Kisspeptin modulates the activity of the stress system and associated behaviours, the body temperature and nociception

Ph.D. Thesis Summary

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1. Publications

Original publications the presented work is based on:

I Csabafi K, Jászberényi M, Bagosi Z, Lipták N, Telegdy G. *Effects of kisspeptin-13 on the hypothalamic-pituitary-adrenal axis, thermoregulation, anxiety and locomotor activity in rats.* Behav Brain Res 2012;241C:56-61. **IF:3.391**

II Tanaka M, Csabafi K, Telegdy G. *Neurotransmissions of antidepressant-like effects of kisspeptin-13.* Regul Pept 2013;180:1-4. **IF:2.014**

III Csabafi K, Kincses B, Bagosi Z, Telegdy G. *Kisspeptin alters the acute effects of morphine in mice* In preparation, 2014

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Other original papers published during the PhD fellowship:

I Bagosi Z, Csabafi K, Palotai M, Jászberényi M, Földesi I, Gárdi J, Szabó G, Telegdy G. *The effect of urocortin I on the hypothalamic ACTH secretagogues and its impact on the hypothalamic-pituitary-adrenal axis.* Neuropeptides 2014;48 (1): 15-20. **IF:2.546**

II Jászberényi M, Bagosi Z, Csabafi K, Palotai M, Telegdy G. *The actions of neuropeptide SF on the hypothalamic-pituitary-adrenal axis and behavior in rats.* Regul Pept 2014;188: 46-51. **IF:2.014**

III Bagosi Z, Csabafi K, Palotai M, Jászberényi M, Földesi I, Gárdi J, Szabó G, Telegdy G. *The interaction of Urocortin II and Urocortin III with amygdalar and hypothalamic cotricotropin-releasing factor (CRF) - Reflections on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis.* Neuropeptides 2013;47 (5): 333-338.

IF:2.546

IV Lipták N, Dochnal R, Csabafi K, Szakács J, Szabó G. *Obestatin prevents analgesic tolerance to morphine and reverses the effects of mild morphine withdrawal in mice.* Regul Pept 2013;186: 77-82. **IF:2.014**

V Palotai M, Bagosi Z, Jászberényi M, Csabafi K, Dochnal R, Manczinger M, Telegdy G, Szabó G. *Ghrelin and nicotine stimulate equally the dopamine release in the rat amygdala.* Neurochem Res 2013;38 (10): 1989-1995. **IF:2.551**

VI Palotai M, Bagosi Z, Jászberényi M, Csabafi K, Dochnal R, Manczinger M, Telegdy G, Szabó G. *Ghrelin amplifies the nicotine-induced dopamine release in the rat striatum.* Neurochem Int 2013;63 (4): 239-243. **IF:2.65**

VII Bagosi Z, Csabafi K, Jászberényi M, Telegdy G. *The effects of corticotropin-releasing factor and the urocortins on hypothalamic gamma-amino butyric acid release–the impacts on the hypothalamic-pituitary-adrenal axis.* Neurochem Int 2012;60 (4): 350-354. **IF:2.659**

VIII Lipták N, Dochnal R, Babits A, Csabafi K, Szakács J, Tóth G, Szabó G. *The effect of pituitary adenylate cyclase-activating polypeptide on elevated plus maze behavior and hypothermia induced by morphine withdrawal.* Neuropeptides 2012;46 (1): 11-17. **IF:2.067**

IX Csabafi K, Jászberényi M, Bagosi Z, Tóth G, Wollemann M, Telegdy G. *The action of a synthetic derivative of Met5-enkephalin-Arg6-Phe7 on behavioral and endocrine responses.* Peptides 2011;32 (8): 1656-1660. **IF:2.434**

