L045

DRUG DELIVERY BASED ON GNRH-III AS TARGETING MOIETY

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The local chemotherapy approaches using drug delivery systems, and special strategies such as receptor-mediated targeting have opened a new way to enhance the efficacy of chemotherapy with fewer side effects. Specific targeting ligands for cancer cells usually includes three main components: an apoptosis-inducing anticancer drug, a targeting moiety, and a carrier. As carrier molecule tetratuftsin derivative [TKPKG]4 was applied in this study. GnRH-III (Pyr-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2) having antiproliferative activity itself was used as targeting moiety recognized by GnRH receptors overexpressed on breast, prostate and/or colorectal cancer cells. Drug molecules (e.g. doxorubicin, methotrexate) were attached to the carrier via an enzyme labile spacer (GFLG). Several drug-conjugates with different number and conjugation sites of the GnRH peptide were prepared (e.g. GnRH-K(GnRH)-[TKPK(Drug-GFLG)G]4-NH2 or Drug-GFLG-K(Drug-GFLG)-[TKPK(GnRH)G]4-NH2). Cytotoxicity of the compounds was characterised by MTT assay. Receptor binding and cellular uptake of the conjugates on MCF-7 human breast cancer cells and C-26 mouse colon carcinama cell lines were studied by flow-cytometer.

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L046

N-ACYLATED AMINO ACID DERIVATIVES AS SEROTONINERGIC RECEPTOR LIGANDS

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At a time of identification and determination of biological function of 5-HT1A and 5-HT2A receptors, the development of their novel ligands is still of considerable interest. Perhaps the most thoroughly studies class of ligands are long-chain arylpiperazines, which were shown to posses diversified ago/antagonistic pharmacological profile.1 Continuing our research in that field, we have developed new class of arylpiperazine derivatives containing N-acylated amino acid residues (aspartic acid, glutamic acid, asparagine, proline) in the amide fragment. In first stage a 132 member library was synthesized according to sort-and-combine approach on BAL linker SynPhaseTM Lanterns and 5-HT1A, 5-HT2A receptors affinity was estimated in the preliminary screening protocol.2 QSAR within the library were analyzed with Fujita-Ban method. Subsequently, the most active compounds were re-synthesized in solution, evaluated in full radioligand in vitro assays for 5-HT1A, 5-HT2A, D2 receptors, and their intrinsic activity at 5-HT1A and 5-HT2A was assessed in in vivo functional tests. The most potent 3-N-Cyclohexanoyl-amino-1-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-pyrrolidine-2,5-dione, showing 5-HT1A pre- and postsynaptic agonistic and 5-HT2A antagonistic activity, seemed to be particularly promising regarding its anxiolytic and antidepressant effect.

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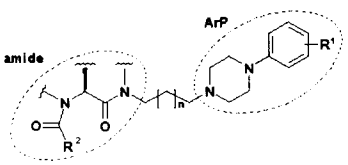

L047

NOVOKININ, AN ANGIOTENSIN AT2 AGONIST PEPTIDE, DECREASES FOOD INTAKE IN MICE

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Novokinin (RPLKPW) is an angiotensin AT2 receptor agonist, which has been designed by replacing 4 amino acids in a vasorelaxing hexapeptide ovokinin(2-7) (RADHPF) derived form ovalbumin. We previously reported that novokinin relaxed blood vessel and decreased blood pressure after oral administration through AT2 receptor. In this study, we found a novel function of novokinin on food intake.

Novokinin decreased food intake after intracerebroventricular and oral administration in fasted mice. The anorexigenic effect of centrally administered novokinin was blocked by a selective AT2 antagonist PD123319. In addition, angiotensin III, an endogenous AT2 agonist, also decreased food intake after central administration. Thus, AT2 signaling might play an important role in food intake regulation in the central nervous system.

Next, we investigated mechanism downstream of AT2 receptor in anorexigenic action of novokinin. The anorexigenic effect of novokinin was blocked by an antagonist for EP4 receptor among four receptor subtypes (EP1-4) for prostaglandin (PG) E2. We also found that an EP4 receptor agonist suppressed food intake in mice. Taken together, novokinin decreases food intake via AT2 receptor followed by PGE2 secretion and EP4 receptor activation.

L048

EXPLORING PROTEIN-LIGAND-INTERACTIONS THROUGH SYNTHETIC MIMICRY OF PROTEIN BINDING SITES

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Protein-mediated biological processes are initiated by specific interactions between proteins and their ligands. The design and generation of molecules capable of mimicking the binding and/or functional sites of proteins, represents therefore a promising strategy for the exploration and modulation of protein function through controlled interference with the underlying binding events. In addition to their basic significance, such proteinmimetics are also useful tools for a range of biomedical applications, in particular the inhibition of protein-ligand interactions.

The binding sites of proteins are often not localized in short, continuous stretches of the amino acid sequence, but rather in sequentially distant fragments of the molecule, which are brought into spatial proximity by protein folding. Synthetic molecules aimed at mimicking such discontinuous protein binding sites should therefore also be conformationally constrained and/or sequentially discontinuous (1).

The synthetic basis of this concept are scaffolded and assembled peptides, in which protein-derived peptide fragments are presented through a molecular scaffold in a non-linear, discontinuous fashion. Recently, we have introduced strategies for the generation of structurally diverse scaffold molecules (2,3). This lecture will present the utilization of these strategies for the synthetic mimicry of discontinuous binding sites of a range of proteins, including interaction domains, cytokines, as well as viral proteins.

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