THE EFFECTS OF THE UROCORTINS AND THE ENDOMORPHINS ON THE MEDIATORS OF THE STRESS RESPONSE

Summary of Ph.D. Thesis

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ABBREVIATIONS

${}^3$HDA = tritium-labelled dopamine
${}^3$H]GABA = tritium-labelled gamma-amino-butyric acid
$\alpha$-MSH = alpha-melanostimulating hormone
ACTH = adrenocorticotropic hormone
AVP = arginine-vasopressin
CNS = central nervous system
CPM = count per minute
CRF = corticotropin releasing factor
CRF-BP = corticotropin releasing factor-binding protein
CRFR = corticotropin releasing factor receptor
DAMGO = Tyr-D-Ala-Gly-MePhe-Gly-Ol
DOR = delta opioid receptor
DPPIV = dypeptidyl-amino-peptidase IV
DV = dorso-ventral
ELISA = enzyme-linked immuno-sorbent assay
EM = endomorphin
GPCRs = G protein-coupled receptors
HPA = hypothalamic-pituitary-adrenal
ICV = intracerebroventricular
IP = intraperitoneal
IR = immunoreactivity
KOR = kappa opioid receptor
ML = medio-lateral
MOR = mu opioid receptor
ORL = opioid receptor-like
POMC = proopiomelanocortin
RC = rostro-caudal
RIA = radio-immuno assay
SCP = stresscopin
SRP = stresscopin-related peptide
UCN = urocortin
1. INTRODUCTION

1.1. CRF receptor agonists

Corticotropin-releasing hormone (CRH), originally named corticotropin-releasing factor (CRF) and occasionally called corticoliberin, is a significant neurohormone of the hypothalamic-pituitary-adrenal (HPA) axis and also an important neurotransmitter released from hypothalamic and extrahypothalamic nuclei in mammals. Since CRF was first characterized, a growing family of ligands and receptors has been discovered. The mammalian family members include CRF, urocortin I (UCN I), urocortin II (UCN II), also known as stresscopin-related peptide (SRP), and urocortin III (UCN III), also known as stresscopin (SCP), along with two CRF receptors, CRFR1 and CRFR2, and a CRF-binding protein (CRF-BP). Previously it has been suggested that CRF-related peptides could play important roles in the regulation of the endocrine, autonomic and behavioral responses to stress. Although the role of CRF, activating CRFR1, is stimulatory upon stress responsivity, the role of UCN II and UCN III, both acting on CRFR2, appears to be inhibitory upon stress sensitivity. As the only ligand with equally high affinity for both receptors, UCN I’s role may be promiscuous.

1.2. CRF receptors

CRFRs belong to the class B subtype of G protein–coupled receptors (GPCRs). CRFR1 and CRFR2 are produced from distinct genes and have several splice variants expressed in various central and peripheral tissues. CRFR1 has α and β isoforms in addition to subtypes designated c-h, which have been detected in human and rodent tissues. Several of these isoforms have been shown to be nonfunctional. CRFR2 is expressed in three functional subtypes, α, β, and γ. Both CRFR2α and CRFR2β have been detected in human and rodents, while CRFR2γ has only been reported in humans. There is nearly 70% identity between CRFR1 and CRFR2 at the aminoacid level. As is consistent with other GPCR family members, the transmembrane and intracellular domains of the CRFRs have the highest homology (over 80% identity). The third intracellular loop is the receptor region thought to interact with the G-proteins for most GPCRs.
1.3. CRF receptor antagonists

The first CRFR antagonist synthesised and studied was α-helical CRF 9-41, that efficiently blocked CRF-induced adrenocorticotropic hormone (ACTH) secretion and stress-induced locomotor activation, followed by D-Phe CRF 12–41, a more potent antagonist of CRF, than α-helical CRF 9-41. Astressin, a novel CRFR antagonist, was found to be particularly potent at inhibiting the HPA axis. It could reverse the CRF- or stress-induced anxiogenic-like behaviour induced, but it could not prevent, CRF or stress-induced locomotor hyperactivity. CP-154,526 and its structurally related analog antalarmin are selective nonpeptidic CRFR1 antagonists used especially to characterize the central actions of CRFR1s. Both compounds were able to penetrate the blood-brain barrier and antagonize endocrine and behavioural effects of CRF, UCN I or specific stressors. Though results with CP-154,526 may seem confusing, studies with antalarmin may prove promising for future anxiolytic and antidepressant research. Antisauvagine 30 and astressin 2B, structurally derived from sauvagine and astressin, respectively, are selective peptidic CRFR2 antagonists used preferentially to scrutinize the peripheral functions of CRFR2s.

