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Totally Synthetic Lipid Containing Polyoxime Peptide Constructs are Potent Immunogens

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A synthetic peptide corresponding to a sequence from influenza hemagglutinin was used as a model antigen to study the immunogenicity of polyoxime constructs. In the absence of any adjuvant, tetrameric forms of different polyoxime constructs did not elicit an antibody response. High and long-lasting levels of antibody were induced, however, by polyoxime constructs to which Pam3Cys was attached. Comparable serum antibody levels were achieved with Tetraoxime-Pam3Cys administered by the i.p. or i.n. routes to those obtained when the monomeric peptide was administered by the i.p. route in complete Freund's adjuvant (CFA). Mice receiving Tetraoxime-Pam3Cys and Pam3Cys-peptide intranasally developed peptide-specific antibody secreting cells (ASCs) in their lungs and mediastinal lymph nodes. At low dose, the Tetraoxime-Pam3Cys induced higher levels of antibody compared to those elicited by the monomeric Pam3Cys-peptide delivered by either route. These results show that lipo-tetraoxime constructs assembled by polyoxime chemistry can be potent inducers of systemic and mucosal immunity.

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A SYNTHETIC PEPTIDE CREATES A NEW STRAIN OF PRION DISEASE IN TRANSGENIC MICE

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Infectious, inherited and sporadic forms of prion disease are attributable to a conformationally plastic membrane-bound designated prion protein (PrP), which can exist in either a normal, cellular form (PrP^C) or a disease-causing isoform (PrP^{Sc}). Gerstmann-Sträussler-Scheinker (GSS) disease is an inherited prion disease in humans and results from substitution of leucine for proline at position 102. GSS was modeled in transgenic mice (Tg (MoPrP,P101L)) by expressing this human mutation in mouse PrP. High-expressing Tg lines develop spontaneous disease between 100-300 days of age, while low expressers (Tg196) rarely succumb to prion illness, even after 600 days. A 55-residue peptide corresponding to MoPrP(89-143), with the P101L mutation, was constructed and refolded into two distinct conformations, one β-sheet and the other random coil. The two conformers of MoPrP(89-143,P101L) were inoculated intracerebrally into Tg196 mice. All 20 mice that received the β-sheet peptide died after 360 ± 13 days, while the 10 mice that received the random coil peptide did not develop clinical signs of CNS dysfunction (Kaneko et al, J. Mol. Biol. 295:997, 2000). Neuropathologic evaluation of the 20 ill mice showed neurohistologic changes similar to those found in GSS patients. Prions from clinically ill Tg196 mice that had received the β-sheet form of the peptide were repassaged into Tg196 mice. All 10 Tg196 mice in this group became ill after ~365 days, which is similar to the incubation period for the peptide inoculation. When Tg196 mice were inoculated with prions from the brains of spontaneously ill, high-expressing Tg(MoPrP,P101L) mice, the incubation time was ~240 days. These incubation times in Tg196 mice, together with neuropathologic results, suggest that a new strain of prion was generated by the β-sheet peptide and represent the most convincing evidence to date that a chemically synthesized molecule, refolded into an appropriate conformation, is able to initiate prion disease.

NEW CHOLECYSTOKININ CCK_2 AGONISTS DISPLAYING HIGHLY FAVOURABLE « CCK_{2B} » PROPERTIES IN VIVO

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The issue of CCK_2 receptor heterogeneity has been discussed for more than ten years, on the basis of in vitro as well as in vivo studies. The latter have allowed to associate with CCK2 agonists two separate pharmacological profiles, now denominated CCK_{2A} and CCK_{2B} (formerly CCK-B1 and -B2). The CCK_{2B} profile associates stimulant and memory reinforcing properties with an ability to stimulate dopamine release in some brain areas and a lack of anxiogenic effects. Only one such agonist, BC264 (Boc-Tyr(SO_3^-)-gNle-mGly-Trp-NMeNle-Asp-Phe-NH_2) had been shown to exhibit this profile until the development of RB400 (HO_2C-CH_2CO-Trp-NMeNle-Asp-Phe-NH_2) which was proved also to be a CCK_{2B} agonist, but whose effects were much weaker than those of BC264.

We report here the development of the tetrapeptidic series based on RB400, with a general structure R-Trp-NMeNle-Asp-Phe-NH_2, and the characterization of BBL454, an extremely potent CCK_{2B} agonist, sharing all the properties of BC264 in vivo but devoid of its drawbacks (size, difficulty of synthesis, bell-shaped pharmacological responses). BBL454 proved to be active after i.p. injection at doses as low as 0.03 μg/kg in vivo, that is to say 10 to 100 times lower doses than BC264.

Further development starting from BBL454 has prompted us into modifying its C-terminus by introducing structural features known to convert CCK_2 agonists into antagonists. Biochemical studies proved some of the resulting compounds to be potent agonists of arachidonate production in CHO cells transfected with the rat CCK_2 receptor, and weak antagonists of inositol phosphate turnover in these cells. One of the resulting compounds, BBL534 was chosen for extended survey. Injected i.p. in rats, BBL534 evoked CCK_{2B}-agonist-like stimulant properties in the open-field, and had CCK_2-antagonist-like anxiolytic effects in the elevated-plus-maze. This suggests a link between some effects in vivo and a particular signalling pathway triggered by the activation of CCK_2 receptors.

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SINGLE AMINO-ACID SUBSTITUTIONS IN A MIMETIC TETRADECAPEPTIDE AGONIST OF THE HUMAN $P2Y_2$ RECEPTOR PREVENT SECOND MESSENGER ACTIVATION.