Total impact factor: **26.886**

2. Abbreviations

aCRF - α-helical CRF (9-41)
Amy - Amygdala
ANOVA - Analysis of Variance
AVP - Arginine Vasopressin
AVP ANT - Arginine Vasopressin receptor 1 antagonist
AVP1R - Arginine Vasopressin receptor 1
CNS - Central Nervous System
CRH - Corticotropin-Releasing Hormone
EPM - Elevated Plus Maze
FST - Forced Swimming Test
GnRH - Gonadotropin Releasing Hormone
HPA - Hypothalamic-Pituitary-Adrenal
HPG - Hypothalamic-Pituitary-Gonadal
icv. - intracerebroventricular
ip. - intraperitoneal
KISS1R - Kisspeptin Receptor 1
KP-10 - Kisspeptin-10
KP-13 - Kisspeptin-13
KP-54 - Kisspeptin-54
NPAF - Neuropeptide AF
NPFF - Neuropeptide FF
NPFF1R - Neuropeptide FF Receptor 1
NPFF2R - Neuropeptide FF Receptor 2
OF - Open Field
PrRP - Prolactin Releasing Peptide
PVN - Paraventricular Nucleus
sc. - subcutan

3. Introduction

The tetrapeptide FMRFamide was isolated from the ganglia of the clam as a cardioexcitatory peptide about 30 years ago. Later on it proved to be the first member of a family of peptides that share an N-terminal sequence homology. Since then, the Arg-Phe (RF)-amide motif was found throughout the animal kingdom and till now, a total of five RF-amide peptide genes have been discovered in mammals, which give rise to neuropeptides AF and FF, prolactin releasing peptide (PrRP), RFamide-related peptides, kisspeptins and the most recently found pyroglutamylated RFamide peptide. They are widely distributed in the central nervous system (CNS), but they vary in their structure and receptor preference binding to either one or several G-protein coupled receptors. Literature shows that the effects of RF-amide peptides partially overlap, but in case of some physiological parameters they exert opposite actions. Several studies show, for example, that RF-amide peptides play a prominent role in nociception: intracerebroventricular (icv.) administration cause hyperalgesia and inhibition of morphine-induced analgesia, which effect is shared by all RF-amide peptides. Furthermore, PrRP activates the hypothalamic-pituitary-adrenal (HPA) axis, increases stereotyped locomotion and pressor response. Neuropepide AF (NPAF), on the other hand, also induces the HPA axis and locomotor activity, however, it causes a decrease in heart rate and core temperature. These discrepancies between the biological actions of the individual peptides may be attributed to the difference in their receptor selectivity and/or place of release and action. Nonetheless, in light of the above-mentioned data, other members of the RF-amide family, more specifically kisspeptin, might also have a wider range of function then so far assumed.

The discovery of the kisspeptin system started with the identification of the gene KISS1, named after its place of discovery: Hershey, Pennsylvania, home of the Hershey's Kisses sweets, in 1996. KISS1 overexpression was found in metastasis suppressed melanoma cells suggesting a role for this gene in tumor progression. However, the as of yet most important physiologic function of the kisspeptin system was realised in 2003, when the inactivating mutations of the gene encoding the receptor for kisspeptins (KISS1R/GPR54) was identified to be the cause of some forms of the isolated hypogonadotropic hypogonadisms. This observation led to an immense interest in the field to catagorise the putative roles of the kisspeptin system in neuroendocrine control and since then kisspeptin was proved to be the central regulator of GnRH secretion and puberty.

Kisspeptin, itself, was first isolated from the human placenta as the endogenous ligand of the orphan G-protein coupled receptor GPR54, later designated as KISS1R. In humans the KISS1 gene encodes a 145 aminoacid long precursor peptide, which through multiple proteolytic steps will generate the major product consisting of 54 aminoacids (KP-54), but its alternative cleavage gives rise to other biologically active derivatives containing 14, 13 or 10 aminoacids, christened kisspeptin-14 (KP-14), kisspeptin-13 (KP-13) and kisspeptin-10

(KP-10), respectively. All fragments share the C-terminal part of the KP-54, and possess the $Arg - Phe - NH_2$ motif distinctive of the RF-amide family. Both kisspeptin mRNA and protein were detected in peripheral tissues and CNS. In the mouse and rat brain, Kiss1 mRNA expression is most prominent in the hypothalamus: arcuate nucleus (Arc) as well as in the anteroventral periventricular area (AVPV) and the adjacent periventricular nucleus (PeN), however it was also detected in brain structures relevant to the stress system, for example the medial amygdala (Amy) and the bed nucleus of stria terminalis (BNST). Similarly, abundant expression of the KISS1R mRNA has been found in different brain regions including several hypothalamic nuclei, the hippocampus, locus coeruleus, Amy and the periaqueductal gray.

