Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 12, pp.4062-4065, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DYNAMICS OF NEUROSTEROIDS IN THE BRAIN: SPECIAL REFERENCE TO STEROIDOGENIC ENZYME 3α-HSD GENE EXPRESSION IN THE BRAIN OF AN INDIAN MAJOR CARP *LABEO ROHITA* (HAM.)

[1]*Saravanan, N., [1]Moses Inbaraj, R. and [2]Uma, T.

[1]Department of Zoology, Madras Christian College, Tambaram, Chennai-600 059, India
[2]Department of Zoology, Pachaiyappa's College for Women, Kanchipuram

ARTICLE INFO

Article History:
Received 07th September, 2013
Received in revised form
15th October, 2013
Accepted 29th November, 2013
Published online 25th December, 2013

Key words:

Steroidogenesis, Neuroactive steroid, 3alpha-HSD, Aldo-keto reductase, *Labeo rohita.*

ABSTRACT

3α-Hydroxy steroid dehydrogenase (3α-HSD) is a member of the aldo-keto reductase family. The enzyme is used in the synthesis of neuroactive steroids in steroidogenesis. The 3α-HSD catalyzes the conversion of 5α-dihydroprogesterone (5α-DHP) and 5α-dihydrotestosterone (5α-DHT) into 3α,5α-tetrahydroxyprogesterone (3α,5α-THPROG) and 3α,5α-tetrahydroxytestosterone (3α,5α-THT) respectively. The progesterone metabolite of neuroactive steroid, 3α,5α-THPROG is synthesized in different regions of brain. In the present study, the gene expression of 3α-HSD in the brain of *Labeo rohita* was identified. The gene was observed by PCR amplification with a specific primer. The result showed has formed a basis for future investigations on the regulation and function of these enzymes in the fishes.

INTRODUCTION

The steroids synthesised in the brain tissues or neural tissues are generally called as 'neurosteroids'. Pregnenolone (PREG) and progesterone (PROG) can be synthesized in the nervous system. A crucial biochemical reaction in vertebrates is the progesterone conversion into neuroactive metabolites such as 5α-dihydroprogesterone (5α-DHP) and 3α, 5α-tetrahydro progesterone (3α,5α-THPROG), which regulate several neurobiological processes, including stress, depression, neuroprotection, and analgesia. 3α,5α-THPROG is a potent stimulator of type A receptors of γ-amino butyric acid (GABA), the main inhibitory neurotransmitter (Belelli and Herd, 2003; Belelli and Lambert, 2005; Pinna, *et al.,* 2006; Belyaeva, *et al.,* 2007). GABAergic neurons of the reticular thalamic nucleus express high levels of 5α-RI and 3α-HSD and their nerve endings may secrete allopregnanolone and release GABA in the proximity of postsynaptic $GABA_A$ receptors located on the dendrites and somata of glutamatergic thalamocortical output neurons (Pinault, 2004). Similar considerations are also expected for allopregnanolone synthesized by medium spiny GABAergic neurons in the caudate or putamen and also very likely by Purkinje cells that modulate GABAA receptors expressed postsynaptically on cell bodies or dendrites of deep cerebellar nuclei neurons (Agis-Balboa *et al.,*2006). The aldo-keto reductase superfamily is involved in the metabolism of endogenous substrate, such as steroid hormones, prostaglandins, bile acids and xenobiotics,such as drugs and environmental carcinogens (Khanna *et al.,* 1995). 3α,5α-THPROG with a steroid chemical structure and low molecular weight of 318.49, easily penetrates the blood-brain barrier to induce central nervous system (CNS) effects, including anxiolytic and sedative hypnotic properties (Gee *et al.,* 1998; Brinton, 1994). Functional analyses indicate that 3α,5α-THPROG induces myelin formation in both the CNS and the peripheral nervous system (Baulieu and Schumacher 2000; Schumacher *et al.,* 2003) and promotes neuron survival. In humans, recent advances in functional brain imaging have identified critical neural circuits in cortico-limbic structures involved in the modulation of fear responses, aggressiveness, anxiety, and sexual behaviors that appear to be affected in mood disorders (Carlson *et al.,* 2006). These circuits include the amygdala, hippocampus, and the medial prefrontal cortex (mPFC) (Carlson *et al.,* 2006; Nelson and Trainor, 2007). In rodents, the olfactory system has also been implicated (Mandiyan *et al.,* 2005). 3α-HSD gene expression has been reported in some other vertebrates like humans, rat, and cattle (Penning *et al.,* 1984; Qin *et al.,* 1993; Lin *et al.,* 1997).