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P2Y receptors mediate the biological actions of extracellular nucleotides such as ATP and UTP via activation of phospholipase C and the subsequent release of Ca^{2+} from intracellular stores. The detailed characterisation of P2Y receptors is hindered by the lack of subtype-selective ligands, especially antagonists [1]. Previous studies with the V_{1a} vasopressin receptor indicated that mimetic peptides of extracellular receptor domains inhibited ligand binding, therefore V_{1a} mimetic peptides acted as functional antagonists [2]. Our previous studies of the $P2Y_2$ receptor revealed, surprisingly, that mimetic peptides designed to extracellular domains of the receptor acted as agonists [3]. One mimetic peptide in particular, L247, (RSLDLSCHTLNAIN) corresponding to $ECIV^{271-284}$ of the human P2Y2 receptor, stimulated inositol phosphate turnover and mediated a sustained release of intracellular calcium in ECV304 cells.
An alanine scan of L247 revealed that substitution of single residues within the amino acid sequence resulted in a complete loss of calcium mobilisation in ECV304 cells. The effect of three further peptides, incorporating L247 in their sequence, designated M501 ($ECIV^{271-291}$), M504 ($ECIV^{260-284}$) and M507 ($ECIV^{260-291}$) was also investigated. .No intracellular calcium release was observed in response to either M503 or M507. However, addition of M504 (100μM) to ECV304 cells resulted in a transient release of Ca^{2+} from intracellular stores. Our observations indicate an important role of ECIV in $P2Y_2$ receptor activation. Moreover, these findings may important implications in future design of peptide analogues that may act as $P2Y_2$ antagonists.

[1] Tuluc, F., Bultmann, R., Glanzel, M., Frahm, A. W. and Starke, K. Naunyn-Schmiedeberg's Arch. Pharmacol. **357** (1997) 111-120.
[2] Howl, J. and Wheatley, M. Biochem. J. **317** (1996) 577-582.
[3] Brown, J., Brown C. A., Martin, A., Langel, Ü and Howl, J. Peptides for the New Millennium (in Press).

CHARACTERIZATION OF POTENT NEW VASOPRESSIN ANALOGS TO STUDY VASOPRESSIN V_{1b} RECEPTORS

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In contrast to the availability of a wide variety of potent and selective peptide and non-peptide ligands for the vasopressin V_{1a} and V_2 receptors[1], there are no non-peptide and only a handful of peptide ligands for the V_{1b} receptor. None of these are potent or selective. Only the d[D-3(pyridyl)Ala^2]AVP was described to be a potent and specific agonist of the rat V_{1b} receptor[2]. Yet, this compound is not selective for human vasopressin V_{1b} receptor, and it exhibits micromolar affinity for the bovine V_{1b} receptor.
Here we report the synthesis and pharmacological properties of new deamino vasopressin (dAVP) analogs modified at position 2. Among a series of 16 compounds tested, 5 exhibited a nanomolar affinity for the human V_{1b} receptor and exhibited an affinity at least 20-fold lower for the human V_2 and oxytocin receptors. However, they still presented a good affinity for the human V_{1a} receptor. The affinity of d[D-Phe^2]AVP, the most promising compound of this series, was tested on rat and bovine V_{1b} receptors. At variance with d[D-3(pyridyl)Ala^2]AVP, it exhibited a nanomolar affinity for all the V_{1b} receptors tested. More interestingly, it was found to be relatively selective for the human and bovine V_{1b} receptors. We thus decided to examine its ability to stimulate inositol phosphate production in CHO cell lines expressing the different human vasopressin and oxytocin (OT) receptors. d[D-Phe^2]AVP was found to be a full V_{1b} and V_2 agonist, a partial V_{1a} agonist (27% of AVP maximal activity) and a full OT antagonist. These data strongly suggest that d[D-Phe^2]AVP used in combination with a specific V_{1a} antagonist, like $d(CH_2)_5$[Tyr(Me)^2]AVP[3] could be a valuable tool to study the V_{1b} receptors in mammals. Synthesis of new d[D-Phe^2]AVP derivatives are in progress to improve the V_{1a}/V_{1b} selectivity.

1. Barberis, C. et al., *Drug News and Perspect.* **12**:279-292 (1999).
2. Schwartz, J. et al., *Endocrinology* **129**:1107-1109 (1991).
3. Kruszynski, M. et al., *J. Med Chem.* **23**:364-368 (1980).

A Discontinuous HIV-gp 120 C3/c4 Domain Derived, Branched, Synthetic Peptide that Binds to CD4 and Inhibits MP-1 Binding

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The biological effects of HIV envelope protein gp 120, depend on its ability to interact with host cell CD4 and interfere with chemokine receptors. X-ray crystallography has identified both CD4 binding cavity and chemokine receptor sites. We have designed and synthesized using Fmoc solid phase peptide synthesis a (3,4,5) branched peptide p3.7, which incorporates sequence discontinuous residues of HIV gp 120 constant region. P3.7 has 3 arms and contains several residues predicted from the site directed mutagenesis studies and X-ray crystallography to be important in forming the CD4 binding cavity. In addition it contains residues of the CXCR4 and CCR5 chemokine receptor sites of gp 120. We have shown that p3.7 is recognised by antiserum raised against native gp 120, binds to cell surface CD4 and competitively inhibits QS4120, an antibody directed against the CDR2 region of CD4 (8). Furthermore, p3.7 inhibits binding of MIP-1α in MM6 cells, a CD4 positive macrophage cell line that expresses mRNA for both CCR3 and CCR5 chemokine receptors. Thus we have designed and synthesised a peptide that incorporates residues known to be important for the biological effects of gp 120, the confirmation of which can mimic both CD4 binding activity and the chemokine receptor sites predicted from crystallography. More generically this represents a novel approach to the rational design and synthesis of peptides which mimic complex sequence discontinuous ligand binding sites of clinically relevant proteins.

DIFFERENTIAL SIGNALLING PATHWAYS UPON ACTIVATION OF RAT AND MOUSE CHOLECYSTOKININ A RECEPTOR.

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We have previously reported that the third intracellular loop of the rat and mouse cholecystokinin A receptor (CCK-AR) is responsible for different patterns of gene activation. We have concluded that both extrinsic and intrinsic parameters to the CCK-AR can explain the differential behavior of compoud JMV-180, a cholecystokinin analogue, which has been shown to act as an agonist on low- and high-affinity sites of the CCK-AR while in the rat this compound acts as an agonist on high-affinity sites and as an antagonist on low-affinity sites. Here, using the reporter gene strategy, we have analyzed the effect of the rat and mouse CCK-AR on the activation of several proteins known to interact with the regulatory part of the c-Fos gene. HeLa cells were thus transiently co-transfected with plasmids encoding the rat or mouse CCK-AR in presence of a plasmid leading to expression of a protein containing the DNA binding domain of Gal-4 fused to the transactivating domain of the protein of interest (Elk-1, ATF-2, c-Jun, CREB). Under these experimental conditions, the reporter plasmid placed the firefly luciferase structural gene downstream from a repeat of the 17M sequence recognized by Gal-4. The transactivating domain of each of these proteins of interest corresponded to the domain which is the target of one or several kinases pathways. Under these experimental conditions, the measure of luciferase activity, after treatment of cells with cholecystokinin or JMV-180 provided information on the signalling pathway(s) upon CCK-AR activation. Our results clearly indicated that the rat and mouse CCK-AR are distinguishable by their ability to activate cJun N-ter kinase (JNK), and thus confirmed that the mouse and rat CCK-AR are differentially involved in activation of kinase(s) pathway(s) leading to activation of proteins interacting at the regulatory part of the human c-Fos gene.

