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Research Paper

BRAIN STEROIDOGENIC ENZYMES AND PROTEIN ENCODING GENE EXPRESSION OF AN INDIAN MAJOR CARP *Cirrhinus mrigala (Ham.)*

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Abstract

Brain is a steroidogenic organ which produces steroids. Our study has been focused to analyze the steroidogenic protein and enzyme encoding gene expression in the brain of an Indian major carp (IMC), *Cirrhinus mrigala.* This is the first attempt to report the possible neurosteroidogenic pathway regulating enzymes and protein encoding gene expression of StAR, P450scc, P450c17, 3α-HSD and 3β-HSD in the brain of *C. mrigala*. The brain samples were collected and Total RNA was isolated. The isolated RNA was reverse transcribed into cDNA using RT-PCR. The synthesized cDNA was then added with the gene specific primers and subjected to PCR for gene specific amplification. Separate bands were obtained which confirms the targeted protein and enzyme encoding gene expression. The present results provide a clear idea for the synthesis of brain steroidogenesis and its regulatory mechanisms.

Key words: Steroidogenesis, Neurosteroids, Cirrhinus Mrigala, StAR, Brain.

INTRODUCTION

Steroid hormones play a crucial role in the development and functioning of the central nervous system (CNS).Neurosteroids are steroids that can be synthesized de novo in the brain from the sterol precursors. The term neurosteroids was coined by Baulieu, (1981).Neurosteroids exert a large array of biological activities in the brain (Belelli et al., 2006;Lapchak et al., 2001;Majewska 1992;Paul and Purdy,1992; Strous et al., 2006). Mechanism of steroid action occurs via a conventional genomic action or through interaction with membrane receptors. Regardless of organ or tissue, the initial process in steroidogenesisis the movementof cholesterol across the mitochondrial membrane (i.e. from the outer to the inner mitochondrial membrane), which is a rate-limiting stepin steroid biosynthesis. It is mediated by Steroidogenic acute Regulatory Protein (StAR).Subsequent conversion of cholesterol to pregnenolone is mediated by P450 side-chain cleavage enzyme (P450scc).The enzyme 3β –HSD catalyses the synthesis of progesterone from pregnenolone, as well as the conversion of 17-hydroxypregnenolone to hydroxyprogesterone. Thus, 3β -HSD is essential for the biosynthesis of all classes of steroid hormones, mainly progesterone, androgens, estrogens, glucocorticoids and mineralocorticoids. The conversion of pregnenolone and progesterone into their 17α–hydroxylatedproducts i.e.17α -hydroxypregnenolone and 17α -hydroxyprogesteroneand then to either DHEA or androstenedione, respectively, is mediated by a single microsomal enzyme,, P450c17 (Nakajin et al., 1981; Zuber et al., 1986) encoded by a

single gene CYP17 (Givens e al., 1994; Picado-Leonard and Miller, 1987, Uma et al., 2013 and 2015). Androstenedione is then converted to testosterone by the enzyme 17β-HSD and testosterone is converted into estradiol by aromatase (Mellon et al., 2001).PROG and testosterone can be converted respectively to 5α-dihydroprogesterone (5α-DHPROG) and to 5α-DHT by the steroid 5α-R (two distinct isoenzymes 1 and 2) and then to 3α, 5α tetrahydroprogesterone (3α, 5αTHPROG) and 3α, 5αtetrahydrosterone (3α, 5α-THT) by the 3α-HSD (Saravanan et al., 2013). This paper deals with the identification and expression of the steroidogenic protein and enzyme encoding genes in the brain of an Indian major carp, *Cirrhinus mrigala*. The reports were confirmed by semi quantitative analysis of Reverse Transcriptase-PCR technique.

MATERIALS & METHODS

Animal model for our work is *Cirrhinus mrigala*, which was obtained from the Sathanur Dam, located in Tiruvannamalai District. Fishes were captured in the early morning in alive condition. Fishes were dissected out to get the brain samples. Brain samples were stored in RNA Later. Samples were taken to the laboratory and stored at -70°C until analysis.