Recently it has been reported that both allopregnenolone and isopregnenolone are also synthesized from PROG in CNS tissues throughout the embryonic and early postnatal period of development in the rat (Pomata *et al.,* 2000). However, there is no evidence for the identification of 3α-HSD gene in fishes. This is the first attempt to know the 3α-HSD enzyme in brain of fish, particularly among the Indian carp. *Labeo rohita*

***Corresponding author: Saravanan, N.** Department of Zoology, Madras Christian College, Tambaram, Chennai-600 059, India.*

(commonly called as Rohu) is an animal model for the present research work and it is an economically important species among the Indian major carps.

MATERIALS AND METHODS

Sample collection

Fish samples of *Labeo rohita* were collected from the Lake Sevilimedu located at Kanchipuram. Fishes were caught in live condition and they were dissected to collect brain samples. The brain tissues were fixed in a sterilized vials containing RNAlater and they were stored at 4˚C until analysis.

Total RNA isolation

Total RNA was isolated by homogenizing the whole brain sample (200mg) with 500µl of TriReagent (Sigma) and 200µl of DEPC water. After homogenizing the sample, it was then incubated at -20˚C for 5 minutes. 0.2ml of chloroform was added and incubated at -20˚C for 5 minutes. It was then centrifuged at 12,000rpm (4˚C) for 15 minutes. The supernatant was collected in a fresh tube. RNA was precipitated by adding equal volume of isopropanol and it was stored at -20˚C for 45 minutes. The sample was centrifuged again at 12,000 rpm (4˚C) for 15 minutes. Total RNA was obtained as a pellet and 75% ethanol (7.5ml of Ethanol was mixed with 2.5ml of DEPC water) was added. It was centrifuged at 12,000 rpm for 5 minutes and air dried and dissolved by adding 40µl of DEPC water. This was frozen and stored at -20˚C for half-an-hour. The sample was then tested with the 1.2% agarose gel for its purity.

Synthesis of first strand cDNA

Total RNA was isolated from the brain of *L. rohita*. The separated RNA has been reverse transcribed into cDNA using RT-PCR method. A clean PCR tube was taken to this 1µl of the sample, 1 µl of Oligo (dT) $_{18}$ primer, 9.5µl of de-ionized water was added and it was spinned gently for few seconds in a micro centrifuge. This mixture was incubated at 70˚C for 5 minutes. After incubation, 4µl of 5X reaction buffer, 0.5µl of Ribonuclease inhibitor (40µl/dl), 2µl of 10mM dNTP mix were added. This was mixed gently, centrifuged and incubated at 42˚C for 5 minutes. After incubation, it was added with M-MuLV reverse transcriptase (20U/µl) to make it around 20 µl volumes. This was incubated finally at 42˚C for 60 min and 25˚C for 10 min.

The reaction was stopped by heating at 70˚C for 10 min and chilled on ice. PCR products were then tested with agarose gel electrophoresis.

Polymerase Chain Reaction

2µl of cDNA was taken in a sterilized PCR tube with 1µl of 3α-HSD primers of sense and antisense. The following primers were used at different reactions. Sense- 5'CTGTGCCTGAGA AGGTTGCT3', antisense-5'CATGTGTCACAGATATCCAC 3', sense-5' GGAGGCCATGGAGAAGTGTA3', antisense 5'CACCCATGTTTTGTCTCGTG3'and sense- 5'ACAAG CGATGGATTCCATAT3' 25µl PCR master mix consists of all basic components : Taq DNA Polymerase, dNTPs and reaction buffer(1.5mM Magnesium chloride) were added. The PCR amplification was used to check the expression of the mRNAs of 3α-HSD enzyme in the brain sample. The temperature adopted in the amplification is as follows: 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, the finally holding temperature was 4°C. The PCR products of gene specific primer of 3α-HSD along with 100bp DNA Ladder were then subjected to 1.2% agarose gel electrophoresis. After running the gel, the image of specific bands were captured by using UV trans illumination under the JH Bio geldoc system.

Sequencing

The PCR product was sequenced and the mRNA sequences of 3α-HSD obtained for *L. rohita* were shown in the result. The mRNA sequences of 3α-HSD of *L. rohita* were multiple aligned with vertebrate organisms to know the similarity, relationship and evolutionary significance of *L. rohita* with other organisms.

