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VASOPRESSIN V_2 AGONISTS AND ANTAGONISTS : DESIGN, SYNTHESIS AND ACTIVITIES.

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The characterization of kidney vasopressin V_2 receptors responsible for the antidiuretic action of arginine vasopressin (VP) has been made possible by the availability of specific agonists and antagonists with high affinity and selectivity for the rat vasopressin V_2 receptor[1]. However species differences between the rat and other mammalian receptors, particularly the human VP V_{1a} and V_2 receptors have been shown[2,3], which compelled the developpement of new ligands with good affinities and selectivities for the human VP V_2 receptor.

Here we report the design, synthesis and activities of new D-Thi[2], L-Thi[2] and L-Thi[3] analogs of dVDAVP and of dDAVP. The affinities of these compounds for the rat liver V_{1a} and for the rat kidney V_2 VP receptors were compared to their affinities for the human V_{1a} and V_2 VP receptors expressed in CHO cells. Their ability to stimulate cAMP production by rat V_2 receptors and human V_2 receptors was examined and compared to their antidiuretic potency estimated in the rat *in vivo*[4]. One of these ligands, d[Thi[3]]VDAVP[4] has a good affinity for the rat and human VP V_2 receptors (0,3 and 5nM respectively). On CHO cells expressing human V2 VP receptors this coupound behaved as a full AVP agonist. It has a very high antidiuretic activity. It has also a very weak affinity for the rat liver VP V_{1a} receptor (1,7µM). These new ligands are promising tools and may lead to the development of therapeutic agonists and antagonists for human use.

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A ROLE FOR THE LINKER BETWEEN TWO BINDING SITES OF SINGLE-CHAIN PEPTIDE HORMONE IN RECEPTOR INTERACTION

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The conformational freedom of single-chain peptide hormones in water is a major obstacle to the determination of their biologically relevant conformation, and thus hampers insights into the mechanisms of ligand-receptor interaction. Under structure-inducing conditions many peptide ligands of class 2 G protein-coupled receptors, such as corticotropin releasing factor (CRF), exhibit an α-helical conformation which is considered essential, but is functionally not understood. We show, by structure simplification of CRF, the existence of two segregated binding/activation sites at the N- and C-termini, connection of which by an appropriate model peptide led to the most potent CRF analogue which adopted, in contrast to naturally occuring CRF receptor ligands, a stable, monomer conformation in aqueous solution. An α-helix functions as the most appropriate connector between the two receptor binding sites. Connection of the two binding sites by highly flexible ε-aminocaproic acid residues resulted in CRF analogues which remained full, although weak agonists (EC_{50}: 100-300 nM) independent of linker length. Connection of the two sites by helical linkers of different lengths resulted in potent agonists (EC_{50}: 0.6-50 nM); their significantly different biopotencies, however, suggest the relative orientation between the two binding sites rather than the maintenance of a distinct distance between them to be essential for a high potency. Furthermore, the fact that analogues bearing connector units of very different lengths between the two sites exhibited full intrinsic activity (receptor activation) indicates an unexpected, high flexibility of the receptor for ligand recognition.

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THE S'-REGION OF HEPATITIS C VIRUS NS3/4A PROTEASE AS A DRUG TARGET

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Hepatitis C is a slowly progressing viral infection that over two decades can lead to liver cirrhosis or liver cancer. Infection by Hepatits C virus (HCV) is widely recognized today as a huge public health concern, with more than 170 million people infected worldwide, most of them unknowingly, while neither a generally effective treatment nor a preventive vaccine are available. Currently one of the most promising approaches to anti-Hepatitis C Virus therapy is the development of inhibitors of the virally encoded protease NS3/4A, which is essential for the maturation of the viral polyprotein. Recently we reported on the discovery of potent peptide inhibitors derived from the P-region of the cleaved substrate [1] whose binding mode of is very similar to the ground-state binding of the corresponding substrates. Structural studies with these inhibitors unraveled that NS3/4A is an induced fit enzyme, requiring both the cofactor and the substrate to acquire its bioactive conformation [2]. At variance with the P region, the P' region of the substrate is not used for ground-state binding to the enzyme. This notwithstanding, inspection of the S' region of NS3 shows the presence of binding pockets which might be exploited for binding. Any ligand taking advantage of S'-binding would therefore display a range of interactions different from the ones used by the substrate, and represent a novel class of NS3 inhibitors. We have optimised S'-binding in the context of non-cleavable decapeptides spanning P6-P4'. Binding was sequentially increased by introduction of the previously optimised P-region, change of the P4' residue, and combinatorial optimisation of positions P2'-P3'. The best decapeptide derived from this optimisation showed IC_{50}< 200 pM. Binding of the most potent compounds to NS3 has been characterised by CD, NMR and limited proteolysis/mass spectrometry, in combination with computer modelling.

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CELLULAR UPTAKE OF AN ANTISENSE OLIGONUCLEOTIDE CONJUGATED AND COMPLEXED WITH PEPTIDES DERIVED FROM AN α-HELICAL AMPHIPATHIC MODEL PEPTIDE

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Peptides of varying amphipathicity and helicity were derived from the membrane permeable α-helical amphipathic model peptide KLALK LALKA LKAAL KLA-NH_2 and were conjugated to a 15-mer antisense phosphorothioate oligonucleotide directed against the starting region of the V2-receptor m-RNA. The uptake of the conjugates into bovine aortic endothelial cells and CHO-cells transfected stably with the V2-receptor was compared to that of complexes of the oligonucleotide and the respective vector peptides and that of the oligonucleotide alone. Confocal laser scanning microscopy revealed an up to tenfold enhanced cellular uptake of conjugates and complexes as compared to that of the oligonucleotide. Analogously, a significantly reduced expression of the V2-receptor was observed after treatment of the CHO-cells with 0.5 µM of the antisense oligonucleotide in the presence of the vector peptides, whereas the antisense oligonucleotide alone remained without effect. No significant differences were observed between the cellular uptake of conjugates and complexes, although the oligonucleotides from the latter were translocated into the nucleus to a slightly greater extent. Gel-capillary electrophoresis of the cell extracts revealed only marginal cleavage of the oligonucleotide and of the disulfide bridge of the conjugates after 60 min incubation. No clear correlation was found between the amphipathic or structure forming properties of the respective vector peptides and the cellular uptake of the conjugates or complexes, suggesting that helical amphipathicity is not an essential structural requirement for peptides to translocate oligonucleotides through mammalian plasma membranes. Our results raise the possibility of exploiting non-amphipathic synthetic peptides without particular structural requirements as oligonucleotide vectors, thereby circumventing substantial obstacles connected with the use of amphipathic vector peptides, such as membrane toxicity and solubility problems.

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DESIGN, SYNTHESIS AND ANTITHROMBOTIC ACTIVITY OF CYCLIC RGD ANALOGUES.

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The crucial step of the platelet-rich thrombous formation process is believed to be the binding of fibrinogen (Fg) to activated GPIIb/IIIα receptor found on the membrane of the platelets. It has been shown that, two copies of the Arg-Gly-Asp tripeptide sequence (95-97 and 572-574) of the fibrinogen α chain contribute to the recognition event. A great effort has been made to synthesize RGD containing peptides and non-peptide small molecules that could exhibit antiaggregatory properties suitable for using as antithrombotic agents. The small size of the RGD sequence and therefore its great flexibility, as well as the presence of this sequence in a variety of proteins results to a difficulty of designing specific antagonists. In this work we report on the design, synthesis and antithrombotic activity of a new class of Cys containing cyclic RGD analogues. This new class of antiaggregatory agents has as common element the replacement of the Gly residue by Cys. The position of the second Cys, which participates in the disulfide bond formation, varries, resulting in different biological activities. Based on this concept, strong inhibitors of the platelet aggregation process, induced by ADP, have been synthesized. The total synthesis of the analogues, including the disulfide bond formation, has been performed by the solid phase method. Sythetic problems related to disulfide bond formation will be also discussed.
(Grants from GGSRT).

