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Local injection of D-lys-3-GHRP-6 in the rat amygdala, dentate gyrus or ventral tegmental area impairs memory consolidation

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ABSTRACT

It is well known that the hormone ghrelin affects learning and memory in different experimental models of learning. Though, the effect of antagonism of ghrelin receptor type 1a (GHS-R1a) in various regions of the brain and on different stages of learning has not been examined. In this study the effect of injection of a GHS-R1a selective antagonist (D-Lys-3-GHRP-6) into the basolateral amygdala, dentate gyrus or ventral tegmental area was examined on memory consolidation in the passive avoidance task. Adult male Wistar rats weighing 230–280 g were used. Animals underwent stereotaxic surgery and cannulated in their amygdala, dentate gyrus or ventral tegmental area. One week after surgery, the rats received different doses of D-Lys-3-GHRP-6 (0.08, 0.8, and 8 nM), immediately after training. The control groups received solvent of the drug. Twenty four hours later in the test day, memory retrieval was assessed. In all groups, post-training injection of D-Lys-3-GHRP-6 decreased step-through latency and increased entries into the dark compartment and time spent in the dark compartment, significantly and in a dose-dependent manner. The results indicate that antagonism of the GHS-R1a in the rat amygdala, dentate gyrus or ventral tegmental area impairs memory consolidation and show that the ghrelin signaling has a widespread influence on cognitive performance.

1. Introduction

Ghrelin is a stomach-derived hormone that was introduced to the scientific community by Kojima et al., 1999. It is the endogenous ligand for the growth hormone (GH)- secretagogue receptor (GHS-R) (Kojima, 2008). Following synthesis of the hormone in the stomach, it circulates in the bloodstream in substantial amounts, of which > 90% consists of des-acyl ghrelin and < 10% acyl ghrelin (Patterson et al., 2005). Though, the acyl group of ghrelin is central for its binding to GHS-R (Kojima et al., 1999).

The ghrelin receptor named GHS-R is a G-protein coupled receptor (GPCR) (Howard et al., 1996). GHS-R1a expresses in various regions of the rat brain including hypothalamic hunger regions, the cerebral cortex, CA_1, CA_2 and CA_3 regions of the hippocampus, raphe nuclei and substantia nigra (Ferrini et al., 2009; Guan et al., 1997; Zigman et al., 2006). It is also expressed in the basolateral complex of the amygdala (Alvarez-Crespo et al., 2012), dentate gyrus or ventral tegmental area (VTA) (Guan et al., 1997).

In the central nervous system, ghrelin is recognized to affect feeding behavior, potentially acting on the hypothalamus (Abtahi et al., 2017; Qi et al., 2015). However, it is now known to affect widespread functions including cognition in general and memory processes in particular. There is accumulating data that show ghrelin can affect memory formation in rodents in different models of memory (Atcha et al., 2009; Babri et al., 2013; Beheshti and Shahrokhi, 2015; Carlini et al., 2010a, 2008, 2002, 2010b; Chen et al., 2011; Diano et al., 2006; Goshadrou et al., 2013; Goshadrou and Ronaghi, 2012; Kajbaf et al., 2012; Li et al., 2013; McNay, 2007; Toth et al., 2010, 2009; Wang et al., 2013; Zhu et al., 2013). Recognition memory, by means of the novel object recognition task, was significantly diminished in ghrelin Knockout (KO) mice. Also, spatial memory was significantly impaired in ghrelin KO mice. These deficits could be prevented by acyl ghrelin injections for 7 days (Santos et al., 2017). In the step-down passive avoidance task, i.c.v. injection of ghrelin increased memory retention (Carlini et al., 2002). Post-training intramygdaloid microinjection of the acylated ghrelin improved memory consolidation in the step-through passive avoidance task (Toth et al., 2009).

Formation of memory occurs in a number of stages counting acquisition, consolidation, retention and retrieval (Abel and Lattal, 2001). The impact of antagonism of the ghrelin receptors on each of the

Abbreviations: CNS, Central nervous system; CTA, Conditioned taste aversion; GHS-R, Growth hormone secretagogue receptor; GPCR, G-protein coupled receptors; i.c.v., intracerebroventricular; LTP, Long-term potentiation; NST, Number of step-through into the dark compartment; PAT, Passive avoidance task; STL, Step-through latency; TDC, Time spent in the dark compartment; VTA, Ventral tegmental area

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particular stages of memory formation and on various regions of the brain is not completely known.

We have previously indicated that blocking the ghrelin receptor type 1a in the rat brain by means of i.c.v. injection of D-Lys-3-GHRP-6, a selective GHS-R1a antagonist impaired memory consolidation (Beheshti and Shahrokhi, 2015). However, it was not evident that the antagonism of the GHS-R1a in which regions of the brain affected the memory performance. Amygdala, dentate gyrus and ventral tegmental area are crucial sites for the consolidation of memory in the passive avoidance task (Mahmoodi et al., 2011; McGaugh, 2002; Shahidi et al., 2008). Meanwhile, they all express GHR-1a with the highest density found in the dentate gyrus (Guan et al., 1997). In the present study, we have assessed the effects of local injection of D-Lys-3-GHRP-6 in the rat amygdala, dentate gyrus or ventral tegmental area on memory consolidation using a passive avoidance paradigm.