RESULTS

Total RNA was isolated from the entire male brain of *L. rohita*. Two RNA bands as 28S and 18S were obtained and shown in Fig.1A. The isolated RNA was reverse transcribed into cDNA using RT-PCR. The single strand cDNA was obtained and it appeared as a smear. The PCR was made to run at specific temperature to analyze the gene expression of 3α-HSD by adding the gene specific primer to the cDNA. The PCR product shows a single band which is represented in Fig.1B and Fig.1C. The PCR product and the 100bp DNA Ladder

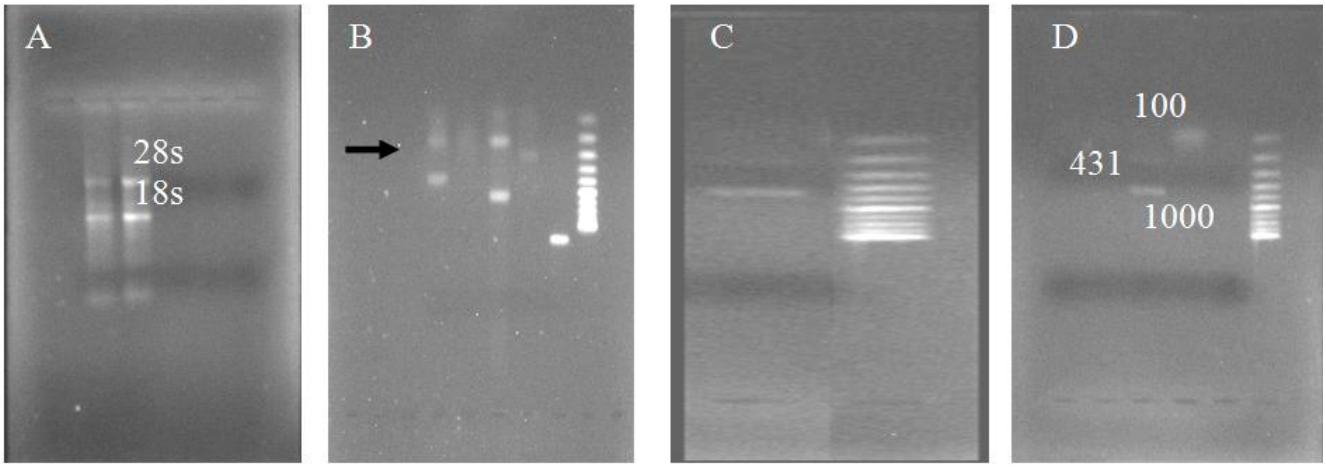

Fig.1. A. Shows the Two RNA bands as 28S and 18S were obtained from brain tissue of *Labeo rohita*, B and C. Shows the PCR products from cDNA amplified along with specific primer of 3a-HSD, D. Shows the 100bp marker DNA and 431bp expression of 3a-HSD gene in the brain of Indian major carp, *Labeo rohita*

were loaded in separate lanes in the gel. Single band at 431bp was obtained and is shown in Fig.1D. The mRNA sequence of 3α-HSD obtained for *L. rohita* are shown below. The obtained mRNA sequence of 3αHSD of *L. rohita* were multiple aligned with different species of vertebrates to know the similarity of *L. rohita* with other organisms. Relative score table and multiple sequence alignment of complete mRNA sequence of 3α-HSD of vertebrates along with the obtained sequence of *Labeo rohita* showing the percentage of similarity shown in Fig. 2.

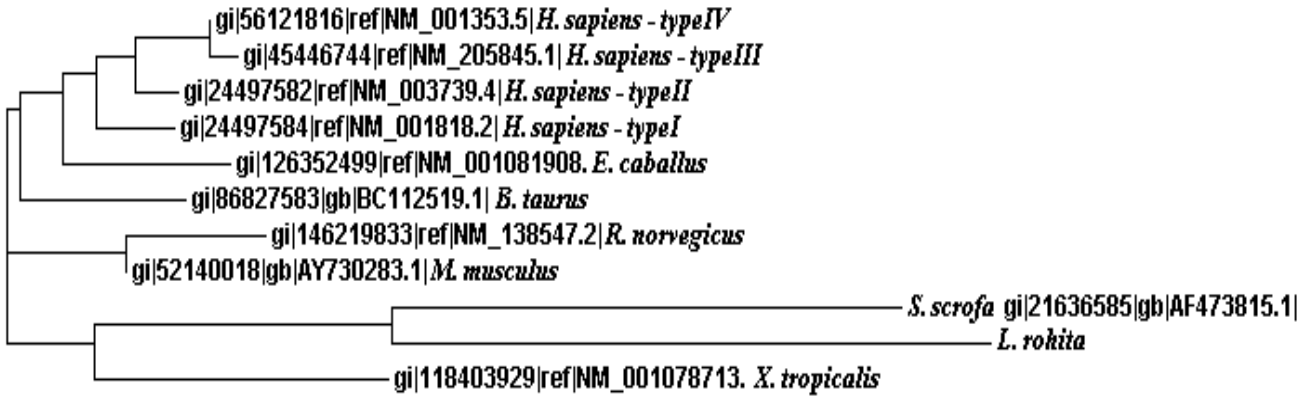

Fig. 2. Phylogenetic tree of mRNA sequences of 3α-HSD of different classes of vertebrates along with the obtained sequences from *Labeo rohita* showing the relationship and evolutionary distance among the organisms when compared with each other

