P C93 - *In vivo* activity of some nociceptin analogues containing mercapto acids.

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Nociceptin/orphanin FQ is an endogenous neuropeptide for opioid receptor OP4 [1]. Its structure (FGGFTGARKSARKLANQ) resembles to some extend other opioids. Although nociceptin is involved in pain transmission its molecular mechanism of is still far from being understood [2]. Nociceptin has pain potentiating properties (an adverse effect to morphine) and analgesic spinally.
We have previously shown that the Cys^1-NC-(1-13)-NH_2 may have antagonistic properties, [3] whereas Cys^5 or Cys^7 analogues are agonists.
The Cys^1 analogue was designed in order to bind irreversible to nociceptin via the formation of an S-S bridge with the receptor.
Continuing our efforts we have synthesized and tested *in vivo* further analogues. To better understand the physiological role of nociceptin and its mechanism of action we have introduced mercapto group near the N-terminal fragment of the peptide in so-called message domain.
The analogues contain at position 1 of the peptide chain thioglicolic acid, mercaptopropionic acid, D or L-Pen, D-Cys.
We will show and discuss the results of *in vivo* study on mice of the analogues.

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P C94 - Anorectic and antiobesic properties of a new CCKA-agonist in rodents

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The anorectic properties of CCK have first been described in 1973. This property has been proven in rodents, man, monkey and dog. Recently highly effective, orally active CCKA- agonists have been developed. However, one have to keep in mind that CCK has numerous physiological functions on gut and gallbladder motility and on pancreatic secretion.
The acute and chronic anorectic effects of a CCKA-agonist (SR146131) in rats was measured continuously using an 'on line' device, measuring feed consumption for 24 h. The acute anorectic effect in mice was determined by measuring milk consumption. In the chronic rat experiment the drug was given for 10 days and the body weight of the animals was measured daily.
In female Wistar rats, SR146131 showed a dose-dependent effect with an ID_{50} of 0.15 mg/kg po. The onset of the anorectic effect was rapid and the effect of a single oral dose lasted for 24 h. In male NMRI mice, milk consumption was also dose-dependently inhibited; the ID_{50} was 2.7 mg/kg po, being much weaker, than the effect observed in rats. In the 10 days rat study the compound was given at a daily dose of 1.5 mg/kg po. On the first day a 70 % reduction of feed consumption was seen. While in the following days the anorectic effect of the compound faded out rapidly and disappeared completely at day 8. At the beginning of the study, body weight of controls and drug-treated rats was 285±16 g (N=16). In the first 2-3 days of treatment body weight of the drug-treated animals decreased by ~3 %. While at the end of the study, body weight of controls and drug-treated animals was identical 279±14 g, which could be expected from the lack of the anorectic activity. At autopsy serum lipase was significantly elevated by 62 %. Pancreatic weight rose from 540±7 mg in controls to 3,640±1,150 mg in drug-treated rats, which corresponds to an increase of 570 % ($p<0.001$).

P C95 - Human Coupling Factor-6 prescursor (55-108)-NH_2 is the active fragment of Coupling Factor-6

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Coupling Factor-6 (CF-6) is a polypeptide with 76 amino acids (Coupling Factor-6 precursor 33-108) from the ATP synthase coupling factor-6, Mitochondrial precursor protein. Recently, rat recombinant CF-6 was reported [1] as a potent endogenous vasoconstrictor.
The total synthesis by solid-phase method and purification of human CF-6 (^{33}NKELDPIQKLFVDKIREYKSKR^{55}QTSGGPVDASSEYQQELERELFKLKQM FGNADMNTFPTFKFEDPKFEVIEKPQA^{108}-NH_2)
and its possibly enzymatic`cleavage C-terminal fragment (pCF-6) pGlu^{55}-CF-6 precursor (55-108)-NH_2 were performed and accomplished. Purification of the HF cleavage peptides were carried out by ion-exchange and HPLC to afford the pure peptide with approximately 5% yield based on the crude peptides. Corrected molecular ion by mass spectra were also obtained (8959.18 for hCF-6 and 6211.59 for pCF-6). After prazosin injection, the mean MAP and HR in urethane-anesthetized rats (n=10) were increased by human CF-6 and pCF-6 (from 0.3μg/kg to 10μg/kg, iv) in a dose-dependent response manner. Statistical analyses showed that both peptides significantly increased HR when compared to saline, but there were no significant differences between these two peptides. On the other hand, there were some differences between the human CF-6 and pCF-6 on MAP. For example, human pCF-6 seemed to have a stronger effect on MAP than CF-6. In conclusion, the C-terminal fragment of human CF-6 is the active site of the whole protein.

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P C96 - Fluorescent form of the oxytocin inhibitor Atosiban.

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In order to prepare fluorescently labelled oxytocin and vasopressin analogues suitable for studies at molecular level it is necessary to choose the right analogues and use appropriate labels. Fluorescein labelling has been described recently [1]. When we tried labelling oxytocin and vasopressin with fluorescamine, we found that the resulting compounds were not sufficiently stable (Barth, unpublished). We described the preparation of basic amino acids fluorescently labelled on their side chain by the NBD reagent (7-chloro-4-nitrobenzo-2-oxo-1,3 diazol) [2]; the modified amino acids were then used in the construction of the peptide chain. In this contribution we describe the labelling of a peptide with a free NH_2-group of in the side chain of ornithine by the direct action of NBC. We chose the peptide Atosiban [3],

Mpa-D-Tyr(OEt)-Ile-Thr-Asn-Cys-Pro-Orn-GlyNH_2

one of the most potent oxytocin inhibitors, which has a free δ-amino group in the side chain of ornithine. Atosiban was repeatedly exposed to the action of NBD in a mildly alkaline medium. After 30 hours the reaction mixture was diluted with water and acidified, the product was isolated by preparative HPLC (Vydac 218 TP 510, 25 x 1 cm, 1 - 80% ACN gradient in 0.1% TFA) and freeze-dried. The t_R value of modified Atosiban was 14.22 min, of free Atosiban 10.82 and NBD 15.75. The product was characterized by MS an CZE. Its excitation maximum was at 480 nm and maximum emission at 520 nm.

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P C97 - Synthesis and stuctural studies of new analogues of the decapeptide luteinizing hormone–releasing hormone (LHRH)

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The hypothalamic decapeptide luteinizing hormone–releasing hormone (LHRH) (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) plays a key role in the control of mammalian reproduction. It is secreted in pulses from the hypothalamus and stimulates the anterior pituitary gland to release the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Oncological uses of LHRH analogues are based on the inhibition of the pituitary gonadal function, the suppression of gonadal steroid hormone secretion leading to inhibition of the growth of sex hormone dependent tumours. Agonistic analogues of LHRH, represented by Leuprolide ([DLeu6, desGly10]-LHRH-ethylamide), have been widely used in oncology and gynaecology for nearly two decades. In this study, we report the synthesis of new analogues of the LHRH. Key considerations in the synthesis of the LHRH analogues were on the basis that the bioactive conformation of LHRH includes a β-turn involving residues 5-8 {Tyr5-Gly6-Leu7-Arg8}. 1D and 2D NMR experiment for one of the synthesized analogues, [Aib6, desGly10]-LHRH ethylamide, were carried out in 2-2.5mM solution of DMSO-d6. The 234 NOESY peaks were transformed into upper distance limits and used as structural constrains in calculations. 300 random structures were generated using simulated annealing protocols and the best 20 structures, in terms of target function, were collected. The average structure has been calculated from the family of 20 refined structures and restrained energy minimization (REM) has been also carried out. The skeleton of the peptide, as calculated using NMR data, was found to form a β-turn at the region covered by residues Ser4 up to Arg8. Moreover, detected signals between the amide protons of the Trp3 and the terminal ethylamide clearly indicate that the two termini of the peptide are in close proximity. Thus, the whole molecule is characterised by a U-shape tertiary structure, while the Aib residue found at the middle of the peptide chain characterises the turn of the peptide backbone (Figure 1). Synthesized analogues are currently tested for their biological activity and binding affinity on the LHRH receptor. The present conformational studies are expected to provide the structural basis for the development of new synthetic analogues (agonists and/or antagonists) through rational modification of the physical properties, such as hydrophobic/hydrophilic character, number and position of charged residues. Synthesis of new analogues varying in positions 6, 8 and 10 is currently under way in our laboratory.

