Origin and molecular evolution of LPXRFamide peptides and PQRFamide peptides in the brain of vertebrates

Tomohiro OSUGI

Department of Sciences for Biospheric Coexistence, Graduate School of Biosphere Science, Hiroshima University Higashi-Hiroshima 739-8521

脊椎動物の脳におけるLPXRFamideペプチドとPQRFamideペプチドの起源と分子進化

大　杉　知　裕

広島大学　生物圏科学研究科　東広島市 739-8521

Key words: PQRFamide peptide, LPXRFamide peptide, molecular evolution, neuropeptide, agnathan, cartilaginous fish

Among the RFamide peptide groups, PQRFamide peptides, such as neuropeptide FF (NPFF) and NPAF, share a common C-terminal Pro-Gln-Arg-Phe-NH_2 motif. LPXRFamide (X = L or Q) peptides, such as gonadotropin-inhibitory hormone (GnIH), frog growth hormone-releasing peptide (fGRP), goldfish LPXRFamide peptide and mammalian RFamide-related peptides (RFRPs), share a C-terminal Leu-Pro-Leu/Gln-Arg-Phe-NH_2 motif. Such a similar C-terminal structure suggests that these two groups may have diverged from a common ancestral gene. In this study, I sought to clarify the evolutionary origin and divergence of these two groups, by identifying novel RFamide peptides from the brain of lower vertebrates.

At the start, I used the brain of lamprey (*Petromyzon marinus*), one of the only two extant representative species of the oldest lineage of vertebrates, the Agnatha, which diverged from the main line of vertebrate evolution approximately 450 million years ago. A novel RFamide peptide was identified by immunoaffinity purification using the antiserum against LPXRFamide peptide. The identified RFamide peptide did not contain a C-terminal LPXRFamide motif, but had the sequence SWGAPAEKFWMRAMPQRFamide (lamprey PQRFa). A cDNA of the precursor encoded one lamprey PQRFa and two related peptides. These related peptides, which also had the C-terminal PQRFamide motif, were further identified as mature endogenous ligands. Phylogenetic analysis revealed that lamprey PQRFamide peptide precursor belongs to the PQRFamide peptide group. *In situ* hybridization demonstrated that lamprey PQRFamide peptide mRNA is expressed in the regions predicted to be involved in neuroendocrine and behavioral functions. This is the first demonstration of the presence of RFamide peptides in the agnathan brain. Lamprey PQRFamide peptides are considered to have retained the most ancestral features of PQRFamide peptides. On the other

hand, I could not identify the homolog of LPXRFamide peptide from the brain of lamprey.

I next used the brain of hagfish (*Paramyxine atami*), another representative species of agnathans. I identified three novel RFamide peptides containing a C-terminal PQRFamide sequence and a novel RFamide peptide containing a C-terminal LPQRFamide sequence from the brain of hagfish. I cloned two cDNAs encoding these hagfish PQRFamide peptides and a LPQRFamide peptide. Interestingly, one cDNA encoded three identified PQRFamide peptides, whereas the other cDNA encoded two identified PQRFamide peptides and one identified LPQRFamide peptide. The latter cDNA had a nucleotide substitution resulting in a codon change from TTT (Phe) to TTG (Leu) compared to the former cDNA. The localization of hagfish PQRFamide peptides was analyzed by immunohistochemistry and *in situ* hybridization. The immunoreactive cell bodies were found in the infundibular nucleus (IN) of the hypothalamus. Hagfish PQRFamide peptide mRNA signal was detected only in the IN, supporting the immunohistochemical observations. Immunoreactive fibers were mainly found in the hypothalamus, ventral medulla and around the fourth ventricle, suggesting multiple functions of hagfish PQRFamide peptides. Because two hagfish PQRFamide peptide precursors are similar to that of mammalian PQRFamide peptide, the origin of PQRFamide peptides can date back at least to the emergence of agnathans. Although, hagfish LPQRFamide peptide is similar to LPXRFamide peptides, further study is needed to clarify whether the precursor encoding hagfish LPQRFamide peptide is the homolog of LPXRFamide peptides.

The results of the study of lamprey and hagfish suggest that PQRFamide peptides exist in the brain of agnathans, whereas LPXRFamide peptides may not exist in their brain. Cartilaginous fish are higher than agnathans in the phylogenetic tree, but there are no information about PQRFamide peptides and LPXRFamide peptides. Therefore, I next sought to clarify whether cartilaginous fish have both LPXRFamide peptides and PQRFamide peptides or not. I used two cartilaginous fish: Japanese banded dogfish *Triakis scyllium* (Elasmobranchii) and Elephant shark *Callorhinchus milii* (Holocephali). I first used a genome database analysis of elephant shark. By using BLAST program, a genome sequence which encodes PQRFamide peptides was found in the database. Based on the genome sequence of elephant shark, degenerate primers were made and used for PCR cloning. A cDNA encoding two PQRFamide peptides was identified in the brain of Japanese banded dogfish. Phylogenetic analysis suggests that the dogfish RFamide peptides belong to the PQRFamide peptide group. On the other hand, cDNA(s) encoding LPXRFamide peptides could not be identified in the present study. These results suggest that LPXRFamide peptides arose after the divergence of Osteichthyes (bony fish) and Chondrichthyes (cartilaginous fish).

To obtain some insights into the evolutionary relationship of LPXRFamide peptides and PQRFamide peptides, I analyzed the chromosomal location and synteny of each peptide gene. The LPXRFamide peptide genes are located adjacent to homeobox (HOX) gene cluster A, whereas PQRFamide peptide genes are located adjacent to HOX gene cluster C. As HOX gene clusters are considered to have arisen through the two rounds of whole-genome duplication, it is highly possible that LPXRFamide peptides and PQRFamide peptides arose from a common ancestral gene in a chromosomal duplication event. Further studies are needed to clarify the evolutionary history of LPXRFamide peptides and PQRFamide peptides.