A Novel Adrenomedullin Family Identified in Fish

Maho Ogoshi and Yoshio Takei
Ocean Research Institute, the University of Tokyo

Adrenomedullin (AM) is a novel peptide initially isolated in 1993 from the human pheochromocytoma of adrenal medulla origin. Since its discovery, a large number of studies have been carried out on this hormone mostly in mammals as AM molecule has been identified only in this vertebrate species. Those studies have revealed that AM has divergent biological functions such as cardiovascular regulation and body fluid regulation. We have been pursuing a novel hormone that plays an important role in fish osmoregulation to draw the whole picture of endocrine control of this homeostatic regulation. From this point of view, we noticed that AM is promising because of its effects on body fluid regulation. We attempted to identify AM in the pufferfish, *Takifugu rubripes*, because the whole genome sequences are available. As a result, we identified five distinct AMs in pufferfish (TrAM-1 through -5) that are expressed as mRNAs and make an independent hormone family, although AM was initially recognized as one of the members of structurally related peptides named calcitonin gene-related peptide superfamily. Among the five TrAMs, TrAM-1 was most similar to mammalian AM in the structure of gene and tissue distribution pattern. Conserved synteny of the genes neighboring human AM and TrAM-1 gene proved that TrAM-1 is the ortholog of mammalian AM. TrAM-4 and -5 were similar to TrAM-1 in tissue distribution of mRNA and the exon-intron structure of the gene. However, TrAM-2 and -3 were different from the other three in those features, suggesting that the five TrAMs can be divided into two groups. Thus we hypothesized that there might be an ortholog of TrAM-2/-3 group in mammals, and searched for it in genome database of mammals. As a result, we identified the orthologs of TrAM-2 in human, rat and mouse and named them adrenomedullin 2 (AM2). AM2 exhibited potent hypotensive effect and caused weak antidiuresis and antinatriuresis in mouse. In rat, however, AM2 promoted diuresis and natriuresis as AM does. Examination of the vasodilating effect using isolated porcine arteries revealed that it was most potent in the coronary artery. Further, immunohistochemical study showed that strong AM2 immunoreactivities are localized in the mouse kidney and heart. In the kidney, AM2 immunoreactivity was present in glomerulus and in endothelial cells of vasa recta, and in the heart immunoreactive AM2 appeared in the endothelial cells of coronary artery and vein. All these results indicate that multiple AM peptides make an independent hormone family not only in fish but also in mammals, and that newly identified AM2 plays an important role in cardiovascular and body fluid regulation in mammals.

Structures and Functions of Novel Hypothalamic RF-Amide Peptides: Their Unity and Diversity

Kazuyoshi Tsutsui[1,2] and Kazuyoshi Ukena[1,2]
[1]Lab. of Brain Sci., Fac. of Integrated Arts and Sci., Hiroshima Univ., Higashi-Hiroshima 739-8521, [2]CREST, JST, Tokyo 150-0002

Recently, we identified a novel hypothalamic dodecapeptide, Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH_2, in the quail brain. This avian neuropeptide was shown to be located in neurons of the paraventricular nucleus (PVN) and their terminals in the median eminence (ME) and inhibit gonadotropin release from the cultured quail anterior pituitary. This is the first hypothalamic peptide inhibiting gonadotropin release reported in a vertebrate. We therefore termed it gonadotropin-inhibitory hormone (GnIH). We also characterized a cDNA encoding the GnIH precursor in the brain of quail and sparrow. The GnIH precursor encoded one GnIH and two GnIH-related peptides (GnIH-RP-1 and -RP-2) that included Leu-Pro-Xaa-Arg-Phe-NH_2 (Xaa = Leu or Gln) at their C-termini. Based on this structural feature, GnIH and GnIH-RPs are considered to be LPXRF-amide peptides as a new member of the RF-amide peptide family. After the identification of GnIH in birds, we further sought to identify novel hypothalamic LPXRF-amide peptides similar to GnIH and GnIH-RPs in other vertebrates. The isolated LPLRF-amide peptide from bullfrog hypothalami possessed growth hormone (GH)-releasing activity, and was designated as frog GH-releasing peptide (fGRP). The fGRP precursor also encoded one fGRP and three related peptides (fGRP-RP-1, -RP-2 and -RP-3), which were identified as mature LPXRF-amide peptides. fGRP-RP-2 also stimulated the release of GH and prolactin (PRL). In addition, we characterized a cDNA that encoded three LPXRF-amide peptides (gfLPXRFa-1, -2 and -3) from the goldfish brain and identified gfLPXRFa-3 as a mature peptide. gfLPXRFa-3 stimulated gonadotropin release from the fish pituitary. Turning to mammals, cDNAs that encode LPXRF-amide peptides have been detected in mammalian brains with a gene database search.