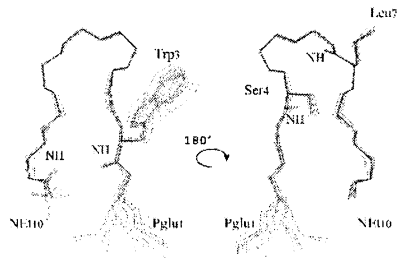

Fig. 1 - Backbone of the best 20 structures of [Aib6, desGly10]-LHRH-ethylamide. Side chains of Trp3, NEt10 (left) and Ser4, Leu7 (right) are also shown.

P C98 - Pitfalls of neurotoxicity investigation of β-amyloid 1-42 peptide in vitro and in vivo

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β-amyloid (Aβ) peptides play important role in the development of Alzheimer`s disease. The neurotoxic effect of Aβ 1-42 can be measured with in vitro and in vivo methods. Aβ 1-42 has very high tendency for aggregation forming diffuse aggregates and fibrils. The aggregated Aβ 1-42 has a very poor solubility in pure water or in aqueous buffers and cell culture media. As a consequence, many research grups use organic solvents (e. g. DMSO, fluorinated alcohols, etc.) for dissolving Aβ 1-42, both for in vitro and in vivo experiments.
The neurotoxic effect of Aβ 1-42 can be measured very easily by an in vitro spectrofotometric assay [3-(4.5-dimethylthiazol-2-yl)-2.5 diphenyltetrazolium bromide, MTT assay]. We have applied MTT assay using SH-SY5Y neuroblastoma cells for Aβ-neurotoxicity measurement. We have found, that the presence of 1 % or 0.1 % organic solvent in the cell culture media cause a dramatic change in formasane formation in the MTT test. Very low concentration of organic solvents, give false results, decreasing the mitochondrial activity in the neurons. According to the literature, the Aβ-cell membrane interaction plays crucial role in the neurotoxicity: A peptides cause a long-lasting disturbance in the cell membrane structure and signalization. In vitro application of organic solvents interfere with this effect and causes false results (the organic solvents themselves are "neurotoxic"). Fortunately, Aβ 1-42 lyophilized from trifluorethanol has lower aggregation grade; it can be directly dissolved in cell culture media without addition of any organic solvent. Ultrasonation helps in dissolving Aβ 1-42 in aqueous solutions. The same method of dissolving process ought to be used also for in vivo experiments with Aβ 1-42. Although the DMSO-content of the probe will be diluted after in volume administration, however, local injection of organic solvent containing samples into the brain causes false results disturbing neuron membranes.

P C99 - New analogues of arginine vasopressin with prolonged inhibition of vasopressor responses to this hormone.

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It is believed that the tyrosine residue at position 2 seems to play a role in the initiation of the pressor response. Recently, we described the synthesis and some pharmacological properties of arginine vasopressin (AVP) analogues having L-1-naphthylalanine (L-1-Nal) or its D enantiomer in position 2. Two of the peptides, [Mpa[1], (L-1-Nal)[2]]AVP and , [Mpa[1], (D-1-Nal)[2]]AVP (where: Mpa = 3-mercaptopropionic acid) which appeared not to interact with V_{1A} and V_2 receptors were exceptionally selective oxytocin antagonists in vitro. These results prompted us to investigate further the influence of naphthylalanine on pharmacological properties of AVP analogues. We synthesized and evaluated the biological activity of the two peptides: [Cpa[1],(L-1-Nal)[2]]AVP and [Cpa[1],(D-1-Nal)[2]]AVP (where: Cpa = 1-mercaptocyclohexaneacetic acid). Both peptides were highly potent V_{1A} antagonists. In addition the first analogue exhibits a protracted antivasopressor effect greater than any we have previously observed (approximately 3 hours). The second compound also shows prolonged inhibition of vasopressor responses by rats to AVP. However, this effect lasts slightly shorter (approximately 2 hours). With their potent ability to antagonize the vasopressor effects of AVP, both peptides should have potential for use as pharmacological tools with which to probe the possible role of AVP in cardiovascular regulation in normal and pathophysiological states.

Table 1. Some pharmacological properties of new analogues (IU/mg or pA2). For comparison the values of the peptides synthesized previously by others are also given.

Compound	Activity	
	Pressor	Antidiuretic
[Cpa[1],(D-Phe)[2], Ile[4]]AVP[a]	pA_2 = 7.86[a], (7.42[b])	pA_2 = 8.24[a]
[Cpa[1],(Tyr(Me))[2]]AVP[a]	pA_2 = 8.35[a]	0.33
[Cpa[1],(L-1-Nal)[2]]AVP	pA_2 = 7.98	$3x10^{-3}$
[Cpa[1],(D-1-Nal)[2]]AVP	pA_2 = 8.50 (preliminary result)	not active

[a] *Data for these peptides were published previously by others elsewhere.*
[b] *Data in parentheses were obtained by us in the conditions of our test.*

P C100 - Pituitary adenylate cyclase activating polypeptide inhibited the β-amyloid-induced neurotoxicity and activation of caspase-3

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The neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP) modulates neurotransmission in the central and peripheral nervous systems. *In vitro* and *in vivo* studies have shown the potent protective effects of PACAP against neuronal damage induced by ischemia and the agonists of glutamate receptors, including N-methyl-D-aspartate (NMDA), kainate and glutamate. Vasoactive intestinal peptide (VIP), a closely PACAP-related neuropeptide in terms of sequence, the solution structure and physiological functions, also shows the potent neuroprotective effect against the above neuronal stimulants. They are members of the VIP/secretin/glucagon family, and they modulate neurotransmission via stimulation of protein kinases including cAMP-dependent protein kinase and protein kinase C in the nervous system. PACAP and VIP share at least three types of receptors (PAC1, VPAC1 and VPAC2) that belong to a subfamily of the seven-transmembrane-spanning G protein-coupled receptors. It is well-established that PACAP/VIP exert their actions through these widely distributed receptors in the central and peripheral nervous systems. Their physiological actions in the nervous system are yet to be fully elucidated. Here, we investigated the effect of PACAP on neuronal toxicity induced by Aß, the aggregation of which is a causative factor of Alzheimer's disease. Two sets of experiments were performed to measure cell damage, which included lactose dehydrogenase (LDH) and WST-8 assays. Exposure of PC12 cells to Aß resulted in a marked lactose dehydrogenase release and a decrease of the WST-8-reducing activity, indicating that Aß-induced neuronal damage with a loss of mitochondrial function. In addition, the caspase-3-like protease activity in PC12 cells treated with Aß was measured using the fluorometric substrate Ac-DEVD-AMC. As reported previously, significant cleavage of the caspase-3 substrate occurred after treatment of the PC12 cells with Aß. In this investigation, PACAP (10^{-9} M) rescued 80% of the decreased cell viability and 50% of the elevated caspase-3 activity that resulted from the exposure of PC12 cells to Aß. PACAP was at least 10^4-fold more effective than other neuropeptides including vasoactive intestinal peptide (VIP) and humanin, which correlated with the level of cAMP accumulation. These results, taken together with the observations of the RT-PCR experiment, indicated that the effect of PACAP is mediated through activation of PAC1 receptors in PC12 cells. PACAP demonstrated a potent neuroprotective effect over a long period (at least 72 hr) and at an extraordinarily low concentration, suggesting that PACAP could act as a neuroprotective/neurotrophic factor. Thus, the results suggest that PACAP attenuates Aß-induced cell death in PC12 cells through an increase in cAMP and that caspase-3 deactivation by PACAP is involved in the signaling pathway for this neuroprotection.