2. Materials and methods

2.1. Drugs

D-Lys-3-GHRP-6 was purchased from Sigma (USA). Ketamine and xylazine were purchased from Alfasan (Netherland). D-Lys-3-GHRP-6 was dissolved in sterile saline.

2.2. Animals

Adult male naïve Wistar rats weighing 230–280 g were used. Rats were obtained from the breeding colony of Department of Biology, University of Isfahan. Animals were housed four per cage in a temperature (24 ± 2 °C) controlled room that was maintained on a 12:12 light cycle (light on at 07:00 a.m.). Following surgery rats were housed separately and had free access to food and water in their home cage. All experiments were carried out in accordance with the guide for the care and use of laboratory animals (USA National Institute of Health publication No. 80-23, revised 1996) and were approved by the graduate studies committee of Department of Biology, University of Isfahan.

2.3. Surgical procedures

Seven days prior to initiation of the behavioral experiments, the rats were anesthetized with a mixture of ketamine (100 mg/kg, i.p) and xylazine (10 mg/kg, i.p). Two guide cannulas were implanted (22-gauge) at site 1 mm dorsal to the dentate gyrus and 2 mm dorsal to the VTA or amygdala (AP: − 3.8, − 4.8 or − 2.4 mm from bregma, ML: ± 2.4, ± 0.9 or ± 4.8 mm from the midline, and DV: 3, 8 or 8.3 mm from dura, respectively), bilaterally and according to the atlas of Paxinos and Watson (Paxinos, 2007). One screw was inserted into the skull. Before cannula fixation appropriate amount of doxycycline powder was spread on the skull surface to avoid probable infections. The cannulas were fixed to the skull with dental cement. The cannulas were locked with a stainless steel stylet (27-gauge) smeared with mineral oil to prevent clogging with blood.

2.4. Microinjection procedure

Intracerebral injections were made via guide cannula with injection needles (27-gauge) that were associated by polyethylene tubing (PE20, Stoelting) to a 2 µl Hamilton microsyringe. The injections (1 µl total volume, 0.5 µl for each side) in each side were delivered over 2 min and the injection needle was left in place an extra minute before it was slowly withdrawn. Injections were done between 10 a.m. to 14 p.m. in order to prevent variations induced by circadian rhythms (Beheshti and Shahrokhi, 2015).

2.5. Passive avoidance task

A step-through passive avoidance apparatus (Borj Sanat Co, Iran) with two opaque white and black compartments was used in the experiments. The white compartment was lightened by a lamp. The two distinct compartments, each with the interior dimensions of $30 \times 25 \times 25\ cm^3$ were separated by a sliding door of $8 \times 25\ cm^2$. The floors of both compartments were made of stainless steel rods with 2.5 mm diameter and 1 cm space between the rods. The experiments were performed in a silent room.

The passive avoidance task consists of two trials: the training and the test, which are performed in two separate days. The training trial consists of two stages: “habituation training” and “acquisition training”. During habituation training, each rat was positioned in the white compartment facing the sliding door. Five seconds later the door was elevated to let the animal enter the second, black compartment. When the animal walked into the black compartment with all four paws, the door was closed and the rat remained there for 20 s. Then the animal was placed in a temporary cage. 30 min later during acquisition training, the rats were again placed in the white start compartment for 5 s, then the door was elevated to let the animal enter the black compartment and the door was closed, but this time an electrical shock of 0.8 mA lasting for 3 s was delivered. After 20 s, the rat was placed into the temporary cage. 2 min later,to assay short-term memory the rats were again placed in the white start compartment for 5 s, and then the door was elevated to let the animal enter the black compartment. If the animal did not enter the black compartment for 2 continouos minutes, the training was considered successful and terminated. If it entered the black compartment for a second time, the rat had a foot shock again. After 20 s, the rat was placed into the temporary cage. 2 min later, short-term memory was assessed again. The rats were again placed in the white start compartment for 5 s and then the door was elevated to let the animal enter the black compartment. When the rat remained in the white compartment for a 2 continouos minutes and did not enter the black compartment, the training was terminated. On the second day, a retrieval test was done to evaluate long-term memory. Each animal was placed in the white start compartment for 20 s, then the door was raised and the step-through latency (STL), the time spent in the dark compartment (TDC) and the number of step-through into the dark compartment (NST) were recorded, up to 600 s.

2.6. Experiments

Four groups of animals (n = 8) received D-Lys-3-GHRP-6 (0.08, 0.8, and 8 nM) or saline at each of the brain regions: basolateral amygdala, dentate gyrus or ventral tegmental area, immediately after training (post-training). Twenty four hours later in the test day, memory retrieval was measured. The doses of the drug were chosen according to the results of our previous study (Beheshti and Shahrokhi, 2015).

2.7. Statistical analysis

Group data were expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons post-test was done using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com. In all experiments, the “P” values < 0.05 were considered statistically significant.

3. Results

3.1. Verification of cannula placements

After completion of the experiments, each animal was euthanized with an overdose of chloroform. The brains were removed and fixed in a 10% formalin solution at least 48 h before sectioning. Sections were