STEROIDOGENIC GENE EXPRESSION IN THE BRAIN OF AN INDIAN MAJOR CARP, *LABEO ROHITA* (HAM.)

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Introduction:

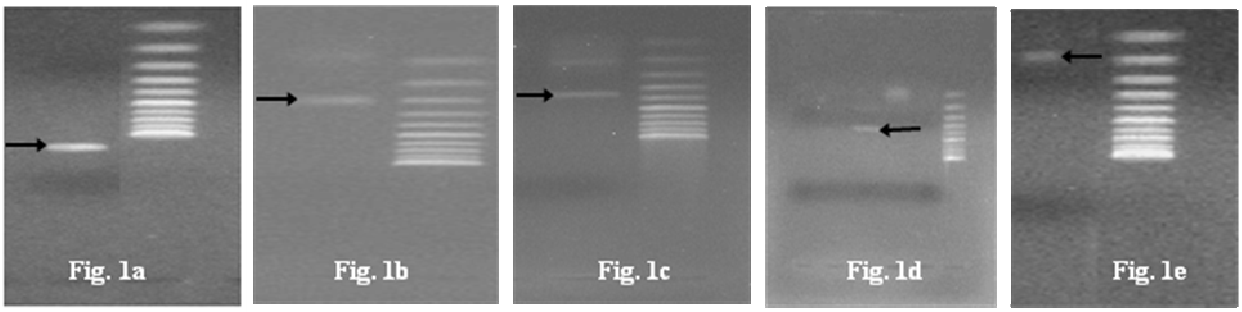
Brain is a steroidogenic organ that produces steroidogenic enzymes [1]. These enzymes play an important role in the pathway of steroidogenesis. The internalization of cholesterol by the mitochondria is the rate-limiting step for the general steroidogenic pathway, and is mediated by Steroidogenic acute regulatory protein (StAR). Once inside the mitochondria cholesterol is converted to pregnenolone by the enzyme P450 side chain cleavage (P450scc). Pregnenolone is converted to progesterone by 3beta-hydroxysteroid dehydorgenase (3β-HSD). The conversion of pregnenolone and progesterone to their 17α-hydroxylated products and then to either DHEA or androstenedione, respectively is mediated by a single enzyme P450c17α-hydroxylase (P450c17). Pregnenolone is the first steroid in the pathway and is the common precursor for all species and all tissues, from this point the converted cholesterol is committed to becoming a steroid. Such steroids synthesized de novo in the brain, as well as other areas of the nervous system, are called neurosteroids. The enzyme 3alpha-hydroxy steroid dehydrogenase (3α-HSD) catalyzes the conversion of 5α-dihydroxytestosterone (5α-DHT) and 5α-dihydroxyprogesterone (5α-DHP) into 3α-androstanediol and 3α,5α-tetrahydroxyprogesterone (3α,5α-THPROG), respectively. The pathways of steroidogenesis differ from organism to organism. Steroids play an important role in the onset of sexual characters and development of gametes [2]. To understand the enzyme pathway and neurosteroid actions in the brain, we need data on the specific gene expression of steroidogenic enzymes in the brain of an Indian major carp, *Labeo rohita*. We have identified the gene expression of StAR protein and enzyme P450scc, P450c17, 3beta-HSD and 3alpha-HSD in the brain of *L. rohita*. This is the first report of gene expression study in this fish brain.

Methods:

Labeo rohita was collected from Sathanoor reservoir. The total RNA extraction was done in brain tissue by guanidine thiocyanate method. The RNA was reverse transcribed into cDNA using M-MuLV, RT-PCR kit (Medox). The gene specific primers were constructed and obtained from sigma (USA). RT-PCR product with gene specific primer of StAR forward 'GGTACAGTGAAACTGCGAATGG', reverse 'TGGTGCCCTTCCGTCAATTCC', P450scc forward 'GAGGAGGGTAGGAGCCA', reverse 'CCTTGTGGGACTCTGGT', P450c17 forward 'CCAGAGAGGTTCTCCTGCTG', reverse 'TGGACAACAGCTCCTCACAG', 3α-HSD forward 'CTGTGCCTGAGAAGGTTGCT' reverse CATGTGTCACAGATATCCAC, 3β-HSD forward 'CTCTGCAGGAACATCCCAAT', reverse 'TGATCCACAGCATCCACACT' was subjected to PCR by Taq polymerase kit (Genei, Bangalore). The PCR products were visualized by gel electrophoresis using ethidium bromide. The DNA amplified through PCR was quantified before sending for sequencing. The PCR amplified samples were sent for sequencing to Bangalore Genei, India.

Results and Discussion:

Total RNA was isolated and reverse transcribed to synthesized first strand cDNA. Using the RT-PCR samples the ordered primers was used to identify and amplify StAR protein, enzymes P450scc, P450c17, 3α-HSD and 3β-HSD responsible gene. After the PCR they were loaded into the agarose gel. The result of PCR products along with 100bp marker DNA is shown in Fig. 1a-e. The results confirm the presence and expression of



steroidogenic enzymes in the brain of *Labeo rohita*. The formation of neurosteroids in the brain was originally demonstrated in mammals and subsequently in other vertebrates, such as birds, amphibians and fish. Thus *de novo* neurosteroidogenesis in the brain from cholesterol is a conserved property of vertebrates [3]. This is the first demonstration of steroidogenic enzyme gene expression in the brain of *L. rohita*. Our studies on steroidogenic protein and enzyme gene expression have provided the opportunity to understand biosynthesis of steroidogenic protein and enzymes in the brain of *L. rohita*.

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