1.4. µ Opioid receptor agonists

There are three classical opioid receptors designated µ (MOR), δ (DOR), and κ (KOR), and one orphan opioid-receptor like receptor (ORL) found in the central and peripheral nervous systems of mammals mediating the biological actions of exogenous and endogenous opioids. Until lately, no mammalian peptide was found and proved to be highly selective for MOR. Endomorphin 1 (EM1) and endomorphin 2 (EM2) were the peptides extracted from bovine brain and isolated later in human brain, that were proposed finally as selective endogenous ligands for MOR. Radioimmunological and immunohistochemical analyses revealed that EM immunoreactivities are distributed throughout the human, bovine, and rodent CNS in similar manner that the MORs. The neuroanatomical localization of these peptides reflect their potential participation in many physiological processes, such as antinociception and modulation of stress.
1.5. µ Opioid receptors

The MORs belong to the superfamily of heterotrimeric, guanine-nucleotide binding, G-protein-coupled receptors (GPCRs). In the CNS, EMs, although specifically positioned to activate the MORs, are not selectively associated with the regions expressing these binding sites. MORs have also been detected outside the CNS, in the enteric nervous system and throughout the immune tissues, where they were found to be coexpressed with EMs. Numerous in vitro studies clearly demonstrated that the EMs selectively, but not exclusively bind to MORs. In classic binding assays on the rat and mouse brain membrane preparations, both peptides displaced naloxone, Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO), and other MOR-selective ligands in a concentration-dependent manner. The efficacy of EMs in many bioassays is slightly lower than that of DAMGO, but higher than that of morphine. Several in vivo studies also demonstrated that EMs are specific ligands and partial agonists of MORs. The relation between binding efficacy and biological activity of these peptides has yet to be elucidated.

1.6. µ Opioid receptor antagonists

Naloxone and naltrexone are non-selective opioid antagonist drugs commonly used in clinical practice. Naloxone and naltrexone are partial inverse agonists, being used in the treatment of drug abuse (opioids, nicotine, alcohol). Naltrexone is administered to reverse the symptoms of opioid withdrawal and naloxone is preferred to reduce the consequences of opioid intoxication. Several studies suggested that EM-1 and EM-2 produce their biological effects by stimulating different subtypes of MORs, MOR1 and MOR2, which might be responsible for their distinct pharmacological activity. Administration of selective MOR1 antagonist naloxonazine and selective MOR antagonist β-funaltrexamine differentially attenuated spinal and supraspinal antinociception induced by EM1 and EM2. Results revealed that MOR1 was stimulated only by EM-2, whereas MOR2 was stimulated by both EM1 and EM2 and that MOR1 mediate supraspinal analgesia and modulate acetylcholine and prolactin release, whereas MOR2 mediate spinal analgesia, respiratory depression, and inhibition of gastrointestinal transit.
2. PURPOSES

Though there are relevant reviews demonstrating the function of CRF, arginine-vasopressin (AVP), ACTH, β-endorphin and glucocorticoids in the regulation of stress response, there are discrepant results concerning the implication of the UCNs and the EMs in stress-induced mechanisms and stress-related disorders. The aim of the present study was to determine the actions of these neuropeptides on hypothalamic neurohormone (CRH and AVP) and extrahypothalamic neurotransmitter (DA and GABA) release in rats for a better understanding of the physiology and pathophysiology of stress response.

The activation of the HPA axis is characterized by release of CRF and AVP from hypothalamic nuclei inducing release of ACTH from the anterior pituitary and consequent release of corticosterone from the adrenal gland into the blood. Hence, our first interest was to determine the CRF and AVP concentrations by immune assays from homogenised hypothalamic tissues and corticosterone concentrations by chemical assays from plasma samples collected from rats after in vivo administration of UCNs and EMs.