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THE ROLE OF OPIOID RECEPTORS AND NMDA RECEPTORS IN ANALGESIC ACTION OF TRH

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Thyrotropin-releasing hormone (TRH) not only stimulates the release of thyroid-stimulating hormone (TSH) but also has a wide range of central effects. The CNS action of TRH is independent of its hormonal activity but is likely to be mediated through the TRH receptors located on neurons in different regions of CNS. TRH is extensively distributed through the central and peripheral nervous system (hypothalamus, extrahypothalamic brain regions, brain stem, spinal cord etc.) but its physiological role in these compartments is not clearly known. One of the possible function of TRH in spinal cord is modulating of pain signal input and transmission. It is known that central or peripheral application of TRH may produce analgesic or hyperalgesic action depending on experimental conditions. It was shown that opioids and NMDA receptors may be involved in some central TRH actions but mechanisms of these effects remains to be fully elucidated. The aim of this study was to estimate possible role of endogenous opioid system and NMDA receptors in analgesic action of TRH and its synthetic analogue Dihipramine with low endocrine activity. The results presented were obtained on male mice. Different nociceptive stimuli where used - acetic acid induced writhing test and tail-flick test. Intraperitoneal administration of TRH in doses 2, 5 and 10 mg/kg and analogue in doses 1, 2 and 5 mg/kg produced a dose-dependent analgesia as assessed by writhing test. Both TRH and analogue in doses used showed no changes in tail-flick latency. In dose 5 mg/kg the analog was more effective than TRH. It was shown that significant analgesic activity of TRH was lasting for 30 minutes and analgesic activity of the analog was lasting for 1 hour after i. p. injection. Naloxone (2 mg/kg) did not attenuate the effects of TRH and Dihipramine. MK-801 (0.1 mg/kg, i.p.) an NMDA receptors antagonist also did not change the number of writhings in mice previously treated with TRH or Dihipramine. However it was noted that both TRH or Dihipramine and MK-801 in doses used induced significant hyperactivity. The data shown that: 1) Analgesic activity is not due to endocrine action of TRH; 2) The mechanism of antinociceptive action of both substances is not opioid or NMDA dependent.

ISOLATION OF GENES DIFFERENTIALLY OVER-EXPRESSED ON GASTRIN TREATED ASTROCYTOMA CELLS AND THEIR IMPLICATION ON CELL MOTILITY

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Prognosis for astroglial brain tumors that are not amenable to surgical resection remains poor. This type of tumor is characterized by a marked propensity to diffuse into large areas of normal brain parenchyma. This invasion relates mainly to cell motility. The heptadecapeptide Gastrin-17 is know to inhibit U373 astrocytoma cell line motility whereas no CCKB/G receptors are presents on these cells (unpublished results).

In this report, genes differentially expressed in G17-treated U373 cells have been cloned using a substractive hybridization technique. A differential screening allowed us to eliminate false positive clones. Sequencing yielded 59% sequences corresponding to known genes among which 21% corresponded to genes related to cell motility. The remaining 41% corresponded to unknown genes (some of them already present in the EST database). A competitive RT-PCR allowed evaluation of the overexpression of *tenascin-C*, a gene encoding an extracellular matrix protein, and of *calcyclin*, a gene encoding a calcium binding protein, both related to cell motility.

These results provide another evidence for the role of gastrin on astrocytoma cell motility not related to CCKB/G receptors. This study focused on new genes as potential targets for the inhibition of cell motility in this type of tumors.

MOLECULAR AND BIOLOGICAL STUDIES OF THE MIDDLE PORTION OF ENDOTHELIN-1.

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Endothelin (ET) is a vasoactive 21-amino acid peptide folded by two disulfide bridges in position 1-15 and 3-11. To better understand the role of the amino acids of the middle segment of ET, we have synthesized [Cys(Acm)^{3,11}, Trp(For)^{21}](3-11)-Aca-(17-21)ET-1, a structurally reduced analogue acting as an agonist in ET_B receptor preparations. To further explore the middle stretch, we synthesized similar analogues but with a shorter spacer, aminobutyric acid (Abu), preceded or followed by a functionalized amino acid residue. The peptides were synthesized using solid phase Boc chemistry. Pharmacological assays were carried out using the lung parenchyma (ET_B) and rat aorta (ET_A) preparations. All analogues were inactive in the ET_A receptor bioassay. Peptides containing the Val, Ala or Tyr residues were also inactive in the ET_B preparation. However when the lateral chain of the residue contained a functional group, hydroxyl (Ser), amine (Lys), amide (Asn, Gln) or carboxylic acid (Glu, Asp), we observed a tissue contraction. This part of the molecule might therefore interact with the C-terminal amino acid residue in order to stabilize a tridimensional conformation.