COMPARISON OF THE PROPERTIES OF LINEAR CATIONIC ANTIMICROBIAL PEPTIDES THAT FORM ALPHA-HELIX VS. BETA-SHEET STRUCTURE

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Most small cationic antimicrobial peptides related to linear host defense peptides like magainins and PGLa are capable of forming highly amphipathic alpha-helical structures. We have compared the activity of PGLa with that of two related 21-residue amidated peptides containing the following heptamer repeats: KIAGKIA and KLAGLAK. Both of these peptides are considerably more antimicrobial than PGLa. Like PGLa, they can form an amphipathic alpha-helix when bound to the membrane surface. Recently, we designed an 18-residue amidated peptide containing the hexamer repeat, KIGAKI. This peptide has no ability to form an amphipathic alpha-helix, but it can form a highly amphipathic beta-sheet. The antimicrobial activity of this peptide is equal to or better than that of the alpha-helical peptides. In this study, we examine the structure and activity of several other linear beta-sheet-forming peptides. The conformation of the peptides bound to lipid bilayers was assessed by Fourier transform infrared and circular dichroism spectroscopy. The peptides were compared for their ability to release calcein from large unilamellar vesicles (LUVs) composed of varying mixtures of neutral (PC or PE) and anionic (PG and DPG) lipids, or of *E. coli* polar lipid extract. Interactions of these peptides with LUVs was monitored by fluorescence changes of analogs containing a single Trp. These results are compared to the antimicrobial and hemolytic activities of the peptides. We conclude that (1) activity is not dependent upon a particular secondary structure; (2) the composition of the lipids in the membrane plays a major (and predictable) role in the ability of the peptide to permeabilize the lipid bilayer; and (3) the nature of the association of the peptide with the polar-nonpolar boundary of the lipid bilayer is critical to the induction of leakage in the membrane. We now are beginning a systematic examination of two families of peptides that were designed with identical charge and overall hydrophobicity, but that differ in their potential to form amphipathic alpha-helical and beta-sheet secondary structures, to further explore the relationship of amphipathicity and secondary structure with antimicrobial activity.

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A NOVEL GROUP OF PEPTIDIC BIOLOGICAL RESPONCE MODIFIERS

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Several hundreds of endogenous peptides ranging from 45 to 2 amino acid residues in length have been isolated from various tissue extracts and sequenced. In contrast to the majority of classical peptide hormones, neurotransmitters and neuromodulators, the peptides studied in that work are formed by *in vivo* proteolysis of proteins with well-established functions, such as hemoglobin, actin, cellular enzymes, etc. The sets of these peptides in tissues are tissue-specific on one hand and conservative in a given tissue at normal conditions on the other. Their levels are sensitive both to pathologies due to alterations of tissue metabolism and to prolonged physiological changes. More than a hundred of such peptides have been evaluated for bioactivity *in vitro* and the majority of them was shown to inhibit or stimulate proliferation, to induce cytolysis of tumor cells or to restore proliferation of normal cells treated with drugs *in vitro*[1-3].
In the present work a more detailed study was carried out of the patterns of action of tissue-specific peptides at cellular level. The typical mechanism of action of the growth inhibitory peptides both in tumor and normal cells is due to the reversible arrest of proliferation. The latter leads to the ability of such peptides to protect the rapidly dividing cells from the toxicity of chemiopreparations. Both the antitumor activity and the cell-protective effect in the case of application of chemiopreparations have been confirmed *in vivo*. The latter results point to an important biological role of growth inhibitory peptides in the organism. On the other hand, the growth-stimulatory peptides were shown to restore proliferation of normal cells after treatment with chemiopreparations or in the case of growth factors deprivation. They also accelerate the rate of cell division at intermediate cell density, which can be interpreted as a tissue restoration model. We believe that these peptides replace growth factors in the case of their deficiency and participate in tissue regeneration processes. On the basis of the data obtained, we suggest that tissue-specific peptides participate in regulation of cell number in tissues, i.e., in maintenance of tissue homeostasis.

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STRUCTURE / ACTIVITY RELATIONSHIP OF THE ANTIBIOTIC NUCLEOTIDE - PEPTIDE MICROCIN C51

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Microcins are antibiotic peptides produced by enteric bacteria which are among the scarce representatives of the ribosomally-synthesized peptides from microorganisms. Microcin C51 is a nucleotide-heptapeptide isolated from an *Escherichia coli* strain[1)]. The plasmid DNA fragment containing the genes involved in production and immunity of Mcc C51 was cloned into multicopy vector[2)]. Using the *E. coli* strain carrying recombinant plasmid, we developed a large-scale production and purification procedure of the microcin and reinvestigated its structure. The Mcc C51 structure was determined using two-dimensional NMR spectroscopy, including homonuclear (COSY, TOCSY, ROESY) and heteronuclear ^{1}H-^{13}C and ^{1}H-^{31}P experiments. It appears similar to that of microcin C7 previously determined[3)]. It is an N-formylated linear heptapeptide (f-Met-Arg-Thr-Gly-Asn-Ala-Asp), the amide group of Asp7 being linked to the phosphodiester of 5'-adenylic acid and to *n*-aminopropanol by a phosphoramide bond. We also isolated Mcc C52, the methionine sulfoxide derivative of Mcc C51. Otherwise, we generated a set of plasmids deleted in different microcin operon genes. One of these plasmids specified the production of the antibiotic substance Mcc C51p, that we postulate to be a precursor of Mcc C51. MICs of Mcc C51 and its analogues were measured for different sensible strains. The results are discussed on the basis of the structural motifs involved in the antibiotic activity of this nucleotide-peptide.

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A STRUCTURE - ACTIVITY STUDY OF DYNORPHIN-(1-13)-PEPTIDE AMIDE. SYNTHESIS OF ANALOGUES WITH UNUSUAL AMINO ACIDS IN POSITIONS 0, 2, 3, 4

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Dynorphin A, (Dyn A) a 17-amino acid peptide, is postulated to be an endogenous ligand for κ opioid receptor. Dyn A shares a common N-terminal tetrapeptide sequence (the "message" sequence) with other mammalian opioid peptides, while containing a unique C-terminal "address" sequence which imparts selectivity for κ opioid receptors. Dyn A(1-13) exhibits a similar receptor binding profile to Dyn A, therefore Dyn(1-13)NH_2 has been chosen as the parent structure for developing analogues. A series of dynorphin analogues was synthesized with following modifications: 1)amino acid residue in position 2 was replaced by amino acids Phe, cPrAla, Tic, Oic, Nal2; 2)N-terminal extention with Lys^0; 3)Gly^3 was substituted with cPrAla, Tic, 4)exchange of Phe^4 for Tic 5)Gly^2-Gly^3 was replaced by cPrAla-Phe, Tic-Phe, Oic-Phe and Nal2-Phe in analogues with double replacement. The analogues were synthesized by solid-phase methodology using Fmoc-amino acid pentofluorophenyl esters. Side chains were protected with Boc- for Lys, Mtr- for Arg. Cleavage of the peptide from the solid support and deprotection of side chains was performed simultaneously in trifluoroacetic acid with scavengers. The resulting peptides were purified by RP-HPLC and characterised by mass spectrometry. The purified dynorphin analogues were assayed *in vitro* for their μ-,δ-,κ- and ORL-1 binding activity, as well. New dynorphin analogues synthesized were κ-selective. All of them retained modest μ activity and were essentially inactive at the δ-type of opioid receptor. Incorporation of cPrAla in position 3 results in compound, which showed the highest κ-activity (Ki=0,07 nM). Double substitution of Gly-Gly by Tic-Phe afforded analogue which showed the modest δ-activity. Most of compounds exhibited modest activity at the ORL-1 receptor, as well.

INVOLVEMENT OF ENDOGENOUS NITRIC OXIDE IN NOCICEPTION OF NEWLY SYNTHESIZED ANALOGUES OF MELANOCYTE-INHIBITING FACTOR IN ACUTE PAIN IN THE RATS SPINAL

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Melanocyte-inhibiting factor (MIF) is a tripeptide (Pro-Leu-Gly-NH_2) isolatedin traditional way from bovine hypotalamus tissue. It inhibits the release of melanocyte-stimulating hormone (MSH) and exhibits a range of behaviooural and pharmacological effects, after central and peripheral administration and alters opiate action in several system.

Nitric oxide (NO) appears to play a role a variety of biological events in the peripheral and central nervous system. Recent data has also suggested the involvement of NO in pain processing at peripheral, spinal and supraspinal levels.

Our study was designed to determine the involvement of NO in the nociception in the rat spinal cord of Cav^2-MIF and $sLeu^2$-MIF. The effect of intrathecally (i.th.) injection of L-NAME and the synthesized analogs of MIF was evaluated in acute pain using tail/flick and paw pressure tests.