The activation of the HPA axis is associated with release of CRF, DA and GABA from extrahypothalamic sources of CRF. On one hand, the DA released from the striatum, part of both mesolimbical and nigrostriatal pathways, may mediate the stress-related locomotor hyperactivation; on the other hand the GABA release from the amygdala, part of the extended amygdala circle, may transmit the stress-induced stereotype behaviour of the animals. Thus, our second purpose was to determine the radio-labelled DA and GABA concentrations from superfused striatal and amygdalar slices isolated from rats after in vitro administration of UCNs and EMs.

The participation of the CRFR subtypes and MOR subtypes in these processes is not fully elucidated; brain slices were pretreated in vitro with selective CRFR antagonists, antalarmin (CRFR1 antagonist) and astressin 2B (CRFR2 antagonist) and selective MOR antagonists, naloxonazine (MOR1 and MOR2 antagonist) and β-funaltrexamine (MOR1 antagonist) to clarify the possible physiological and potential therapeutical roles of these receptors and their ligands.
3. MATERIALS AND METHODS

3.1. Materials

Corticotropin-releasing hormone, CRH (Bachem, Switzerland);
Urocortin I (Bachem, Switzerland), non-selective CRFR agonist;
Urocortin II (Bachem, Switzerland), selective CRFR2 agonist;
Urocortin III (Bachem, Switzerland), selective CRFR2 agonist.
α-helical CRF 9-41 (Sigma-Aldrich, Austria), non-selective CRFR antagonist;
Antalarmin (Sigma-Aldrich, Austria), selective CRFR1 antagonist;
Asstressin 2B (Sigma-Aldrich, Austria), selective CRFR2 antagonist.
Endomorphin 1 (Bachem, Switzerland), selective MOR agonist;
Endomorphin 2 (Bachem, Switzerland), selective MOR agonist.
β-funaltrexamine (Bachem, Switzerland), selective MOR1 antagonist;
Naloxonazine (Bachem, Switzerland), selective MOR antagonist.
$[^3]$H]DA (Amersham, USA), tritium labelled excitatory neurotransmitter;
$[^3]$H]GABA (Amersham, USA), tritium labelled inhibitory neurotransmitter;
Diprotin A (Bachem, Switzerland), DPPIV enzyme inhibitor;
Krebs solution: NaCl, KCl, MgSO₄, NaHCO₃, glucose, KH₂PO₄ and CaCl₂ (Reanal, Hungary);
Ringer solution: NaCl, KCl, Na₂HPO₄, MgCl₂, glucose, CaCl₂ (Reanal, Hungary);
Saline solution (NaCl inj. of 0.9 %, Biogal, Hungary);
Ultima Gold (Perkin Elmer, USA), scintillation fluid;
Mixture of 5 % CO₂ and 95 % O₂ for continuous gassing of the tissues;
Nembutal (CEVA-Phylaxia, Hungary) for general anesthesia of the rats;
Acetic acid of analytical grade (Reanal, Hungary)
Ethyl alcohol, methylene chloride and sulfuric acid of analytical grade (Reanal, Hungary)
CRF ELISA kit (Cosmo Bio Co. Ltd. Japan)
AVP RIA kit (Diagnostics Systems Laboratories Inc., USA)
3.2. Animals

The animals were kept and handled during the experiments in accordance with the instructions of the University of Szeged Ethical Committee for the Protection of Animals in Research. Male Wistar rats weighing 150-200 g were used. The rats were kept in their home cages at constant room temperature (23°C) on a standard illumination schedule, with 12-h light and 12-h dark periods (lights on from 6:00 a.m.). Commercial food and tap water were available ad libitum. The rats were allowed a minimum of 7 days to acclimatize before surgery, and, to minimize the effects of nonspecific stress the rats were handled daily.

3.3. Surgery

For the intracerebroventricular (ICV) administration of neuropeptides, the rats were implanted with a stainless steel Luer cannula (10 mm long) aimed at the right lateral cerebral ventricle under Nembutal (35 mg/kg, ip) anesthesia. Cannulas were secured to the skull with dental cement and acrylate. The rats were used after a recovery period of at least 5 days. The implantation of the cannula and the isolation of different brain regions were made according to the Stereotaxic Atlas Of The Rat Brain (Pellegrino et al. 1979).