*Table 1 - Sequence of synthetic neuropeptides. *, amidated at the C-terminus.*

Peptides	5	10	15	20	25
PACAP27	H S D G I F T D S Y	S R Y R K Q M A V K	K Y L A A V L *		
VIP	H S D A V F T D N Y	T R L R K Q M A V K	K Y L N S I L N *		
Humanin	M A P R G F S C L L	L L T S E I D L P V	K R R A		

P C101 - Somatostatin (srif) analogs that contain β-methyl-2-naphthyl-alanine in position 8 selectively bind to human SST_4

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Over the last three decades hundreds of somatostatin (SRIF) analogs were reported and tested for their affinity and selectivity towards their five known membrane-associated receptor subtypes (sst_s). Few compounds showed sst_4 selectivity, mainly non peptide ligands. The lack of selective analogs has limited progress in locating sst4 receptors in normal and diseased human tissues and tumors and in understanding the physiological and pharmacological functions associated with this receptor. Constraining the structure of SRIF analogs could generate more receptor subtype-selective peptides with distinct pharmacokinetic properties both peripherally and centrally from those of non peptide ligands. Goodman and his co-workers have published that cyclic hexapeptide analogs of somatostatin c[Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}] displayed considerable conformational flexibility around the backbones of Phe^7 and Thr^{10} and the side chains of Phe^7, Trp^8, and Phe^{11}. To lock a favorable "bioactive conformation", they synthesized a series of analogs containing α- and β-methylated amino acids and found that their β-$MeTrp^8$ analog exhibited a 'folded' conformation and significant changes in the receptor binding affinity. However, the different sst_s were not discovered at that time, so the affinities and selectivities to the five sst_s could not be determined. Based upon the above findings and the fact that the non-proteinogenic amino acid D-2Nal is also a suitable substitution in position 8 of the somatostatin analogs, we have synthesized the conformationally biased β-methyl derivatives of naphthyl-alanine and introduced them into our earlier reported c[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys] scaffold. We will show a series of new analogs with β-Me-2Nal substitutions at position 8 that can shift selectivity away from sst_3 to sst_4. These agonistic analogs show >30-fold selectivity over the other four sst_s and their binding affinities are in the low nM range

P C102 - Synthesis and *in vitro* opioid activities of cyclic dermorphin analogues containing a carbonyl bridge

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We recently reported the synthesis, biological activity and conformational studies of enkephalin analogues containing a carbonyl bridge [1]. The carbonyl bridge was constructed by incorporation of ω-diamino acid residues in positions 2 and 5 of the peptide sequence. Some of these analogues were extremely potent μ agonists as determined in the guinea pig ileum (GPI) assay.
In this contribution we used the same approach to obtain new dermorphin(1-4) analogues in which dibasic amino acids of various side chain lenght were introduced in positions 2 and 4 to form 14-18 membered ring structures. Cyclization was accomplished using bis(4-nitrophenyl)carbonate or bis(succinimidyl)carbonate. The opioid activity profiles of these peptides determined in the GPI and mouse vas deferens (MVD) assays will be presented and compared with those previously obtained with the enkephalin series. Conformational properties in aqueous solution have also been investigated and will be discussed.

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P C103 - Effects produced by alterations in the cyclic portion of the vasoactive peptide, urotensin-II

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Urotensin II (UT-II), was first isolated and characterized from the urophysis of the fish *Gillichthys mirabilis* (goby). Subsequently, UT-II was also characterized in other species and recently, after cloning the human cDNA, hUT-II was described as an 11-amino acid peptide containing a disulfide bond (H-ETPDCFWKYCV-OH). UT-II is a potent vasoeffector in mammals and it was discovered as the endogenous ligand of the orphan G-protein coupled receptor, GPR-14. As UT-II might be involved in the regulation of cardiovascular homeostasis, we carried out a structure-activity relationship study focusing on the cyclic domain CFWKYC which is highly conserved in evolution from fish to human. Various strategies were applied in order to analyze this region such as the Ala-scan, successive deletions, inversion of the chirality and substitution with, for instance, isofunctional residues. Each analog was evaluated using a binding assay on membranes from a stable HEK-293 cell line containing the human or rat UT-II receptor, a functional assay for Ca^{2+} mobilization on transiently transfected CHO-K1 cells with the human or rat UT-II receptor and, a biological activity paradigm (rat thoracic aorta). Binding studies showed that human and rat UT-II are equipotent to displace ^{125}I-hUT-II from the human receptor but rUT-II is about twice as potent as hUT-II in the rat receptor assay. The binding potency on rat receptors is analogous to that observed with the pharmacological paradigm as rUT-II is 1.7 times more potent than hUT-II in eliciting the contraction of the aorta ring. Surprisingly, in the aorta bioassay rUT-II was much less potent than hUT-II to induce a maximal effect. Most alterations led to a major or even a complete loss of binding and activity thus confirming the major role of the conserved cyclic structure. However, two analogs *i.e.* [Tyr^6]hUT-II and [Phe^9]hUT-II exhibited significant binding and bioactivity properties suggesting that the aromaticity and hydrophobicity of these two positions are very important but that chemical alterations are tolerated. The functional Ca^{2+} assays gave data rather difficult to interpret as they do not fully correlate with binding and/or activity. Many analogs exhibiting no binding nor activity appeared as potent inducers of Ca^{2+} mobilization. Nevertheless, rat and human UT-II, as well as the two active analogs, are among the most potent peptides in this assay. We believe that UT-II and many of its various analogs, because of a structural similitude with a rodent ligand, are able to interact with a receptor located on the CHO-K1 cells.

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P C104 - Two neuropeptides from invertebrates - allatostatin and proctolin modulate spontaneous neurotransmitter release in mouse neuromuscular junction

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Allatostatin and proctolin were originally identified in insects and crustaceans and they are regarded as potent inhibitory (allatostatin) and excitory (proctolin) modulators of intestinal and skeletal muscles in crustaceans and insects. The aim of our study was to investigate possible effects of allatostatin-1 (Dip-Ast 7) and proctolin (Proc) on the amplitude, frequency and time-course parameters of spontaneous (miniature) end-plate currents (mEPCs) in mouse hemidiaphragm neuromuscular junctions. Spontaneous mEPCs were recorded using standard two-electrode voltage clamp technique. Dip-Ast 7 and Proc in 1 μM concentrations were tested during their applications by bath solution exchange in experimental chamber. Dip-Ast 7 caused gradual increase in mEPCs amplitude from control level by average 105±25 % (SEM, n=12). There were no significant changes in the shape of mEPCs amplitude distribution histogram, the histogram was shifted into the area of higher means of amplitude. Rise-time and half-decay time of mEPCs were not affected after application of 1 μM Dip-Ast 7. For concentration 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 1 μM Dip-Ast 7 didn't significantly affect mEPCs frequency during the whole period of Dip-Ast 7 presence in bath solution (up to 60 minutes). We found that Dip-Ast 7 increased the integer area below mEPCs mostly at the expense of rising of mEPCs amplitude. Also we did measurements of muscle fibers input resistance in control and after application of 1 μM Dip-Ast 7. Dip-Ast 7 caused no significant changes in muscle fibers input resistance. These results suggest a possible presynaptic mechanism of Dip-Ast 7 action due to increase of quantal size. Experiments with vesicular acetylcholine pump blockers and acetylcholine iontophoresis will be required for definite answer. Application of 1 μM Proc in normal bath solution didn't cause any significant changes in amplitude, frequency and time-course parameters of mEPCs. In next experiments we depolarized presynaptic membrane by increasing concentration of potassium ions in bath solution from 4mM up to 20 mM, which caused an increase of mEPCs frequency. Application of 1 μM Proc in these sustained depolarizing conditions of presynaptic membrane caused further increase of mEPCs by average 80±31 % (SEM, n=13). In our previous works on crustaceans we have shown that Proc can increase evoked postsynaptic currents by positive modulation of P/Q and N-types of voltage-dependent calcium channels which causes an increase in quantal content of evoked neurotransmitter release. Due to our results on mammalian neuromuscular junction we can suggest possibly the same mechanism of Proc action on presynaptic membrane as it was with invertebrates – positive modulation of voltage-dependent calcium channels. Experiments with evoked neurotransmitter release and calcium channel blockers will be required for definite answer. In conclusion, our results suggest that peptide neuromodulators which were found in invertebrates can also act on mammalian neuromuscular junction, modulating neurotransmitter release.

