in orthopedics such as calcium phosphate (Fig. 1).

The focus of the infection in the bone treated with AMP mixed with carrier was eradicated much more effectively then the focus treated with antibiotics such as vancomycin or gentamicin mixed with the same carrier. Furthermore, AMPs incorporated into model implant made from poly-methylmethacrylate based bone cement prevented the formation of bacterial biofilm on its surface after the implant was inserted inside the infected bone.

Fig. 1. Infected spongy part of the bone sample filled with a peptide mixed with local carrier (a), local carrier alone (c), and local carrier loaded with antibiotic (d).

Fig. IX - 225
TRIDECAPIN A, SELECTIVELY BINDS TO GRAM-NEGATIVE LIPID II
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The number of bacterial strains resistant to current classes of antibiotics is increasing and has led some to warn that we are approaching an “antibiotic apocalypse.” Therefore, there is an increasingly urgent need for new structurally and mechanically distinct classes of antibiotics. The tridecaptins, non-cholesterol synthesized peptides produced by Bacillus and Paenibacillus species. Although they have been known for decades, their antimicrobial activity was left mostly unexplored until their serendipitous re-discovery by our group. In particular, analogues of tridecaptin A (TriA), show selectivity against Gram-negative bacteria, including against multidrug resistant Klebsiella pneumoniae both in vitro and in vivo (mouse model). Furthermore, no persistent resistance develops against Escherichia coli exposed to low concentrations of octyl-tridecaptin A (Oct-TriA) over a one-month period. Our studies have shown that TriA, selectively binds to the Gram-negative analogue of peptidoglycan precursor lipid II. Nuclear Magnetic Resonance (NMR) and molecular docking studies were used to determine the NMR solution structure of the Oct-TriA lipid binding motif for an antimicrobial peptide. Our studies suggest that TriA, exerts its bactericidal effect against Gram-negative bacteria through disruption of the proton motive force at the inner-membrane. Furthermore, we have used in vitro assays to show that the presence of Gram-negative lipid II in artificial membranes significantly accelerates the disruption of a proton motive force. Based on our studies, we believe tridecaptin A could be an excellent antibiotic candidate.

Fig. IX - 226
BIOLOGICAL EVALUATION OF RADIIODINATED AMINATED KYTOPHIN
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Kytophin (KTIP) is an endogenous analgesic neuropeptide (T-LyT-LyArg) with limited ability to cross the blood-brain barrier. Thus, KTIP is limited by its systemic administration. This behaviour, which narrows its pharmaceutical applications, has been ascribed to both insufficient lipophilicity and susceptibility to enzymatic degradation. With the aim of increasing the lipophilicity of KTIP, several new derivatives have been prepared using chemical modification of the basic structure. The kytophin analogue KTIP-amine (KTIP-NH2) presents improved lipophilicity and analgesic activity following administration in animal models. Apart from the ability to cross the BBB, other relevant issues in the development of drugs for the central nervous system are related with the assessment of toxicity and determination of the biological fate. Thus, with the aim of addressing such issues, in this communicative study, we report on the radioiodination of KTIP-NH2 with 125I as well as on the assessment of the interaction of the resulting radioiodinated peptide with the BBB through permeation studies in blind 3 cell lines. We will also describe the biodistribution studies of the radioiodinated peptide in Sprague-Dawley male rats. In addition, to check whether the incorporation of iodine into the tyrosyl residue of KTIP-NH2 would affect its analgesic efficacy, KTIP-NH2 was iodinated with “cold”, non-radioactive iodide, and the analgesic efficacy of the resulting mono-iodinated (MIK) and di-iodinated (DIK) peptides was evaluated in acute pain models. The main conclusion drawn from these studies is that although the radiodiiodinated peptide could translocate the cellular model of the BBB, the accumulation of that species in the brain was not relevant. Significant accumulation of “125I was found in the thyroid, probably reflecting the hydrolysis of the iodine-tyrosine bond by liver deiodinases. The analgesic activity of mono- and di-iodinated KTIP-NH2 evaluated by the hot plate assay, showed a delayed peak of maximal efficacy compared to non-iodinated KTIP-NH2 (30 s vs. 15 minutes). Overall, the peripheral effects of the peptides cannot be excluded.

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Diseases such as diabetes and obesity have become major health concerns worldwide. To address this issue, our group is attempting to contribute through a focus on neurexins U (NMU), a highly conserved neuropeptide regulator of feeding, energy homeostasis and glycemic control. It exerts its biological effects via two G protein-coupled receptors, NMU1R and NMU2R. NMU1R is mostly found in the peri- phery whereas NMU2R is most abundant in the central neuro- nous system. Both central and peripheral administration of the peptide reduce food intake and body weight in rodents. The anorexigenic effect of NMU renders NMU agonists attractive as potential therapeutics in the treatment of diabetes and obesity [1].

