decrease in pituitary levels of DOPAC as well as in the DOPAC/DA ratio. On the other hand, the acute administration of E\(_2\) induced an increase in pituitary 5-HT levels as well as a decrease in the 5-HIAA/5-HT ratio. In a second experiment, 20 mg MT per kilogram body weight was implanted for 10, 20 or 40 days into sexually immature male rainbow trout. Implanted rainbow trout showed increased testosterone and decreased E\(_2\) levels. In the pituitary, MT induced long-term decreases in NE, DA, DOPAC and 5-HT levels, as well as in the DOPAC/DA ratio. Hypothalamic and telencephalic DA, NE and 5-HT levels were not affected by MT implantation. However, 5-HIAA levels and the 5-HIAA/5-HT ratio were reduced by MT implantation in both brain regions. These results show that chronic treatment with MT exerts both long-term and region-specific effects on NE, DA, and 5-HT contents and metabolism, and thus that this androgen could inhibit pituitary catecholamine and 5-HT synthesis. A possible role for testosterone in the control of pituitary dopaminergic activity and gonadotropin II release is also discussed.

Keywords Hypothalamus · Pituitary · Testosterone · Dopamine · Rainbow trout Oncorhynchus mykiss

Abbreviations DA dopamine · DOPAC 3,4, dihydroxyphenylacetic acid · E\(_2\) 17\( \beta \)-estradiol · GSI gonado-somatic index · GTH II gonadotropin II · 5-HIAA 5-hydroxyindoleacetic acid · HPLC-EC high performance liquid chromatography with electrochemical detection · 5-HT serotonin · GnRH gonadotropin-releasing hormone · MT 17\( \alpha \)-methyltestosterone · NE norepinephrine · T testosterone

Introduction

In teleosts there is gonadal steroid feedback on hypothalamo-hypophysial gonadotropic activity (Peter and Yu 1997), with endogenous steroids participating in the up- and down-regulation of gonadotropin II (GTH II) secretion, depending on stage of gametogenesis (Bommelaer et al. 1981; Breton and Sambroni 1996). These steroids may control the preoptic-hypothalamic-pituitary axis by regulating hypothalamic inputs to the pituitary (Yu et al. 1998) or directly controlling GTH secretion in the pituitary (Crim et al. 1981; Amano et al. 1994; Breton and Sambroni 1996). Studies have demonstrated that 17\( \alpha \)-methyltestosterone (MT) and testosterone (T) stimulates GTH II release in masu salmon and rainbow trout (Amano et al. 1994; Breton and Sambroni 1996) whereas no effects were observed after 17\( \beta \)-estradiol (E\(_2\)) administration (Breton and Sambroni 1996).

Steroids may modulate the release of pituitary hormones acting on monoaminergic neurotransmitters (Saliga et al. 1992; Trudeau et al. 1993b, 1993c; Senthilkumar and Joy 1994; Tsai and Wang 1997a, 1997c). In teleosts, the effect of estrogens (E\(_2\)) on the activity of brain monoaminergic systems is better
understood than that of androgens. Thus, E2 administration is known to increase pituitary dopamine (DA) activity, through an enhancement of the inhibitory action of DA on the release of pituitary GTH II (Saligaut et al. 1992, 1998; Trudeau et al. 1993c; Linard et al. 1995), as well as to induce a decrease in both hypothalamic levels of norepinephrine (NE; Manickam and Joy 1993b), whereas it triggered an increase in hypothalamic levels of norepinephrine (NE; Manickam and Joy 1993b, 1995), as well as to induce a decrease in hypothalamic NE contents in male tilapia fry (Tsai and Wang 1997a, 1997b) and to induce an increase in pituitary GTH II content (Amano et al. 1994). MT can also induce and synchronize gonadal development in teleosts (Lee et al. 1986; Tamaru et al. 1988).

Materials and methods

Animals

Rainbow trout (Oncorhynchus mykiss) were obtained from a fish farm in Soutorredondo (Noia, Spain). In the first experiment previtelligenic female trout (251 ± 12 g body weight, gonadosomatic index, GSI = 0.16 ± 0.01%) were used. In the second experiment, immature male trout (139 ± 5 g body weight, GSI = 0.07 ± 0.02%) were used. In both experiments, the fish were randomly allocated to 250-l tanks and were acclimatized for 1–2 weeks (photoperiod 10L:14D, water temperature 14.0 ± 0.5 °C, pH 6.5 ± 0.1). Fish were fed daily ad libitum with commercial trout food.