ION CHANNEL ACTIVATION BY SPC3, A SYNTHETIC PEPTIDE INHIBITOR OF HIV INFECTION

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SPC3 is a multibranched peptide containing eight GPGRAF motifs which are derived from the HIV-1 gp120 V3 loop consensus sequence. This molecule was reported to prevent the infection of $CD4^+$ cells by various HIV-1 and HIV-2 strains. However, the mechanism of action of SPC3 remains unclear. We investigated the possibility that SPC3 could interact with α/β-chemokine receptors following the observations that, first, the V3 loop is likely to be involved in the α/β-chemokine receptor-dependent HIV entry and, second, natural ligands of these receptors are potent inhibitors of cell infection. To address this point, we examined the effects of SPC3 onto *Xenopus* oocytes either uninjected or expressing exogenous human CXCR4 receptors. Extracellular applications of SPC3 onto *Xenopus* oocytes trigger potent inward chloride currents which can be inhibited by increasing extracellular Ca^{2+} concentration. This effect can be blocked by chloride channel antagonists and is highly specific to SPC3 since it is not triggered by structural analogs of SPC3. The SPC3-induced chloride conductance in oocytes is α/β-chemokine receptor-dependent since (i) SPC3 alters the sensitivity of this channel to external applications of human recombinant MIP-1α, a natural ligand of human CCR5 receptor, and (ii) the amplitude of the inward current could be increased by the expression of exogenous human CXCR4 chemokine receptor. The effect of SPC3 appears to rely on the activation of a phospholipase A2 signalling pathway, but is neither affected by changes in cytosolic Ca^{2+} concentration, nor by alterations in Gi/Go protein, adenylate cyclase, phospholipase C or protein kinase C activity. Altogether, the data indicate that SPC3 is capable of activating a surface α/β-chemokine-like receptor-mediated signalling pathway in competent cells, thereby triggering -either directly or indirectly- a Ca^{2+}-inactivated chloride conductance.

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THE EFFECT OF DIVALENT IONS ON BINDING AND SIGNAL TRANSDUCTION IN CELLS HAVING STABLY EXPRESSED OXYTOCIN RECEPTOR

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The role of magnesium and other divalent cations in oxytocin (OT) action was investigated throughout the whole period oxytocin and vasopressin have been known. The role of magnesium in the action of oxytocin was studied on the level of isolated organs (uterus, mammary gland, arteries) and on the level of receptor membrane fractions. It has been shown that magnesium strongly influences biological activity of oxytocin analogs, the agonistic activity being usually enhanced, the antagonistic activity decreased. In this aspect analogs modified in *p*-position of the amino acid in position 2* are interesting because of changing agonistic, partial agonistic and antagonistic qualities according to the presence or absence of magnesium.

At present, cell lines having stably expressed OT receptors (e.g., human embryonal kidney oxytocin receptor - HEK OTR – cells[1]) are available and it is thus possible to study these problems in simpler system and differentiate binding (determined using ^{125}I-oxytocin antagonists and ^{3}H-OT) and transduction of the signal, in our case determined as quantity of IP_3 in the cells after stimulation by oxytocin or analogues. Intact cells grown on polylysine-coated plates at about 70% confluency were used. The binding of both tracers was studied in magnesium and mangan concentrations 0.05-5mM and displacement curves for oxytocin and its analogues modified in positions 2 were determined. Strong ion concentration dependence of the displacement was found. The binding data were correlated with the quantity of IP_3.

1. Gimpl G., Burger K., Fahrenholz F., Biochemistry 36, 10959 (1997).

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VASOACTIVE INTESTINAL PEPTIDE RECEPTOR (VPAC1) IS DESENSITIZED AND INTERNALIZED INDEPENDENTLY OF ITS PHOSPHORYLATION AT CONSENSUS SITES FOR PKC AND PKA

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Vasoactive intestinal peptide or VIP readily increases cellular cAMP after binding to VIP receptors (VPAC1 & VPAC2) present in many tissues.The turning off of such a VIP-signaling pathway implies desensitization and internalization of VPAC1 receptors. Phosphorylation has an important role in desensitization or internalization of G protein-coupled receptors. Thus, we have investigated if certain Ser residus present in intracellular domains of VPAC1 have a role in these processes. The Ser 250 and Ser 447 which are respectively putative phosphorylation sites for PKC and PKA were substituted to Ala by directed mutagenesis. The resulting VPAC1 receptor mutants with an N-terminal FLAG epitope and a C-terminal green fluorescent protein (GFP) were stably transfected in CHO cells. All these mutants were as biologically active as the wild FLAG/GFP receptor type when challenged with VIP (EC50: 0.2 nM for stimulating adenylyl cyclase). The GFP fluorescence of CHO cells observed under microscope gave an indication of the presence of VPAC1 in both the cytoplasm and the plasma membrane. In contrast, VPAC1 receptors in the plasma membrane of non-permeabilized CHO cells were detected by primary anti-FLAG antibodies and secondary antibodies coupled to rhodamine (Rho). Thus the ratio of Rho/GFP fluorescence allows us to directly estimate a % of cellular VPAC1 receptors present in the plasma membrane and hence the % of their internalization. By this method, we showed that VIP (0.1-50 nM) had a dose-dependent effect on the internalization of wild type VPAC1 receptors, in agreement with results obtained by receptor binding studies. When VIP (50 nM) was incubated for 12 h with CHO cells expressing wild type VPAC1 receptors, it induced receptor internalization by 90% as compared to cells incubated in absence of VIP. Similar results as above were obtained with all the VPAC1 mutants. We next evaluated how VIP induced desensitization of wild type and mutant VPAC1 receptors. The corresponding CHO cells were incubated overnight at 37°C in presence and in absence of 50 nM VIP. Then, the cells were challenged with increasing concentrations of VIP and the amount of cellular cAMP was measured. With all mutants and wild type VPAC1 receptors there was a 100-fold rightwards shift in the dose-response curves when cells were pretreated with VIP versus control cells. Thus, VPAC1 receptors are desensitized and internalized independently of phosphorylation at PKC and PKA consensus sites. The role of GRK phosphorylation sites in these processes is currently under investigation.

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INHIBITION OF VASOPRESSIN BINDING BY C-TERMINAL PHOSPHORYLATED PEPTIDE SEGMENT OF Gαq/α11 GTP-BINDING PROTEIN

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αq/α11 G proteins are known to couple V1a vasopressin receptor to phospholipase C β (PLC). Recently[1], it has been shown that C-terminal tyrosine phosphorylation of Gαq/Gα11 could occur during hormonal activation and be important for transduction mechanisms. To study the importance of G protein phosphorylation, we prepared synthetic peptides mimicking the C-terminal part of the αq/α11 G protein either in its native form (unphosphorylated) or phosphorylated on the tyrosine residue in position – 4 (αq, αq-P) and tested their activities.