Taking the special importance of kisspeptin in the regulation of the HPG axis into account, and the fact that recent data suggests kisspeptin neuronal projections to the PVN, it seems plausible that kisspeptin may take part in the control of the HPA axis, the interaction between the two systems and may exert further integrative activities in autonomic and endocrine control. Therefore, in the present study, we investigated the central action of KP-13 on the stress response, behaviour and thermoregulation, which processes are controlled by the hypothalamus and the limbic system, where kisspeptin and its receptors are found in abundance. As an index of the activation of the HPA system the corticosterone response was used. The spontaneous locomotion and core temperature were monitored continuously with a telemetric system, while the exploratory and anxiety-associated behaviour was observed in open field (OF) and elevated plus maze (EPM) tests. Furthermore, antidepressant-like effects of KP-13 were studied and the potential involvement of the adrenergic, serotonergic, cholinergic, dopaminergic and gabaergic receptors in its antidepressant-like effects was investigated in a modified FST in mice.

NPFF and analogues were found to have analgesic, pronociceptive and antinociceptive, and furthermore morphine modulating activities. Evidence suggests that these effects are mediated by the activation of the neuropeptide FF receptors 1 and 2 (NPFF1R and NPFF2R), respectively. Additionally, all endogenous RF-amide peptides seem to target not only their cognate receptors, but the NPFF1R and NPFF2R receptors as well, which argue for their role in pain modulation. Although much evidence has accumulated in the past couple of years that ascribes a critical role for the NPFF1R and NPFF2R receptors for the pain-modulating effects of RF-amide peptides, however, recent distribution data draws attention to other receptors, such as KISS1R and GPR10, and their endogenous ligands that are expressed in several brain areas involved in the control of pain. For instance, both KISS1R and kisspeptin mRNAs and proteins have been detected in the dorsal horn of the spinal cord and in the dorsal root ganglia in rats. Therefore, in the present thesis we also investigated the possible interaction of kisspeptin with the acute effects of morphine on nociception and the potential involvement of kisspeptin in acute morphine tolerance and withdrawal in adult male CFLP mice.

4. The aim of our experiments

Taking into account the distribution of the kisspeptin system in the CNS and the available literature on other RF-amide peptides we have hypothesised that kisspeptin has a wider range of function in the CNS then so far assumed. Therefore, we have investigated,

I if centrally administered KP-13 has any impact on the stress response and associated behaviours, general activity and thermoregulation:

- We measured corticosterone response indicative of the endocrine HPA axis activity. After establishing the overall effect, we set out to identify the possible mechanism of action by applying CRH and AVP antagonist pretreatments as they are the most prominent activators of the endocrine axis.
- To assess the effect of KP-13 on anxiety-related behaviour we registered the explorative locomotor activity of animals in a novel environment (OF test) and anxiety in the EPM test. Again, after determining the overall effect of kisspeptin we continued experiments with combined treatments with antagonists (AVP receptor 1 (AVP1R) antagonist and KISS1R antagonist) to explore how kisspeptin exerts it's effects on these parameters.
- We also investigated the effect of KP-13 on thermoregulation and general activity by continuously monitoring core temperature and spontaneous locomotor activity, both of which via a telemetric system.

II if KP-13 influences depressive behaviour in mice:

- For this purpose we observed the swim stress-induced behavioural despair in the modified forced swimming test (FST) in mice. Furthermore, to investigate the neurotransmitters involved in mediating the effect of kisspeptin different antagonist pretreatments were preformed.

III if KP-13, as a member of the RF-amide family also might play a role in pain modulation:

- Thus, in the present experiments we have studied the effect of KP-13 on pain sensitivity in the tail-flick test and the interaction between kisspeptin and acute morphine actions.

5. Materials and Methods

5.1. Animals

Adult male Sprague-Dawley rats (Domaszék, Hungary), CD1 and CFLP mice were used. They were housed under controlled conditions (12/12-h light/dark cycle, lights on from 6:00 a.m., at constant room temperature) and were allowed free access to commercial food and tap water. 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. They were implanted with cannula aimed at the right lateral cerebral ventricle and the telemetric radio transmitter was placed in the abdominal cavity of rats under pentobarbital anesthesia.