The partial mRNA Sequences of 3α-HSD of *Labeo rohita* obtained from the present study

>gi|326325828|gb|JF683819.1| Labeo rohita 3-alpha hydroxy steriod dehydrogenase (AKR1C) mRNA, complete sequence
GCGATGGTGCTGTGCTCTTGGATACCGTCATTTTTCAT
CGTCGCGAGCGTACTTAGGTGACCTGCCTGGCCACTT
GCATCTGCACGGCATTGCTTGGCTGGTCTGTCCAATA
TATTTGTGGGATTTTGAGAGTGAAGTAGCTCCTCTGTT
GGCTGCATTTGTAACTGCCATTCTGGCTGCAAATGAA
CCTGCTGGGAATGATTTCTAGTCTGCAAGTGCTGCGA
TCTAAGCTGCATGCGTTCATGTCTATTTGCATCGAAGC
TACGAGAGTGGTGTGGAACCATGTTTGGCAACAGCGT
TTCTGACATGATTCTACCAGGTAGAGGTGCCGTCCTT
CCGTTGTCTCGATCTAACCTTGGCCTACCTCTCCTGCC
GGTACTTACTCTACCTCGTTGAGAATGTATGGACAGT
GGATATCTGGTGACACATGA

DISCUSSION

Our results confirm the presence of 3α-HSD in the brain of an Indian Major Carp, *L. rohita*. Results from the present experiment indicates the expression of the mRNAs of 3α-HSD, the enzyme involved in the conversion of 5α-DHPROG into 3α, 5α-THPROG in the brain of *L. rohita*. Aldo-keto reductases interconvert weak androgens, estrogens, progestin, mineralocorticoids and glucocorticoids to their more potent counterparts by catalyzing the reduction and oxidation of keto- and hydroxysteroids, respectively, thereby regulating a wide range of physiological processes involved in development, homeostasis and reproduction (Bauman *et al.,* 2004). In this manner, AKRs regulate the occupancy and transactivation of several steroid receptors in target tissues leading to transcription of hormone-responsive genes (Penning *et al.,* 2004). The products of AKR activity have been implicated in prostate disease, breast cancer, obesity, poly cystic ovary disease and delay in the onset of puberty in humans (Stanbrough *et al.,* 2006; Rittner *et al.,* 1997; Nonneman *et al.,* 2006). They reported the AKR1C gene expression in different tissues of pig. From the report it is noted that spleen, ovary, lung, adrenal, kidney, and endometrial expressed all the five genes. AKR1C2 and AKR1C4 were the most widely expressed genes. AKR1C2 was expressed in all tissues except pancreas and brain. Unlike human AKR1C4, pig AKR1C4 expression was not specific to the liver but was expressed in all tissues and AKR1C2 was the only other AKR1C gene expressed in brain. Because of their location in the swine genome and their implication in reproductive physiology, this gene cluster was characterized and evaluated for effects on reproductive traits in swine. Results of the present experiment confirm the presence of the gene AKR1C in the brain tissue of *L. rohita*. The mRNA sequence obtained for *L. rohita* was partial and it was multiple aligned with *B. Taurus, M. musculus, R. norvegicus*, *X. tropicalis* and *H. sapiens*. The phylogenetic analysis revealed that the tree was very well bifurcated into two branches with *H. sapiens* at the top and *L. rohita* at the bottom. *H. sapiens* and *B. tarus* arise from a same point of origin whereas *R. norvegicus* and *M. musculus* have arisen from a different branch which reveals that they share quite conserved regions. *L. rohita*, *S. scrofa* and *X. tropicalis* have risen from a common point which reveals that *L. rohita* is more closely related to *S. scrofa* and distantly related to *H. sapiens*. The present identified gene might be the AKR1C2 which is specific to brain of Indian major carp. The identification of other genes AKR1C1, AKR1C3, AKR1C4 and AKR1C5 need to be studied in this carp to state whether the 3α-HSD expression is tissue specific. Further studies are required to understand the expression of the enzyme in the brain which will highlight the presence or absence of AKR1C2 or AKR1C1.

REFERENCES

Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. 2006. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci USA*, 103:14602–14607.