P C105 - Potent and selective peptide agonist/antagonist analogues at human melanocortin receptor-4

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Melanocortin peptides regulate a variety of physiological processes and these peptides are mediated through seven transmembrane GPCRs (MC1-MC5). Human MC3 and MC4 receptors are both found in the brain and these receptors have been implicated to play a role in the control of feeding behavior and energy homeostasis [1]. The aim of this study is to identify structural requirements for the ligand that determine alpha-MSH selectivity for the hMC3R versus the hMC4R. Substitution in the MTII template (Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2) with *D*Nal for Nle-4, Pro for His-6 and extending the carbon chain by introducing Gly in position 10, increased selectivity 25-100 fold greater for the hMC4R (IC_{50}=11.3nM). Alternatively, when *D*Phe is substituted for Nle-4, Pro for His-6 and *D*Nal for Trp-9 in MTII, an antagonist of hMC4R is obtained, We find that the 26 membered lactam ring in alpha-MSH analogues under investigation is favored over 23 membered ring for being a selective agonist of hMCR4. Thus, selective and potent hMC4R agonists may find therapeutic use for treating metabolic diseases such as obesity.

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P C106 - New potent GH-RH analogues containing homoarginine residues

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Extensive experimental and clinical findings demonstrate the essential role of growth hormone-releasing hormone (GH-RH) and its GH-RH(1-29)-NH_2 analogue in the control of growth hormone (GH) secretion and the regulation of linear growth. It has been suggested that GH-RH(1-29)-NH_2 could be applied instead of GH in order to accelerate the growth velocity in GH-deficient children. As this peptide is rapidly hydrolysed in subcutaneous tissue and plasma, there is a great demand for more stable analogues, which would allow a reduction in the doses and frequency of administration. In this contribution we present solid phase synthesis (Boc strategy) of four new GH-RH(1-29)-NH_2 analogues in which Arg (positions 11, 20 or 29) or Lys (positions 12 or 21) residues are substituted by homoarginine (hArg). A distinctive feature of this synthesis was the use of a commercially available Lys derivative in which the side chain function was protected by the FMOC group, the protection was removed after completing the peptide chain and the amino group was converted by guanidine group. It was observed that peptide bonds formed by hArg were resistant to tryptic digestion. In tests *in vivo* in rats, after subcutaneous administration, the analogues displayed a much higher potency compared to GH-RH(1-29)-NH_2. This high potency could be attributed to the increased resistance of the analogues to digestion by trypsin-like enzymes.

P C107 - Analogues of arginine vasopressin modified in position 2 or 3 with 1-aminocyclohexane-1-carboxylic acid

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It is believed that L-phenylalanine residue at position 3 in arginine vasopressin (AVP) is mainly involved in recognition of this hormone and its binding to the receptors. On the other hand the tyrosine residue at position 2 seems to play a role in the initiation of the pressor response. So far, most of the analogues modified in these positions still contained an aromatic amino acid residue. Our previous results indicated that introduction of conformationally restricted elements into position 2 and/or 3 resulted in analogues with interesting pharmacological properties. As a continuation of our efforts to elucidate the role of these positions in AVP or its analogues, we designed, synthesized and pharmacologically evaluated new peptides in which we replaced Tyr or Phe with 1-aminocyclohexane-1-carboxylic acid (Ac_6c). This aliphatic, non-coded amino acid is conformationally constrained. Thus four peptides with Acc in position 3, i.e. [Ac_6c^3]AVP, [Ac_6c^3,D-Arg]^{8}VP, [Cpa^1, Ac_6c^3]AVP and [Mpa^1, Ac_6c^3]AVP were synthesized (where Cpa=1-mercaptocyclohexaneacetic acid, Mpa=3-mercaptopropionic acid). In the next three analogues the Ac_6c was placed in position 2: [Ac_6c^2]AVP, [Ac_6c^2,D-Arg^8]VP and [Cpa^1, Ac_6c^2]AVP were prepared. All new peptides were tested for pressor and uterotonic *in vitro* activities. In addition two analogues we checked for their activity in the antidiuretic test. Meanwhile substitution of position 3 with Acc results in almost complete loss of the activities, the same substitution in position 2 means differentiated change of the activities (see the Table 1).

Table 1. Some biological activities of the new analogues (IU/mg or pA_2). For comparison values of the mother compounds are also given.

Compound	Activity	
	Uterotonic *in vitro* no Mg^{2+}	Pressor
AVP	17	412
[Mpa^1]AVP	27-63	346-370
[D-Arg^8]VP	0.4	4.1
[Cpa^1]AVP	pA_2=8.15	pA_2=8.35
[Ac_6c^2]AVP	0	0.25 IU/mg
[Mpa^1, Ac_6c^3]AVP	0.04 IU/mg	0.45 IU/mg
[Ac_6c^3,D-Arg^8]VP	0	0
[Cpa^1, Ac_6c^3]AVP	0	0
[Ac_6c^2]AVP	pA_2~5.6	62.5 IU/mg
[Ac_6c^2,D-Arg^8]VP	pA_2~5.7	0
[Cpa^1, Ac_6c^2]AVP	pA_2=7.33	pA_2=7.1

P C108 - Conformationally constrained analogues of neuropeptide Y bind to the NPY Y1-receptor

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One peptide can interact via the same binding site with multiple receptors either with one defined conformation or with different conformations, respectively. This results in different orientations of its side chains at the respective receptor subtype. If the region responsible for recognition and bioactivity has no defined structure in solution its flexibility could be reduced and a proposed bioactive conformation could be fixed for further structural investigations. This can be done by substitutions with suitable building blocks. Thus constrained but still bioactive structures can then further be analyzed to gain more insights into the bioactive structure of the native ligand at the respective receptor subtype. A new tool to study certain bioactive conformations are the conformationally constricted β-aminocyclopropane carboxylic acid (β-ACC) derivatives. Because of its small size and its *cis*-standing amino and carboxyl group this amino acid might be a promising tool to induce and to stabilize certain backbone conformations at peptide parts that are sensitive to affinity and selectivity. In order to obtain stable building blocks the β-ACC residue was introduced with the respective preceding amino acid as dipeptide into the C-terminal sequence of truncated neuropeptide Y (NPY)-analogues. Subsequently the sequence can be elongated and worked-up by standard SPPS-protocols to get peptides with an intact cyclopropane ring. Depending on the chirality of the introduced β-ACC's and the position in the C-terminal NPY-sequence several truncated analogues showed biological activity at the Y_1-receptor, whereas at other receptors they were nearly inactive. Interestingly, the same truncated but unsubstituted NPY-analogue or analogues with the natural conformationally constricted amino acid proline or with a β-amino acid at this position showed no biological activity at this receptor subtype. Furthermore from CD-studies it can be concluded that β-ACC substitutions influence the peptidic structure and the tendency to induce certain conformations. Accordingly we found that the observed biological activities of the shortened NPY-analogues were exclusively evoked by substitutions with the conformationally constricted (1*R*,2*R*,3*R*)-β-ACC-derivative and can neither be induced with natural amino acids nor with unconstrained -amino acids so far. Therefore we assume that this amino acid could be a useful tool to constrain flexible peptidic ligands and to gain further insights into the bioactive conformations. Future studies will show more details about the secondary structure motif that will be stabilized by β-ACC substitutions and is probably necessary for Y_1-receptor selectivity.

