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Research report

Effects of kisspeptin-13 on the hypothalamic-pituitary-adrenal axis, thermoregulation, anxiety and locomotor activity in rats

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HIGHLIGHTS

- Effects of kisspeptin-13 on the HPA axis, temperature and behavior were studied.
- Kisspeptin-13 activated the HPA axis in rats.
- Also stimulated spontaneous locomotor activity, exploratory behavior and anxiety.
- Furthermore, kisspeptin-13 induced hyperthermia.

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ABSTRACT

Kisspeptin is a mammalian amidated neurohormone, which belongs to the RF-amide peptide family and is known for its key role in reproduction. However, in contrast with the related members of the RF-amide family, little information is available regarding its role in the stress-response. With regard to the recent data suggesting kisspeptin neuronal projections to the paraventricular nucleus, in the present experiments we investigated the effect of kisspeptin-13 (KP-13), an endogenous derivative of kisspeptin, on the hypothalamus-pituitary-adrenal (HPA) axis, motor behavior and thermoregulatory function. The peptide was administered intracerebroventricularly (icv.) in different doses (0.5–2 μg) to adult male Sprague-Dawley rats, the behavior of which was then observed by means of telemetry, open field and elevated plus maze tests. Additionally, plasma concentrations of corticosterone were measured in order to assess the influence of KP-13 on the HPA system. The effects on core temperature were monitored continuously via telemetry. The results demonstrated that KP-13 stimulated the horizontal locomotion (square crossing) in the open field test and decreased the number of entries into and the time spent in the open arms during the elevated plus maze tests. The peptide also caused marked elevations in the spontaneous locomotor activity and the core temperature recorded by the telemetric system, and significantly increased the basal corticosterone level. In conclusion, our data indicate that icv. administered KP-13 stimulates the HPA axis, induces hyperthermia, activates motor behavior and causes anxiety in rats.

1. Introduction

Kisspeptin, classified as a member of the Arg-Phe (RF)-amide family [1], is a C-terminally amidated neurohormone and is a key regulator of the hypothalamic-pituitary-gonadal (HPG) axis [2–4]. The kisspeptin related peptides are neuropeptide FF and AF, prolactin releasing peptide (PrRP), RFamide-related peptides, and the most recently found, pyroglutamylated RFamide peptide [1]. They all share an N-terminal sequence homology and are widely distributed in the CNS, but they vary in their structure and receptor preference [1] binding to either one or several G-protein coupled receptors [5]. Literature shows that the effects of RF-amide peptides partially overlap, but in case of some physiological parameters they exert opposite actions. For example, PrRP activates the hypothalamic-pituitary-adrenal (HPA) axis [6], increases stereotyped locomotion [7] and pressor response [8]. Neuropepide AF (NPAF) also induces the HPA axis and locomotor activity, however, it causes a decrease in heart rate and core temperature [9]. Thus, in light of the above-mentioned data, kisspeptin might also have a wider range of function then so far assumed and may influence the same biological parameters as other RF-amide peptides.

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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 [10,11]. Kisspeptin is the product of the *KiSS-1* gene; the peptide consists of 54 aminoacids (KP-54), but its cleavage can give rise to biologically active derivatives containing 14, 13 or 10 aminoacids, christened kisspeptin-14 (KP-14), kisspeptin-13 (KP-13) and kisspeptin-10 (KP-10), respectively [2,10]. Kisspeptin and its receptor are abundant in the central nervous system (CNS), especially in the limbic system, the striatum, the pituitary and the hypothalamus, including the paraventricular nucleus (PVN) [10,12–14]. Recent evidence suggests that kisspeptin, beside the KISS1R, also activates the neuropeptide FF2 receptor [15], which mediates autonomic, endocrine, behavioral and nociceptive processes [9,16].

The first biological action associated with kisspeptin was the suppression of metastasis in melanoma [17], but recently a number of publications [4,18,19] has demonstrated the pivotal role of the kisspeptin system in the regulation of the reproductive axis. Kisspeptin is necessary for the normal secretion of gonadotropin releasing hormone (GnRH) [20,21] and subsequently luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [21], meanwhile, it may also control the onset of puberty [20,22] through its activity on the biological clock of the CNS [23,24]. These seemingly disparate activities can be attributed to the ability of the peptide to stimulate diverse intracellular signal transduction cascades involving the activation of phospholipase C (PLC), mitogen activated protein kinase (MAPK), calcineurin and NFκB [25]. These pathways can influence hormone secretion, chemotaxis, and the organization of the cytoskeleton, neuronal activity and plasticity [24–26].