5.2. Treatments

In protocol 1 animals were injected with different doses of KP-13 (Bachem, Switzerland) icv. in a volume of 2 μl. The doses applied were 0.5, 1, 2 or 5 μg dissolved in 0.9% saline. Thirty minutes after peptide administration, the rats were decapitated to obtain trunk blood for corticosterone measurement or were subjected to behavioural testing. In experiments with combined treatments, animals were subjected to pretreatment with one of the following: AVP1 receptor antagonist (AVP ANT) (Bachem, Switzerland); kisspeptin-234 (Sigma); α-helical CRF(9-41) (aCRF) (Bachem, Switzerland), phenoxybenzamine-HCl (Smith Kline French, UK); prazosin-HCl and yohimbine-HCl (Tocris, Germany); propranolol-HCl (ICI Ltd., UK); methysergide hydrogenmaleate (Sandoz, Germany); cyproheptadine-HCl (Tocris, UK); atropine sulfate (EGYS, Hungary); haloperidol (G. Richter, Hungary); bicuculline methiodide (Sandoz, Switzerland), morphine-HCl (Sigma) and naloxone-HCl (Sigma). Thirty min after the antagonist pretreatment the animals were treated with the dose of KP-13 that had proved most effective in protocol 1.

5.3. Plasma corticosterone measurement

In order to determine plasma corticosterone concentrations, trunk blood was collected in heparinized tubes. The plasma corticosterone concentration was measured by the fluorescence assay described by Zenker and Bernstein as modified by Purves and Sirett.

5.4. Telemetry

Different doses of KP-13 (1, 2 μg) or saline alone were injected icv. into conscious rats, between 8:20 and 8:35 a.m. The animals had previously been implanted with an E-mitter (Mini Mitter, USA), which recieves power from the radiofrequency field generated by an energizer-reciever placed below the home cage. The system recorded the motor activity and

core temperature every 10 min, the output of which then was processed by the VitalView program provided by the manufacturer.

5.5. Open field test

In the OF test novelty-induced locomotor activity was assessed. The rats were removed from their home cages and placed at the center of a white wooden open field box (60 x 60 cm, marked into 36 10 x 10 cm squares). The observed parameters were horizontal locomotion, vertical locomotion, grooming and the number of defecations. The horizontal locomotor activity was characterized by the total number of squares crossed during a 5-min test session, the vertical locomotion was determined by the number of rearings, and the grooming activity was established by observing face washing, forepaw licking and head stroking.

5.6. Elevated plus maze test

The EPM apparatus is a plus-shaped platform elevated 50 cm above the floor. It consists of two opposing arms (50 cm x 10 cm each) with 10 cm high enclosing walls (closed arms) and two arms with no walls (open arms). Naive rats were placed in the center of the maze facing toward an open arm, and the number of entries per arm and the times spent in the various arms were recorded for a 5-min period by an observer who was blind to the experimental groups, sitting approximately 1.5 m away from the apparatus.

5.7. Forced swimming test

The mice were forced to swim individually in a glass cylinder 12 cm in diameter and 30 cm in height, filled with water to a height of 20 cm. The temperature of the water was adjusted to $25 \pm 1\,^{\circ}\mathrm{C}$. The water was changed between the individual mice. A 15-min pretest session was followed 24 h later by a 5-min test session. Phenoxybenzamine (2 mg/kg, ip.), prazosin (62.5 μg/kg, ip.), yohimbine (5 mg/kg, ip.), propranolol (5 mg/kg, ip.), methysergide (5 mg/kg, ip.), cyproheptadine (3 mg/kg, ip.), atropine (2 mg/kg, ip.), haloperidol (10 μg/kg, ip.) or bicuculline (2 mg/kg, ip.) was administered 1 h before the test session, followed 30 min later by KP-13 (2.0 μg/2 μl, icv.). Physiological saline was used for control. A time-sampling technique was applied to score the durations of climbing, swimming and immobility. Climbing time was measured when the mouse was participating in active vertical motion with its forelegs above the water level; swimming time was recorded when the mouse was moving horizontally on the surface of the water; and immobility time was registered when the mouse was in a upright position on the surface with its front paws together and making only those movements necessary to keep itself afloat.