P C109 - Glutamic acid-10: a key-residue for the structural stability and vasoactive activity of endothelin agonists specific to ET-A receptor

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Endothelin is among the most potent vasoactive peptides ever described and this effect is mainly mediate through an interaction with ET-A receptors. So far, structure-activity studies carried in our lab led us to the identification of the two first endothelin fragments, N-Ac hET-1 (9-21) and Ala^{11,15} hET-1 (9-21), which exhibit biological activity on rat thoracic aorta (ET-A) but not on lung parenchyma strips (ET-B). Both analogs appeared as full agonists on the ET-A paradigm and their activity was fully abolished with BQ-123, an ET-A specific antagonist. The replacement of Glu^{10} with glutamine in N-Ac hET-1 (9-21) allowed us to demonstrate the crucial role of this residue for the binding and the activation of the ET-A receptor. In fact, the loss of the negative charge in the N-terminal segment of the 9-21 fragment results in an inactive peptide. Moreover, conformational studies using circular dichroism, carried on N-Ac hET-1 (9-21) showed the adoption of a β-sheet pattern by the peptide. While the modification of the glutamic acid residue appears to destabilize this secondary structure. These results suggest that the negative charge in position 10 stabilizes the conformation of the endothelin in order to allow a correct interaction between the peptide and its ET-A receptor.

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P C110 - Structure-activity relationship of rat octadecaneuropeptide: importance of the leucine residues

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The octadecaneuropeptide (ODN; QATVGDVNTDRPGLLDLK), which has been originally characterized as an endogenous ligand of benzodiazepine receptors (BZ-R), is a potent inhibitor of food intake in rodents [1]. However, we have recently shown that the anorexigenic effect of ODN is not mediated through BZ-R. We have also demonstrated that ODN increases intracellular calcium concentration ($[Ca^{2+}]_i$) in cultured rat astrocytes through activation of a G protein-coupled receptor (GPCR) [2], and we have found that the C-terminal octapeptide OP (RPGLLDLK) is the shorter isoactive fragment [3,4]. In order to further investigate the structure-activity relationship of ODN on its GPCR, several novel analogues of OP were synthesized and their effects on $[Ca^{2+}]_i$ were assessed in cultured rat astrocytes. Homodetic backbone cyclization of OP yielded an analogue ($cyclo_{1-8}$ OP) that was 1.4-fold more efficacious than OP on $[Ca^{2+}]_i$. In addition, $cyclo_{1-8}$ OP mimicked the stimulatory effect of both OP and ODN on polyphosphoinositide turnover. Structural analysis by two-dimensional ^{1}H-nuclear magnetic resonance and molecular modeling of $cyclo_{1-8}$ OP showed that the amide proton (N-H) of the Leu^4 residue is involved in an intramolecular hydrogen bond stabilizing a γ-turn (-Pro^2-Gly^3-Leu^4-) and the carbonyl (C=O) of the Leu^5 residue is implicated in a type-III β-turn (-Leu^5-Asp^6-Leu^7-Lys^8-). Replacement of the amide proton of Leu^4 in OP with a methyl group led to an analogue ([*N*MeLeu^4]OP) that was 1.7-fold less efficacious than OP on $[Ca^{2+}]_i$, suggesting that the N-H of Leu^4 is involved in an intramolecular hydrogen bond stabilizing the folding of OP. Moreover, *N*α-methylation of Leu^5 alone ([*N*MeLeu^5]OP) or dual methylation of Leu^4 and Leu^5 ([*N*MeLeu^{4,5}]OP) yielded analogues that were totally devoid of agonistic activity on astrocytes. [*N*MeLeu^5]OP and [*N*MeLeu^{4,5}]OP (10^{-6} M each) did not affect ODN-evoked $[Ca^{2+}]_i$ increase either. Since the amide proton of Leu^5 is not involved in an intramolecular interaction in $cyclo_{1-8}$ OP, these data indicate that the amide proton of Leu^5 plays a crucial role in the interaction of OP with the GPCR. Taken together, these results indicate that *(i)* N-H of Leu^4 is involved in an intramolecular hydrogen bond which partially stabilizes the conformation of bounded peptides and *(ii)* N-H of Leu^5 establishes a hydrogen bond with a generic C=O donor group of GPCR. Our data also confirm the crucial role of the Leu^5 residue of OP (Leu^{15} in ODN) for GPCR binding and activation. New OP analogues incorporating γ- and/or β-turn mimetics may contribute to the development of selective and potent ligands of the ODN-GPCR that could modulate feeding behavior.

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P C111 - Synthesis, biological activity and conformational study of new oxytocin analogues

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Oxytocin (OT), a nonapeptide hormone and neurotransmitter, is involved in regulation of several physiological functions, as milk ejection, uterine contractions, vascular and cardiac relaxation, salt and water balance etc. It is also known to play a role in sexual, maternal and social behavior. Hundreds synthesized oxytocin analogues have been the subject of extensive pharmacological investigations. As oxytocin antagonists, in contrast with currently used tocolytics, afford greater specificity and can be expected to exhibit better efficacy and risk profiles, potent peptide inhibitors of oxytocin action have been synthesized with the potential of using them for arresting preterm labor. The design of OT antagonists has been mainly based on data from structure-activity studies. Observations in bibliography suggested that the C-terminal tripeptide sequence and especially the proper orientation of the C-terminal glycine carboxamide appear to be critical for obtaining high potency oxytocin analogues. Furthermore, the configuration and the hydrophobicity of the aromatic amino acid in position 2 are important for the antagonistic activity. On the basis of these findings, we set out the synthesis of new oxytocin analogues containing the conformation constraining amino acid residue L-α-t-butylglycine (Gly (But)) in positions 8 or 9 and the α-helix inducing amino acids 2-aminoisobutyric acid (Aib) or pipecolic acid (Pip) in position 7. The analogues [Gly(But)9]OT (I), [Mpa1, Gly(But)9]OT (II), [Mpa1, D-Tyr(Et)2, Gly(But)8]OT (III), [Mpa1, D-Tyr(Et)2, Gly(But)9]OT (IV), [Mpa1, D-Tyr(Et)2, Aib7]OT (V) and [Mpa1, D-Tyr(Et)2, Pip7]OT (VI), where Mpa=β-mercaptopropionic acid and D-Tyr(Et)=O-ethyl-D-tyrosine, were synthesized by Fmoc solid phase methodology utilizing a 2-chlorotrityl chloride resin as solid support bearing a Rink-Bernatowitz linker to provide the peptidic amide and diisopropylcarbodiimide/1-hydroxy-benzotriazol (DIC/HOBt) as coupling agent. The analogues were tested for their potency in two pharmacological tests, i.e. uterotonic in vitro test in the absence of magnesium ions on an isolated strip of rat uterus and in the pressor test on phenoxybenzamine treated male rats. The results suggest that replacement of Gly9 by Gly(But) in OT and [Mpa1]OT (analogues I and II) decreases biological activity significantly, while the same substitution in the case of potent antagonist [D-Tyr(Et)2]OT (analogue IV) has no deteriorating effect on the antagonistic potency (pA2 8.1). The introduction of Aib or Pip into position 7 also decreases the agonistic potency of agonists however has no influence on the antagonistic potency of antagonists. Important feature of these analogues is that they are devoided of antipressor activity. The conformations of the analogues I, III and IV were also studied by a combined use of 1D & 2D NMR spectroscopy in DMSO-d6.