NMU-8 (H-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-NH₂), a natural occurring fragment of NMU, is taken as a lead molecule for the synthesis of novel analogues. A first batch of analogues is prepared on basis of the available structure-activity relationships described in the literature [2,3]. Mainly two types of modifications were initially performed, namely chirality switches and the introduction of different N-capping groups. In a second set of NMU-ligands, more advanced modifications were performed, such as the introduction of unnatural/constrained amino acids or N-alkylated glycines (‘peptoid’) analogues in the NMU-sequence. A third generation of compounds was synthesized and contains analogues in which the most promising modifications of the previous generations were combined. The in vitro characterization of these peptides has been performed by an inositol phosphate accumulation assay. Additionally, the plasma stability of these analogues has been investigated.

The results of the in vitro characterization present the discovery of high potency agonists. Compared to NMU-8, more active agonists on both NMU1R and NMU2R were discovered. Our experiments revealed, for instance, that acetylation of the N-terminus leads in general to an increase of activity. When replacing the Tyr² by 7-OH-Tic, Dmt, Oic, 1’Nal or 2’Nal leads to ligands with a comparable activity to NMU-8, but an increased plasma stability emerged. The most promising ligands were tested in an in vivo model to study their effect on food intake, and promising results were obtained.

The translocator protein (TSPO, 18 kDa) plays an important role for the synthesis of neurosteroids by promoting the transport of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in neurosteroidogenesis. Stimulus of TSPO by appropriate ligands increases the level of neurosteroids [1].

The present study describes design, synthesis and inves- tigation of anxiolytic-like effects of novel dipeptide TSPO ligands on the basis of lead compounds. The design is based on a non-targeted LC-HR-MS/MS approaches combined with non-targeted LC-HR-MS/MS approaches combined with the anorexigenic effect of NMU renders NMU agonists attractive as potential therapeutics in the treatment of diabetes and obesity [1].

The anxiolytic activities were investigated in Balb/C mice using the illuminated-open field test [4] and elevated plus- maze test in CD1 mice. The activating effect on locomotor activity in mice was taken as a measure of the anxiolytic ac- tivity of compound. GD-23 significantly increases the level of neurosteroids [1].

The anxiolytic-like effect of GD-23 was abolished by PK11195, a specific TSPO antagonist. It was found that by pretreatment with tri- losast, a selective inhibitor of 3β-HSD, or finasteride, a se- lective 5α-reductase inhibitor, anxiolytic effect of GD-23 was not registered. Results were evaluated as the ratio of the time spent in the open arms of maze to the total residence time of the animals in the open and closed arms. The obtained results demonstrate that the anxiolytic effect is mediated by interaction of the compound GD-23 with TSPO receptor. Hence GD-23 can provide a basis for a new peptide class of fast anxiolytics without side effects of benzodiazepines.

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3. Cappelli K et al., Brain Res. 2006, 1120, 3428-3437

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THE MECHANISM OF ANXIOLYTIC-LIKE EFFECT OF GD-23, THE DIPEPTIDE TSPO LIGAND
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Peptide-based vaccines would appear as the ideal alterna- tive to conventional (e.g., inactivated whole-virus) vaccines, because they are safe (no infectious agent involved), versatile (readily adaptable to emergent outbreaks) and cost-effective (reliable, reproducible production and scale-up by chemical synthesis; simple storage and transport). These advantages are however offset by problems such as the difficulty in de- fining the optimal chemical structure of a peptide or a protein ligand, the usually low immunogenicity of peptides, or the often intricate relat- ion between host-pathogen interaction and immune response, all of which partly explains why only a handful of peptide vaccines have attained therapeutic status.

Among the different pathogens targeted by peptide vac- cines, foot-and-mouth disease virus (FMDV), arguably the economically most devastating animal disease worldwide, has received considerable attention. We have recently de- scribed vaccine candidates, genetically known as B, T, con- sisting of a T-cell epitope branching out via a Lys tree into the peptide by immune cells and on its adjuvanticity as well as provide insights on the uptake of adjuvants. Our presentation will illustrate important aspects in the development of this synthetic vaccine, particularly issues such as epitope orientation and multiplicity, chemical ligation methods or adjuvanticity as well as provide insights on the uptake of the peptide by immune cells and on its in vivo localization.

Our presentation will describe design, synthesis and inves- tigation of anxiolytic-like effects of novel dipeptide TSPO ligands on the basis of lead compounds. The design is based on a non-targeted LC-HR-MS/MS approaches combined with non-targeted LC-HR-MS/MS approaches combined with