5.8. Tail-flick test

Kisspeptin effect on morphine-evoked analgesic response was tested by the tail-flick system (IITC Life Science, California, USA). All experiments were started with an initial tail-flick latency measurement. Tail stimulation was delivered at different sites in consecutive measures to prevent tissue damage. The analgesic effect was expressed according to following equation: analgesic effect (%) = (TFn - TF0)/ TFmax - TF0) x 100, where TF0 is the tail-flick latency in the preliminary test mentioned above or (in tolerance studies) before morphine injection. TFn is the value of a repeated corresponding measurement n (15, 30, 60 or 60, 90, 120 min) after KP-13 or/and morphine injection, and TFmax indicates the cutoff (20 s). The following experiments were carried out: The effect of KP-13 on pain sensitivity were measured. In experiments with KP-13 on the acute antinociceptive effect of a single dose of morphine, the peptide was administered 30 min prior to the test dose of morphine (2.4 mg/kg sc.), and the pain sensitivity was measured 30 and 60 min later. In acute tolerance studies, animals were pretreated with KP-13 and 60 min later a tolerance-inducing dose of morphine (60 mg/kg sc.) was administered, 24 h after of which a test dose of morphine (4 mg/kg sc.) were injected to assess the antinociceptive effect. In acute withdrawal studies, 30 min after KP-13 pretreatment a tolerance-inducing dose of morphine (60 mg/kg sc.) was administered, 3 h after of morphine injection animals received naloxone to precipitate withdrawal signs. The precipitated abstinence syndrome was assessed by scoring the latency of the stereotyped jumping from a circular platform with a diameter of 35 cm placed 70 cm high for 15 min. Meanwhile, body temperature and weight of the animals were measured before naloxone treatment, 15 min, 30 min and 60 min after, of which the changes were calculated.

5.9. Statistical analysis

Statistical analysis of the results was performed by analysis of variance (ANOVA). In case of single treatments one-way ANOVA was employed, followed by the Holm-Sidak post hoc test for multiple comparisons when the test prerequisites were fulfilled. When the test of the homogeneity of variances was not satisfied, nonparametric ANOVA on ranks (Kruskal-Wallis) was performed, followed by Dunn's test for multiple comparisons. For the evaluation of the telemetric recordings, repeated measure ANOVA was performed. For the assessment of the experiments with combined treatments two-way ANOVA was used followed by the Holm-Sidak post hoc test. A probability level of less then 0.05 was accepted as indicating a statistically significant difference.

6. Results

6.1. Effects of KP-13 on corticosterone secretion

The icv injection of KP-13 induced a dose-dependent elevation in basal plasma corticosterone level. The corticosterone level following the 2 μg dose proved to be statistically different from the control. In KP-13-treated rats, pretreatment with the aCRF did not reverse the KP-13-induced elevation of corticosterone, however pretreatment with AVP ANT resulted in a marked decrease of KP-13 evoked corticosterone elevation.

6.2. Effects of KP-13 on spontaneous locomotion and core temperature

After the KP-13 treatments increases in both locomotor activity and core temperature were observed in the home cages of the animals. In the case of locomotion, this effect was present only for approximately the next hour and the activity of the rats then returned to the level of the control animals, whereas in the case of the core temperature the hyperthermic action of KP-13 persisted for several hours after peptide administration.

6.3. Effects of KP-13 on open field behaviour

KP-13 evoked a marked increase in the number of square crossings in the open field test, but did not affect the other recorded parameters: rearing activity, grooming or defecation. The effect of KP-13 administered in a 1 μg dose on the number of square crossings proved to be statistically significant.

6.4. Effects of KP-13 on elevated plus maze behaviour

KP-13 reduced both the number of entries into and the time spent in the open arms, this action proving to be dose-dependent. A statistically significant change in the time spent in the open arms was caused by the 2 μg dose of KP-13, while as concerns the number of entries into open arms, both the 1 and 2 μg doses induced significant reductions. There was no difference in the number of total entries between the tested groups. In KP-13-treated rats, pretreatment with the AVP ANT partially reversed the KP-13-induced decrease in both time spent and entries into open arms and pretreatment with kisspeptin-234 resulted in an increase the time spent and the entries into open arms.