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 [13,27], 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, behavior and thermoregulation, which are processes controlled by the hypothalamus and the limbic system, where kisspeptin and its receptors are found in abundance [13]. 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 behavior was observed in open field and elevated plus maze tests.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Domaszék, Hungary) weighing 150–250 g were used at the age of 8 weeks. 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, which approved these experiments. Approximately 160 animals in total were used in our experiments. Every experiment was carried out separately; the same animal has never been used for different experimental procedure.

2.2. Surgery

The animals were allowed 1 week to acclimatize before surgery. Subsequently, they were implanted with a stainless steel Luer cannula (10 mm long) aimed at the right lateral cerebral ventricle under pentobarbital (35 mg/kg, intraperitoneally) anesthesia. The stereotaxic coordinates were 0.2 mm posterior and 1.7 mm lateral to the bregma, and 3.7 mm deep from the dural surface, according to the atlas of Pellegrino et al. [28]. The cannula was secured to the skull with dental cement and acrylate. The rats were used after a recovery period of 5 days. All experiments were carried out between 8:00 and 10:00 a.m.

For implantation of the telemetric radio transmitter (E-Mitter: a temperature-activity transponder), the rats were anesthetized with pentobarbital (35 mg/kg, intraperitoneally). The abdomen was opened by making a 2-cm midline incision along the linea alba. The E-Mitter was placed in the abdominal cavity, along the sagittal plane, in front of the caudal arteries and veins, but dorsal to the digestive organs. The abdominal wound was then closed with absorbable suture material, while the skin was closed with stainless steel suture clips. After a recovery period of 5 days, the rats were implanted with the stainless steel Luer cannula for intracerebroventricular (icv.) administration.

At the end of the experiments, the correct position and the permeability of the cannula were checked. In the behavioral studies, each rat was sacrificed under pentobarbital anesthesia, and in the endocrinological experiments the head was collected after decapitation. Methylene blue was injected via the implanted cannula and the brains were then dissected. Only data from animals exhibiting the diffusion of methylene blue in all the ventricles were included in the statistical evaluation.

2.3. Treatment

Rats were injected with different doses of KP-13 (Bachem Ltd., Switzerland) icv. in a volume of 2 μl over 30 s with a Hamilton microsyringe, immobilization of the animals being avoided during handling. The doses applied were 0.5, 1, 2 or 5 μg dissolved in 0.9% saline. Control animals received saline alone. Thirty minutes after peptide administration, the rats were decapitated to obtain trunk blood for corticosterone measurement or were subjected to behavioral testing.

2.4. 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 [29] as modified by Purves and Sirett [30].

2.5. 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.

2.6. Open field test

In the open field 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, the floor area of which measured 60 cm × 60 cm, marked into 36 10 cm × 10 cm square. The standard source of illumination was a 60 W bulb at a height of 80 cm. 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 (square crossing), the vertical locomotion was determined by the number of rearings (standing on the hind legs), and the grooming activity was established by observing face washing, forepaw licking and head stroking. Every episode of face washing, forepaw licking and head stroking was counted as a separate grooming session, independently of how long it actually lasted.

2.7. Elevated plus maze test

The elevated plus maze apparatus is a plus-shaped platform elevated 50 cm above the floor. It consists of two opposing arms (50 cm × 10 cm each) with 10 cm high enclosing walls (closed arms) and two arms with no walls (open arms). A 60 W light bulb at a height of 80 cm provided the illumination. The maze was cleaned between each session with 96% ethyl-alcohol and all experiments were conducted between 8:00 a.m. and 10 a.m. 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. The test is designed to assess anxiety based on the concept that the open arms are more aversive, and anxious rats therefore spend less time in them [31]. In the figures the ratio of time spent in open arms to total time spent in all arms, the ratio of entries to open arms to total number of entries and the total number of entries into all arms are presented.

2.8. Statistical analysis

Data are presented as means ± SEM. Statistical analysis of the results was performed by analysis of variance (ANOVA). For the corticosterone measurements, open field and elevated plus maze tests, one-way ANOVA was employed, followed by the Holm–Sidak post hoc test for multiple comparisons when the test prerequisites were