Total RNA isolation

Total RNA was isolated by homogenizing the whole brain sample (approximately 200mg) using 500μl of Tri Reagent (Tri Reagent T9424) by adopting the procedure of Medoxkit.

Synthesis of cDNA first strand

The first strand of cDNA was synthesized by using the procedure followed in Medox Kit (Filichkin, Gelvin, 1992; Rolfs, 1992). The separated RNA has been reverse transcribed into cDNA using RT-PCR. RT product was then subjected to 1.2% agarose gel electrophoresis to test its purity.

Reverse transcription-polymerase chainreaction

Primers targeting the cDNA sequence of StAR (Sense 5'-GGTACAGTGAAACTGCGAATGG -3' and antisense-5'TGGTGCCCTTCCGTCAATTCC -3), P450scc(Sense -GAGGAGGGTAGGAGCCA and antisense - CCTTGTGGGACTCTGGT)P450c17(Sense CCAGAGAGGTTCTCCTGCTG and antisenseTGGACAACAGCTCCTCACAG),3α-HSD(Sense CTGTGCCTGAGAAGGTTGCT and antisense CATGTGTCACAGATATCCAC)and 3β-HSD (Sense CTCTGCAGGAACATCCCAAT antisenseTGATCCACAGCATCCACACT)were designed using primer 3tool and purchased from Sigma Aldrich.

The temperature adopted in the amplification is as follows: StAR- 95°C for 2 minutes in 1 cycle, 95°C for 30 sec, 60°C for 30 sec and 72°C for 1 minute in 35cycles, the finally holding temperature was 4°C. P450scc- 95°C for 3 minutes in 1 cycle, 95°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec in 40 cycles, temperature for 3α-HSD and 3β-HSD are same 94°C for 2 minutes in 1 cycle, 94°C for 1 minute, 56°C for 1 minute and 72°C for 1 minute in 35cycles, and P450c17- 95°C for 2 minutes in 1 cycle, 95°C for 30 sec, 48°C for 30 sec and 72°C for 1 minute in 35cycles. After 35 cycles of amplification, the PCR products were isolated by electrophoresis using ethidium bromide and visualized under Gel Doc.

RESULTS

Total RNA was isolated from the brain of *C. mrigala* and checked its purity. The brain steroidogenic mRNA preparation with gene specific primers activity were identified with the gel electrophoresis used suitable DNA marker. The Gel picture (Fig. 1)shows the PCR products bands around the 1100bp of StAR gene, 250bp of P450scc, around 600 and 200bp products for P450c17 genes, 150bp product of 3β-HSD the band not clear and 3α-HSD gene specific band shows in 450bp. The concentration of mRNA in the brain of *C. mrigala* and its purity results represent in the bar diagram (Fig.2).