We found that the Cav^2-MIF (200-400μg) and $sLeu^2$-MIF (200μg) enhanced antinociception in the tail-flick and paw pressure tests compared with MIF-1.

L-NAME (1 500μg) co-administration with Cav^2-MIF and $sLeu^2$-MIF elicit a profound and long-lasting antinociception in the tail-flick and paw pressure tests. More pronounced was the antinociceptive activity of Cav^2-MIF and $sLeu^2$-MIF in the tail-flick test. These results suggest that nitric oxide is involved in spinal nociceptive events.

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SYNTHESIS AND SPECTROSCOPIC CHARACTERISATION OF TRYPTOPHAN CONTAINING PEPTIDES LABELLED WITH 4-ETHOXY-METHYLENE-2-[1]-NAPHTHYL-5(4H)-OXAZOLONE OR 4-[7-HYDROXYCOUMARYL]ACETIC ACID

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In order to analyse the sensitivity of newly developed fluorescent group for the detection of aromatic amino acid residue in its vicinity two groups of tryptophan containing oligopeptides and their derivatives were prepared. Synthesis of Q–W and of Q–WS, Q–X_1WS and Q–X_1X_2WS peptides (where Q represents fluorescent group) corresponding to the interleukin-6 receptor α-chain were performed by solid phase procedures on p-benzyloxy-benzylalcohol-resin using suitable protected N^{α}-Fmoc amino acid derivatives and DIC/HOBt coupling method. After the removal of the blocking group the terminal α-NH_2 was reacted with 4-ethoxymethylene-2-[1]-naphthyl-5(4H)-oxazolone (naOx-OEt). 4-[7-Hydroxy-coumaryl]acetic acid (Hca) was introduced by using DIC/HOBt coupling method in DMF. The resin bound peptide derivatives were treated with TFA in the presence of scavangers. Alternatively N^{α}- amino group of free peptides was labelled with naOx-OEt or with Hca in solution. The crude products were purified by RP-HPLC. The homogeneity, amino acid composition and chemical stability of peptides were assayed by RP-HPLC, amino acid analysis and mass spectrometry. Comparative analysis of absorption and fluorescence properties (absorption and emission spectra, lifetime of excited state, quantum yield of fluorescence) of naOx-OEt and of Hca derivatives was performed in MeOH as well as in aqueous buffer solution under various pH. Based on these data the chromophore interaction between Q-group and side chain of Trp as well as the effect of their relative position has been investigated. An intense and pH-sensitive emission band in aqueous solution has been discovered. This feature might be useful for monitoring receptor-ligand interaction.

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PEPTIDIC ANALOGUES OF CHEMOKINE RANTES : SYNTHESIS AND ACTIVITY.

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Chemokines (*chemo*tactic cyto*kines*) are a large superfamily of microproteines (8-10 kDa) involved in the recruitment and activation of a variety of cell types in inflammation. They are subdivided into two principal families : the α chemokines or CXC-chemokines and β chemokines or CC-chemokines based on the arragement of the amino-terminal two of the four conserved cysteine residues. They are now identified to play an important role in the infection of cells by the human immunodeficiency virus type 1 (HIV-1). Indeed, CD4 and CXC or CC-chemokine receptors (CXCR or CCR) are required for virus infection and especially CCR5, natural receptor of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) which is utilized as the principal coreceptor by the macrophage tropic viruses.

The use of chemokine-derived molecules constitutes a potential therapeutic approach to prevent infection by HIV-1. Therefore, based on the structural analysis by modelisation of RANTES potential interaction sites with CCR5 (12-21 loop and β3 sheet) were defined. Thus a series of cyclic and non-cyclic peptide analogues of RANTES was synthetized and their anti-HIV-1 activity was evaluated as new ligands of CCR5.

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LEVOCABASTINE-NEUROTENSIN CONJUGATES: AN INVESTIGATION OF THE MESSAGE-ADDRESS CONCEPT FOR NEUROTENSIN RECEPTORS

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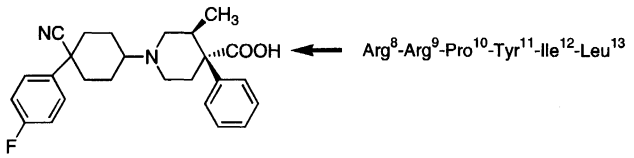
A high affinity neurotensin receptor (NT_H, NTS1) and a low affinity receptor (NT_L, NTS2) have been characterized.[1]
The latter binds the potent antihistaminic compound Levocabastine (LC),which is devoid of any NT-like pharmacological properties. We have prepared hybrid compounds containing LC and various partial sequences of Neurotensin (NT) (8-13):



Levocabastine (LC)

According to the message-address concept, the attachment of a NT_H address to LC might produce an antagonist for the NT_H receptor. Binding assays revealed high NT_H affinity for LC-NT(8-13) and LC-NT(9-13), but considerable loss of affinity for LC-Gly-NT(9-13), LC-NT(9-11) and LC-Gly-NT(9-11). A positive PI turnover for LC-NT(9-13) indicated however agonist character for this analog.

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Synthesis, Characterization, and Biological Relevance of a New Class of Constrained Heterocycles for Drug Design.

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Dioxopiperazines, also known as diketopiperazines are often formed as a by-product in solid phase synthesis, especially at the C-terminus when glycine, proline or N-alkylated amino acids are present. Because of their rigid structures, DKPs are potentially useful scaffolds for drug lead discovery, especially if the problem of their insolubility can be overcome. In this work, we describe a solid phase synthesis of N-methylated DKPs that feature a resin-bound simultaneous cyclization and cleavage. By comparison with their non N-alkylated versions, we demonstrate their improved physical properties including increased solubility, even in chloroform.
The dipeptides in this study were prepared either by solution methods or by our solid phase methylation/cyclization procedure. The solid phase synthesis has been performed using available Fmoc-N-Me amino acids and/or Boc amino acids using a resin bound process for producing their corresponding N-methylated derivatives, (S. Miller and T. Scanlan, *J. Am. Chem. Soc.*, **1997**, 119, 2301-2302). As expected, the methylation improved significantly the solubility: for example, cyclo(N-Me-Phe-N-Me-Ala) dissolves in a wide range of solvents, including methanol, acetic acid, acetonitrile and chloroform as well as DMF.
In order to prove the structural and chiral integrity of these procedures, we have selected a variety of new and unusual chiral amino acids as building blocks. By the use of D as well as L amino acids, we provide important structural motifs with respect to their biological action and other properties based on side chain orientation. Furthermore, through the synthesis of diastereomeric products, we were able to show that chiral integrities were retained in many although not all of our synthetic transformations. Finally, we describe the utility of these methods for the production of other N-alkylated cyclic dipeptides as well as cyclic pseudopeptides that may provide new leads for drug discovery. Supported by IMD[3] and NIH GM 33376.

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MONOCYCLIC ANALOGS OF MAST CELL DEGRANULATING PEPTIDE

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Mast cell degranulating (MCD) peptide has potent immunological properties such as histamine-releasing activity at low concentrations and anti-inflammatory activity at higher concentrations. These properties are associated with its high basicity and its disulfide bridges. Structurally MCD peptide is a 22-amino acid residue bicyclic peptide characterized by two disulfide bonds between cysteines 3-15 and 5-19, respectively: IKCNCKRHVIKPHICRKICGKN-NH_2 .
To determine the role of these disulfide bonds on the biological activity and conformation of MCD peptide, we synthesized monocyclic MCD peptide analogs lacking one disulfide bond by selectively replacing each pair of these disulfide bonds with alanine residues. Peptides were synthesized by solid phase synthesis with Boc/Bzl protection on a BHA resin. They were cleaved, purified and characterized by standard protocols used in our ongoing studies with MCD peptide analogs. The peptides were tested for histamine-releasing activity with peritoneal rat mast cells using a microplate histamine assay. Both monocyclic analogs showed significantly decreased histamine-releasing activity compared to that of the parent MCD peptide. Structural changes between the analogs were followed by CD spectrometry. The results suggest that the bicyclic structure of MCD peptide is an absolute requirement for biological activity.

