Peptides 62 (2014) 155–158

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

The role of apelin-13 in novel object recognition memory

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ARTICLE INFO

Article history:
Received 4 September 2014
Received in revised form 7 October 2014
Accepted 7 October 2014
Available online 17 October 2014

Keywords:
Apelin-13
APJ
Object recognition
Short-term memory
Long-term memory

ABSTRACT

Apelin and its receptor APJ (apelin receptor) are prominently expressed in brain regions involved in learning and memory. However, the role of apelin in cognition was largely unclear. Here, the role of apelin-13 in memory processes was investigated in mice novel object recognition task. Post-training injection of apelin-13 (0.3 and 1 nmol) dose-dependently impaired short-term memory (STM), however, pre-training infusion of apelin-13 (1 nmol) did not affect STM, suggesting apelin-13 blocks formation but not acquisition of STM. Apelin-13 (1 nmol) administered immediately, 30, 60 or 120 min post-training impaired long-term memory (LTM) in a time-dependent manner (30 min), however, both pre-training and pre-test infusion of apelin-13 (1 nmol) did not affect LTM, suggesting apelin-13 impaired consolidation but not acquisition and recall of LTM. Taken together, for the first time, our results indicate that apelin-13 blocks STM formation and LTM consolidation in novel object recognition task.

Introduction

Neuropeptide apelin, the endogenous ligand of the G-protein-coupled receptor APJ (apelin receptor) [19,25], exhibits several biologically active forms, including apelin-36, apelin-17 and apelin-13 [15]. Apelin is extremely conserved among different species, and the peptide apelin-13, studied in our present study, is completely conserved across all species investigated [13]. Both apelin and its receptor are widely expressed in both the CNS and peripheral tissues [5,10,13,16,23,24]. Therefore, lots of physiological and pathophysiological roles for apelin/APJ system have been reported, including regulation of cardiovascular system, immune system, fluid homeostasis, and adiposity [7,12,22].

Apelin and APJ are strongly expressed in the learning- and memory-associated brain regions, including hippocampus, amygdala and cerebral cortex [10,13,16], suggesting apelin/APJ system may take part in the regulation of memory processes. This possibility is also raised by the following evidences. First, apelin/APJ system could negatively regulate the cAMP pathway [25], which is crucial for learning and memory [6]. Second, apelin has been reported to attenuate N-methyl-D-aspartate (NMDA) receptor-induced intracellular Ca^{2+} accumulation and excitotoxicity in the cortical and hippocampal neurons [4,18,28], suggesting that apelin could inhibit the NMDA receptor pathway, which plays an essential role in learning and memory [14]. However, the role of apelin/APJ system in cognition was still unclear; except that, most recently, Telegdy et al. reported that intracerebroventricular (icv) injection of apelin-13 facilitated passive avoidance memory consolidation [26].

Novel object recognition (NOR) task is a non-aversive learning paradigm which is based on animals' spontaneous preference for the novel object, thus it has the advantage of avoiding the potential confounds of using differential rewards or punishments, and is widely used to evaluate the effects of various drugs on learning and memory processes (see review [2]). The present study was undertaken to investigate whether apelin-13 could regulate short-term memory (STM) and long-term memory (LTM) in mice NOR task.

Materials and methods

Animals

Male Kunming strains of Swiss mice were obtained from the Experimental Animal Center of Lanzhou University, China. Animals were housed in an animal room that was maintained at 22 ± 2 °C with a 12-h light: 12-h dark cycle. Food and water were available *ad libitum*. All the protocols in this study were approved by the Ethics Committee of Lanzhou University, China.

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http://dx.doi.org/10.1016/j.peptides.2014.10.003
0196-9781/

Surgery

Each mouse (20–24 g) was anesthetized with sodium pentobarbital (75 mg/kg; Sigma–Aldrich, USA) and placed in a stereotaxic frame (Leica, Germany). According to the atlas of Paxinos and Franklin [21], a 9 mm 26-gauge stainless-steel guide cannula was implanted over the right lateral ventricle (0.5 mm posterior to bregma, 1.0 mm lateral to midline, 2.0 mm ventral to skull surface). After surgery, mice were housed individually and were allowed to recover 5–7 days.

NOR task

The procedure of NOR task was based on our previous reports [8,9], and that described by Okamura et al. [20]. Briefly, each mouse was tested in its home cage in a sound-attenuated room with somber lighting. The general procedure consisted of a training phase and a retention phase, separated by a delay. Each mouse was handed 3 min per day for three consecutive days prior to training. During the training phase, two identical objects were placed in the opposite sides of the home cage. The sample phase ended when mouse had explored two identical objects for a total of 20 s. Mouse showing a total exploratory time (TET) < 20 s within 10 min was excluded. In the test phase, a familiar object from the training phase and a novel object were placed in the same locations as in the training phase. The test phase was ended when mouse had explored two objects for a total of 25 s, or after 5 min had passed, whichever came first. All objects were made of plastic or glass, similar in size (4–5 cm high) but different in color and shape. There were several copies of each object for use interchangeably. Throughout the experiments, the objects used as novel or familiar were counterbalanced to exclude possible preference for special object. Moreover, the locations of the novel and familiar objects were also counterbalanced in the test to exclude possible spatial bias. Objects were cleaned thoroughly between trials to ensure absence of olfactory cue. Exploration was defined as sniffing or touching object with nose and/or forepaws. Resting against or turning around object was not considered exploratory behavior. The time spent exploring each object was recorded by an observer blind to the treatments. A discrimination index (DI) in the test phase was calculated as a percentage of the time spent exploring the novel object over the total time spent exploring both objects. A DI of 50% corresponds to the chance level and a higher DI reflects intact recognition memory.

