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known to aggregate in PolyQ length dependent manner. It is believed that aggregation is connected to cytotoxicity and neurodegeneration. Therefore, inhibiting polyQ aggregation is one of the therapeutic strategies in HD. *In vitro* studies of PolyQ peptides show that aggregation proceeds *via* nucleation and elongation steps. But, 17 amino acid segment at N-terminus of PolyQ enhances aggregation through a non-nucleated pathway. These mechanistic details have given rationale for development of peptide inhibitors targeting aggregation. To validate their clinical outcome in HD, they need to be delivered through blood brain barrier to brain. Besides, peptides in general are susceptible to degradation *in vivo* conditions. To overcome these challenges, as a first step, we have synthesized poly-D, L-lactide-co-glycolide nanoparticles containing a PolyQ aggregation inhibitor peptide PGQ₉[P²], by nanoprecipitation method. This process yielded less than 200 nm, spherical, uniformly distributed nanoparticles. Characterization studies by FTIR and HPLC based assays show the presence of PGQ₉[P²] in nanoparticles. *In vitro* release kinetics demonstrates that nanoparticles release PGQ₉[P²] by erosion and diffusion processes. After release, PGQ₉[P²] arrests aggregation of a fragment of N-terminal mHtt. Now these nanoparticles will be tuned to cross blood brain barrier and validated in cell lines and mouse model of HD.

O25.

THE DRIVING FORCE BEHIND THE IN VIVO EFFICACY OF A LEPTIN ANTAGONIST PEPTIDE IS VERY TIGHT BINDING TO THE RECEPTOR

L. Otvos, S. W. Vetter, J. D. Wade, D. Knappe and R. Hoffmann

Temple University, Philadelphia, USA, North Dakota State University, Fargo, USA, Florey Neurosciences Institutes, Melbourne, Australia, and Leipzig University, Leipzig, Germany

At the previous European Peptide Symposium we presented the remarkable efficacies of the leptin receptor antagonist peptide Allo-aca in various animal models. Then most inquiries targeted the pharmacological background of the *in vivo* activity. To answer these questions, we studied the pharmacological properties and receptor binding of Allo-aca. Allo-aca decomposes in minutes in human serum and is undetectable by nano-high performance liquid chromatography techniques beyond 0.5 hours from mouse plasma during pharmacokinetic measurements. The C_{max} of 8900ng/mL at 5 min corresponds to approximately 22 % injected peptide. The peptide exhibits picomolar anti-proliferation activity against a chronic myeloid leukemia cell line and addition of a C-terminal biotin label reduces the *in vitro* activity by approximately 200-fold. In surface plasmon resonance measurements with the biotin-labeled Allo-aca immobilized to a NeutrAvidin-coated chip, exceptionally tight binding to the human leptin receptor is observed. The half-life of the peptide-receptor complex is about 2 hours. Allo-aca is an excellent example of receptor response modulating peptides that demonstrate high activity and selectivity to their targets, and activate/deactivate receptor functions considerably longer than molecular turnovers take place *in vitro* or *in vivo*.

Key words: designer antagonist, leptin receptor, pharmacokinetics, surface plasmon resonance

O26.

DECIPHERING THE STRUCTURE-FUNCTION OF APELIN WITH UNNATURAL AMINO ACIDS AND MACROCYCLES

E. Marsault, A. Murza, É. Besserer-Offroy, P. Bérubé, J.-M. Longpré, P. Sarret

Institut de Pharmacologie de Sherbrooke, Université de Sherbrooke, Sherbrooke (Québec), Canada

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Apelin is the endogenous peptide of the APJ receptor. It recently emerged as a promising target for the treatment of various pathophysiological conditions, including regulation of fluid homeostasis, cardiovascular function and obesity-related disorders. To date, there is little information concerning the structure-activity relationships (SAR) of apelin-13. However, two epitopes appear to play a central role in the biological activity of this tridecapeptide. The C-terminal Phe¹³ of apelin-13 is crucial for receptor internalization as well as for its hypotensive effects, while the N-terminal moiety is critical for receptor interaction. In order to better understand the structure-function of apelin, we introduced a number of modifications in apelin, including replacements of the C-terminal Pro-Phe moiety with unnatural amino acids, and synthesized a series of macrocyclic analogs. We then studied the binding, plasma stability, and intracellular signaling pathways downstream of the APJ, and finally the hypotensive effects of the resulting molecules. First, our results led to the most potent APJ agonists reported to date on the cAMP signaling pathway, with affinities as low as 40 pM. Then, we discovered that keeping the C-terminal amino acid of apelin-13 exocyclic is very important for affinity and for biased activation of the APJ signaling pathways, ultimately leading to blood pressure regulation. Finally, we discovered macrocyclic antagonists of APJ with low nM affinities. Due to their improved stability, these new biased ligands represent very promising pharmacological tools to link the intracellular signatures to desired physiological responses.

Key words: apelin, structure-activity relationships, signalling, macrocycles

[1] O'Dowd *et al*, *Gene*, (1993); Reaux, *J. Neurochem.* (2001); Murza *et al*, *Chem.Med.Chem.* (2012); Marsault *et al*, *J. Med. Chem.* (2011); *Peptide Science (in revision)*

O27.

PEPTIDE BINDING PATHS ON CLASS B G-PROTEIN COUPLED RECEPTORS

MAPPED BY GENETICALLY ENCODED CHEMICAL PROBES

I. Coin^{a,d}, V. Katritch^b, T. Sun^a, Z. Xiang^a, F. Y. Siu^b, M. Beyermann^c, R.C. Stevens^b, L. Wang^a

^aThe Salk Institute for Biological Studies;

^bThe Scripps Research Institute, La Jolla, CA, USA; ^cLeibniz-Institute of Molecular Pharmacology, Berlin, Germany; ^dInstitute of Biochemistry, University of Leipzig, Germany

Class B G-protein coupled receptors (GPCRs) bind medium-length polypeptide hormones to regulate essential physiological functions. To date, full-length 3D structures have been resolved only for class A GPCRs mostly bound to small-molecule ligands. Using a new surface mapping approach, we have investigated directly in live cells the binding of the 40-mer neuropeptide hormone Urocortin-I (Ucn1) to its cognate corticotropin releasing factor class B GPCR type 1 (CRF1R).

Using the expanded genetic code technology, we have systematically incorporated photochemical and novel chemical crosslinking probes [1] throughout about 150 positions of the juxtamembrane region of CRF1R [2, 3]. We have obtained an unprecedented panoramic map of the peptide binding pocket in the CRF1R transmembrane domain and pinpointed intermolecular pairs of ligand-receptor proximal residues, which yielded 44 independent experimental constraints.

We have derived a complete conformational model for the Ucn1-CRF1R complex, which provides molecular insights on the mechanism of receptor activation and on the basis for discrimination between agonist and antagonist function. Moreover, we have identified hallmarks of structural elements of the full-length post-translationally modified receptor embedded in the native cellular membrane, which is not accessible to reductionist biophysical approaches [3, 4].