3.4. Administration

The in vivo administration of CRF agonists or MOR agonists was made ICV through the cannula implanted in the right lateral cerebral ventricles 30 min before the animals were sacrificed. The CRF antagonist α-helical CRF was injected ICV 30 min before MOR agonist treatment. The in vitro administration of CRF agonists was made 12 min before the electrical stimulation of the brain slices. When antagonization of a previous effect was intended, the slices were pretreated with selective CRF antagonists, antalarmin or astressin-2B 22 minutes before the electrical stimulation. The administration of MOR agonists and MOR antagonists was performed in similar way. When DPPIV inhibition was needed, the slices were pretreated with diprotin A 15 min before the administration of EMs.
3.5. Methods

3.5.1. In vitro homogenisation assay

The rats were decapitated and their brains were rapidly removed. The hypothalami were isolated in a Petri dish filled with ice-cold Krebs solution and dissolved in 500 µl acetic acid of 2M concentration in Eppendorf tubes and immersed in boiling water for 5 min and then homogenised with ultrasonic homogeniser (Branson Sonifier 250) at 0°C for 30 sec. The homogenisates were centrifuged twice at 10000 rpm at 4°C for 20 min after of which the supernatants were separated and lyophilisated for CRF and AVP determination.

3.5.2. Enzyme-Linked Immuno-Sorbent Assay (ELISA)

For the determination of the amount of CRF in the brain homogenisate we used the mouse/rat CRF-high sensitivity ELISA kit provided by CosmoBio Company, Ltd., Japan. This ELISA kit for determination of mouse/rat CRF is based on a sandwich enzyme (horseradish peroxidase) immunoassay and shows no crossreactivity to UCNs (mouse/rat and human). The CRF concentrations of the extracted samples were corrected for extraction recovery and expressed in ng/ml.

3.5.3. Radio Immuno Assay (RIA)

For the detection of the amount of AVP in the brain homogenisate we used solid-phase extraction and competitive radioimmunoassay used for human determination by the Department of Endocrinology, University of Szeged. This assay uses a rabbit anti-AVP antiserum and a radioiodinated AVP [125I] tracer assured by Diagnostics Systems Laboratories Inc., USA. The AVP concentrations of the extracted samples were corrected for extraction recovery and expressed in ng/ml.

3.5.4. Chemical fluorescence assay

The plasma corticosterone concentration was determined by the chemical-fluorescence assay, using ethyl alcohol, methylene chloride and sulfuric acid of analytical grade. The amount of corticosterone secreted was measured by a Hitachi 204-A fluorescent spectrophotometer expressed in terms of 100 mg adrenal tissue, for a period of 1 hour (µg/100 mg hour).
3.5.5. In vitro superfusion assay

The striata and amygdalae were dissected in a Petri dish filled with ice-cold Krebs solution. The dissected tissue was cut with a McIlwain tissue chopper and slices of 300 µM were produced. The slices were incubated for 30 min in 8 ml of Krebs solution, submerged in a water bath at 37 °C and gassed through a single-use needle with a mixture of 5% CO$_2$ and 95% O$_2$. During the incubation, the slices were labeled with 0.15 mM of [³H]DA or [³H]GABA. Two tritiated slices were transferred to each of the four cylindrical perspex chambers of the superfusion system (Experimetria Ltd.). A multichannel peristaltic pump (Gilson Minipuls 2) was used to maintain a constant superfusion rate of 300 µl/min. The slices were superfused for 30 min to allow tissue equilibrium, and the superfusates were then collected in Eppendorf tubes by a multichannel fraction collector (Gilson FC 203B). 2 min after the sample collecting had started, electrical stimulation consisting of square-wave impulses (total duration: 2 min, voltage: 100 V, pulse length: 5 ms, frequency: 10 Hz) was delivered to each of the four chambers by ST-02 electrical stimulator (Experimetria Ltd.). The total collecting time was 32 min (4x16 samples, 2 min each).

3.5.6. Radio scintillation assay

The remnants of superfused brain slices were solubilized in 200 ml of Krebs solution, using an ultrasonic homogenizer (Branson Sonifier 250). The radioactivity in the fractions and the homogenized tissue samples was measured with a liquid scintillation spectrometer (Tri-carb 2100TR, Packard) after the addition of 3 ml of scintillation fluid (Ultima Gold, Packard). The fractional release was calculated as a percentage of the radioactivity (count per minute, CPM) present in the collected sample compared to the total radioactivity of the correspondent tissue.

