NEGATIVE IMPACT OF STRESS ON REPRODUCTION: ROLE OF BRAIN AND GILL DURING SALINITY RESPONSE IN TILAPIA

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Introduction:

Negative impact Stress is considered to be the physiological resultant of demands that exceed an organism's regulatory capacities. Response to stressors is vitally important for normal response, allowing the organism to avoid or cope with challenges to homeostasis. Sex steroids actually have profound influence on stress perception in the brain. Stress manifests centrally in the nervous system, converging at the hypothalamus and the final product of stress hormone, cortisol is believed to contribute to the stress-induced reproductive suppression during stress by central actions on the pituitary or hypothalamus. Suppression of reproductive hormones reduced gamete viability and gonadal growth retardation. Therefore, the aim of the present study was to test the hypothesis that stress has a negative impact on reproduction. To address this, we examined the differential expression pattern of stress hormones (corticotrophin releasing hormone, CRH; CRH-receptor, CRH-R; adrenocorticotropic hormone, ACTH and beta-Na+/K+ ATPase, β-NKA) and reproductive hormones (luteinizing hormone, LH; follicle stimulating hormone, FSH; gonadotropin releasing hormone, GnRH1, 2 and 3) in freshwater (FW) and seawater (SW) acclimated tilapia in the forebrain, midbrain, hypothalamus, pituitary and gill during salinity stress.

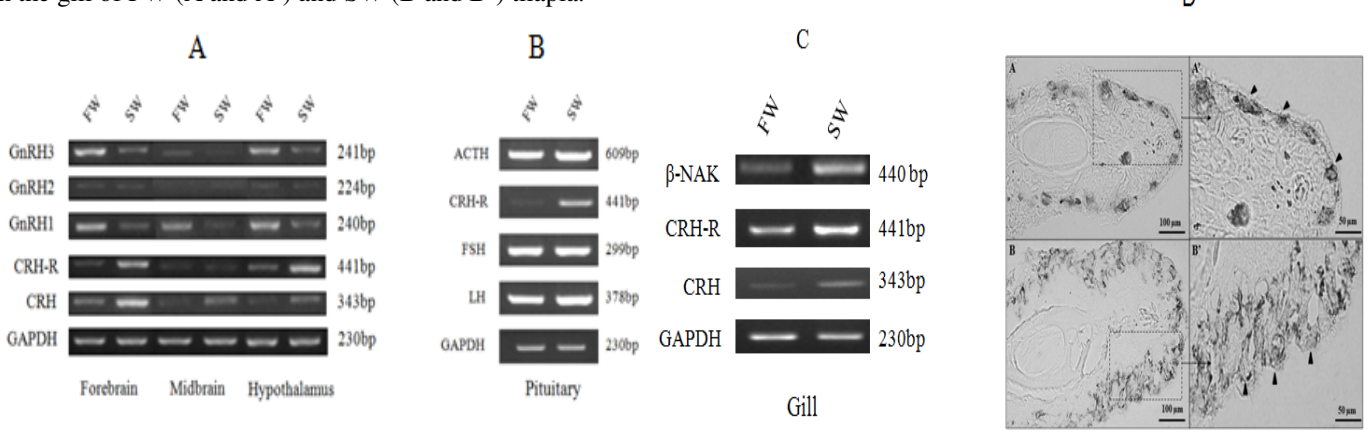
Methods:

RT-PCR analysis was performed to find out the mRNA expression of CRH, CRH-R, ACTH, β-NKA, GnRH, LH, and FSH in the brain, pituitary and gill of tilapia during salinity stress. *In situ* hybridization study was also performed to localize the transcripts of hypothalamic neuropeptide (CRH, GnRH1 and GnRH3) in the tilapia brain, and immunolocalization also carried out by using α-Na+/K+-ATPase (α-NKA) antibody to differentiate the mitochondria rich cells in the gill at FW and SW.

Results and Discussion:

The present results demonstrated that the intensity of the band of CRH and CRH-R was strongly detected in the forebrain, midbrain and hypothalamus of SW fish. However, the mRNA expression of GnRH1 and GnRH3 was weak in the forebrain, midbrain and hypothalamus of SW fish compared to their respective FW fish (Fig.1A). In addition, there was no significant difference observed in LH, FSH and ACTH in the pituitary, whereas increased expression of CRH-R was found in the SW fish (Fig. 1B). Stress increased the expression of CRH, which led to a suppression of GnRH1 and GnRH3 in the hypothalamus. Similarly, the transcripts of gill CRH [1], CRH-R, β-NKA [2] (Fig. 1C) and α-NKA protein (Fig. 1D) were increased in the SW fish to maintain the homeostasis and balance the acid-base regulation. Furthermore, the CRH, GnRH1 and GnRH3 transcripts hybridization signals were detected in the tilapia brain especially in the telencephalic ventricle (TelV) (Fig. 2B, E and H) and preoptic area (POA) (Fig. 2C, F and I), which implied that the stress induce CRH neuron and this may activate GnRH, which then

Fig.1. RT-PCR analysis of (A) CRH, CRH-R, GnRH1, GnRH2 and GnRH3 in the forebrain, midbrain and hypothalamus, (B) LH, FSH, CRH-R and ACTH in the pituitary, (C) CRH, CRH-R and β-NKA in the gill and (D) immunolocalization of α-NKA in the gill of FW (A and A') and SW (B and B') tilapia.



suppress reproduction. Stress exerts profound inhibitory effects on reproductive function by suppressing the pulsatile release of GnRH.

Conclusion:

These results suggested that the activation of stress axis (hypothalamus- pituitary- interrenal axis, HPI) suppressed the hypothalamus- pituitary- gonadal (HPG) axis to affect reproduction in tilapia during salinity stress. The elevated transcripts of CRH and CRH-R in both brain and gill suggest that it may be essential for tilapia SW acclimation. Further, the expression of β-NKA transcripts and α-NKA proteins were increased in the gill of SW fish than the FW fish, which implied that the gill play an essential key role during salinity stress response. The *in situ* hybridization study demonstrated that the transcripts of CRH and GnRH3 were more abundant in the POA of brain than the GnRH1.

References:

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Fig. 2. Localization of CRH, GnRH1 and GnRH3 mRNA in the tilapia brain by *in situ* hybridization analysis.