P C112 - Design and synthesis of vasopressin agonists with high affinities and selectivities for the human V_{1b} receptor

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The search for vasopressin (VP) agonists with a high affinity for the V_{1b} (pituitary) receptor and low affinity for both the V_{1a} (vascular) and the V_2 (renal) receptors has proved to be an elusive and highly challenging goal [1,2]. Previous studies have shown that modifications of deamino-arginine vasopressin (dAVP) at position 2 leads to analogs like d[D-Phe^2]AVP, which exhibit a good affinity for the V_{1b} receptor, some V_{1b}/V_2 selectivity but a lack of V_{1b}/V_{1a} selectivity [2]. Based on the pharmacological properties of [Val^4]AVP and d[Val^4]AVP which have a good V_{1b}/V_{1a} selectivity but a weak V_{1b}/V_2 selectivity, we decided to pursue other modifications of dAVP at position 4. In this study we report the synthesis and binding affinities for the human V_{1b}, V_{1a}, V_2 and OT receptors of 22 d[X^4]AVP analogs where X = 1, iso-Gln; 2. Gly, 3. Ala, 4. Thi, 5. Cha, 6. Nal, 7. Nva, 8. N-Me-Val, 9. Leu, 10. Nle, 11. t-Leu, 12. Ile, 13. Asn, 14. Glu, 15. Lys, 16. Ser 17. Thr, 18. Phe, 19. Tyr, 20. Trp, 21. Abu, 22. Val (A number of these had previously been examined in rat vasopressor and antidiuretic assays but not in rat pituitary assays). 19 of these peptides exhibit nanomolar affinities for the human V_{1b} receptor. Three of these are the first peptides to exhibit high affinity for the human V_{1b} receptor and high selectivities with respect to both V_{1a} and V_2 receptors. These are d[Cha^4]AVP, d[Leu^4]AVP and d[Lys^4]AVP. Their V_{1b} affinities are respectively 1.2, 0.23 and 1.8 nM. Their V_{1b}/V_{1a} selectivities are respectively 257, 192 and 126. Their respective V_{1b}/V_2 selectivities are 6206, 1065 and 627. By comparison, dAVP has a V_{1b} affinity of 0.26 nM. However its V_{1b}/V_{1a} selectivity = 15; its V_{1b}/V_2 selectivity = 5. These new potent and selective V_{1b} agonists are very valuable as research tools for studies on VP V_{1b} receptor pharmacology, physiology and for investigations on V_{1b} receptor structure and function.

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P C113 - Cox17p, a novel mammalian copper trafficking peptide

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Recently, we have isolated a novel 62-mer polypeptide from porcine heart extract [1]. Since there is no secretion signal in the N-terminus, this peptide was supposed to function at cytoplasm. Structural analysis showed that it possessed a copper binding motif (-KPCCXC-) and high sequence homology to yeast copper chaperone Cox17p. Although Cox17p has been implied to be involved in copper recruitment to mitochondria for the functional assembly of cytochrome oxidase in yeast, its role in mammalian cells is unknown. To investigate the physiological function of this peptide in mammals, the following studies have been performed: 1) analysis of the expression profiles; 2) analysis of the copper binding activity; 3) determination of the transcription mechanism; 4) generation and analysis of COX17 gene deficient mice [2, 3, 4]. The results obtained in this study suggested that mammalian Cox17p is essential not only for the delivering copper to mitochondria and the cellular respiration but also for the embryonic development. In this paper recent advances in the Cox17p peptides are presented.

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P C114 - A new generic caspase substrate for fluorescence-based assays

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Caspases play a crucial role in the programmed cell death. The targeting of caspases and their regulation will offer new therapeutic ways for the treatment of numerous human diseases. Thus, the development of potent assay tools for caspase activity is of highest interest.
Fluorogenic substrates have become a powerful tool for the qualitative and quantitative assaying of proteases. A drawback of this technique is the limited choice of suitable dyes. Furthermore, the linkage between the dye and the recognition sequence of the substrate leads to a reduced affinity of the fluorogenic substrate towards the enzyme. As consequence, high concentrations of the fluorogenic substrate are to be applied which are not always compatible with the readout of the assay.
Here we describe a generic fluorescent substrate for caspase 3 and caspase 8 assays with fluorescence polarization as readout.

P C115 - Tetraamine functionalized neurotensin analogues labeled with ^{99m}Tc with potential application in the diagnosis of tumours

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The overexpression of neurotensin (NT) receptors by neoplastic cells, in particular those of ductal exocrine pancreatic carcinoma, reported recently, is instrumental for the detection of NT receptor positive neoplasms in patients employing SPECT (single photon emission computed tomography). As a result, the search for useful radiolabeled synthetic NT analogues has been recently intensified. Owing to the fact, that ^{99m}Tc remains the gold standard of Nuclear Medicine Diagnosis (a consequence of its superior nuclear properties, wide availability and cost effectiveness), the development of target-specific, ^{99m}Tc based NT tracers is highly desirable. In this work, two novel ^{99m}Tc labeled NT analogues have been synthesized and evaluated. The original NT(8-13) peptide motif required for effective NTR1 receptor binding of the native hormone has been modified in an effort to increase the in vivo stability of the peptides and enable binding of the radiometal. Thus, after coupling of a tetraamine chelator to the N-terminal, the following analogues were produced, NT1: N4-βAla-Arg-Dab-Pro-Tyr-Ile-Leu-OH and NT2: N4-βAla-Arg-Dab-Pro-Tyr-Tle-Leu-OH. The respective ^{99m}Tc NT1 and 2 radiopeptides form almost quantitatively at a high specific activity under mild conditions. The two analogues preserve their binding capabilities for the NTR1 receptor with IC_{50}s at the lower nM range, as estimated by competition binding experiments in WiDr cell preparations. Both ^{99m}Tc radiopeptides are rapidly internalized in WiDr cells by incubation at 37 °C. After administration in mice, ^{99m}Tc NT1 and 2 show specific localization in both the intestines and the experimental WiDr tumours, tissues reported to contain the NTR1 receptor at a high density. This fact, paralleled by their rapid clearance from background tissues over the kidneys into the urine, constitutes a very attractive biological profile for further application of ^{99m}Tc NT1 and 2 in the diagnosis of NT receptor positive tumours in patients.

P C116 - Delta sleep inducing peptide (DSIP) prevented hypoxia-induced reduction of respiratory activity in rat brain mitochondria.

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The endogenous bioregulator DSIP was proved in our previous experiments to have the unique and strong ability to facilitate the resistance of animals to various stressful stimuli and mitigate some pathological stress-induced deviations from normality. Physiological and biochemical data obtained provided the basis for development of a new stress-protective DSIP-based medicine Deltaran®, registered already (July, 1998) in Russia.
We suggested that the realization of the numerous stress-protective effects of DSIP could be associated with its regulatory action on the mitochondrial energetic machinery. Mitochondria are the essential cellular site for production of reactive oxygen species under physiological and pathological conditions and represent sensitive target of impairments resulting from stresses and leading to cell death through activated pathways of apoptosis and necrosis.
In this work we investigate the influence of DSIP and mentioned above drug Deltaran® on processes of oxidative phosphorylation and ATP production in isolated rat brain mitochondria and rat brain homogenates. A polarographic measurement of oxygen consumption was applied to evaluate the impact of DSIP on maximal rates of mitochondrial respiration and coupling of respiration to ATP production. We found that DSIP enhanced the efficiency of oxidative phosphorylation on isolated rat brain mitochondria. This peptide significantly increased the rate of phosphorylated respiration V_3 without an alteration of the rate of uncoupled respiration V_{DNP}, it also enhanced the respiratory control ratio RCR and the rate of ADP phosphorylation. DSIP and Deltaran exhibited the same action in rat brain homogenates. We examined also the influence of DSIP under hypoxia when mitochondrial respiratory activity is altered. In rats subjected to hypoxia we detected a significant stress mediated reduction of V_3 and ADP/t values. Pretreatment of rats with DSIP at the dose of 120μg/kg (i.p.) prior to their subjection to hypoxia completely inhibited hypoxia induced reduction of mitochondrial respiratory activity. The revealed capacity of DSIP to enhance the efficiency of oxidative phosphorylation found in vitro experiments could contribute to understanding pronounced stress protective and antioxidant action of this peptide *in vivo*.