Baulieu EE, Schumacher M. 2000. Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Hum Reprod*, 15: 1-13.

Bauman DR, Steckelbroeck S, Penning TM. 2004. The roles of aldo-ketp reductases in steroid hormone action. *Drug News Perspective,* 17: 563-578.

Belelli D, Herd MB. 2003. The contraceptive agent Provera enhances $GABA_A$ receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids? *J Neurosci*, 23:10013-10020

Belelli D, Lambert JJ. 2005. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nat Rev Neurosci*, 6:565–575.

Belyaeva OV, Chetyrkin SV, Clark AL, Kostereva NV, SantaCruz KS, Chronwall BM, Kedishvili NY. 2007. Role of Microsomal Retinol/Sterol Dehydrogenase-Like Short-Chain Dehydrogenases/Reductases in the Oxidation and Epimerization of 3α-Hydroxysteroids in Human Tissues. *Endocrinology* May, 148(5):2148-2156.

Brinton RD. 1994. The neurosteroid 3α-hydroxy-5α-pregnan-20-one induces cytoarchitectural regression in cultured fetal hippocampal neurons. *J Neurosci*, 14: 2763-2774.

Carlson PJ, Singh JB, Zarate CA Jr, Drevets WC, Manji HK. 2006. Neural circuitry and neuroplasticity in mood disorders: insights for novel therapeutic targets. NeuroRx: *J Am Soc Exp NeuroTher*, 3:22-41.

Gee KW, Bolger MB, Brinton RE, Coirini H, Mc Ewen BS. 1998. Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. *J Pharmacol Exp Ther,* 246: 803-812.

Khanna M, Qin KN, Wang RW, Cheng KC. 1995. Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3α-hydroxysteroid dehydrogenases. *J Biol Chem,* 270: 20162-20168.

Lin HK, Jez JM, Schlegel BB, Peehl DM, Pachter JA, Penning TM. 1997. Expression and characterization of recombinant type2 3α-hydroxysteroid dehydrogenase (HSD) from human prostate: demonstration of bifunctional 3α/17β-HSD activity and celluar distribution. *Mol Endocrinol*, 11: 1971-1984.

Mandiyan VS, Coats JK, Shah NM. 2005. Deficits in sexual and aggressive behaviors in Cnga2 mutant mice. *Nat Neurosci,* 8:1660–1662.

Nelson RJ, Trainor BC. 2007. Neural mechanisms of aggression. *Nature Rev Neurosci*, 8:536-546.

Nonneman DJ, Wise TH, Ford JJ, Kuehn LA, Rohrer GA. 2006. Characterization of the aldo-keto reductase 1C gene cluster on Pig chromosome 10: possible associations with reproductive traits. *BMC Veterinary Reasearch*, 2: 1-11.

Penning TM, Jin Y, Steckelbroeck S, Lanisnik Rizner T, Lewis M. 2004. Structure-functions of 3 alpha hydroxysteroid dehydrogenases: genes and proteins *Mol Cell Endocrinol*, 215(1-2): 63-72.

Penning TM, Mukharji I, Barrows S, Talalay P. 1984. Purification and properties of a 3α-hydroxysteroid dehydrogenase of rat liver cytosol and its inhibition by anti-inflammatory drugs *J Biochem*, 222: 601-611.

Pinault D. 2004. The thalamic reticular nucleus: structure, function and concept. *Brain Res Rev*, 46:1-31.

Pinna G, Costa E, Guidotti A. 2006. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. *Psychopharmacology (Berl),* 186:362-372

Pomata PE, Colman-Lerer AA, Baranao JL, Fiszman ML. 2000. In vivo evidences of early neurosteroid synthesis in the developing rat central nervous system and placenta. *Brain Res Dev Brain Res,* 120: 83-86.

Qin KN, New MI and Cheng KC. 1993. Molecular cloning of multiple cDNAs encoding human enzymes structurally related to 3α-hydroxysteroid dehydrogenases. *J Steroid Biochem Mol Biol*, 46: 673-679.

Rittner HL, Lee PD, Blum WF, Doerr HG, Steiss J, Kreuder J, Rascher W, Kiess W. 1997. Developmental patterns of serum 3alpha-androstanediol glucuronide. *Journal of Endocrinological Investigation*, 20: 138-143.

Schumacher M, Weill-Engerer S, Liere P, Robert F, Franklin RJ, Garcia-segura LM, Lambert JJ, Mayo W, Melcangi RC, Parducz A, Suter U, Carelli C, Baulieu EE, Akwa Y. 2003. Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog Neurobiol,* 71: 3-29.

Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP. 2006. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Research*, 66: 2815-2825.

****