Drug infusion

Apelin-13 (Glu-Arg-Pro-Arg-Leu-Ser-His-Lys-Gly-Pro-Met-Pro-Phe) was synthesized and purified as described in our previous report [3]. Apelin-13 was dissolved in artificial CSF containing (in mM) 126.6 NaCl, 27.4 $NaHCO_3$, 2.4 KCl, 0.5 KH_2PO_4, 0.89 $CaCl_2$, 0.8 $MgCl_2$, 0.48 Na_2HPO_4, and 7.1 glucose, pH 7.4. For icv infusion, the infusion cannula extended 0.5 mm beyond the tip of the guide cannula. Apelin-13 (0.3 or 1 nmol) or vehicle (2 μl) was infused over a period of 2 min via a 25 μl Hamilton syringe (Hamilton) mounted on a microdrive pump (KD Scientific). The infusion cannula remained in place for 1 min after infusion to allow for drug diffusion. After completion of experiment, the placement of cannula was verified by histological examination and the animal with misplaced icv injection was excluded.

Experiment design

STM and LTM were determined at a delay of 30 min and 24 h, respectively. Initially, three groups of mice (vehicle, 0.3 and 1 nmol apelin-13) were used to determine whether apelin-13 injected immediately post-training could regulate the formation of STM.

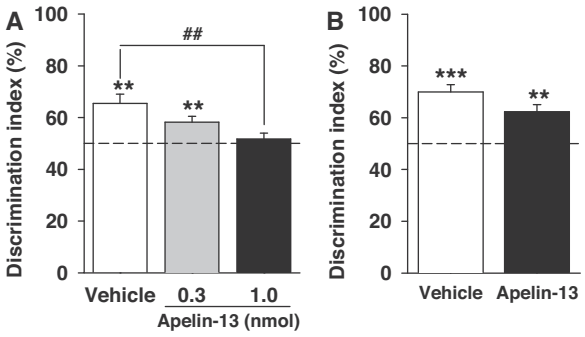

Fig. 1. The effect of apelin-13 on short-term memory (STM). (A) Apelin-13 (0.3 and 1 nmol) injected immediately after training dose-dependently impaired STM formation. Vehicle, $n = 10$; apelin-13 0.3 nmol, $n = 8$; apelin-13 1 nmol, $n = 10$. (B) Apelin-13 (1 nmol) infused 5 min before training did not affect STM acquisition. $N = 8$ for both group. The dashed line indicates 50% chance level. $^{\#\#}p < 0.01$ compared with vehicle; $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with chance level. Vertical lines represent SEM.

Then, two groups of mice (vehicle and 1 nmol apelin-13) were utilized to detect whether apelin-13 could influence STM acquisition when it was infused 5 min before training. Furthermore, four vehicle groups and four apelin groups of mice were adopted to investigate whether apelin-13 (1 nmol) could modulate LTM consolidation when it was delivered immediately, 30, 60 or 120 min after training. Finally, two vehicle groups and two apelin groups of mice were used to determine the role of apelin-13 in LTM acquisition and recall by infusion of apelin-13 (1 nmol) 5 min before training and test, respectively.

Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was conducted using SPSS 17.0. One-sample *t*-test was used to determine whether the DI differed from the chance level (50%) for each group. Differences between two groups were determined by unpaired Student's *t*-test. Differences among more than two groups were determined by one-way ANOVA followed by Bonferroni post hoc test. $P < 0.05$ was considered significance.

Results

The role of apelin-13 in STM

When tested 30 min after training, the mice treated with vehicle or 0.3 nmol apelin-13 immediately post-training showed significant preference for the novel objects, as indicated by the DI of both groups was significantly higher than 50% chance level ($p < 0.01$ for both groups; Fig. 1A). However, when a higher dose of apelin-13 (1 nmol) was infused immediately after training, the DI of the mice was almost equal to the chance level (Fig. 1A). One-way ANOVA analysis indicated that post-training injection of apelin-13 (0.3 and 1 nmol) dose-dependently impaired STM expression ($F_{2,25} = 0.007$; $P < 0.01$; Fig. 1A). Further *post-hoc* analysis revealed that the DI of 1 nmol apelin-13 group was significantly lower than that of the control mice ($p < 0.01$; Fig. 1A). However, when apelin-13 (1 nmol) was delivered 5 min before training, the DI of the mice was significantly higher than the chance level ($p < 0.01$; Fig. 1B), and no significant difference was detected between the DI of vehicle and apelin-13 groups (Fig. 1B). There is no significant difference between treatments in the training phase duration, as well as in the duration and TET of the test phase (Table 1).