別紙様式５(Attached Form 5)

学位論文要旨 Abstract of Thesis

所属専攻 Field: New Frontier Science 専攻(Field)
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Title of Thesis
The Role of Brain Aromatase and Estrogen in Zebrafish Nervous System
(ゼブラフィッシュ神経系における脳アロマテースとエストロゲンの役割)

Abstract（within 1600 words）

Aromatase, a product of *cyp19a1* gene, is a rate-limiting enzyme for estradiol biosynthesis. Unlike mammals, zebrafish has two isoforms of *cyp19a1* gene, *cyp19a1a* and *cyp19a1b*, encoding ovarian and brain aromatase, respectively. The increase in brain aromatase (AroB) expression after 12 hour post-fertilization (hpf) in zebrafish suggests a possible role of brain-formed estradiol in early development, however the functions remain unclear. Serotonin is a neuromodulator that appears to play a critical role in modulating brain neural activity and neurophysicological functions. Here, we used zebrafish to elucidate the function of brain-formed estradiol in regulation of serotonergic neurons by exposing the embryos to exogenous estradiol and by knocking down AroB using morpholino oligos (MO). To assess the effects the serotonin contents and expression of tryptophan hydroxylase (*tph*), heart rate, and anxiety level were measured. Lower dose of estradiol (≤ 0.005 μM) increased serotonin levels and relative exression of *tph* isoforms followed by increased heart rate and decreased anxiety level. While, higher dose of estradiol (≥ 1 μM) acted contrary. All the parameters were reversed by addition of estrogen receptor blocker suggesting that these effects were mediated by estrogen receptor. Injection of AroB MO to fertilized eggs decreased serotonin levels, relative expression of *tph* isoforms and heart rate, while the anxiety level was increased. All the parameters were rescued either by co-injection of AroB mRNA or exposing injected embryos to estradiol, suggesting that brain-formed estradiol specifically regulates serotonergic neuron in zebrafish. Taken together, first part of the study demonstrated that estradiol has a biphasic manner toward serotonergic neurons and brain-formed estradiol has an important role in modulating serotonergic neurons during early development of zebrafish.

Aromatase is known to be expressed in retina, a part of the central nervous system. In zebrafish it has been reported that AroB is expressed in the eye. Therefore, possible functions of AroB in zebrafish eye were investigated by MO-mediated knockdown of AroB. Injection of AroB

MO to fertilized eggs decreased the eye size (eye/body length ratio) and diameter of optic nerve, while increasing apoptosis in the eye. The effects of AroB MO were rescued either by co-injection of AroB mRNA or exposure to 1 μM of E_2. AroB MO decreased the response in visual background adaptation (VBA) at 5 dpf and optomotor response (OMR) at 7 dpf. Since MO injection eliminated the AroB immunoreactivity found in retina, the effects of MO injection are likely to be due to the MO-mediated knockdown of AroB. To examine the role of estrogen receptor (ER) in the eye development, embryos were exposed to ER blockers. While MPP (ERα antagonist) did not change the eye size and diameter of optic nerve, CYC (ERß antagonist) decreased them. ICI (ERα and ß antagonist) decreased the eye size but not diameter of optic nerve. Apoptosis in the eye at 24 hpf was increased by ICI or CYC, but not by MPP. Exposure to ICI or CYC, not to MPP, resulted in decreased response in VBA. On the other hand, OMR was decreased by ICI or MPP, but not by CYC. The results indicate that AroB in retina has an important role in development of the eye, and the functions of estradiol on visual function may be differentially regulated by different ERs. In addition, blocking 5-HT signaling by ritanserin (5-HTR2 antagonist) resulted in small eyes and disrupted the visual functions, similar to the effects observed in AroB MO-injected embryos and larvae. Interestingly we showed that ritanserin disrupts the expression of AroB in the eye, indicating that one of the possible pathways for 5-HT to exert effects on eye development may be through modulating AroB expression.

Zebrafish has an ability to generate new neurons in the retina throughout its life. It has been known that brain aromatase is presence in zebrafish eye. In this study, the cellular localization and possible function of brain aromatase in adult zebrafish eye was shown for the first time. It was shown that brain aromatase is not exclusively expressed in Müller glia cells, but also in interneuronal cells, protoreceptor cells, and lens epithelial cells of zebrafish eye. Furthermore, estradiol increases the expression and changes the distribution of brain aromatase with additional expression in ganglion cells. It was shown that estradiol acts as a neuroprotective and a neuroregenerative agent by decreasing the number of cell death and increasing the number of proliferative cells in the injured retina. The study provides the evidence that brain aromatase is involved in activation of Müller glia cell in response to retinal injury. The data strongly suggest that estradiol produced in the eye mediates a robust capacity of zebrafish retina to regenerate retinal neurons in the injured condition.

Taken together, the study provides the significant evidence that brain aromatase and estradiol play an important role in nervous system of zebrafish especially in regenerative capacity by modulating 5-HT neuron in the brain and by modulating the activity of Müller glia cells in the retina.