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A FLUORESCENCE AND CD STUDY ON THE INTERACTION WITH PHOSPHOLIPID VESICLES OF A SYNTHETIC IMMUNOGENIC PEPTIDE ANALOGUE OF mt1 MELATONIN RECEPTOR N-TERMINAL EXTRACELLULAR SEQUENCE

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Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced mainly by the pineal gland in most vertebrate species including humans. The physiological role of melatonin is related to both chronobiology and modulation of the body hormonal milieu. Moreover, this hormone was shown to be a potent free radicals scavenger.
Our attention is focused on characterisation of melatonin interaction with its high affinity membrane-bound receptors (mt1 and MT2) which belong to the G protein-coupled receptor superfamily. Within the scope of this research, we have developed an antibody directed against the N-terminal extracellular sequence of murine mt1 melatonin receptor, using the Nle[1][1-32]mt1 peptide sequence as antigen.
The successful presentation of peptide antigens to the immune systems depends on the peptide conformation, besides on the presence of B-cell and helper T-cell epitopes. Furthermore, it is known that the membrane plays a crucial role in inducing the biologically active conformations and orientations on peptides.
The aim of the present study was to get more insight into the interaction of the Nle[1][1-32]mt1 immunogenic peptide with lipid bilayers employing liposomes as model membranes. Moreover, to enhance the affinity for the membrane and, potentially, the immunogenicity of N-terminal mt1 moiety, we achieved the esterification of the carboxylic terminal group of the Nle[1][1-33]mt1 peptide sequence with cetyl alcohol. To evaluate the role of hydrophobic and electrostatic interactions in peptides binding with membrane both zwitterionic (net neutral) and anionic phospholipid were employed to construct variably charged vesicles. Fluorescence techniques were employed to study the topology of peptides in lipid bilayer by monitoring the fluorescence of tryptophan residue placed in position 28 of peptide sequences. In addition, circular dichroism measurements were used to detect the influence of phospholipids on the secondary structures of peptides. Our results are in agreement with the hypothesis that peptide-lipid interaction may play a significant role in peptide-receptor recognition mechanism by capturing the peptide from the surrounding fluid and by imposing conformations and topological arrangements that are favourable for receptor interaction.

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SYNTHESIS, PHYSICOCHEMICAL CHARACTERISATION AND BIOLOGICAL ACTIVITY OF THROMBOSPONDIN PEPTIDE DERIVATIVES.

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Targeting of drugs to specific organs/cells is one of the main aims of cancer therapy. The search for chemical entities suitable to achieve this goal is based in the following data. Increased expression of integrins has been demonstrated for many tumour entities. The αVβ3 Integrin is a binding partner for Laminin, fibronectin and thrombospondin (TSP). Several sequences of TSP have been already identified as binding sites to αVβ3 integrin[1]. These sequences can be good candidates to target liposomal Doxorubicin to tumoural cells avoiding in this way unwanted side effects and increasing patient compliance.
In the present work four different TSP related peptide sequences have been synthesised following Solid Phase Methodology, purified by HPLC and identified by Mass spectrometry. The physicochemical interactions of these peptides with membrane models were soft and the percentage of induced fusion as well as destabilisation of liposomes indicate that it could be used safely to coat Doxorubicin loaded liposomes . "In vitro" biological studies were carried out on MCF-7 breast cancer cells. Results obtained indicate that two TSP1 peptides (TSP1-B1 and TSP1-C1) significantly promote cell proliferation, while a TSP2 sequence has no activity on cell growth.
These results suggest the presence of receptors for TSP1 peptides in the tumour cells line assayed. The interaction of liposomes (loaded with doxorubicin and coated with these peptides) with tumoural cells is now been studied.

[1]N. Sheibani and W.A. Fraizer, Histopathol (1999), 14, 285-294

CYSTEINE PROTEINASE INHIBITORS BASED ON THEIR PROPEPTIDE INTERACTION MODEL: A RETROENANTIOMER APPROACH.

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A common feature, in the general structure of the cysteine proteinases from the papain family , is the presence of an inhibitory propeptide, whose size is variable but has a common folding pattern. Interestingly, the direction of the peptide chain, in the region responsible for the active site blocking, is in the reverse direction (N→C) relative to a genuine substrate, and this striking feature seems to render resistant to self-processing this particular portion of the propeptide. Considering that if the peptide was "seen" in the correct substrate orientation it would correspond to the *all-L inverso* peptide, we have decided to verify the inhibitory potential of the *all-L* (normal L), *all-L-inverso* (retro-L), *all-D* (normal D) and *all-D-inverso* (retro-D) peptides containing the sequences of the propeptides that are directly interacting and blocking the active sites of rat cathepsin B, human cathepsin L and cruzipain – the major cysteine proteinase of *Trypanosoma cruzi*. The *all-L, retro-L* and *retro-D* sequences derived from the pro-region of rat catB, KLCGTVL, are μM inhibitors of rat catB and human cat L but showed a markedly reduced affinity for human catB. The *all-D* sequence was the only able to inhibit cruzain. Peptides with sequences derived from human cat L (F RQ V M N G F Q), *all-L, reto-L and retro-D,* behaved either as poor inhibitors or high affinity substrates for both cat B and cat L. Curiosly, the *all-D* sequence was a μM inhibitor for the three enzymes. All the four peptides showed high affinity for cruzain, the *all-L* and *retro-all* as high affinity substrates and the *all- D* and *retro-D* as μM inhibitors. Pro-cruzipain related sequences showed a clear distinction between both cat B and cat L or cruzipain. For cat B all peptides related to RYHNGAA have shown a relatively low affinity. Unexpectedly a 0.3μM Ki was obtained for the *retro-L* sequence with cat L and the same value, 0.3M for the *retro-D* sequence with cruzain. The results indicate that very selective high affinity inhibitors for the parasite enzyme can be designed based on the sequence of the corresponding pro-peptide.

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EVALUATION OF THE RXP407 POTENCY AND SELECTIVITY, A N-DOMAIN SELECTIVE INHIBITOR OF HUMAN ACE, ON RAT AND MICE ACE.

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By screening phosphinic peptide libraires, we recently reported the discovery of RXP407, a N-domain selective inhibitor of recombinant human ACE able to differentiate the two active sites of this enzyme. The aim of this study was to assess the potency of RXP407 towards mice and rat ACE, as well as to determine the selectivity of this inhibitor to discriminate the N- and C-domain of these ACE enzymes. By comparing the potency of RXP407 to block purified somatic and germinal ACE from mice, we concluded that RXP 407 is a potent N-selective inhibitor of mice somatic ACE, a behaviour similar to the one observed with human somatic ACE. In contrast, RXP407 appeared less potent toward purified ACE from rat. Furthermore, RXP407 was almost inactive toward ACE activity present in crude rat plasma. This study demonstrated that for further evaluation of the RXP407 *in vivo* efficacy mice animal model, but not rat, should be used.

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COUPLING OF HUMAN VPAC1 RECEPTOR FOR VIP TO ADENYLYL CYCLASE : IMPLICATION OF MULTIPLE INTRACELLULAR DOMAINS.

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The vasoactive intestinal peptide (VIP) is a neuropeptide widely distributed in central and peripheral nervous systems where it is implicated in many biological functions mediated by specific receptors (VPAC1 and VPAC2) coupled to adenylyl cyclase (AC) transduction system.The human VPAC1 receptor is a glycoprotein of 457 amino acids containing 7 transmembrane domains which belongs to the G-protein coupled receptor class II sub-family. In the present study, we determined the role of intracellular domains (loops (I-III) and C-terminal tail) in the coupling of the VPAC1 receptor to AC activation by directed mutagenesis and stable transfection of mutants in CHO cells. These results show that : 1) The highly conserved residue K322 (intracellular loop III) and E394 (intracellular C-terminal tail) are important for AC activation by VIP. Mutation of K322 or E394 residues into alanine induces 50 % decrease of maximal AC activation (EC_{50}= 1 nM and 0.3 nM respectively) as compared to wt receptor (EC_{50}= 0.3 nM). 2) Substitution of V243, S244 and F245 (intracellular loop II) residues into alanine cause a 50 % decrease in efficacy of VIP to stimulate AC (EC_{50}= 0.3 nM). All these mutants display the same binding parameters as compared to wild type (wt) VPAC1 receptor (K_d= 0.3 nM) with the exception of A322K mutant (K_d= 23 nM). 3) The double (A322K/A394E) and quintuple (A322K/A394E/A243V-A244S-A245F) mutants display a 80 % decrease of AC activation inducing by VIP (EC_{50}= 0.3 nM). Analysis of ^{125}I-VIP binding in presence of increasing concentration of GTP (10^{-8}-10^{-4} M) to membrane prepared from CHO cells transfected by A322K, A394K or A243V-A244S-A245F mutants reveals a drastic decrease of GTP sensitivity as compared to wt receptor. Indeed, half-maximal inhibition of ^{125}I-VIP binding was observed for 3 μM GTP for wt receptor, 30 μM GTP for A322K and A394K mutants and > 1mM GTP for A243V-A244S-A245F mutants strongly suggesting the implication of these different domains in coupling of the VPAC1 receptor to the Gs protein.
In conclusion, three domains (intracellular loops II and IIII and N-termina domain of C-terminal tail) are responsible of coupling of human VPAC1 receptor to adenylyl cyclase transduction system.