3.6. Statistics

The differences between groups were tested by one-way ANOVA analysis of variance followed by Tukey's post hoc comparison test in cases of homogenisation study and the differences between samples were determined by two-way ANOVA with repeated measures in cases of superfusion study. A probability level of 0.05 or less was accepted as indicating a statistically significant difference.
4. RESULTS

4.1. The hypothalamic CRF and AVP release induced by the UCNs

Equimolar doses (2 µg) of UCNs produced important but different effects on hypothalamic hormone contents; UCN I produced an increase of CRF and AVP, UCN II produced a decrease of CRF, but not AVP amount, UCN III induced an increase of CRF, but not AVP content. However, UCN II and UCN III displayed similar bell-shaped dose-response curves, with lower doses decreasing and higher doses increasing the CRF levels. Most evident reductions were assessed with 2 µg of UCN II and 0.5 µg of UCN III. Most significant elevations were achieved with 5 µg of both UCN II and UCN III.

4.2. The hypothalamic CRF and AVP release induced by the EMs

Similarly to UCN I, EM1 and EM2 elicited considerably the CRF response, but contrary to the CRFR ligand, the MOR ligands did not influenced significantly the AVP response. The changes of hypothalamic CRF production were mirrored by the corticosterone secretion as well, however only the most effective doses of EM1 (5 µg), EM2 (0.5 µg) and UCN I (2 µg) were tested and represented in the relation of CRF and AVP release.

4.3. The adrenal corticosterone release induced by the UCNs

CRF and UCN I induced an important increase of adrenal corticosterone release represented by the plasma corticosterone level elevated considerably by equimolar doses of CRF and UCN I. A stimulating tendency (125-150%) of glucocorticoid release was assessed even at lower doses (0.5-1 µg), but the highest efficacy (200%) was achieved by administration of 2 µg CRFR agonist. UCN II and UCN III had biphasic effects. 2 µg UCN II and 0.5 µg UCN III tent to decrease corticosterone concentration by 100%. Lower or higher doses (0.5 or 5 µg) of UCN II seemed ineffective, while higher doses (2 and 5 µg) of UCN III increased the corticosterone concentration by 200-300% as compared to control.
4.4. The adrenal corticosterone release induced by the EMs

Both EM1 and EM2 induced a significant increase of the adrenal corticosterone release mirrored by the elevated plasma corticosterone level. 5 µg EM1 in a dose of elevated the corticosterone level by 160% as compared with control. 0.5 µg of EM2 elevated the corticosterone level with 150% more than saline and in a statistically significant manner. The adrenal responses induced by either EM1 or EM2 were inhibited by pretreatment with 1 µg α-helical CRF 9-41.

4.5. The extrahypothalamic DA and GABA release induced by the UCNs

Both CRF and UCN I increased significantly the striatal [3H]DA release evoked by the electrical stimulation, though a difference between the stimulatory effects of CRF (175%) and UCN I (275%) was clearly visible. However both were inhibited by antalarmin and uninfluenced by astressin 2B. UCN II and UCN III appeared to be ineffective. CRF and UCN I also increased significantly the amygdalar [3H]GABA release elicited by electrical stimulation. The stimulatory effects of CRF and UCN I were blocked by the selective CRFR1 receptor antagonist antalarmin, but not by the selective CRFR2 antagonist astressin 2B. The effects of the selective CRFR2 agonists UCN II and UCN III proved to be insignificant as well.

4.6. The extrahypothalamic DA and GABA release induced by the EMs

EM1 increased significantly the striatal [3H]DA release induced by electrical stimulation. The DPPIV inhibitor, diprotin A did not potentiate this effect. The stimulatory effect of EM1 was antagonized only by β-funaltrexamine, but not by naloxonazine. EM1 inhibited significantly the striatal [3H]GABA release increased by electrical stimulation; the inhibitory effect of EM1 was antagonized by β-funaltrexamine, but not by naloxonazine. In contrast, the increasing effect of EM2 on striatal DA release was significant only when striatal slices were pretreated with diprotin A. This effect of EM2 was antagonized by both β-funaltrexamine and naloxonazine, at least when the slices were pretreated with diprotin A.
5. DISCUSSION

