PROJECT SUMMARY
The aim of the project is to provide a detailed molecular and functional description of novel communication pathways that are thought to modulate a number of physiological and pathological processes including pain, mood, and autonomic functions in the central nervous system. Novel peptide and non-peptide ligands for the opioid, nociceptin and cannabinoid receptors, including structural analogues, selective antagonists, radioprobes will be developed and studied by means of synthetic-chemical and functional-biochemical methods. Numerous studies have established the presence of G-protein coupled receptors (GPCRs) as dimers in heterologous cell expression systems but also in vivo. In this context, heterodimers of opioid receptors would constitute new targets of specific interest, each entity possessing original properties in terms of function and pharmacology. Mu opioid receptor (MOPr) activation induces analgesia, while NOPr activation would produce hyperalgesia. Thus, in order to fight pain, it would be important to synthesize one molecule combining the agonist effect for the MOP and antagonist for the NOPr receptors, in order to reinforce the synergistic effect on analgesia. The objective of this project is to conceive new analgesic molecules by combining two pharmacophores targeting the MOPr/NOPr and other heterodimers. These bivalent ligands would be part of a new generation of drugs, at least as efficient as morphine and maybe deprived of some side effects.

BACKGROUND OF THE STUDY
The increasing incidence of drug addictions and the limited success in the treatments of inevitable pain calls for a better knowledge of the physiopathology of drug abuse and of the therapeutic action of opioids. Dispite considerable advances over the past decades, our understanding of the pathophysiology of drug abuse is still far from complete. This notably relates the heterogeneity of receptor proteins involved in different pain states and euphoria. This research should speed up comprehension of the physiological roles of the endogenous neuuropeptides and may provide new targets and tools to develop novel, potent, non-opiate and non-addictive analgesic drugs. For better understanding the interaction of opioid peptides and receptors, novel directions of research is required, using up to date model systems. The bivalent ligands would be part of a new generation of drugs, at least as efficient as morphine and maybe deprived of some side effects. For fundamental sciences, these molecules will also be a new tools for understanding molecular mechanisms involved in formation and pharmacology of the MOPr/NOPr heterodimers. The ultimate goal is to give new guidelines for designing novel and highly effective compounds for the treatment of pain and addiction, which remains a major health and social issue in Hungary, the European Community and world-wide. Grasping the overall response to opioids, with an integrated overview of the receptor systems,
signaling cascades and neural circuits at play, should help to design more potent and selective therapies.

RELEVANT RESEARCH IN THE HOST LABORATORY

The research team is investigating the reaction partners of receptor ligand binding and activation. A number of scientific papers has been published in the field of ligand design and development. Nociceptin/orphanin FQ (NOPr) receptor specific hexapeptides have been synthetised, radiolabeled and characterised by biochemical and pharmacological means (Bojnik et al. 2009, 2010a). Novel, exotic enkephalin derivatives (penta-, hexa- and heptapeptides) have been identified in genomic libraries, representing a wide range of different animal species. The new enkephalins have been detailly characterised (Bojnik et al. 2009, 2010b, 2011 and 2014). Design and development of various ligands targeting functional receptor dimers are in progress with preliminary results as follows: opioid – neuropeptide hybrid peptides and their shorter metabolic fragments (Kleczkowska et al. 2013); endomorphin – DAMGO fusion peptide analogues (Mollica et al. 2013); bivalent ligands combined from opioid agonists and Ca2+-ion channel blockers (Mollica et al. 2014a); cyclic biphAPH peptide derivatives with improved antinociceptive properties (Mollica et al. 2015).

SPECIFIC AIMS

One of the significant achievements of the project is to synthetise and test bivalent ligands targeting NOPr-MOPr receptor heterodimers. Up to the 90’s, the GPCRs were referred as monomeric entities in the cell membrane. Numerous studies have established the presence of GPCRs as dimers or oligomers in heterologous cell expression systems but also in vivo. More, the existence of heterodimers, meaning the association of two different types of GPCRs has been demonstrated. In this context, heterodimers of opioid receptors would constitute new targets of specific interest, each entity possessing original properties in terms of function and pharmacology. Our working hypothesis asks the following question: are bivalent ligands targeting receptor heterodimers useful tools in the course of G-protein coupled receptor research? Positive answer is expected, i.e., bivalent (dimeric) ligands are good tools studying G-protein coupled receptor function. One of the gold standard for this research is biphalin, a tail-to-tail conjugated double tetrapeptide ligand. Our aim is to extend the range of bivalent ligands. For this reason enkephalin peptides will be fused with i) nociceptin antagonists by different directions, and ii) with cytostatic molecules, such as taxol, vinblastine or daunomycin. Other peptide analogues include cyclic peptides based on the structure of biphAPH, or peptides with a diversely substituted guanidine bridge (see international cooperation file).

![G-protein assay](image)

**G-protein assay**

![Agonist induced receptor internalization](image)

Agonist induced receptor internalization (right)
MATERIAL AND METHODS

- Laboratory animal handling, tissue dissections, cell membrane preparation by differential centrifugation
- Tissue culture maintenance. Fluorescence and confocal microscopy of EGFP-tagged opioid and NOP receptors
- Sample preparation and protocols for studying radioligand binding and ligand-receptor interactions
- G-protein activation studies by the use of [35S]GTP S binding assays

SUGGESTED READINGS


SNAPSHOTS FROM THE HOST LABORATORY

Significant publications


Representative recent research grants

Some of the latest students in the laboratory
Erdei A, Ph.D., 2014-recent; "Combined peptide and non-peptide ligands for the opioid- and the nociceptin/orphanin FQ (NOP) receptors"

Samavati R, Ph.D., 2014-recent; "Interaction of multiple opioid receptors with the kynurenine system in brain membranes"

Zador F, Ph.D., 2006-2014; "Rimonabant: a CB1 receptor antagonist as a direct interactional partner for mu-and delta-opioid receptors"

Rapavi R, B.Sc. and M.Sc., 2010-2014; "Binding properties and receptor activation of oxymorphone and oxycodone in mouse brain membranes"

Bojnik E, Ph.D., 2005-2009; "Biochemical and pharmacological characterizations of the novel endogenous opioid peptide motifs and synthetic nociceptin hexapeptide sequences"