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PHARMACOLOGICAL STUDY OF CONSTRAINED ANALOGUES OF BOMBESIN.

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Analogs of bombesin which incorporate dipeptide or turn mimics have been synthesized. One of them containing a seven-membered ring lactam revealed a good affinity for GRP/BN receptors on rat pancreatic acini (Ki value of 1.7 ± 0.4 nM) and on Swiss 3T3 cells (Ki value of 1.0 ± 0.2 nM)[1]. On this observation, antagonists containing the same dipeptide mimic were obtained by modification of the C-terminal part of the bombesin analogs. The most potent constrained compounds were able to antagonize 1 nM bombesin-stimulated amylase secretion from rat pancreatic acini with high potency (Ki = 21 ± 3 nM and 3.3 ± 1.0 nM respectively) and 10^{-7} M bombesin stimulated [^{3}H]-thimidine incorporation into Swiss 3T3 cells (Ki = 7.8 ± 2.0 nM and 0.5 ± 0.1 nM respectively).
These results and a conformational study by molecular modelisation and dynamic will be presented.

1. Cristau et al., J. Med. Chem., 2000, in press.

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STRUCTURE-ACTIVITY ANALYSIS OF TRUNCATED OREXIN-A ANALOGUES AT THE OREXIN-1 RECEPTOR

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Truncated peptide analogues of orexin-A were prepared and their biological activity assesed at the orexin-1 receptor (OX_1). Progressive *N*-terminal deletions identified the minimum *C*-terminal sequence required for maintaining a significant agonist effect. An alanine scan and other pertinent substitutions identified key side-chain and stereochemical requirements for receptor activation. Peptide activity was determined by recording functional potency, as reflected by changes in intracellular calcium concentration measured using fluorometric imaging technology. In addition, the binding affinity of some sequences was investigated using laser scanning cytometry.

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AGONIST ACTIVITY AT THE KININ B_1 RECEPTOR: STRUCTURAL REQUIREMENTS OF THE CENTRAL TETRAPEPTIDE

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We have previously described a series of desArg^9-Lys-BK (Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe) analogues where the central tetrapeptide -Pro-Pro-Gly-Phe- is replaced by alkyl spacers [Tancredi et al., Bioorg. Med. Chem. Lett., 1997, 7:2661-2664]. These analogues displayed significant agonist activity, selective for the kinin B_1 receptor. The most active compound of this series (Lys-Arg-Ado-Ser-Pro-Phe), contains the 12-aminododecanoic acid (Ado) in place of the central portion and has a pD_2 of 6.0 in the rat ileal longitudinal smooth muscle, a preparation selective for the kinin B_1 receptor. Subsequent conformational studies addressed the relative topological arrangement of the N- and C-terminal residues of this peptide, as well as the role of the hydrophobicity of its central portion. [Pellegrini et al., J. Med. Chem., 1999, 42:3369-3377]. These studies were in part hampered by the expected flexibility of the long alkyl chain of the Ado residue. To address this problem, we have designed a series of analogues based on C^α-tetrasubstituted α-amino acids of the family of 1-aminocycloalkane-1-carboxylic acids (Ac_nc, n = 6, 7, 8, 9, 12). In each analogue the cyclic residue, known to promote a costrained, folded conformation, replaces the central position of Ado. To optimize the distance between the termini of the peptide, we incorporated in the spacers additional residues of Gly, βAla or γAbu.
In this communication we describe the design, synthesis, and pharmacological characterization of this new series of desArg^9-Lys-BK analogues.

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NEUROPEPTIDE Y ANALOGUES, THAT CONTAIN SPIN- AND FLUORESCENT-LABELLED AMINO ACIDS SELECTIVELY BIND TO Y-RECEPTORS

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G-Protein coupled proteins constitute a super-family of integral membrane proteins encompassing hundreds of receptors for all types of chemical messengers as well as, for example, peptide hormones and neurotransmitter. Due to their complicated organisation with the characteristic seven transmembrane segments (7TM) it has as yet been almost impossible to structurally characterise these proteins and especially their ligand-binding domain by crystallography or magnetic resonance. However, a number of indirect methods to study the structure, the ligand binding and the signal transduction capacity of these proteins are known.
In order to study the change of conformation during the process of ligand binding, two approaches are presented. Peptides, containing the spin-labelled amino acid TOAC[1] at different positions were synthesized by solid-phase peptide synthesis using Fmoc/tBu strategy and investigated. A modified procedure was required because TOAC is instable versus trifluoroacetic acid. Furthermore, fluorescent labels were placed at the N- and the C-terminus of neuropeptide Y, a 36-amino acid peptide amide that binds to at least three G-protein coupled receptors. By FRET (fluorescence resonance energy transfer) the dynamic of the ligand in different vicinities has been investigated and significant differences between the distance of fluorophore donator and acceptor were identified. Interestingly, the compounds were still able to interact with the receptor as investigated in competition binding assays with transfected cell lines, that selectively express recombinant NPY receptors. Accordingly, this approach allows the following of the change of conformation during the process of ligand binding.
[1]*Toniolo, C., Crisma, C., Formaggio, F., (1998) Biopolymers., 47, 153-158.*

FLUORESCENT OXYTOCIN AGONISTS AND ANTAGONISTS : DESIGN, SYNTHESIS AND ACTIVITIES .

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Arginine vasopressin (AVP) and oxytocin (OT) are two nonapeptides which participate in the regulation of numerous physiological functions. To date, three vasopressin receptor subtypes and one oxytocin receptor subtype have been characterized[1]. Because of the diversity of AVP and OT receptors and of the numerous analogues which have been synthesized[2], the AVP and OT system constitutes a good model to study ligand-receptor interactions. The structural bases of their ligand recognition have been investigated using both selective ligands and site-directed mutagenesis[3]. Photoactivatable ligands have also been used to map binding domains in the human V_{1a} vasopressin receptor[4] and in the bovine V_2 vasopressin receptor[5].

Here we report the design, synthesis and activities of a series of eight fluoresceinylated analogs of two known OT antagonists, two known OT agonists and 4 putative OT agonists. Fluoresceinyl was introduced on residue 8 of the oxytocin analogs because it was shown that coupling a fluorophore in position 8 of vasopressin antagonists only sligthly affect the characteristics of the ligands[6]. All the analogs synthesized but one have a very good affinity and selectivity for human OT receptors expressed in CHO cells. These fluorescent ligands could be powerful new pharmacological tools for cellular and molecular studies on human AVP and OT receptors.

1. Barberis, C. and Tribollet, E., *Crit. Rev. Neurobiol* . **10**:119-154 (1996) and refs therein.
2. Manning, M. et al., *Adv. Exptl. Med. Biol.* **396**:559-583 (1995) and refs therein.
3. Barberis, C. et al., *J. Endocrinol.* **156**:223-229 (1998) and refs therein.
4. Phalipou, S. et al., *J. Biol. Chem.* **272**:26536-26544 (1997).
5. Kojro, E. et al., *Biochemistry* **32**:13536-13544 (1993).
6. Durroux, T. et al., *J. Med. Chem.* **42**:1312-1319 (1999).

SYNTHESIS, ^{1}H-NMR STRUCTURE AND ACTIVITY OF A THREE DISULFIDE-BRIDGED MAUROTOXIN ANALOG DESIGNED TO RESTORE THE CONSENSUS MOTIF OF SCORPION TOXINS.

