

Index	Page
1. Introduction.....	001
1.1. Serotonin system.....	001
1.1.1. Discovery of serotonin.....	001
1.1.2. Biosynthesis and metabolism of serotonin.....	001
1.1.2.1. Biosynthesis.....	001
1.1.2.1.1. Tryptophan hydroxylases.....	002
1.1.2.1.2. Characteristics of TPH1 and TPH2.....	002
1.1.2.2. Metabolism of serotonin.....	004
1.1.2.2.1. Monoamine Oxidase (MAO).....	004
1.1.2.2.2. Arylalkylamin-N-Acetyltransferase (AANAT).....	004
1.1.3. 5HT receptors.....	005
1.1.3.1. Classification.....	005
1.1.3.2. Mechanism of action.....	005
1.1.3.3. Localization.....	006
1.1.4. Serotonin transporter.....	007
1.2. Serotonin system in the brain.....	009
1.2.1. Localization of serotonin in the brain.....	009
1.2.1.1. Raphe nuclei.....	009
1.2.1.2. Hypothalamus.....	010
1.2.1.3. Thalamus.....	011
1.2.2. Development of the brain serotonin system.....	011
1.2.2.1. Transcription factors involved in serotonergic neurons differentiation.....	012
1.2.2.1.1. Phox2b.....	012
1.2.2.1.2. Mash1.....	012
1.2.2.1.3. Nkx2.2.....	012
1.2.2.1.4. Lmx1b.....	012
1.2.2.1.5. Pet1.....	013
1.2.2.1.6. Gata2 and Gata3.....	013
1.3. Developmental role of 5HT during embryogenesis.....	014
1.3.1. Invertebrates.....	015
1.3.2. Vertebrates.....	016
1.4. The pharyngeal arches.....	016
1.4.1. Neural crest cells.....	017

II

1.4.1.1. Cranial neural crest cells.....	019
1.4.1.2. Trunk neural crest cells.....	020
1.4.2. Endodermal pharyngeal pouches.....	021
1.5. Relationship between serotonin system and neural crest cells.....	021
1.5.1 MAO.....	021
1.5.2. Gbx2.....	022
1.5.3. SHH.....	022
1.5.4. FGF8.....	022
1.5.5. BMPs.....	022
1.5.6. Mash1.....	023
1.5.7. Phox2b.....	023
1.6. Aims.....	025
2. Materials and Methods.....	026
2.1. Materials.....	026
2.1.1. Chemicals.....	026
2.1.2. Cell culture media.....	026
2.1.3. Enzymes.....	027
2.1.4. Kits.....	027
2.1.5. Equipment for molecular biology.....	027
2.1.6. Solutions and buffers.....	028
2.1.7. Sterilization of solutions and equipments.....	031
2.1.8. Media, antibiotics, and agar-plates.....	031
2.1.9. Vectors used for the cloning and sequence analysis.....	032
2.2. Methods.....	033
2.2.1. Animals.....	033
2.2.2. Removing the chorions from zebrafish embryos.....	033
2.2.3. Extraction and purification of RNA from zebrafish embryos.....	033
2.2.4. RT-PCR analysis.....	034
2.2.5. DNA transformation in bacteria.....	034
2.2.5.1. Preparation of competent E.coli bacteria.....	034
2.2.5.2. Ligation of DNA fragments.....	034
2.2.5.3. Transformation of competent bacteria.....	034
2.2.5.4. Isolation of DNA.....	035
2.2.5.4.1. Isolation of genomic DNA from mouse tails.....	035

III

2.2.5.4.2. Isolation of genomic DNA from cultured cells.....	035
2.2.5.4.3. DNA extraction from agarose gel.....	035
2.2.5.4.4. Isolation of plasmid DNA from Escherichia coli.....	036
2.2.5.4.4.1. Mini preparation.....	036
2.2.5.4.4.2. Maxi preparation.....	036
2.2.6. DNA sequencing.....	036
2.2.7. TPH2 mRNA synthesis and microinjection.....	036
2.2.8. Riboprobes synthesis for in situ hybridization.....	037
2.2.9. Whole mount in situ hybridization.....	037
2.2.10. Immunohistochemistry.....	038
2.2.11. Alcian blue staining.....	038
2.2.12. DASPEI live staining.....	038
2.2.13. Tunnel assay (apoptosis test).....	039
2.2.14. Enzyme activity assay.....	039
2.2.14.1. Phenylalanine hydroxylase (PAH).....	039
2.2.14.2. Tryptophan hydroxylase (TPH).....	040
2.2.15. Western Blot.....	041
2.2.16. Cell culture.....	041
2.2.16.1. Embryonic stem cells (ES cells).....	041
2.2.16.2. COS7 cells.....	042
2.2.17. Synthetic oligonucleotide.....	043
2.2.17.1. Primers.....	043
2.2.17.2. Morpholino antisense oligonucleotides.....	046
3. Results.....	047
3.1. Serotonin system in the Zebrafish.....	047
3.1.1. Early stages.....	047
3.1.2. The brain.....	049
3.1.3. 5HT single cells.....	049
3.1.3.1. How 5HT single cells get serotonin?.....	051
3.1.3.2. The origin of 5HT single cells.....	055
3.2. Cloning and sequencing of TPH isoforms in the zebrafish.....	059
3.3. Expression pattern of TPH isoforms in the zebrafish embryos.....	060
3.3.1. TPHD1.....	060
3.3.2. TPHD2.....	061

IV

3.3.3. TPH2.....	062
3.4. Phenotyping the TPHD1 and TPH2 knockdown zebrafish embryos.....	064
3.4.1. TPHD1 morphants.....	064
3.4.2. TPH2 morphants.....	064
3.4.2.1. Ventral pharyngeal arches.....	064
3.4.2.2. Pigment cells.....	066
3.4.2.3. Neural crest induction.....	068
3.4.2.4. Cranial neural crest cells migration.....	069
3.4.2.5. Myocardial function.....	070
3.4.2.6. Peripheral nervous system.....	071
3.4.2.7. Motor neurons in the spinal cord region.....	072
3.4.2.8. Endodermal pharyngeal pouches.....	073
3.5. 5HT2B receptor in zebrafish.....	074
3.5.1. Cloning of the 5HT2B receptor.....	074
3.5.2. Expression pattern of 5HT2B receptor in zebrafish embryos.....	077
3.5.3. Loss-of function test of 5HT2B receptor.....	078
3.5.3.1. Pharmacological loss-of function test.....	078
3.5.3.2. Genetic loss-of function test.....	078
3.6. Cloning tryptophan hydroxylase in <i>Drosophila melanogaster</i>	080
3.7. Knock out of TPH2 in the mouse.....	083
4. Discussion.....	087
4.1. 5HT in early stages of zebrafish.....	087
4.2. Defects of the pharyngeal arches.....	088
4.3. 5HT in the hindbrain.....	091
4.4. 5HT in the hypothalamus.....	092
4.5. 5HT single cells.....	093
4.6. Independent genes encoding TPH and PAH in <i>Drosophila</i>	097
4.7. Generation of TPH2 Knock out mouse.....	099
5. References.....	101
6. Appendix.....	115
• Curriculum Vitae.....	115
• Publications.....	115
• GenBank Entries.....	116
• Summary.....	117

- Acknowledgement..... 119