Added on WRK1 cells, genisteine, a tyrosine kinase inhibitor, reduced by 42 % the vasopressin-stimulated inositol phosphate accumulation, showing the importance of tyrosine phosphorylation in these processes. αq and αq-P peptides, added to membrane preparations expressing V1a vasopressin-receptors completely inhibited [^{3}H]AVP specific binding with IC_{50} values of 6 and 40 μM, respectively. Such inhibitions were non competitive since αq and αq-P peptides reduced the affinity of [^{3}H]AVP for its receptor without affecting its maximal binding capacity. As a control, we showed that αs and αs-P peptides (the C-terminal part of αs G-protein which couples V2 vasopressin receptor to adenyl cyclase) were inefficient.

We also tested the abilities of these peptides to modulate GTPγ-S sensitive PLC activity. Added to WRK1 membranes, αq-P and, to a lesser extent, αq dose-dependently inhibited GTPγ-S stimulated PLC activities (K_i = 11 and 18 μM, respectively).

Such results show that both αq and αq-P peptides interact with the V1a receptor and PLC. As αq was more efficient in inhibiting [^{3}H]AVP specific binding and αq-P in inhibiting PLC activity. We can suggest that phosphorylation of αq/α11 G proteins induce the dissociation of the hormone/receptor/G-protein complex, which release the active form of αq/α11 G proteins able to interact with PLC.

[1] H. Umemori et *al.*, *Science*, 1997, **276**, 1878-1881.

LIPOPHILIC MODIFICATIONS OF BRADYKININ AGONISTS

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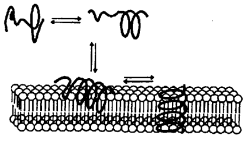
The model represented in the figure [L. Moroder *et al.*, *Pure & Appl. Chem.*, (1994), *66*, 2111-2114; R. Schwyzer, *Biopolymers* (1991) *31*, 785-792] hypothesizes a catalytic role of the membrane with respect to the peptide-receptor interaction. Accordingly, the lipophilic moiety of a lipopeptide drives its interaction with the plasma membrane, increasing the possibility of recognition by the membrane receptors. Based on this hypothesis, we synthesized a series of lipophilic peptides derived from bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (KD, Lys-BK), two peptide hormones recognized by the kinin B2 receptors. Different lipophilic moieties were N-terminally added to these peptides: palmitic acid (Pam), 2-aminohexadecanoic acid (Ahd, a large scale synthesis of the two enantiomers protected for Fmoc/tBu strategy was previously set up by our group) and the less flexible C^{α}-tetrasubstituted amino acids 2-amino-2-methyloctanoic acid (αMe)Aoc and 2-amino-2-methylundecanoic acid (αMe)Aun. Moreover, linear alkyl spacers of increasing length [Gly, β-Ala, ω-aminopentanoic acid (ωApe) and ω-aminoundecanoic acid (ωAun)] were introduced between the lipophilic moiety and the peptide sequence in order to separate the modified region from the active part of the agonist. Binding tests on Chinese hamster ovary cells transfected with the human kinin B2 receptor show that (αMe)Aoc-KD retains the highest affinity for the kinin B2 receptor (pK_i = 8.1, in comparison with BK, pK_i = 9.45). A good affinity was also shown by the peptide Pam-ωAun-KD (pK_i = 7.8). These studies could find an important application in the recently investigated role of BK in the development of small cell lung cancer. Our approach will be extended to the synthesis of lipopeptide BK antagonists as new anti-inflammatory and anti-cancer drugs.



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An Analogue of the Glycine-Extended Bombesin Induces its Biological Activity by Interacting with the GRP/Bombesin Receptor

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1- Alpha-amidation of a peptide is required to produce biologically active amidated hormones, such as gastrin releasing peptide/bombesin (GRP/BN). Such amidation takes place from their glycine-extended precursors. This terminal post-translational processing step was thought to be essential to obtain biological actions of most amidated peptide hormones.

2- It was shown that glycine-extended gastrin mediates mitogenic effects on various cell lines by interacting with a specific receptor, different from the classical CCK-A or CCK-B receptors. On the basis of this observation, we have extended the concept of obtaining active glycine-extended forms of others amidated peptides to produce new active analogues.

3- In this study, we have tested the biological behavior of an analogue of the glycine-extended bombesin (JMV-1458) on various *in vitro* models.

4- We showed that compound JMV-1458 was able to inhibit specific [^{125}I]-GRP binding in rat pancreatic acini and in Swiss 3T3 cells with Ki values of approximately 10 nM. In isolated rat pancreatic acini, we found that JMV-1458 induced inositol phosphates production and amylase secretion in a dose-dependent manner with EC_{50} values respectively of 46 ± 38 nM and 0.5 ± 0.7 nM. In Swiss 3T3 cells, the glycine-extended bombesin analogue dose-dependently produced [^{3}H] thymidine incorporation with an EC_{50} value of 3.0 ± 1.7 nM.

5- By using potent GRP/BN receptor antagonists, we showed that this glycine-extended bombesin analogue induces its biological activities *via* the classical GRP/BN receptor.

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CHARACTERIZATION OF NON CCK-A, NON CCK-B BINDING SITES USING THE C-TERMINAL HEPTAPEPTIDE OF GASTRIN (G-7) ON HUMAN ASTROCYTIC TUMORAL CELL LINES

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Gastrin and other neuropeptides are able to bind to their own receptors through their amidated C-terminal part. Over the last years, authors have hypothesized the presence of new gastrin binding sites, using modified peptides related to gastrin on gastrointestinal cell lines. No study has been carried out on brain, where Gastrin and Gastrin precursors are co-localized. Astrocytic tumor cell line U373 responds to gastrin in term of biological activity. However, using RT-PCR and binding studies we have shown that U373 cells do not possess any classical Gastrin receptors. We have tested a number of peptides related to Gastrin for their ability to present specific binding. We have shown that the C-terminal heptapeptide of Gastrin (G-7) was able to bind U373 cells with an IC_{50} value of 0.2 μM. This specific binding was not inhibited by neither CCK-A nor CCK-B/Gastrin receptors agonists or antagonists. Structure-activity relationships and second messenger studies have been carried out on this new binding site. Moreover, on this cell line we showed that G-7 was able to inhibit cell motility in a dose dependant manner.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW h/rCRH PEPTIDE ANALOGS