P C117 - Galanin and its new analogues; synthesis and biological activity in rat isolated gastric smooth muscles

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Galanin (GAL), a 29-amino-acid-residue C-terminally amidated neuropeptide, modulates gastric smooth muscles activity by acting at specific receptors. However due to the lack of specific antagonists in the gastrointestinal (GI) tract the actual level of GAL involvement in GI motility remains largely unknown. Both N- and C-terminal GAL sequences are important for specific types of biological responses and different GAL fragments might be recognised as ligands by different receptors in a species and locus-specific manner. In our studies we have performed structure activity studies of two porcine galanin fragments and several 15-amino-acid-residue galanin analogues modified in positions: 2, 3 ,4, 6, 8 or 14, investigating their contractile action on rat isolated gastric fundus strips, employed as in vitro assay of peptides activity. Thus we intended to characterize the molecular domains of GAL responsible for binding and activation of GAL receptors in rat gastric smooth muscle cells. All tested peptides, except for [des-Trp^2,Val^4]GAL(1-15)NH_2 and GAL(16-29)NH_2 (which were inactive and did not act as an antagonist of GAL receptors in rat gastric fundus) contracted gastric smooth muscles in a concentration-dependent manner with significantly increased or decreased activities as compared to GAL(1-15)NH_2. Our studies showed that the N-terminal fragment 1-15 plays a key role in high-affinity binding of GAL to its receptors, but the C-terminal fragment 16-29 is required for full activation of galanin receptors in gastric smooth muscle cells. Moreover, our results indicate that the integrity of the first four N-terminal amino acids of GAL molecule is essential for the full excitatory myogenic action of the peptide in rat gastric fundus. We showed that Trp^2 in the amino sequence of GAL plays a key role in the recognition and/or stimulation of GAL receptors in rat gastric fundus. Substitution with D-Trp or D-Tic, addition of a second L-Trp residue or deletion of amino acid residue in position two can considerably influence the ability of GAL analogues to interact with GAL receptors. Similarly, substitution or deletion of amino acid residues in position three, four, six or eight can considerably influence the ability of GAL analogues to interact with GAL receptors. Interestingly all GAL(1-15)NH_2 analogues modified in position 14 have shown significantly increased activities as compared to non-modified N-terminal fragment, GAL(1-15)NH_2. The most active analogue was [Cit^{14}]GAL(1-15)NH_2 with a 7.5–fold higher relative potency than GAL(1-15)NH_2. These data acquired in the course of our structure-activity study suggest that both N- and C-terminal fragment of GAL molecule contribute towards the affinity and activity of GAL gastric smooth muscle cell receptors. Moreover, we concluded that positions: 2, 3, 4, 6, 8 and 14 in the amino acid sequence of GAL play an important roles in binding and activation of GAL receptors in rat gastric smooth muscle cells. Consequently it seemed clear that data obtained in the course of such studies might be helpful in a purposeful search for specific GAL receptor antagonists in the GI tract.

P C118 - Proliferative products of *in vivo* hemoglobin proteolysis as tissue growth promoters

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The *in vitro* effects of the group of proliferative peptides arising from proteolysis of the α-hemoglobin segment (133-141) were studied and compared with those of growth factors. All peptides α(133-141), α(134-141), α(135-141), α(137-141), α(134-140), α(137-140), α(133-138) and α(137-138) were shown to stimulate proliferation of murine normal and tumor cells (transformed fibroblasts L929, melanoma cells (M3) and embryonic fibroblasts (MEF) in the absence of fetal bovine serum (FBS). The maximal effects varied in the 30-50% range, depending on the structure. The effects of peptides α(137-141) (neokyotorphin) and α(134-141) during prolonged co-incubation in serum-deprivation conditions where studied in tumor (L929 and M3) and normal cells (primary cultures of murine red bone marrow and spleen cells and MEF). Both peptides supported survival/proliferation of the tumor (at least for 96 h) and normal cells (72 h for bone marrow and spleen cells and 96 h for MEF), which points to the ability of the peptides to substitute for growth factors.

The dependence of the action of peptides α(137-141) and α(134-141) on initial cell density was studied in transformed and normal fibroblasts (L929 and MEF) and in M3 cells. In the absence of FBS both peptides stimulated cell proliferation independently on initial cell density. In the presence of 10% FBS the effect of the peptides depended on initial cell density, the stimulating activity being observed for both peptides at cell density range corresponding to slightly damaged tissue, while in dense or sparse cultures it was exhibited by one of them (complimentary action). The data obtained speaks of possible participation of the peptides in tissue regeneration. The ability of α(137-141) to induce the progression of cell cycle was demonstrated by flow cytometry in L929 cells, along with the reduction of the cell volume induced by neokyotorphin. The latter points at the involvement of the mechanisms differing from those of growth factors. Basing on the data on neokyotorphin ability to increase intracellular calcium level, we tested its activity in tumor cells in the presence of Ca channel inhibitors. Verapamil fully inhibits proliferative effect of neokyotorphin, which points at the involvement of L-channel Ca fluxes in peptide action.

Summarizing, the tested hemoglobin fragments replace growth factors in their absence and increase the rate of cell proliferation in cell density dependent manner. The results obtained allowed to outline the role of the titled peptides in regulation of cell proliferation in tissues and in tissue regeneration.

P C119 - Mammalian copper chaperone Cox17p is indispensable for cellular respiration and embryonic development

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Previously, Munakata et. al. have reported the isolation of a novel polypeptide from porcine heart [1]. A structural analysis showed that it is a mammalian homologue of Cox17p, thought to be involved in copper recruitment to mitochondria and in the functional assembly of cytochrome c oxidase in yeast. Although the human, mouse and porcine homologs of this peptide have already been cloned or purified, the function of Cox17p in the mammalian cells is unknown. To investigate the physiological function of Cox17p in mammals, we have analyzed the expression patterns [2] and determined the genomic structure, chromosomal localization [3] and transcriptional mechanisms [4] of this peptide to date. The expression patterns and transcriptional regulation of the COX17 are characteristic for other respiratory genes (e. g. cytochrome c, COX IV, Vb, IVc, IIVa), which indirectly implies that the mammalian Cox17p peptide is involved in cellular respiration.

In this study, we generated mice lacking COX17 and analyzed them. The null mutant embryos were retarded in development, died between embryonic day 8.5 to 10. It was shown that the severe reductions in cytochrome c oxidase activity. Succinate dehydrogenase activity and anti-COX I, II, and IV immunoreactivity were normal in the mutant embryos indicating that this defect was due not to a deficiency of other complexes and/or subunits but to impaired mitochondrial copper delivery. Most recently, gene disruption of Ctr1, a high-affinity copper transporter involved in copper uptake, was also reported to result in embryonic death. The relationship between these molecules and Cox17p is discussed in light of the developmental mechanism. At this symposium, we will present the results of biochemical, morphological and histochemical analyses of the mutant embryos and discuss the importance of Cox17p for cellular respiration and embryonic development.

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P C120 - The somatostatin receptor type 1: chemical synthesis and conformational studies of the 56-residues n-terminal fragment

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The biological activities of the well known peptide hormone somatostatin (SST) are mediated through a recently identified family of G-protein coupled receptors. Five subtypes, sstr1-sstr5, of receptors are known. The five receptor subtypes bind the natural SST peptides, SST-14 and SST-28, with low nanomolar affinity. Short synthetic octapeptide and hexapeptide analogs, in particular the well known cyclic octapeptide named octreotide, bind well to only three of the subtipes, 2, 3 and 5. Selective nonpeptide agonists and putative peptide antagonists have been recently identified for sstr2 and sstr5. All these ligands, the natural somatostatin peptides as well the peptide and nonpeptide analogs, bind the receptors within the trans-membrane domains III and VII, with a potential contribution by the second extracellular loop. We have observed that the major differences among the five subtype receptors are displayed by the extracellular N-terminal fragments. In particular the 56 residue N-terminal fragment of sstr1 presents peculiar features for lenght and sequence. A detail knowledge of the structural properties of this receptor fragment could allow the developing of a new class of peptide selective ligands for the type 1 somatostatin receptor. In particular, new radioactive labelled peptides as well labelled policlonal or monoclonal antibodies could be developed with the aim to identify the overexpression of the type 1 receptor that occour in many tumors.

We present results on the solid-phase chemical synthesis of the 56 residue N-terminal fragment of sstr1 and its preliminary structural study, in different solvents and in micelles, by circular dichroism and NMR.