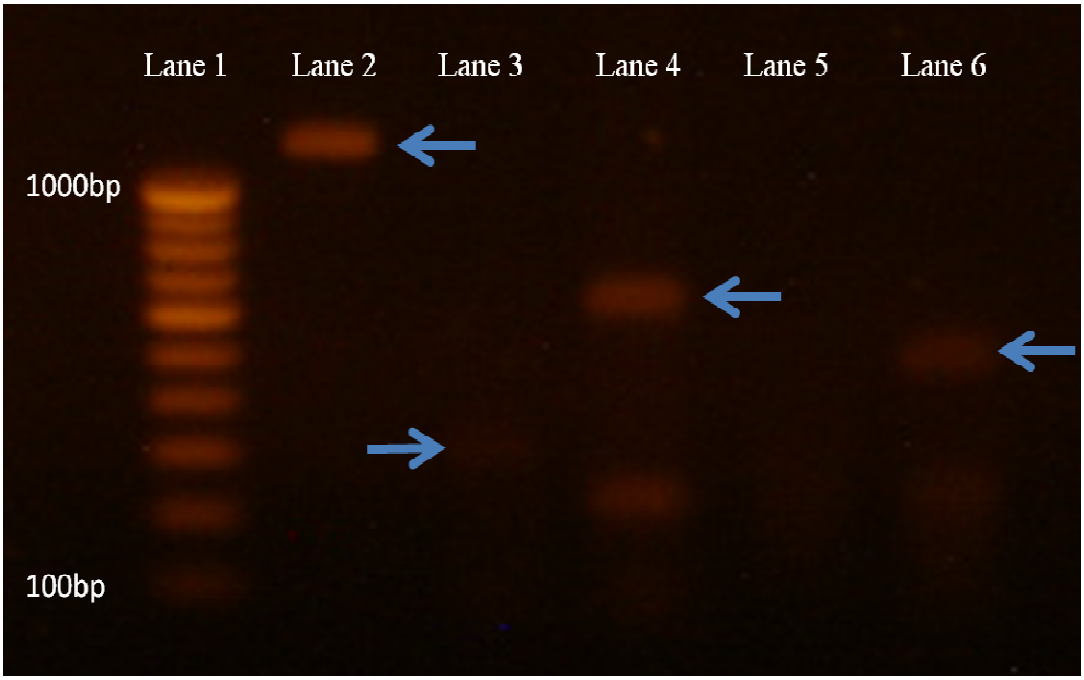

Fig. 1. The arrow indicating the steroidogenic enzymes encoding gene expression in the ovary of *C. mrigala,* along with DNA marker. **Lane1-**100bp DNA marker; **Lane2**-PCR product of StAR gene; **Lane3**-PCR product of P450scc gene; **Lane4-**PCR product of P450c17 gene, **Lane5-**PCR product of 3β-HSD gene; **Lane6-**PCR product of 3α-HSD gene.

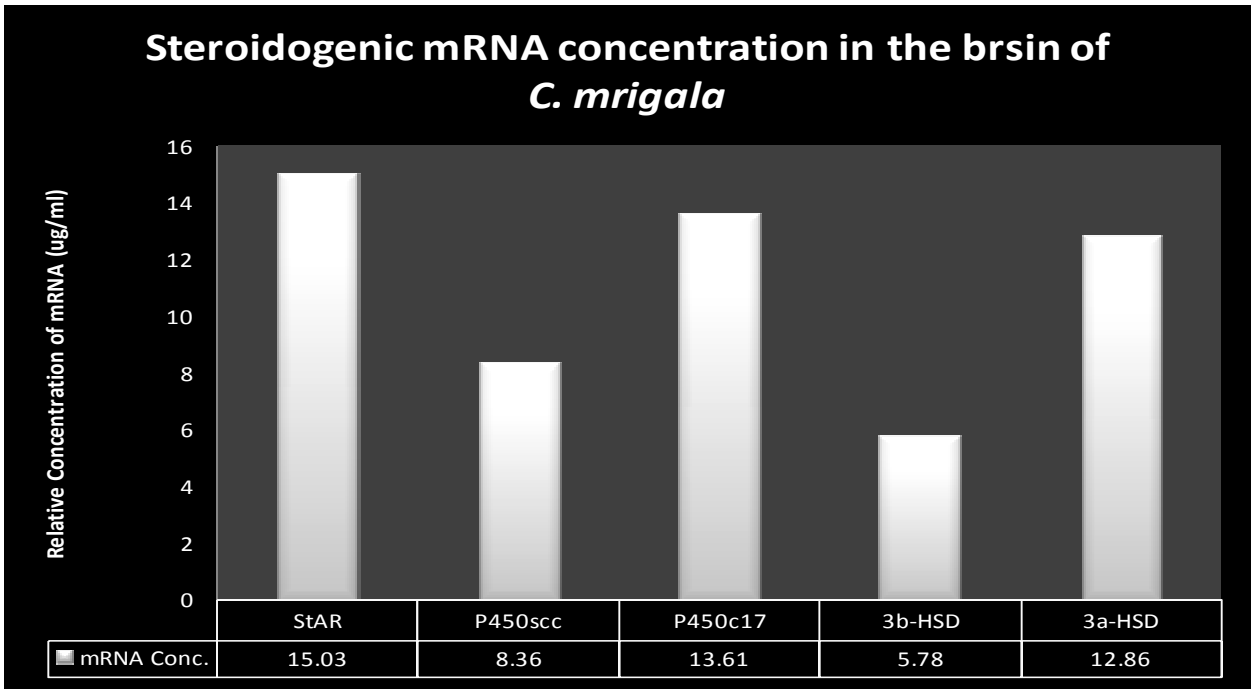

Fig.2. The bar diagram shows the relative expression and concentration of steroidogenic enzymes and protein encoding mRNA in the brain of *C. mrigala.*