6.5. Effects of KP-13 on forced swimming test

Relative to the control, both the 1 and 2 μg dose of KP-13 significantly decreased the immobility time, significantly increased the climbing time and swimming time. The most

effective dose of KP-13 proved to be the 2 μg dose.

6.6. Effects of antagonist treatments on the KP-13-induced antidepressive behaviour

All applied antagonist treatments alone did not affect the immobility time, climbing time or swimming time. In KP-13-treated mice, pretreatment with phenoxybenzamine, yohimbine and cyproheptadine partially reversed the KP-13- induced change in the immobility time, and decreased the changes in the climbing and swimming times. Whereas, pretreatments with prazosin, methysergide, atropin and bicuculline did not affect the increased immobility time or the decreased climbing and swimming times elicited by KP-13 injection. The above results reveal that the antidepressant-like effects of KP-13 in this modified mouse FST are mediated, at least in part, by α_2-adrenergic receptors and $5 - HT_2$ serotonergic receptors.

6.7. Effects of KP-13 on tail-flick latency

Icv injection of KP-13 dose dependently decreased the tail-flick latency of CFLP mice, of the different doses applied the 1 μg proved to be the most effective. The action of kisspeptin on nociception was observed both 30 and 60 min after peptide administration.

6.8. Effects of KP-13 on challenge dose of morphine

A single dose of 2.4 mg/kg sc. morphine induced an appr. 80 % analgesia. KP-13 in a 1 μg dose significantly lowered the analgesic effect of morphine 30 and 60 min after the narcotic challenge.

6.9. Effects of KP-13 on acute morphine tolerance

Acute tolerance was observed 24 h after a tolerance inducing dose of morphine was applied sc. Our results showed that KP-13 treatment 30 min before tolerance induction prevents the development of acute morphine tolerance. The KP-13-treated animals that received the tolerance inducing dose of morphine showed a significantly higher antinociceptive effect then tolerant animals both 30 min, 60 min and 120 min after injection of the 4 mg/kg sc. test dose of morphine.

6.10. Effects of KP-13 on naloxone-precipitated acute morphine withdrawal

In the naloxone-precipitated withdrawal studies KP-13 caused a marked decrease in the jumping latency of animals from the platform. The two-factor analysis of variance on weight

changes and temperature changes revealed no significant difference between the morphine tolerant and the KP-13-treated morphine tolerant groups. Only a slight tendency for KP-13 to further reduce the weight loss and hypothermia was observed. Of note is the result that KP-13 alone caused a marked elevation in body temperature compared to the control animals within all levels of time.

7. Conclusion

The thesis was set out to explore if kisspeptins have a more widespread function in the CNS then the regulation of the HPG axis. We have proposed a role for kisspeptin in the organisation of the stress response and stress-associated behaviours. The study has also sought to know whether kisspeptin, similarly to other RF-amide peptides can modulate pain sensitivity and can impact the acute actions of morphine on nociception.

Our data indicate that icv. administered KP-13 stimulates the HPA axis through the release of AVP, induces hyperthermia, activates motor behaviour and causes anxiety in rats by the activation of KISS1R and by AVP secretion. Our results also demonstrate that, in mice, the antidepressant-like effects of KP-13 in a modified mouse FST are mediated, at least in part, by an interaction of the α_2-adrenergic and $5 - HT_2$ serotonergic receptors. Finally, our data underlies kisspeptin's hyperalgesic effect and suggest that central KP-13 administration can modify the acute effects of morphine.

Kisspeptin and the stress response Our findings are in complete harmony with the growing body of evidence suggesting that kisspeptin may play a more general role in autonomic, neuroendocrine and behavioural regulation. The peptide takes part in cardiovascular and metabolic functions, pregnancy and cognitive processes. Clearly, the control of the aforementioned processes necessitates integration with gonadal activities. The gender-dependent nature of the stress response and stress tolerance, the interactions between the HPG axis and the HPA system have been well described in the literature. Sexual steroids influence the expression of CRF and AVP in the hypothalamus, whereas chronic stress suppresses the reproductive function. However, a series of experiments demonstrate that glucocorticoid release from the adrenal gland, in actuality, preserves the HPG activity during stress. In light of this and the effect of kisspeptin, a prominent stimulator of GnRH release, on the HPA axis activity, the concept on the interplay between the HPG and HPA axises needs to be revisited in order to truly understand how integration between them is managed. Kisspeptin has also already been implicated in sensing and relaying information about energy stores to the HPG axis. Animal studies revealed reduced expression of KISS1 mRNA and gonadotrophin secretion in fasting states. Also, leptin, a critical adipose hormone essential for pubertal onset and fertility, may signal metabolic state of the body through kisspeptin neurones. Furthermore, a number of other metabolic modulators have been linked with kis-