5.1. The effects of the UCNs on the HPA neurohormones

Our results are in agreement with previous studies which demonstrated that both CRF and UCN I induce increase of corticosterone production in rats in dose-dependent manner. Previous experiments indicated that CRF and UCN I share common receptors and pathways but show different potencies in their abilities to stimulate ACTH and/or glucocorticoid production and/or secretion. Indeed, the higher affinity of UCN I for CRFR1 than that of CRF could explain a higher potency of UCN I than that of CRF, but an equal affinity of UCN I for both CRFR1 and CRFR2, with putative antagonistic actions on the HPA axis, could invalidate these discrepancies. Though there is no doubt about the role of CRFR1 and its principal agonists in the activation of HPA axis, there is still a debate about the role of CRFR2 and their preferential agonists in the modulation of stress response. Our studies are clearly discordant with studies in mice suggesting that central administration of UCN II or UCN III does not evoke HPA responses. They are partly concordant with agonist studies in rats, demonstrating that activation of CRFR2s provokes activation of HPA axis, and partly concordant with antisense oligonucleotide studies in rats, suggesting that the antagonization of CRFR1 and CRFR2 provokes antagonistic changes of ACTH and corticosterone release.

5.2. The effects of the UCNs on striatal and amygdalar neurotransmitters

Our studies demonstrate that both CRF and UCN I increase DA release from the striatum and GABA release from the amygdala following electrical stimulation, through the activation of CRFR1, in accordance with studies indicating a strong correlation of CRF with DA and GABA in brain regions like the paraventricular and arcuate nuclei of hypothalamus, the ventral tegmental area, the basal ganglia, the basolateral and central nuclei of amygdala. The CRFR2 agonists, UCN II and UCN III, did not influence the neurotransmitter release. This is not surprising since distribution of CRFR2 in the basal ganglia and in the central amygdala is very low. However these results do not rule out the possibility that activation of CRFR2 in other regions of the brain may affect DA or GABA function in the basal ganglia or in the extended amygdala, respectively.
5.3. The significance of antalarmin

Previous studies demonstrated that acute administration of CRF and UCN I induce locomotor hyperactivation and anxiogenic behaviour through the activation of CRFR1 receptors. The present study claims that these behavioural changes might be mediated or associated by biochemical processes, such as, the DA release from the striatum and GABA release from the amygdala. Chronic stress would induce a sensitisation of the HPA axis and increase of ACTH and glucocorticoids and through this, desensitisation of the glucocorticoid receptors and decrease of the negative feedback effects exerted by glucocorticoids upon CRF and ACTH. Chronic stress would induce a stimulation of the amygdala with the release of CRF and UCN I and GABA that would overactivate the HPA axis directly (through CRFR1 activation) or indirectly (through GABA-GABA interaction) leading to the development of stress-related disorders. In this order of thoughts, antalarmin, or any other selective CRFR1 antagonist with similar behavioural and biochemical properties, could represent future therapy of stress-induced disorders, such as major depression and general anxiety disorder.

5.4. The effects of the EMs on the HPA neurohormones

Centrally administered EMs did not stimulate the HPA system and had no effect on corticosterone release at doses which were sufficient to activate other physiological systems suggesting that EMs do not play an important role in mediating the stress response or regulating the HPA axis. However parallel studies described EMs having high affinity for the HPA axis. Thus, our colleagues demonstrated that both EM1 and EM2 elicited corticosterone release exhibiting two dose-response curves with similar inverted U-shape but different dose-ranges. These effects are likely to be mediated through CRF, however the participation of other ACTH secretagogues (with the exception of AVP) cannot be fully excluded. Our experiments validated this second opinion, not the first observation. The discrepancies in dose-effect could be explained more likely by the differences in the receptor activation and signal transduction, than the differences in the diffusion and degradation of EMs.
5.5. The effects of the EMs on striatal and amygdalar neurotransmitters

Our results are in concert with studies evidencing that EM1 and EM2 have stimulating impact on striatal DA release, emphasizing that EM1, but not EM2, have inhibiting effect on striatal GABA release, at least in the presence of enzyme inhibitor and electrical stimulation. These actions of the EMs seem to be mediated by different subtypes of MORs (MOR1 for EM2, and MOR2 for EM1) in the basal ganglia, yet the participation of non-opioid mechanisms can not be fully excluded. We propose an EM-ergic model for the activation of nigrostriatal system and locomotor activity based upon that of mesolimbical system and drug reward. EM1, acting on MOR2 situated on GABAergic neurons may reduce the GABA release from the recurrent axon terminals and induce indirectly DA release from striatal DA-ergic axon terminals. EM2, activating MOR1 located on DAergic neurons may stimulate directly the DA release or eventually with the participation of dynorphynic, enkephalinergic or non-opioidergic mechanisms.