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Human/rat Corticotropin Releasing Hormone (h/rCRH) is a hypothalamic neuropeptide, acting as the principal neuroregulator of the secretion of adrenocorticotropic hormone (ACTH), β-endorphin and other proopiomelanocortin products of the anterior pituitary gland. Due to the role it plays in the endocrine, autonomic, immunological and behavioural responses of mammalian organisms to stress, the synthesis of analogs and the study of their properties is very important and could lead to the development of potent antagonists of the hormone. Towards this direction, a series of synthetic peptide analogs of h/r CRF is synthesised via Fmoc methodology on Solid Phase Peptide Synthesis. On these analogs, namely the [D-Phe^{12}, Cys(S-Et)^{21}] CRH, [D-Phe^{12}, Cys(S-Pr)^{21}] CRH, [D-Phe^{12}, Cys(S-Pr)^{38}] CRH, [D-Phe^{12}, Aib^{14}] CRH and [D-Phe^{12}, Aib^{15}] CRH, minor modifications were induced concerning the hydrophobic character of the side chains of aminoacids in various points on the hormone's primary structure. A novel protocoll was applied for the synthesis enabling us to both shorten the total duration of the synthetic procedure and reduce the cost of synthesis, preserving at the same time the yield and purity of the products at extremely high levels. The peptides underwent a series of analytical procedures aiming to their purification, namely Size Exclusion Chromatography, High Performance Liquid Chromatography and Mass Spectroscopy. We have also tested the biological activity of the above CRH analogs by comparing their effect on the release of catecholamines from the PC12 rat pheochromocytoma cell line to that of authentic synthetic CRH. At least two of the above analogs were found to be devoid of any agonist CRH-like activity and are now under investigation for antagonist activity against synthetic CRH.

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The Third Intracellular Loop of the Rat and Mouse Cholecystokinin-A Receptors is Responsible for Different Patterns of Gene Activation

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It was previously reported that the cholecystokinin analogue JMV-180 behaves differently on the rat and the mouse cholecystokinin-A receptor (CCK-AR). In mice this anologue acts as an agonist on low- and high-affinity sites of the CCK-AR while in rats this compound acts as an agonist on high-affinity sites and as an antagonist on low-affinity sites. In an attempt to understand why the same compound behaves differently on these two CCK-A receptors, we cloned the cDNA encoding the mouse CCK-AR and compared it with the rat CCK-AR. We then investigated a cellular model able to mimic the effect that was observed in rats and mice. HeLa cells were transiently cotransfected with plasmids leading to expression of the rat or mouse CCK-AR in the presence of pFos-Luc as reporter plasmid. Such a plasmid placed the regulatory part of the human c-Fos gene upstream from the firefly luciferase structural gene (Luc) ; we observed that the two CCK-A receptors behaved differently not only in the presence of compound JMV-180 but also in the presence of cholecystokinin or even in absence of ligand. Such a result was confirmed in the same kind of experiment with the reporter plasmid p(TRE)$_3$-tk-Luc. Experiments performed with these reporter genes revealed that rat CCK-AR was two to three times more potent than the mouse CCK-AR in inducing the reporter protein whatever the ligand studied (JMV-180 or cholecystokinin, or even in absence of ligand). In the same kind of cotransfection experiments, we showed that the third intracellular loop of these receptors is responsible for this differential behavior.

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BIOSENSOR ANALYSIS FOR MAPPING THE DISCONTINUOUS INTERLEUKIN-10 / INTERLEUKIN-10 RECEPTOR α BINDING SITE

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In the present study we used mixtures of overlapping soluble peptides spanning the interleukin-10 (IL-10) sequence to identify the regions of IL-10 which bind with high affinity to the interleukin-10 receptor α chain (IL-10Rα). The peptides were prepared by highly parallel automatic nano-synthesis of cleavable peptides on cellulose membranes. The interactions between the soluble IL-10-derived peptides and the receptor were measured by the inhibition of IL-10Rα binding to immobilized IL-10 using BIACORE inhibition in solution assay.

In the assay IL-10 was immobilized to the sensor surface. IL-10Rα at a constant concentration was preincubated with 15 different peptide mixtures to be screened. Each mixture contained 10 overlapping 15mer IL-10-derived peptides in order of the IL-10 scan. The receptor peptide mixture complex was passed over the sensor surface. The signal measured in the BIACORE sensogram reflected the concentration of free IL-10Rα. If one ore more peptides had blocked the specific binding site for IL-10 on the receptor the signal dropped.

We identified three binding regions of the discontinuous IL-10Rα binding site on IL-10. One corresponds to helix A and the AB loop, the second to helix C and the third region is located in Helix F. These results are in agreement with earlier results obtained from molecular modelling of the IL-10/IL-10Rα complex and peptide scanning techniques using solid phase-bound peptide arrays.

FUNCTIONAL RESPONSES TO KININS IN A NOVEL BOVINE HEPATIC ARTERY CELL LINE

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Receptors for bradykinin (BK) and related peptides have typically been characterised by agonist affinity studies in isolated tissue preparations and ligand binding studies. Here, we present evidence for a novel cultured cell model for the study of functional responses to co-expressed B1 and B2 receptors. Bovine hepatic artery endothelial cells(BHAEC's) were screened using a range of G protein-coupled receptor ligands. BK stimulated inositol phosphate turnover with a log EC_{50} value of –8.83 +/- 0.24, and this was abolished by the B2 selective antagonist Hoe 140 (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK), but was unaffected by the B1 antagonist Des arg^9 Hoe 140. The B2 receptor displayed low affinity to Aib^{7}BK, log EC_{50} value –6.49 +/- 0.18, suggesting the presence of the B2a subtype, as previously reported in bovine kidney membranes (Howl *et al.*, 1996). The B1 selective ligands Des arg^{9}BK and Des arg^{10} kallidin were also able to mediate turnover of inositol phosphates with log EC_{50} values of -7.15 +/- 0.16 and –9.25 +/- 0.13 respectively, suggesting that B1 receptors are also present on these cells. However, the B1 receptor antagonist Des arg^{9}Hoe 140 only marginally shifted responses of both agonists to the right, log EC_{50} –7.20 +/- 0.17 vs. –6.57 +/- 0.57 and –9.25 +/- 0.13 vs. –8.69 +/- 0.16 for Des arg^9 BK and Des arg^{10} kallidin in the presence and absence of Des arg^9 Hoe140 respectively. Thus, in BHAEC's, Des arg^9 Hoe 140 does not appear to be an effective antagonist demonstrating non classical B1 pharmacology, and consistent with observations made in other bovine tissue (Wohlfart *et al.*, 1997). Further studies revealed the ability of both BK receptor subtypes to stimulate nitric oxide and prostacyclin in these cells These data demonstrate the usefulness of BHAEC's as a convenient functional model for the study of BK pharmacology.