speptin neuronal activity directly or indirectly like insulin, ghrelin and central hypothalamic regulators. All of these findings point to the possibility that changes in kisspeptin signalling might be responsible for the compromised fertility in altered energy balance. Taking these phenomena and the versatile physiological functions of kisspeptin into account, it is apparent that, besides the well-characterised PrRP, further members of the RF-amide family may play integrative roles in the harmonization of the HPG and HPA activity.

Kisspeptin and nociception On another note, sex differences in pain sensitivity and variations in pain threshold and pain tolerance over the menstrual cycle as well as the overrepresentation of characteristic pain syndromes such as fibromyalgia, migraine, tension headache, trigeminal neuralgia, carpal tunnel syndrome and temporomandibular disorders in women point toward the involvement of the reproductive system in pain modulation. Of additional interest are the changes occurring with the onset of menopause. It has been shown that after menopause a decrease in endogenous opioid production occurs, which have been associated with the symptomatology of post menopause and hormone replacement therapy restored opioid levels. Clearly, there is a connection between the HPG axis and the regulation of pain sensation, however, the underlying mechanism is not well understood and many of the published studies, both animal and human, experimental and clinical reported, at least, partly contradictory results. Gonadal hormones including estrogens and progesterone have been implicated in affecting pain sensitivity, however again results are contradictory as a clear anti or pronociceptive effect could not be demonstrated for either. Our results taken together with the above mentioned data raises the possibility that kisspeptin might provide a link between the reproductive and pain modulatory systems.

Kisspeptin and circadian rhythm The dense expression of kisspeptin in the arcuate nucleus and the innervations of the suprachiasmatic nucleus underlines our findings and argues for the role of the peptide in the circadian regulation of metabolic processes, core body temperature, pain sensation and hormone production. In point of fact, the basal HPA activity shows a circadian rhythm that is provided by input from the suprachiasmatic nucleus, leading to the pulsatile secretion of CRF. The role of kisspeptin in circadian control is further supported by recent publications establishing the kisspeptin system as an important relay center for the integration of environmental cues and the precise timing of puberty, the preovulatory LH surge, and structural plasticity in seasonal reproduction. Similarly, the observed effect in our experiments on motor paradigms and pain sensation can also be attributed to a plausible regulatory role in circadian activity, sleep-wake cycle, arousal and autonomic regulation suggested by the expression of kisspeptin neurons in the suprachiasmatic nucleus and the preoptic nucleus of the hypothalamus.

Limitations of the study and Implications for the future Limitations of the studies on which this thesis was based on must be addressed as well. The interpretation of the above presented results need careful consideration. First, the seemingly contradictory results on anxiety and depressive behaviour in rats and mice draw attention to the differences among animal species. Second, this study did not investigate and therefore could not distinguish if the observed effects of kisspeptin are truly that of KISS1R mediation and not of the more general effects of other RF-amide peptides due to NPFF receptor activation. As a consequence, further investigations are necessary to clarify the mediation and signal transduction of the presented physiological phenomena, with special emphasis on the separation of the unique and overlapping features in the activity profile of the different RF-amides. Furthermore, the mode of kisspeptin administration, as with most of peptides, provides quite a challenge and thus there is a need for the development of non-peptidergic analogues. With these it might be possible to further elaborate the true spectrum of its physiological actions and may provide a new avenue for the development of novel strategies for reproductive endocrine disorders. In point of fact, there are multiple clinical trials underway to investigate kisspeptin's possible effect and therapeutic value in post menopause, diabetes mellitus, disorders of puberty and infertility. The potential ability of kisspeptin to influence pain sensitivity, response to analgesic treatment and mood cannot be disregarded and must be further investigated in both animals and humans.

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