DISCUSSION

These results provide clear evidence that the expression of steroidogenic protein and enzymes encoding genes isolated from the *C. mrigala* brain. The following enzymes and protein StAR, P450scc, P450c17, 3β-HSD and 3α-HSD were met for its designation as a specific role on the pathway of biosynthesis of steroidogenesis in this fish species. The occurrence of StAR in the brain has been first demonstratedin rat by Furukawa et al., (1998). The presence of the StAR

protein has been confirmed by Western blot analysis and immunohistochemical labeling in the brain of rodents and primates (Kimet al., 2004; Kimotoet al., 2001; Kimotoet al., 2002;Sierra 2004; Sierra et al., 2003; Wehrenberg et al., 2001). In particular, the presence of steroidogenic protein and enzymes it may be designated in that pathway and governed the brain steroids synthesis of the fish. The occurrence of StAR mRNA has also been described in non-mammalian vertebrates. In birds, the expression of StAR has been studied in the brain of zebra finch (London et al., 2006).In fish, the StAR gene is expressed, although at relatively low level, in the brain of freshwater stingrays (Nunez et al., 2005), Atlantic salmon (Arukwe 2008;Lyssimachou and Arukwe,2007)and white sturgeon (Kusakabeet al., 2009).

Previously our reported in the steroidogenic pathway regulated enzymes encoding gene expression in the brain of *Labeo rohita* (Saravanan *et al.,* 2013). The presence of P450scc was first detected in the brain of rat by using antibody raised against bovine adrenal P450scc (Le Goascogne et al., 1987). Oonk et al. (1989), isolated cDNA clones encoding rat $P450_{scc.}$ Studies of Compagnone et al. (1995), Mellon and Deschepper, (1993), demonstrated the occurrence of P450scc mRNA in the CNS of mammals. Expression of P450scc is higher in the cerebral cortex of rat and mouse. The presence of the P450scc protein has been confirmed in the brain of birds by Western blot analysis (Leaet al., 2001; Tsutsui and Yamazaki, 1995; Tsutsui et al., 1999; Usuiet al., 1995). The expression of the P450scc gene has also been studied in the brain of zebra finch (London et al., 2006). In mammals, a single form of P450C17 has been described so far (Gyomorey et al., 2000; Lin et al., 1991; Nakajin et al., 1984; Suhara et al., 1984; Zuber et al., 1986) whereas, in the medaka Oryzias latipes, two types of P450C17 encoded by two distinct genes have been recently identified and characterized (Zhou et al., 2007a, b), in the CYP17 gene were identified in the brian tissues of *L. rohtia* and diffent brain regions of *C. mrigala* (Uma et al., 2013 and 2015).In fish, the distribution of 3β-HSD-expressing cells has been investigated in the brain of teleosts and dipnoans (Mathieuet al., 2001;Sakamoto et al., 2001a, b). The presence of a bioactive form of 3β-HSD has also been demonstrated in the brain of birds (Matsunagaet al., 2004; Pignataroet al., 1998; Schlinger andPradhan, 2008; Ukenaet al.,1999; Vansonet al., 1996), amphibians (Inai et al., 2003; . Mensah-Nyaganet al., 1994;Takase et al., 1999)and fish (Sakamoto et al., 2001b). In both zebrafish and lungfish, the 3β-HSD gene is expressed exclusively in neurons (Mathieuet al., 2001;Sakamoto et al., 2001a). The occurrence of 3b-HSD-positive cells has also been detected in the distal lobe and, to a lesser extent, in the neuro intermediate lobe of the pituitary of zebrafish (Sakamoto et al., 2001b)and lungfish (Mathieu et al., 2001). To our knowledge, this is the first report for steroidogenic enzymes and protein encoding gene expression that has been shown to fulfill the criteria for the brain steroidogenic pathway designation in these fish *C. mrigala*. Further, research need to characterice and localize in brain cell and region of the Indian major carp, especially this fish.

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