Howl, J., Yarwood, N.J., Davies, A.R.L., Wheatley, M (1996),. Kidney International, 50:586-592.

Wohlfart, P., Dedio, J., Wirth, K., Scholkens, B.A., Wiemer, G. 1997. J Pharm. Exp. Ther. 280: 1109-1116.

Posters: topic C16

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EFFECT OF A DAUNOMYCIN-POLYPEPTIDE CONJUGATE IN MULTIDRUG RESISTANT TUMOR CELL LINES

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Anthracyclines, like daunomycin (Dau) are widely used in the therapy of cancer. Side effects (e.g. immunosuppression, cardiotoxicity) and multidrug resistance developed during the treatment, seriously limit the therapeutic efficiency. Earlier results have demonstrated that coupling of daunomycin with an acid labile spacer (cAD) to an amphoteric branched chain polypeptide, EAK, significantly increased the survival of L1210 bearing mice [1].
In human tumor cells multidrug resistance is often associated with overexpression of two ATP-dependent transport proteins, the 170 kDa P-glycoprotein (MDR1) and the 190 kDa multidrug resistance-associated protein (MRP1). As daunomycin is the substrate of these two membrane proteins, we have compared the uptake and effect of daunomycin with those of the cAD-EAK conjugate in HL-60/sensitive, as well as in HL-60/MDR1 and HL-60/MRP1 cell lines. The drug resistant cell lines showed decreased daunomycin uptake and sensitivity. In contrast, the cAD-EAK conjugate accumulated and was effective not only in the sensitive but also in the drug resistant cell lines.
Since free daunomycin and its polypeptide conjugate may enter the tumor cells via different routes, we have studied drug uptake as a function of temperature and also in the presence of various endocytosis inhibitors (nigericin, a protonophore; and colchicine, a microtubular inhibitor). In addition, we have examined the uptake of free daunomycin and the cAD-EAK conjugate in ATP-depleted cells. Our data suggest that the conjugate enters the resistant as well as the sensitive cells by an endocytotic mechanism, bypassing the multidrug transporters.

This work has been supported by grants from Hungarian Research Fund (OTKA N° T03838) and from Ministry of Health (N° T115/1996).

[1] Gaál, D., Hudecz, F.: Eur. J. Cancer, 34, 155-161 (1998).

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ANALOGS OF [L-(pEt)PHE^2] OR [D-(pEt)PHE^2]OXYTOCIN HAVING AN α-HELIX INDUCING AMINO ACID Aib OR β-Ala IN POSITION 3 OR 7 OR 9

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Nine new analogues of oxytocin were synthesized, i.e. D and L diastereoisomers in position 2 of d[(pEt)Phe^2,Aib^3]oxytocin (I), d[(pEt)Phe^2,Aib^7]oxytocin (II), d[(pEt)Phe^2,Aib^9]oxytocin (III), d[(pEt)Phe^2,β-Ala^3]oxytocin (IV), and [Aib^7]oxytocin (V). The analogues were synthesized using solid phase methodology on a 2-chloro-trityl resin bearing a Rink-Bernatowitz linker and Fmoc/^{t}Bu chemistry. The coupling was performed upon HOBt/DIC activation. The cyclization was performed in DMSO/H_2O (2:8v/v) for 36-48h. Mixtures of L- and D-[(pEt)Phe^2] diastereoisomers were obtained. The final separation of individual diastereoisomers was then performed on the RP HPLC (VYDAC 218TP510, C18 semipreparative column), in water-TFA / 80%acetonitrile-TFA under isocratic conditions running for 20 min.
The analogs were tested for their biological potency in 2 pharmacological tests, i.e. uterotonic *in vitro* test in the absence and in the presence of magnesium ions and in the pressor test. The presence of Aib amino acid in position 7 of oxytocin (analogue V) decreased its agonistic activities for about 2 orders of magnitude. All the (pEt)Phe^2 analogs showed to be strong inhibitors of the uterotonic action of oxytocin with the exception of β-Ala^3 substituted analogs which showed to be rather low antagonists (pA_2 around 6). The L-diastereoisomers showed usually about one order of magnitude lower inhibitory potency than the D-counterparts with the exception of the Aib^9 analog (III) where both diastereoisomers and even their mixture displayed the same high inhibitory potency (pA_2=8.2).
We can conclude that in the case of the antagonist, the Aib substitution in any of the 3 positions doesn't significantly reduced the inhibitory potency of the analogs studied, the β-Ala^3 substitution on the other hand has deteriorating effect on the inhibitory potency. The Aib substitution has thus different effect on the activity in the case of the agonists and antagonists.
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STRUCTURAL MODIFICATIONS OF HIGHLY POTENT BRADYKININ ANTAGONISTS AND THEIR PHARMACOLOGICAL CONSEQUENCES

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The first peptide bradykinin (BK) B2 receptor antagonist, NPC-349 (DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DPhe-Thi-Arg) (Thi: β-2-thienylalanine) was developed by Stewart and Vavrek in 1984. The second generation antagonists began with Hoechst HOE-140, in which DPhe was replaced by its ringclosure analog D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) at position seven, and it was modified further at position eight with octahydroindole-2-carboxylic acid (Oic). In 1995 the third generation BK antagonist B9430 (DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg) was developed containing the novel amino acid α-2-indanylglycine (Igl). Modification with 2,3,4,5,6-pentafluorophenylalanine (F5F) and with N-cycloheptylglycine (Nc7G) increased the potency, and B10206 (DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-Nc7G-Arg) has 5-10 times higher BK antagonist activity than B9430. These very powerful antagonists, their derivatives and their mimetics have special pharmacological activity. B9430 is a potent general antiinflammatory agent and decreases the secretion of proinflammatory cytokines after spinal cord injury in mice. These BK antagonists that were very potent for inhibiting BK-evoked intracellular Ca^{++} flux failed to inhibit growth of human lung cancer cells even at high concentrations. When these peptides were acylated or dimerized at the N-terminal they became selectively cytotoxic for lung cancer cells *in vitro* and *in vivo*. We have synthesized and evaluated many shorter chain analogs and other bradykinin peptido-mimetics. In many of these new compounds there is no correlation between anti-bradykinin and cytolytic activity. Several of these newly developed compounds impressively inhibit growth of lung cancer and kill cancer cells by apoptosis. In addition, some of them also inhibit the growth of prostate cancer cell lines. These compounds may have opened a new route for the development of highly potent anti-cancer agents for treatment of human lung or prostate cancer and of new drugs for spinal cord injury.