5.6. The significance of diprotin A

The present *in vitro* superfusion study confirmed previous results, as the effects of EM1 on striatal neurotransmitters were not really influenced by DPPIV inhibition, but the effects of EM2 were significant only with the addition of diprotin A. However a similar *in vitro* superfusion study investigating the effects of EM1 and EM2 on norepinephrine release from the tractus solitariud–dorsal motor vagal nucleus complex denied the importance of the enzymatic degradation in this process. Though the EMs and diprotin A were administered in similar conditions, the divergent conclusion may result from the different MOR expression and DPPIV concentration in this region. From our point of view diprotin A, reducing the sensitivity of DPPIV for the EMs, may increase the efficacy of the EM2, especially in the striatal synapses. Furtherly, EM2 analogues resistant to DPPIV, with different chemical structure but similar pharmacological profile, could be as potent antinociceptive or anti-panic drugs as EM1.
6. CONCLUSIONS

6.1. The central administration of UCN I, equipotent agonist of CRFR1 and CRFR2, is associated with CRF and AVP production in the hypothalamus, that ends with the secretion of corticosterone from the pituitary after 30 minutes, proving an activating role for CRFR1 agonists on the HPA axis.

6.2. The central administration of UCN II and UCN III, selective agonists of CRFR2, is associated with a biphasic release of CRF and consequently biphasic release of corticosterone 30 minutes following its administration, showing a dose-dependent or time-dependent tuning role of CRFR2 agonists on the HPA axis.

6.3. The local administration of UCN I, activating CRFR1, but not CRFR2, provokes DA release from the striatum and GABA release from the amygdala, mediating the anxiogenic behaviour observed in CRFR1 agonist administration or stressful conditions.

6.4. The *in vitro* administration of UCN II and UCN III, both selectively activating CRFR2, does not influence DA release from the striatum or GABA release from the amygdala; the anxiolytic behaviour elicited by UCN II or UCN III *in vivo* might be explained by stimulation of other extrahypothalamic sites or mediators.

6.5. The central administration of EM1 and EM2, endogenous agonists of MORs, increase CRF, but not AVP production in the hypothalamus, inducing corticosterone secretion from the pituitary that peaks after 30 minutes, suggesting a modulating role for the EM on the HPA axis.

6.6. The local administration of EM1 and EM2, activating different subtypes of MORs, evokes DA release from the striatum, through GABA-ergic and non-GABA-ergic mechanisms, mediating the locomotor hyperactivity observed in MOR agonist administration.
1. Original publications the present work is based on:


2. Original publications cited in the present work:


III. Jászberényi, M., Bagosi, Z., Thurzó, B., Földesi, I., Szabó, G., Telegdy, G., *Endocrine, behavioral and autonomic effects of neuropeptide AF.* (Hormones and Behavior, 2009; 56:24-34.) IF: 3,401 C:0
3. Poster presentations related to the present work:


II. Bagosi, Z., Jászberényi, M., Bujdosó, E., Telegdy, G., *The effects of corticotropin-releasing factor and urocortins on striatal dopamine release induced by electric stimulation* (MITT 2005)

III. Bagosi, Z., Jászberényi, M., Bujdosó, E., Telegdy, G., *The actions of selective m opioid receptor antagonists on striatal dopamine release increased by endomorphins* (MÉT 2005)

IV. Bagosi, Z., Jászberényi, M., Telegdy, G., *The actions of selective CRF receptor antagonists on striatal dopamine release increased by CRF and urocortin* (MÉT 2006)


VI. Bagosi, Z., Jászberényi, M., Szabó, G., Telegdy, G., *The actions of the selective CRF receptor antagonists on the rat amygdalar transmitter release evoked by electrical stimulation and enhanced by CRF-related peptides* (MÉT 2007)


XII. Bagosi, Z., Csabafi, K., Jászberényi, M., Földesi, I., Gardi, J., Telegdy, G., Szabó, G., *The actions of the endomorphins and the urocortins on the hypothalamic amounts of CRF and AVP in rats* (MÉT 2011)

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