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Maurotoxin (MTX) is a 34-residue toxin from the venom of the scorpion *Scorpio maurus*. The toxin binds onto SK channels and also blocks Kv channels. MTX possesses high sequence identity with HsTx1 and Pi1, two K^+ channel short scorpion toxins cross-linked by four disulfide bridges. These toxins differ from other scorpion toxins cross-linked by either three or four disulfide-bridges by the presence of an extra half-cystine residue in a consensus sequence generally associated with the formation of an α/β scaffold (an α-helix connected to a β-sheet by two disulfide bridges). Because MTX exhibits an uncommon disulfide organization among scorpion toxins (C1-C5, C2-C6, C3-C4 and C7-C8 instead of C1-C4, C2-C5 and C3-C6 for three disulfide-bridged toxins or C1-C5, C2-C6, C3-C7 and C4-C8 for four disulfide-bridged toxins), we designed and synthesized an MTX analog with three instead of four disulfide bridges ([$Abu_{19,34}$]-MTX) and in which the entire consensus motif of scorpion toxins was restored by substitution of the two half-cystines in positions 19 and 34 (corresponding to C4 and C8) by two α-aminobutyrates (Abu). The 3-D structure of [$Abu_{19,34}$]-MTX in solution was solved by ^{1}H-NMR. This analog adopts the α/β scaffold with conventional half-cystine pairings connecting C1-C5, C2-C6 and C3-C7 (with C4 and C8 replaced by Abu). This novel arrangement in pairings that concerns the last disulfide bridge results mainly in a reorientation of the α helix regarding the β-sheet structure. The structural variations are also accompanied by changes in the pharmacological selectivity of the peptide suggesting that the pattern of disulfide bridges should affect the 3-D presentation of certain key residues critical to the blockage of K^+ channel subtypes.

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CHEMICAL SYNTHESIS AND CHARACTERIZATION OF MAUROCALCINE, A SCORPION TOXIN THAT ACTIVATES CA^{2+} RELEASE CHANNEL/RYANODINE RECEPTORS

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Maurocalcine is a novel toxin isolated from the venom of the chactidae scorpion *Scorpio maurus* palmatus. It is a 33-mer basic peptide cross-linked by three disulfide bridges, which shares 82% sequence identity with Imperatoxin A, a scorpion toxin from the venom of *Pandinus imperator*. Maurocalcine is peculiar in terms of structural properties since it does not possess any consensus motif reported so far in other scorpion toxins. Due to its low concentration in venom (0.5% of the proteins), maurocalcine was chemically synthesized by means of an optimized solid-phase method, and purified after folding/oxidation by using both C18 reversed-phase and ion exchange high-pressure liquid chromatographies. The synthetic product (sMCa) was characterized. The half-cystine pairing pattern of sMCa was identified by enzyme-based cleavage and Edman sequencing. The pairings were Cys3-Cys17, Cys10-Cys21, and Cys16-Cys32. *In vivo*, the sMCa was lethal to mice following intracerebroventricular inoculation (LD_{50}, 20 _ g/mouse). *In vitro*, electrophysiological experiments based on recordings of single-channels incorporated into planar lipid bilayers showed that sMCa potently and reversibly modifies channel gating behavior of type 1 ryanodine receptor (RyR1) by inducing prominent subconductance behavior.

VENOMOUS CHIMERIC SECRETAGOGUES

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Mastoparan (INLKALAALAKKIL-NH_2, MP) is an amphiphilic tetradecapeptide that exhibits a range of biological activities including the specific activation of heterotrimeric G proteins. We have designed novel chimeric MP-containing constructs as both ligands for G protein-coupled receptors and selective probes of biological phenomena. Examples of chimeric MP analogues include galparan (galanin(1-13)-MP) and M391 ([PhaaDTyr(Me)^{2}Arg^{6}Tyr^9]AVP-εAhx-MP). These chimeric constructs, combining MP with the N-terminal of galanin or aV_{1a}-selective vasopressin antagonist, are high affinity ligands at galanin and vasopressin receptors respectively. Moreover, galparan and M391 also display biological activities drastically different to their parent compounds. Examples of such phenomena include the inhibition of G proteins and novel effects upon enzyme activity, insulin secretion and calcium homeostasis.

We have utilised the RBL-2H3 cell line as a model for stimulus secretion coupling in mucosal mast cells. RBL-2H3 secretes 5-hydroxytryptamine (5-HT) in response to secretagogues that include MP, the calcium ionophore A23187 and antigen. Comparison of a range of chimeric constructs, including MP-human calcitonin-gene-related peptide (hCGRP) chimeras, revealed that M435 (hCGRP(1-15)-MP) stimulated the secretion of 5-HT with greater efficacy than MP. Similar assays revealed a rank order of efficacy of secretion M435 > M436 (MP-hCGRP(8-18)(28-37)) ≈ MP > M432 (MP-hCGRP(28-37) ≈ galparan ≈ M391. We also determined the ability of chimeric peptides to activate phospholipase D (PLD). The activation of PLD and synthesis of phosphatidate is believed to be intimately involved in the secretory response of RBL-2H3 following antigenic stimulation. Significantly, M391 was a very efficient activator of PLD whilst M436 was inactive at 100 μM. For PLD activation the rank order of efficacy was M391 > galparan > MP ≈ M435 ≈ M432 >> M436. These data indicate that the efficacy of PLD activation does not correlate with the ability of MP-containing chimeric peptides to induce 5-HT secretion from RBL-2H3 cells. Moreover, our findings confirm that chimeric MP analogues can be engineered to target specific intracellular proteins and selectively modify metabolic pathways.

MODULATION OF SPONTANEOUS ACTIVITY OF MOUSE NEUROMUSCULAR SYNAPSE BY PEPTIDE ALLATOSTATIN

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Allatostatins were originally identified in insects as peptide inhibitors of juvenile hormon biosynthesis, as well as potent inhibitory modulators of intestinal and skeletal muscles of crustaceans and insects. The aim of our study was to investigate possible effects of allatostatin (Dip-Ast 7) on the amplitude, frequency and time parameters of spontaneous (miniature) end-plate potentials (mEPPs) in mouse hemidiaphragm neuromuscular junctions. Spontaneous mEPPs were intracellularly recorded using standard electrophysiological microelectrode technique. Different concentrations of Dip-Ast 7 (1 μM and 10 nM) were tested during application to the bath solution. Both concentrations of Dip-Ast 7 caused gradual increase in mEPPs amplitude from control level: by average 67 ± 15 % (SEM, n=11) and by average 108 ± 14 % (SEM, n=16) for 10 nM and 1 μM, respectively. There were no significant changes in the shape of mEPPs amplitude distribution histograms, but histograms were shifted into the area of higher means of amplitude. Integer area below mEPPs was also increased by Dip-Ast 7: by average 49 ± 13 % (SEM, n=11) for 10 nM and by average 114 ± 12 % (SEM, n=16) for 1 μM. Rise-time and half-decay time of mEPPs were not affected after application of 10 nM Dip-Ast 7 and only slightly increased upon application of 1 μM Dip-Ast 7: by average 17 ± 6 % (SEM, n=16) and 13 ± 4 % (SEM, n=16), respectively. So we suggest, that Dip-Ast 7 increased the integer area below mEPPs mostly due to the rising of mEPPs amplitude. For both concentrations of Dip-Ast 7 used, it took approximately 60 minutes for full development of these Dip-Ast 7 effects, which were partially reversed by washing. Application of 10 nM Dip-Ast 7 didn't significantly affect mEPPs frequency during the whole period of Dip-Ast 7 presence in bath solution (up to 60 minutes), but application of higher concentration of Dip-Ast 7 (1 μM) produced a significant gradual decrease in mEPPs frequency – by average 37 ± 3 % (SEM, n=16) from control level. The results presented indicate dual presynaptic modulatory effects of Dip-Ast 7 on spontaneous activity of mice neuromuscular junctions, i.e.dose- and time dependent decrease of mEPPs frequency and increase of integer area below mEPPs, which could be due to increase of mean size of spontaneously released mediator quanta.