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STUDIES OF THE INTERACTION BETWEEN TAT ARGININE RICH DOMAIN PEPTIDES AND TAR RNA HIV-1 BY CAPILLARY ELECTROPHORESIS

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Tat, a regulatory protein, is necessary for HIV-1 virus transcription. Tat binds TAR RNA structure, which is present on the 5'-end of all viral mRNA transcripts. The binding causes a 10- to 1000-fold increase in viral transcription and translation level. Arginine rich domain (ARD) directly binds TAR. Single arginine52 (Arg52) residue is the main source of specificity. Detailed mechanism of the Tat-TAR interaction is still unknown. TAR-Tat complex belongs to the group of the most extensively investigated ones.

The aim of the research project was to investigate the interaction of 27-nucleotide TAR RNA with synthetic Tat peptides using capillary electrophoresis (CE). CE experiments were performed using coating capillary and buffer containing physical gel. A native ARD fragment Tat(49-57) and its analogues substituted in position 52 with D-arginine, L-citruline, L-ornithine, N^G,N^G–dimethyl-L-arginine, N^G–nitro-L-arginine and L-homoarginine were studied. Tat peptides were obtained by the solid phase peptide synthesis using Fmoc-technique and purified by HPLC. All modifications of guanidine group of Arg52 decreased or completely abolished TAR binding. Only the L-Arg → D-Arg substitution seems to cause a slight increase in the peptide binding affinity. We observed specific interactions and complex formation for the native ARD peptide and its analogue even in molar ratio 1:1 (TAR RNA : peptide). The analogue with substitution D-Arg52 would serve as a potential inhibitor of viral transcription. Analysis of the native ARD sequence – TAR interaction in the presence of calcium ions shows that these ions bind TAR. Therefore, the presence of the calcium ion may be an important factor of TAR-Tat complex structure. This suggests that calcium ions should be taken into account when studying the TAR-Tat interaction.

CE analysis of the interaction between Tat(49-57) and equimolar mixture of native 27-nucleotide TAR and its analogue, containing single mutation in bulge (U23→C), indicates that ARD recognises only native TAR RNA. Results have demonstrated that CE is a useful and fast technique to detect and assess binding specificity and affinity of peptides to TAR RNA.

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PHARMACOLOGICAL STUDIES OF PHOTOLABILE LIGANDS DERIVED FROM TTA-386, A SELECTIVE ET_A RECEPTOR ANTAGONIST.

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Actions of endothelin (ET) are usually mediated through the so-called ET_A or ET_B receptors. As part of our ongoing research program, we pursue the characterization of the ET_A receptor using specific photolabile ligands. Starting with the ET_A specific antagonist TTA-386 (hexamethyleneimino carbonyl-Leu-Trp-Ala-βAla-Tyr-Phe), as a leading compound, we developed new ET_A specific antagonists containing the photolabile amino acid derivative, p-benzoyl-phenylalanine (Bpa). Bpa residue was introduced at positions 1,2 or 6 of the peptide with either L- or D-isomer. Pharmacological studies on rat aorta (ET_A receptor preparation) demonstrated that only [D-Bpa^6]TTA-386 and [L-Bpa^6]TTA-386 are analogues showing antagonistic properties with pA2 of 7.7 and 7.5, respectively as compared to 7.9 for TTA-386. No agonistic nor antagonistic properties were measured with these peptides in a ET_B pharmacological preparation (guinea pig lung parenchyma) suggesting that these two photoprobes are specific for ET_A receptors. Thus, these new ligands appear as promising probes for the biochemical characterization of the ET_A receptor using photolabeling techniques.

THE "PYY-PREFERRING" RECEPTOR IN RAT JEJUNAL CRYPT CELLS : A PERIPHERAL Y2 RECEPTOR ?

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Peptide YY and neuropeptide Y have potent antisecretory effects in rat small intestine. Scatchard analysis of ^{125}I-PYY binding revealed a 10-fold higher concentration of a PYY-preferring receptor in rat jejunal crypt cells than in villus cells and no detectable receptors in colonic epithelium. Reverse transcription polymerase chain reaction analysis of all cloned Y receptor (Y1, Y2, Y4 and Y5) mRNA indicated that Y2- and Y5-subtype are expressed in crypt cells. A recent study showed that cryptic PYY receptor and Y5 receptor present very different pharmacological profiles supporting that the intestinal PYY receptor is not a Y5 receptor. In order to determine whether the Y2 receptor could represent the intestinal crypt PYY receptor, the ability of PYY, NPY, pancreatic polypeptide and synthetic analogues to inhibit ^{125}I-PYY binding to membrane prepared from rat crypt cells and CHO cells stably transfected with the rat Y2 receptor cDNA was tested. It appeared that all natural peptides and truncated analogues displayed the same inhibition constants (K_i) in the two binding assays. The previously reported Y2 receptor-specific peptides (PYY(3-36), NPY(3-36), N-α-AC-PYY(22-36) and C2-NPY) were potent agonists in the rat crypt cells and for the recombinant rat Y2 receptor as well. PYY inhibited cAMP production in crypt cells and Y2 receptor-expressing CHO cells with the same EC_{50} values. It is worth pointing out that N-α-AC-PYY(22-36) was very potent in inhibiting cAMP production in both crypt cells and Y2-receptor transfected CHO cells. The new selective non-peptide Y2 antagonist, BIIE0246, displaced ^{125}I-PYY binding to Y2 expressing CHO membranes with high affinity (IC_{50} = 1.5 nM) and abolished the PYY inhibitory effect of cAMP production on Y2 expressing CHO cells. BIIE0246 displayed the same antagonistic properties on "PYY-preferring" receptors expressed in jejunal crypt cells. These data support that the intestinal crypt PYY-preferring receptor is most probably a peripheral Y2 receptor.