P C121 - New endomorphin analogues using β-amino acids as proline mimetics in position 2

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Recently, two new peptides, endomorphin 1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin 2 (Tyr-Pro-Phe-Phe-NH_2) have been isolated from mammalian brain. These peptides activate μ-opioid receptors with high affinity and selectivity [1]. New analogues were designed and synthesized using 2-amino-cyclopentanecarboxylic acid (Ac_5c) [2], and 2-amino-cyclohexanecarboxylic acid (Ac_6c) as proline mimetics in position 2. Both β-amino acids contain *cis* and *trans* isomers (1*S*,2*R*; 1*R*,2*S*;1*R*,2*R* and 1*S*,2*S*). *Cis* and *trans* racemic β-amino acids were used in the peptide synthesis (SPPS) and the diastereomeric peptides were separated by RP-HPLC. The configuration of β-amino acids in the peptides was determined after acidic hydrolysis using GITC derivatization. μ-Opioid receptor affinity and selectivity of the new analogues were investigated by radioreceptor assay in rat brain membrane preparation using selective opioid receptor ligands, ^{3}H-naloxone, ^{3}H-endomorphin-2 for μ, and ^{3}H-Ile^{5,6}-deltorphin-2 for δ-ligand. The analogues containing the (1*S*,2*R*)Ac_5c and (1*S*,2*R*)Ac_6c show high affinity at the μ receptor, resulting high selectivity for the μ receptor. The analogues containing *trans* β-amino acids and (1*R*,2*S*)Ac_5c and (1*R*,2*S*)Ac_6c *cis* residues are less active at μ and δ opioid receptors. The best active peptide was Tyr-(1*S*,2*R*)Ac_5c-Phe-Phe-NH_2. This compound was investigated in *in vivo* test (tail flick test) showing high analgesic activity too.

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P C122 - [Dmt1]DALDA: preparation of a tritiated analogue and structural modifications in positions 1, 3 and 4 of the peptide sequence

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[Dmt1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH2; Dmt = 2',6'-dimethyltyrosine) is a dermorphin analogue with very high binding affinity for the μ opioid receptor (Ki(μ) = 143 pM) and extraordinary μ receptor selectivity (1). It is extremely potent in the rat tail flick test after intrathecal administration (3000 times more potent than morphine) with a duration of action (12 h) four times longer than that of morphine (2). For the preparation of [Dmt1]DALDA in tritiated form, a precursor peptide containing 2',6'-dimethyl-3',5'-diiodotyrosine was prepared and catalytically tritiated (Institute of Isotopes, Budapest, Hungary). The resulting radioligand with a specific radioactivity of 47.18 Ci/mmole displayed high μ affinity (Kd = 149 pM) and a low level of non-specific binding (10-15 %) in the mouse brain membrane binding assay. It thus represents a superior new radioligand for μ opioid receptor binding studies. An analogue of [Dmt1]DALDA containing 2,3-diaminopropionic acid in place of Lys4 showed further enhanced μ receptor affinity (Ki(μ) = 64 pM) and retained very high μ vs. δ and μ vs. κ selectivity. For the preparation of analogues containing a double bond in the 4,5-position of the Lys4 side chain, both *cis*- and *trans*-4,5-dehydrolysine (cDhl and tDhl) were synthesized using a synthetic scheme similar to that described by Davis et al. (3). The cDhl4- and the tDhl4-analogue displayed similarly high μ agonist potency in the guinea pig ileum (GPI) assay and similarly high μ receptor binding affinity and μ selectivity, indicating that the exact location of the positively charged amino group on the side chain is not crucial. The effect of eliminating the positive charge on the N-terminal amino group of [Dmt1]DALDA on the opioid activity profile was examined by performing three different structural modifications. Formylation of the amino group produced a compound which retained full μ agonist activity in the GPI assay. Deletion of the amino group resulted in a weak but pure μ antagonist, whereas replacement of the amino group with a methyl group led to an analogue with partial μ agonist properties. These results indicate that a positively charged N-terminal amino group is not an absolute requirement for the μ agonist activity of [Dmt1]DALDA.

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P C123 - Pseudopeptide foldamers. The homo-oligomers of pyroglutamic acid and *trans*-(4S,5R)-4-carboxy-5-methyl oxazolidin-2-one

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Foldamers are unnatural oligomers or polymers, based on natural or unnatural building blocks, that generate well-defined three-dimensional structures. After the stimulating *manifesto* on foldamers published by Gellman in 1998 [1], the investigation on these new structural scaffolds has blossomed in many laboratories as they are promising tools for addressing chemical, physico-chemical, and biological problems and represent a new frontier in research. As a part of a program aimed at evaluating substituted -lactams as conformationally constrained building blocks of pseudopeptide foldamers, we synthesized by solution methods the homo-oligomers of L-pyroglutamic acid (L-pGlu) and *trans*-(4S,5R)-4-carboxy-5-methyl oxazolidin-2-one (L-Oxd) to the tetramer and pentamer levels, respectively. The preferred conformation of these pseudopeptide series in structure-supporting solvents was assessed by FT-IR absorption, ^{1}H NMR and CD techniques. In addition, the crystal-state structure of the N$^\alpha$-protected L-pGlu homo-dimer was established by X-ray diffraction. High-level DFT computational modeling investigations were also performed.
In these analyses we demonstrated that an αC-H ... O=C intramolecular H-bond is co-responsible for the stabilization of the *trans* -L-pGlu-L-pGlu- and -L-Oxd-L-Oxd- conformations. This effect can be easily detected by ^{1}H NMR, owing to the anomalous chemical shifts of the αCH protons involved in such interaction. In summary, we developed new poly-imide based, foldameric structures [ternary poly(L-Pro)$_n$ II type helices] which, if appropriately functionalized, hold promise as rigid scaffolds for novel functions and properties.

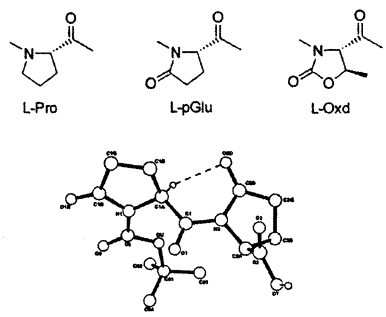

Fig. 1 - X-Ray diffraction structure of Boc-(L-pGlu)$_2$-OH

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P C124 - Targeting VEGF receptors using designed polypeptides

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Vascular Endothelial Growth Factor (VEGF) is a homodimeric protein belonging to the cystine knot growth factor family. It is a potent angiogenic factor and a mitogen specific for vascular endothelial cells. It plays a central role in angiogenesis, pathogenesis of cancer, proliferative retinopathy and rheumatoid arthritis. Its biological activity is mediated through binding to two different membrane receptors: the kinase domain receptor (KDR) and the Fms-like tyrosine kinase (Flt-1). VEGF binds to Flt-1 with higher affinity than to KDR and recent data highlight the different biological roles of these two receptors. In particular, KDR is responsible for the angiogenic and mitotic activity, whereas it has been suggested that Flt-1 is not a signaling receptor but rather a decoy molecule able to regulate in negative fashion the activity of VEGF on the vascular endothelium. VEGF and its receptors are overexpressed in pathological angiogenesis making this system an ideal target for therapeutic and diagnostic applications [1, 2]. Inhibition of tumor growth and metastasis has been achieved by various approaches that interfere with VEGF-receptor binding. Mainly antibodies against VEGF, and/or KDR, or fragment of soluble portion of the receptor have been used. In particular, antibodies against VEGF have shown therapeutic potential as agents capable of suppressing in vivo tumor growth by inhibiting tumor-induced angiogenesis [2]. Since VEGF antagonists may have broad application in diagnostic and clinical treatment of a variety of human diseases where pathological angiogenesis is involved, we are interested in developing peptides/peptidomimetics able to bind to VEGF receptors with high affinity and specificity and interfering with the VEGF binding. The structures of VEGF free and complexed to the second extracellular domain of Flt-1 have been reported [3-4]. Mutagenesis studies[3,5] and structural data have shown the receptor-binding epitope of VEGF for these two receptors. Residues involved in the binding surface extend across the dimer interface and they are localized mainly in two hot spots: a short three stranded β-sheet and loops b1-b2 of one monomer; the N-terminal α-helix and loops b3-b4 of the other monomer. Based on the X-ray structure of the VEGF-Flt-1D$_2$ complex, peptides have been designed and synthesized. Data about binding studies and spectroscopic characterization will be discussed.

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