Chapter 4

Thiourea-induced alterations in the expression of some steroidogenic enzymes in air-breathing catfish

*Clarias gariepinus*
Abstract

Previous study from our laboratory on thiourea-induced thyroid hormone depletion in mature male demonstrated that thyroid hormones play a significant role in testicular function of catfish. In the present study, we aimed to analyze the changes in the expression pattern of several steroidogenic enzyme genes using semi quantitative RT-PCR after thyroid hormone depletion by thiourea in adult male and female catfish. There was a marked decrease in the \(11\beta H\) expression in the testis while no changes were observed in kidney. A marked decrease in \(11\beta\text{HSD2}\) transcript level in testis, liver and kidney were observed in the thiourea-treated males. The observed results corroborate our earlier findings on testicular regression after thyroid hormone depletion. In females, expression of \(cyp19a1\) increased in the experimental group when compared to control. No significant changes were observed in the transcript levels of \(3\beta\)-hydroxysteroid dehydrogenase, cytochrome p450c17\(\alpha\) enzyme, and \(20\beta\)-hydroxysteroid dehydrogenase in both males and females. Thus, thyroid hormones might regulate expression of terminal steroidogenic enzyme genes and thereby reproduction in catfish.

Introduction

Thyroid hormone plays a crucial role in embryogenesis, sex steroid metabolism and reproduction in mammalian and human system (Jannini \textit{et al}., 1995; Krassas \textit{et al}., 2004). In teleosts its role in metamorphosis, embryogenesis and vitellogenesis is well documented (Cyr and Eales, 1988; Weber \textit{et al}., 1992; Liu and Chan, 2002; Reine and Leatherland, 2003). Previous reports demonstrated histological and biochemical changes in testis on
thyroid hormone depletion illustrating significant role of thyroxine in fish reproduction (Misra and Panday, 1985; Matta et al., 2002). Studies from our laboratory on thiourea-induced thyroid (hormone) depletion indicated alteration in hypothalamo-hypophyseal-testicular (HHT) axis by depleting cfGnRH immunoreactivity (ir) neurons in preoptic area vis à vis on LH ir cells in the pituitary (Swapna et al., 2006). In the same study, we also suggested that HHT axis alteration is one of the causative factors for the reduction in tissue and serum levels of testosterone and 11-ketotestosterone (11-KT). Thus, it is plausible that thyroxine exerts a direct or indirect effect on HHT axis by modulating sex steroid levels. To understand this at molecular level, we intended to analyze the effect of thyroid hormone depletion on the expression pattern of some steroidogenic enzyme genes involved in production of sex steroids.

Materials and Methods

Adult (recrudescence) air-breathing male and female catfishes (200-250g) were purchased from local fish markets of Hyderabad, India. They were acclimatized for 3 weeks by maintaining in aquarium tanks filled with filtered tap water under natural photoperiod and ambient temperature (26 ± 2°C) and fed with minced goat liver ad libitum during acclimation and experimentation. Thyroid hormone depletion was induced by adding thiourea (SRL, Mumbai, India) to a final concentration of 0.03% in well-aerated aquarium as per the methodology described in detail by Swapna et al. (2006). For a period of 21 days, group I, control fishes (n=10), were maintained in filtered water whereas group II, male fishes (n=5) and group III, female fishes (n=5) were maintained in filtered water
containing thiourea. Water in control and treated tanks were replenished daily along with thiourea for the treated group alone. After 21 days of treatment, fish from both the groups were weighed and sacrificed. Serum was obtained by centrifugation at 1500g, lyophilized and stored briefly in -80°C ultra low freezer for the estimation of 3, 3, 5’- triiodothyronine (T₃) levels. T₃ levels in control and thiourea-treated fish were measured as per the method described by Swapna et al. (2006). Testes, liver and kidney tissues from male catfish and ovarian tissue from female catfishes were collected for isolation of total RNA using Tri-reagent. Total RNA from respective tissues were reverse transcribed to cDNA using Superscript III (Invitrogen, Carlsbad, CA, USA) and oligoT₁₈ primers following the manufacturer’s instructions. To clone partial cDNA fragments a set of degenerate primers for each steroidogenic enzyme genes were designed from conserved region by aligning the existing sequences (GenBank database) of teleost 11β-hydroxylase (11β-H), 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), 3β-hydroxysteroid dehydrogenase (3β-HSD), cytochrome p450c17α enzyme (cyp17), 20β-hydroxysteroid dehydrogenase (20β-HSD) and ovarian aromatase (cyp19a1) using Lasergene software, (release 3.05; DNASTAR, Madison, WI, USA). 11β-H, 11β-HSD2, 3β-HSD, cyp17 and 20β-HSD partial cDNA fragments were amplified from testis first stand cDNA templates. Similarly, 3β-HSD, cyp19a1, cyp17 20β-HSD, partial cDNA fragments were amplified from ovarian first stand cDNA templates. Following are the degenerate primers for 11β-H, Fw: 5’ CCT NGG SCC CAT WTA CAG GSA G 3’, Rv: 5’ GTC GTG TCC ACY SCY CCB GCC AT3’, 11β-HSD2, Fw1:5’GCG GTS YTC ATC ACM GGY TGT GA 3’, Rv1:5’ CCA AAG
Chapter 4: Thiourea-induced effect on gonadal recrudescence