OPPOSITE MODULATION OF EVOKED POSTSYNAPTIC CURRENTS IN CRAB BY THE PEPTIDES PROCTOLIN AND ALLATOSTATIN

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The nervous system of crustaceans contains a great number of regulatory peptides, among others - the proctolin (Proc) and allatostatins, which were shown to exert sometimes antagonistic influences on activity of central neurons and peripheral targets like smooth and skeletal muscles. The aim of present work was to compare the effects of Proc and allatostatin (Dip-Ast 8) on the activity of crustaceans peripheral motor synapses. The studies were performed on identified fibers of closer muscle of marine crab *Eriphia spinifrons* walking legs. Evoked postsynaptic currents (EPSCs) from individual release boutons were recorded extracellularly using the focal macropatch technique. Application of Proc (1μM) was shown to increase the amplitude of EPSCs by average 31 ± 12 % (SEM, n=4), while 1μM Dip-Ast 8 application decreased the mean amplitude of EPSCs by average 21 ± 6 % (SEM, n=4). During washing the effects of both peptides on the amplitude of EPSCs could be partially reversed. Noteworthy, these inhibitory effects of Dip-Ast 8 on transmitter release were revealed by a such type of muscle fibers, which have no GABAergic inhibitory innervation. By both peptide application, there were no significant changes in the amplitude, rise time or half-decay time of spontaneous postsynaptic currents, which were appeared rarely during recordings. The alternative changes in EPSCs amplitude seemed to be due to increasing (Proc) or decreasing (Dip-Ast 8) probability of evoked quantal release - Proc was shown to decrease, and Dip-Ast 8 – to increase the number of failures of evoked responses in statistically significant manner. In the next serie of experiments, combined effects of Proc and Dip-Ast 8 on EPSCs were tested. The first Proc (1μM) application increased the amplitude of EPSCs by average 45 ± 15 % (SEM, n=3). The further application of Dip-Ast 8 (in the presence of Proc) decreased amplitude of EPSCs below the background control level – to average 76 ± 12 % (SEM, n=3). This inhibitory effect of Dip-Ast 8 was reversed during prolonged washing with normal Ringer solution. After washing the amplitude of EPSCs had reached the level which was after 1μM of Proc was applicated – by average 43 ± 21 % (SEM, n=3). The results obtained show that Proc and Dip-Ast 8 exert opposite presynaptic modulatory action on evoked synaptic transmission in neuromuscular junctions of crab.

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BIOLOGICAL ACTIVITY OF MELANOCYTE GROWTH-INHIBITING TRIPEPTIDE *IN VIVO*

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Melanocyte tripeptide (MTP) pyroGlu-Phe-GlyNH_2 was previously purified from cultured melanocytes and melanoma cells and was shown to influence growth of melanocytes *in vitro*. The present study was focused on investigation of a possible effect of the MTP on *in vivo* growth of melanomas. For this purpose, a relatively slow-growing clone of the original B16 melanoma was established and designated B16A2. B16A2 cells were injected *s.c.* into hairless mice (hr/hr) at four sites (300,000 cells per site). MTP was given by *i.p.* injections three times a week at two concentrations (1 pmole and 1 nmole per animal). The control animals received the equal volume of the solvent. The animals were sacrificed one and two weeks after the tumor transplantation, and individual tumors were weighed. DNA content in the tumors was analyzed by flow cytometry. One week after the transplantation, the animals which received 1 pmole of MTP had fewer tumors and reduced tumor load. Two weeks after the transplantation, the differences between control and treated animals were no longer observed. The results indicate that MTP temporarily delays *in vivo* tumor growth. At present time, it is not clear how the tumor cells are delayed and this will be the subject of future investigation.

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BRADYKININ RELATED COMPOUNDS HAVING IMPROVED ANTI-CANCER ACTIVITY *IN VIVO* COMPARED TO CISPLATIN AND SU5416

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Bradykinin (BK: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and several other neuropeptides (cholecystokinin, gastrin, GRP, neurotensin, vasopressin) were shown to promote clonal growth of small cell lung cancer (SCLC). Bradykinin is one of the most potent peptides and its B2 receptor is the most commonly expressed in almost all lung cancer cell lines. Our highly potent B1 and B2 receptor antagonist (B9430: DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; Hyp: *trans*-4-hydroxy-L-proline), Igl: α-2-indanylglycine, Oic: octahydroindole-2-carboxylic acid) unfortunately did not inhibit the growth of SCLC *in vitro* but showed some inhibition *in vivo*. We have reported that the dimerized B9430 with appropriate cross linkers showed growth inhibition both *in vitro* and *in vivo*. Surprisingly, a simple N-terminal acylation of the antagonist monomers, especially with a bulky, fluorine-rich, hydrophobic group (F5C: 2,3,4,5,6-pentafluorocinnamoyl) increased the growth inhibition of the parent compounds *in vivo*. We have also developed two new classes of smaller mimetic antagonists. Lead compounds are monomeric F5C-OC2Y-Atmp (BKM-570) (OC2Y: O-2,6-dichlorobenzyl tyrosine, Atmp: 4-amino-2,2,6,6-tetramethylpiperidine) and the dimeric DDD-(DArg-Igl-Arg-Matp)$_2$ (BKM-638) (DDD: dodecanedioyl, Matp: 4-(methylamino)-2,2,6,6-tetramethylpiperidine). When injected daily and intraperitoneally at 0.5 to 5 mg/kg, these mimetics effectively suppressed the growth of human small cell lung cancer cells (SCLC) implanted subcutaneously in athymic nude mice (80 to 95% inhibition). Activities of the new compounds will be compared with the well known anti-cancer drug Cisplatin, with the recently developed SU5416 and with those of previously described compounds from this laboratory. These smaller mimetics are cheaper to synthesize and are more potent than our classical BK-peptide dimers. They may have clinical potential for the treatment of human lung cancers.

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BIOSENSOR ANALYSIS IN THE IMPROVEMENT OF ANTIGENICITY OF PEPTIDES FROM C-S30 FOOT-AND-MOUTH DISEASE VIRUS

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In previously reported studies on foot-and-mouth disease virus (FMDV), several variant isolates have been detected and characterized. One of them, isolate C-S30, included four replacements within the main antigenic site A (G-H loop of protein VP1). However, its reactivity with neutralizing monoclonal antibodies (mAb) 4C4 and 3E5 (raised against FMDV C_1-Brescia) was shown to be indistinguishable from that of reference FMDV strain C-S8c1. Peptides reproducing site A have been recognized by mAb 4C4 in previous enzyme-linked immunodot (EID) and in recent structural crystallographic studies. The isolate C-S30 includes a mutation at a critical and highly conserved position, 147Leu→Val, which was previously seen to be detrimental for antibody recognition. These somewhat surprising results (i. e., one mutation abolishing recognition; three additional mutations restoring it) prompted us to study these effects in greater detail. This was accomplished through surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA) studies of synthetic peptides reproducing antigenic site A from C-S30 and C-S8c1 FMDV. Antigenicities of the C-S30 peptides were surprisingly lower than those of the C-S8c1 reference. The high dissociation rate constants measured for the C-S30 by means of the SPR biosensor were judged to be the main cause for such low antigenicity which, however, was substantially increased b decreasing peptide flexibility upon cyclization. Therefore, high mobility appears to be the reason for the low antigenicity of the linear C-S30 peptides. SPR dynamic analysis allowed us to diagnose this problem and to improve peptide antigenic potential through the synthesis of the corresponding cyclic analogue.

RATIONAL DESIGN OF NEW BIOACTIVE LIPOPEPTIDES : STRUCTURE-ACTIVITY RELATIONSHIPS, BIOSYNTHESIS AND APPLICATIONS.

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During the last decades, studies of natural lipopeptides remained somewhat of marginal interest since knowledge on both biosynthesis, structure-activity relationships and potent applications were lacking. As a matter of fact, interest in such compounds, particularly in surfactins and lichenysins, really began after we collected an increasing amount of information involving structure and biosynthesis techniques as well as development of bio-assays. Surfactins and lichenysins, produced by *Bacillus species*, are not only very powerful biosurfactants but also valuable antibacterial, antitumoral and anti-mycoplasma agents. Their recent and successful applications for metal removal, oil recovery and dispersion from contaminated soils are promising for difficult ecological problems. Structural characterization of these attractive lipopeptides is a cyclic heptapeptide with a chiral sequence *LLDLLDL* linked *via* a lactone bond to a β-hydroxy fatty acid C13-C15. The biosynthesis is catalyzed non-ribosomally by a multienzyme complex involving a multicarrier thiothemplate mechanism. By control of nitrogen source of the culture medium, we directed the biosynthesis process towards production of particular peptidic variants. Used in association with the screening of various *Bacillus* strains, this strategy has led to the building and isolation of numerous variants that constitute now surfactin and lichenysin G families. Progress in purification techniques allowed an effective separation of all isoforms while the availability of improved spectroscopic techniques provided easier and faster structure elucidation. Here, fine studies of the structure-activity relationships associated with the 3D structure of surfactin have evidenced the respective role of the polar and hydrophobic domains in interfacial and membrane properties. They have led to the recognition of the specific residues required for an improved activity. The surfactant, chelatant and hemolytic properties of all new compounds were evaluated thus leading to the selection to the most efficient lipopeptide. This comprehensive approach permits advances in rational design of bioactive lipopeptide for medicinal, pharmaceutical and environmental industries. Such applications will be all the easier since now the methodology for chemical synthesis and the tool boxes for genetic engineering are well developed.

