Summary

APJ is a member of seven-transmembrane-domain receptors, but its ligand had not been identified for a long time. I prepared CHO cells expressing APJ (CHO-A10 cells), and searched for the endogenous ligand for APJ by monitoring specific signal transduction in CHO-A10 cells. In our recent study, we purified a peptidic ligand for APJ, designated 'apelin', from bovine stomach extracts by a combination of the microphysiometric assay and various chromatographies. On the basis of this peptide sequence, we isolated bovine and human cDNAs encoding apelin. The cDNAs encoded preproproteins consisting of 77 amino acid residues, and apelin was encoded in the C-terminal regions. Synthetic peptides, apelin-36 and [pGlu]apelin-13, corresponding to the C-terminal portion of bovine preproapelin were capable of specifically promoting the extracellular acidification rate in CHO-A10 cells, indicating that apelin is the endogenous ligand for APJ.

In this study, I subsequently isolated rat *apj* cDNA, and quantitatively analyzed the expression of *apj* mRNA in rat tissues by reverse transcription-polymerase chain reaction. *Apj* mRNA was detected in a variety of rat tissues, and the highest expression was observed in the lung. The expression of *apj* mRNA tended to be higher in some tissues in infants than in adults.

An apelin analogue ([pGlu65, Nle75, Tyr77]apelin-13) labeled with ^{125}I specifically bound to human APJ with high affinity (K_d = 22.3 pM), and its binding was competitively inhibited by [pGlu]Apelin-13 (IC_{50} = 1.4 nM) and human apelin-36 (IC_{50} = 4.8 nM). The patterns of extracellular acidification induced differed between [pGlu]apelin-13 and apelin-36: the effect of [pGlu]apelin-13 was temporary, whereas that of apelin-36 was

sustained. The promotion of extracellular acidification induced by both [pGlu]apelin-13 and apelin-36 was almost completely inhibited by the treatments with purtussis toxin, the inhibitor for Gi, and methyl-isobutyl amirolide, the inhibitor for Na^+/H^+ exchanger, respectively. The binding of [^{125}I][pGlu65, Nle75, Tyr77]apelin-13 to APJ was effectively suppressed by pretreatment of the cells with apelin-36 but not with [pGlu]apelin-13, indicating that [pGlu]apelin-13 dissociated more easily than apelin-36 after binding to APJ. In addition, I found that apelin could induce the migration of CHO-A10 cells, and [pGlu]apelin-13 was more potent than apelin-36 in this activity. Heterogeneous molecular forms of apelin corresponding to apelin-36 and [pGlu]apelin-13 were produced in bovine colostrum. My results suggest that the N-terminal portion of apelin modulates the interaction with the receptor and the biological activity, though the core structure of apelin is situated in the C-terminal portion.

The identification of the endogenous ligand (apelin) for APJ enabled us to characterize the function of APJ. I believe that further studies on APJ and apelin will give us new insights into unknown mechanisms in physiological regulation through APJ-apelin system.

Abbreviations

In this dissertation following abbreviations are used.

7TMRs: seven-transmembrane-domain receptors
BSA: bovine serum albumin
Bmax: number of binding sites
CHO-A10 cells: CHO cells expressing human APJ
CHO: Chinese hamster ovary
DMEM: Dulbecco's modified minimum essential medium
EC_{50}: effective dose of 50%
EDTA: ethylenediaminetetraacetic acid
EST: expressed sequence tag
G3PDH: glyceraldehyde-3-phosphate dehydrogenase
HBSS: Hanks' balanced salt solution
HIV-1: human immunodeficiency virus type 1
IC_{50}: inhibitory dose of 50%
Kd: dissociation constant
MIA: methyl-isobutyl amirolide
NHE: Na^+/H^+ exchanger
Nle: norleucine
PBS: phosphate-bufferd saline
PCR: polymerase chain reaction
PTX: purtussis toxin
PrRP: prolactin-releasing peptide
RACE: rapid amplification of cDNA ends
RP-HPLC: reversed phase-high performance liquid chromatography
RT-PCR: reverse transcription-polymerase chain reaction
SDS: sodium dodecyl sulfate