AAR TTS ACY TCC ATR CA3’, Rv2: 5’GCT GCY TTS GAG GYY CCA TA3’, cyp19a1, Fw: 5’ TGG WYK GGN ATH GGB ACD GC3’, Rv1: 5’GGV CCD GTB RVG CTT TRG 3’. The sequences of degenerate primers for 3β-HSD, CYP17, 20β-HSD were reported by Raghuveer et al. (2005) and Sreenivasulu et al. (2005). PCR was performed at 94°C (7 min), 55-60°C (1.5 min), 72°C (1.5 min), for 30 cycles and 72°C final extension for 10 min using Gene AMP PCR system 9700 ( Applied Biosystems, USA) thermal cycler. The efficiency of the transcription was checked by performing a PCR for β-actin a constitutively expressed gene. All the partial cDNA fragments were cloned in pGEMT-easy vector (Promega, Madison, WI, USA) and the sequence was determined. The identity of amplified partial cDNA was analyzed by NCBI-BLAST. Gene specific primers were designed for 11β-H, 11β-HSD2, cyp19a1, 3β-HSD, CYP17 and 20β-HSD from the sequences obtained from partial cDNA fragments. Semi-quantitative RT-PCR was opted to observe the relative expression pattern of the above mentioned steroidogenic enzyme genes in liver, kidney, testis and ovary by following the method of Kwon et al., (2001). The PCR conditions [94°C (1 min), 50-60°C (1 min), 72°C (1 min), for 30 cycles and final extension at 72°C for 10 min] were standardized for all the templates and their expression patterns studied. PCR product of each gene obtained from specific primers were cloned in pGEM-T Easy vector (Promega) and the amplicon size and identity confirmed by sequencing.
Chapter 4: Thiourea-induced effect on gonadal recrudescence

Results

Thiourea induced thyroid hormone depletion during testicular recrudescence

The T₃ level in the serum of thiourea treated male fish and control were 0.29 ± 0.02 and 0.69 ± 0.01 ng/ml, respectively. On the other hand, T₃ levels in the serum of thiourea treated female fish and control were 0.21 ± 0.02 and 0.57 ± 0.03 ng/ml, respectively. The RT-PCR performed using specific primers amplified 11β-H, 11β-HSD2 and cyp19a1 products of 212, 296, and 262 bp that showed homology to previously reported medaka, the Nile tilapia and channel catfish, respectively. The expression of 11β-H declined in the thiourea treated males both in the liver and testis after 21 days of treatment. There was no significant change in 11β-H in the head kidney. The expression of 11β-HSD2 decreased in testis, liver and kidney following thyroid hormone depletion by thiourea. In adult female fishes, thiourea treatment resulted in significant increase in cyp19a1 transcript levels when compared to the control group. There was no significant change in the transcript levels of, 3β-HSD, CYP17, 20β-HSD in both males and females.
Fig. 1: RT-PCR analysis (representative gel) of the expression pattern of a) 11β-hydroxylase, b) 11β-hydroxysteroid dehydrogenase type 2, d) 3β-hydroxysteroid dehydrogenase, e) cytochrome p450c17α enzyme, and f) 20β-hydroxysteroid dehydrogenase in adult thiourea treated male. c) cyp19a1 in adult thiourea treated female. TC, control testis; TE, thiourea treated testis; KC, male control kidney; KE, male treated kidney; LC, male control liver; LE, male treated liver; OC, ovary control; OE, ovary treated.
Chapter 4: Thiourea-induced effect on gonadal recrudescence

Fig. 2: Graphical representation of the changes in the expression pattern of $11\beta$-hydroxylase, $11\beta$-hydroxysteroid dehydrogenase type 2, $3\beta$-hydroxysteroid dehydrogenase, cytochrome p450c17α enzyme, and $20\beta$-hydroxysteroid dehydrogenase in adult thiourea treated male. cyp19a1, in adult thiourea treated female. Values are mean ± SEM, n=5. Statistical analysis was done by one way ANOVA followed by Students Newman-Keul’s test using Sigma stat 3.5 software. Level of significance was set at $P<0.05$. * Significant over control.
Chapter 4: Thiourea-induced effect on gonadal recrudescence

Discussion

The significant decrease in T3 level in the serum after thiourea treatment confirms the effective depletion of thyroid hormone by thiourea. Physiologically relevant androgen for the initiation of spermatogenesis in catfish is 11-KT and any change in its level alters testicular function (Cavaco et al., 1998). Previous report by Swapna et al. (2006) indicated that thiourea-induced thyroid hormone depletion leading to testicular regression (by reducing 11-KT) might be due to the alteration in transcript levels of 11β-H and 11β-HSD2 in testis. In accordance to this, present study demonstrated a decline in the expression of aforementioned steroidogenic enzyme genes. Further, reduction in the transcript level of 11β-HSD2 in liver, the major site for 11-KT production (Cavaco et al., 1997) and in kidney by thyroid hormone depletion hampered spermatogenesis leading to testicular regression which may be attributed to the decrease in physiological concentration of 11-KT produced by these tissues. Histological results by Supriya et al. (2005) showed that there was not much impact of thyroid hormone depletion on the ovarian structure and function in adults though a considerable increase in the cyp19a1 transcript levels was evident in the treated fish in the present investigation. The results obtained thus hint that the depletion of thyroid hormone either directly or indirectly modulates the steroidogenic enzyme expression in the gonad and peripheral tissues leading to drop in androgen level and testicular regression In females, it may have a variable role which require further studies (see Swapna and Senthilkumaran, 2007) at molecular level though physiological studies show modulation of ovarian function.
Chapter 4: Thiourea-induced effect on gonadal recrudescence

References


Chapter 4: Thiourea-induced effect on gonadal recrudescence


Liu Y.W., Chan W.K., 2002. Thyroid hormones are important for embryonic to larval transitory phase in zebrafish. Differentiation, 70: 36-45.


Chapter 4: Thiourea-induced effect on gonadal recrudescence