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SYNTHESIS AND CONFORMATIONAL STUDIES OF CYCLIC PEPTIDES WITH ANTAGONIST ACTIVITY AT MELANOCORTIN 3 AND 4 RECEPTORS.

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Melanotropins have become immensely important for the treatment of obesity, skin pigmentation disorder, inflammation and other important aspects. We recently have described a novel templates as ligands for melanocortin receptors showing a high activity and selectivity at hMC3, hMC4 and hMC5 receptors. By replacing of His^6 with Pro^6 in potent antagonist SHU9119 (Ac-Nle-[Asp-His-DNal(2')-Arg-Trp-Lys]-NH_2) we have got a potent antagonist at hMC3 and hMC4 receptors and highly selective and potent as agonist at hMC5 receptor, that is, PG-901. Using primarily cyclic conformationally constrained analogues of proline for His^6 position in melanotropins peptides SHU9119 and MTII (Ac-Nle-[Asp-His-DPhe-Arg-Trp-Lys]-NH_2) we have obtained a series of analogues which are highly potent and selective agonists an antagonists for the hMC3, hMC4, and hMC5 receptors. In order to understand which "bioactive conformation" is the most important and specific to interact selectively at hMC3 and hMC4, we carried out a molecular modeling study. Here, we will propose a 3D pharmacophore model for the ligands of hMC3 and hMC4 receptors obtained by molecular modeling which employed low-energy conformations of peptides possessing a pronounced hMC3 and hMC4 selectivity. This model is well supported by synthesis and biological testing of several conformationally constrained cyclic peptides.

REDUCED BOND ANALOGS OF NEUROPEPTIDE PACAP.

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The design of peptidomimetic drugs is essential to determine structural features and metabolic stability of peptide hormone and neuromodulator antagonists. We have developped a chemical fragment coupling strategy to introduce a reduced bond by reductive amination of protected peptide aldehyde with resin bound amino peptide. Synthesis of peptide aldehyde on solid support consists in elongating a peptide from an amino thiazolidine linked to a resin as a masked amino aldehyde [1]. The peptide aldehyde is generated by cleavage of copper salts. The reductive amination of peptide aldehyde with resin bound amino peptide was perfected in acidic methanol. No epimerization was observed during the aldehyde preparation step or the reductive amination step [2].

This strategy allowed the synthesis of analogs of a 38 amino acids neuropeptide, PACAP (Pituitary Adenylate Cyclase Activating Polypeptide), member of the Vasoactive Intestinal polypeptide / glucagon / secretin family of peptides.

We report the total synthesis of the Lys^{21}-Tyr^{22} reduced bond PACAP(6-30) analogue of a selective $VPAC_2$ receptor antagonists.

[1] N. Galéotti, M. Giraud and P. Jouin. *Lett. Peptide Science*, **1997**, *4*, 437-440. *Solid phase synthesis of peptidyl aldehydes from C-terminal thiazolidinyl peptides.*
[2] C. Gros, N. Galéotti, R. Pascal and P. Jouin. *Tetrahedron*, **1999**, *55*, 11833-11842. *Solid phase synthesis of a ψ[CH_2NH] pseudopeptide by ligation of a peptidyl aldehyde with a resin-bound amino peptide.*

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STRUCTURE-FUNCTIONAL SIMILARITY OF THE BRAIN PEPTIDE CYCLO-PRO-GLY AND NOOTROPIC DRUG PIRACETAM

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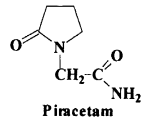
Cyclo-Pro-Gly (cPG) was identified in rat brain by HPLC and gas chromatography-mass spectrometry methods as the endogenous nootropic (Gudasheva et al., 1996). The purpose of this study was to investigate the cognition enhancing properties of cPG and to compare them with these of the nootropic drug Piracetam.

cyclo-Pro-Gly



Piracetam

The profiles of activity for cPG and Piracetam in passive avoidance and active avoidance tests were demonstrated to be quite similar. Both substances facilitate the impute of information, do not effect the consolidation and even inhibit the memory traces retrieval in passive avoidance performance damaged by electroshock. Both cPG and Piracetam facilitate the active avoidance learning improving the performance in animals which failed to reach it before treatment. Similar kind of cPG and Piracetam effects was demonstrated not in these associative forms of learning only, but in the nonassociative learning (habituation of explorative behavior) too. The common features of cPG and Piracetam have been revealed also in electrophysiological experiments: both of them markedly enhance the amplitude of the transcallosal evoiked potential without changing its pattern. The comparision of cPG and Piracetam structures have also discovered their resemblance.

We conclude that cPG is the endogenous prototype of nootropic agent Piracetam.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL SERIES OF GROWTH HORMONE SECRETAGOGUES

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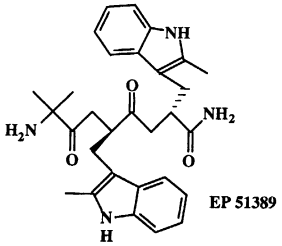
Growth Hormone Secretagogues (GHS) liberate Growth Hormone (GH) acting on receptors distinct from GHRH receptors. In a preliminary study the hexapeptide GHS Hexarelin (His-2-Me-D-Trp-Ala-Trp-D-Phe-Lys-NH2) was downsized to yield, among others, a tripeptide, compound EP 51389, subcutaneously more potent than Hexarelin in a infant rat model and orally active in dogs and man.



EP 51389

The design, synthesis and biological evaluation of a new class of orally bioavailable pseudo-tripeptide analogs will be presented. The lead compound, JMV 1843 was shown to stimulate GH secretion in a infant rat model. It was also orally active in dogs and could be selected as a development candidate.

Life Sciences, 1994, 18, 1321-1328, A. Torsello, C. Battisti, R. Deghenghi, M. M. Müller, V. Locatelli.
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INCREASED ANTIBACTERIAL ACTIVITY OF 15-RESIDUES BOVINE LACTOFERRICIN DERIVATIVES EMPLOYING NON-CODED AROMATIC AMINO ACIDS

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Bovine lactoferricin is a 25-residue highly cationic amphipatic peptide posessing antimicrobial activity. A 15-residues derivative of bovine lactoferricin is shown to contain most of the activity against bacteria(1). Tryptophan residues are believed to play an essential part in the interaction of these peptides with bacterial cell membranes, in which replacement of tryptophan residues with alanine results in decreased ability to inhibit bacterial growth(2). We present here the effects of replacing tryptophan with unnatural aromatic amino acids. These modifications resulted in major increasement of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Selected peptides were tested for activity against *Methicillin Resistant Staphylococcus aureus* and *Methicillin Resistant Staphylococcus epidermidis* and shown to efficiently inhibit growth of these pathogenic bacteria. Measurement of hemolytic activity showed that the peptides are substantially less active against human erythrocyttes.

1) Rekdal, Ø., Andersen, J., Vorland, H. and Svendsen J.S., *J Pep. Sci.*, **1999** 32-45
2) Strøm, M.B., Rekal Ø. and Svendsen J.S., *J. Pep. Res*, **2000**, *submitted*

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A NOVEL METHOD FOR CHARACTERIZING AND OPTIMIZING PEPTIDE INHIBITOR/PROTEASE INTERACTIONS USING SPOT SYNTHESIS

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A new method to characterize a peptide inhibitor/protease interaction by using parallel peptide array synthesis on cellulose is presented. A peptide comprising P5-P4' of the third domain of turkey ovomucoid inhibitor was investigated for both binding and inhibition of porcine pancreatic elastase. Binding was performed directly on the cellulose peptide array, while inhibition was measured by an assay in microtiter plates with punched out peptide spots. The importance of each residue for binding or inhibition was determined by substitutional analyses, exchanging every original amino acid by the other 19 coded ones. From these data together with length analysis we designed new peptides in a step-wise fashion that in the end not only inhibit elastase 250 times better than the original peptide but are highly specific for this enzyme. In addition, the optimized inhibitor peptide was protected against exoproteases by substituting d-amino acids at both termini.