Experiment 1: effects of acute MT and E2 treatment in previtelligenic female trout

This experiment was performed during November 1998. Fish were anesthetized by immersion in 0.05% (p/v) ethyl-maminobenzoic acid methanesulfonate salt (MS-222 buffered pH 7.4) for less than 1.5 min, then weighed. Each fish then received two i.p. injections, separated by 24 h, of either 10 mg kg⁻¹ E2 (Group 1, n = 7), or 10 mg kg⁻¹ MT (Group 2, n = 8), with an injection volume of 2 μl g⁻¹ body weight in all cases. The third group (control) was injected twice with vehicle alone (ethanol/saline, 1:20 v/v). The treated and control fish were randomly distributed among six 250-l tanks. E2 and MT (crystalline form, Sigma Chemicals) were dissolved in ethanol/saline (5/95%, v/v), and were made up fresh before injection. The fish were killed by decapitation 72 h after the first steroid injection and the pituitaries and brains were removed. The brains were dissected into three parts: telencephalon, hypothalamus and remaining brain. The telencephalon, hypothalamus and pituitaries were kept at −77 °C until further analysis. Gonads were also removed for calculations of the GSI.

Experiment 2: effects of chronic MT treatment in immature male trout

This experiment was started in December 1998 and finished in February 1999. Fish were intraperitoneally implanted with MT. Implants were prepared with crystalline MT mixed with the elas-tomer Silastic MDX4-4210 (Medical Grade, Dow Corning) at 36.5 mg g⁻¹ elastomer, according to the method described by Pankhurst et al. (1986). The Silastic implants (2.0×8.0 mm) were introduced into the fish body cavity through a 1–2-mm incision. A total of 87 fish were used. Before implantation, seven fish (time zero) were anesthetized by immersion in MS-222 aqueous solution (0.05%, buffered pH 7.4). The remaining fish were divided into two groups of 41 and 39, respectively. The first group received implants containing 20 mg MT kg⁻¹ body weight, and the second group (control) was implanted with the elastomer alone. The fish were randomly allocated to three 250-l tanks per group. Nine fish per group were killed 10 days after the beginning of the experiment. A second sampling was performed 20 days after implantation, with 10 fish being sampled per group. Finally, the remaining fish were sampled 40 days after implantation. Blood samples (600 μl) were taken using heparinized syringes by caudal vein puncture, and the fish were then killed by decapitation. Pituitaries and brains were removed. The brains were dissected into three parts: telencephalon, hypothalamus and remaining brain. Blood samples were centrifuged at 4,400 g for 15 min, and then the plasma was removed and kept frozen at −77 °C until assay. After dissection, all pieces were frozen in dry ice and kept at −77 °C until analysis for determination of monoamine contents; the gonads were extracted for GSI calculation.

Hormone analysis

T and E2 were directly measured in unextracted plasma using RIA commercial kits (Active Testosterone DSL-4000, and Active Estradiol DSL-4300, for T and E2, respectively, Diagnostic Systems, USA). Kits were validated according to the method described by Mol et al. (1994), i.e., the method was valid if the slope of the plot of measured concentration against known dilution for pooled plasma was close to that of the plot for the corresponding hormone standard. No significant differences between the slopes for standard curves and trout plasma dilutions were observed with either kit (P = 0.568, for T, and P = 0.875, for E2; Student’s t test). Sensitivity and intra- and interassay coefficients of variation (CV %) were 0.08 ng ml⁻¹, 2.05% and 4.2%, respectively, for T and 0.007 ng ml⁻¹, and 2.27% and 8.8%, respectively, for E2. Cross-reactivities of anti-T with different steroids were below 0.5% except for 5α-dihydrotestosterone (5.8%), 11-oxotestosterone (4.2%), androstenedione (2.3%), ethisterone (1.9%), norethindrone (0.92%), and 19-hydroxyandrostenedione (0.86%). Cross-reactivities between anti-E2 and other steroids were also below 0.5% except for estrone (0.86%) and estradiol (0.57%).

Chromatographic analysis

Monoamine and metabolite contents (NE, DA, DOPAC, 5-HT and 5-HIAA) were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-EC) as previously described (Hernandez-Rauda et al. 1996). An electrochemical detector (ESA Coulochem 5100A) with a 5011dual analytical cell set at +50 mV (first, screening cell) and +350 mV (second, analytical cell) was used. Monoamines and their metabolites were extracted...