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Ghrelin and memory: Differential effects on acquisition and retrieval

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ABSTRACT

In a previous paper we have demonstrated that the orexigenic peptide Ghrelin (Ghr), increases memory retention in rats and mice. In the present work we evaluated the Ghr effect when it was administered previous the training session or previous the test session (24 h after training) on the memory performance, using step-down test. The results showed that the intra-hippocampal Ghr administration previous the training session improved the long-term memory in this task, but did not modify the short-term memory. Nevertheless, when the Ghr was administrated previous the test session, no changes were observed in the memory performance. Taking into account these results and other previously published by our group, we could hypothesizes that Ghr may modulate specific molecular intermediates involved in memory acquisition/consolidation but not in the retrieval.

1. Introduction

Ghrelin (Ghr) is a 28 amino acid peptide that induces robust feeding responses in different experimental models [29,36]. Although Ghr comes from both peripheral (stomach) and central sources, its hyperphagic properties, to a large extent, arise from activity at the brain level [22]. Ghrelin receptors (GHS-R) are expressed in several hypothalamic nucleus and other areas such as the hippocampus, substantia nigra, ventral tegmental area, and dorsal and median raphe nucleus [15,29,36].

In the mammals, the ability to seek and find food is crucial for survival. After finding the food and ingesting it, it is important to remember where this food can be found or perhaps – more importantly – to be able to retain the successful approach that was used to find it. Effective learning and memory are thus likely to be crucial for survival during periods of food shortage [30].

We have previously demonstrated that intracerebroventricular (icv) Ghr administration increased, in a dose-dependent manner, the memory retention (measured as the latency time in the step-down test) and induced anxiety-like behavior in rats. This was the first evidence showing that Ghr increases memory retention [9]. This finding prompted further studies where Ghr was administered into other brain regions, such as the hippocampus, amygdala and dorsal raphe nucleus (DRN) [10]. In relation to memory, Diano et al. demonstrated that Ghr causes an increase in synaptic plasticity promoting new synaptic connections between hippocampal neurons [12]. Based on the above mentioned observations, the central Ghr receptor could be considered as a new drug target for therapeutic approaches to treat diseases that affect cognition [2].

Since the different memory processes including acquisition, consolidation and retrieval are well known [1], in the present work we extended previous experiments and analyzed the Ghr participation on memory acquisition/consolidation and retrieval.

2. Materials and procedure

2.1. Animals

Male Wistar rats weighting 260–290 g were maintained under controlled temperature at 21 ± 1 °C and a light/dark cycle (12 h light, 12 h dark) with food and water ad libitum. Rats were handled daily for 7 days before the experiments. All procedures were conducted according to the National Institutes Health (NIH) Guide for the Care and Use of Laboratory Animals (Publications No. 80-23, 1996) and approved by the local Animal Care and Use Committee. Every attempt was made to minimize the number of animals used and their suffering.

2.2. Surgery

The animals were anesthetized with 55 mg/kg Ketamine HCl and 11 mg/kg xylazine (both Kensol könig, Argentina) and placed in a

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stereotaxic apparatus. Then, rats were implanted bilaterally into the CA_1 hippocampus or intracerebroventricullarly (icv) with steel guide cannula, according to the atlas of Paxinos [31]. The coordinates relative to bregma were anterior: −3.6 mm; lateral: ±2.0 mm; vertical: −2.8 mm and anterior: −4.3 mm; vertical: −4.6 mm for hippocampus and icv, respectively. Cannulas were fixed to the skull surface with dental acrylic cement.

2.3. Drugs and infusion procedures

Animals were injected bilaterally with Ghr or artificial cerebrospinal fluid (ACSF) using a 10 μl Hamilton syringe connected by Pe-10 polyethylene tubing to a 30-gauge needle. The infusion volume was 0.5 μl bilaterally or 1 μl for the intra-hippocampal and icv administration respectively, and it was delivered over a 1 min period. The Ghr peptide was purchased from Neosystem, France. Ghr was resuspended in ACSF, aliquoted to obtain final concentrations of 0.03, 0.3 and 3.0 nmol/μl and stored at −20 °C until use. Injections were done between 11:00 am and noon in order to prevent variations induced by circadian rhythms.

Drug was administered 15 min previous the training session of the step down test or 15 min previous the test session (24 h after training) of this test in different animal groups.

2.4. Step-down test (inhibitory avoidance)

One-trial avoidance test in rats involves the activation of two molecular pathways in the CA_1 region of the dorsal hippocampus, one induces a short-term memory (STM) that lasts 3 h, and the other induces a long-term memory (LTM) that takes 3–6 h to be formed and lasts for many days or even months [14,16–20]. The apparatus was a 50 cm × 25 cm × 25 cm plastic box with a 2.5 cm high and 7.0 cm wide platform on the left of the training box apparatus. The animals were placed on the platform, and latency to step down by placing the four paws on the grid was measured. In the training session, immediately upon stepping down, the rats received a 0.4 mA, 2 s scrambled shock to the feet, and were then immediately removed from the training box and placed in their home cages. The test session was identical regarding the procedures, except that no shock was given. A ceiling of 180 s was imposed on the test measurement. Latency time was taken as an index of memory retention. The test session was carried out 1.5 and 24 h after training in order to measure STM and LTM respectively.

2.5. Histology

After the behavioral test, rats were anesthetized with chloral hydrate, cardially perfused with paraformaldehyde (4%) and their brains were removed. Frontal sections were cut in cryostat (Leica), and the cannula position was localized. Only results obtained from animals in which the tips of the cannulas were placed in the hippocampus or in the central ventricle were included.

2.6. Elevated plus-maze

Rats were tested in an elevated plus-maze, in order to determine if the Ghr promotes or reduce anxiety and locomotion under these experimental conditions (pro- or anti-conflict behaviors) [5–7,32]. Briefly, the elevated plus-maze consisted of a central platform (5 cm × 5 cm), two open arms (50 cm × 10 cm), and two closed arms (50 cm × 10 cm × 40 cm). The arms were arranged in such a way that two arms of each type were opposite to each other. The maze was 50 cm above floor level and tests were carried out under a dim red light 24 h after Ghr administration. Animals were placed individually on the central platform facing an open arm. The number of entries in each arm, the time spent in the open and enclosed arms and the number of rearing were recorded during 5 min.

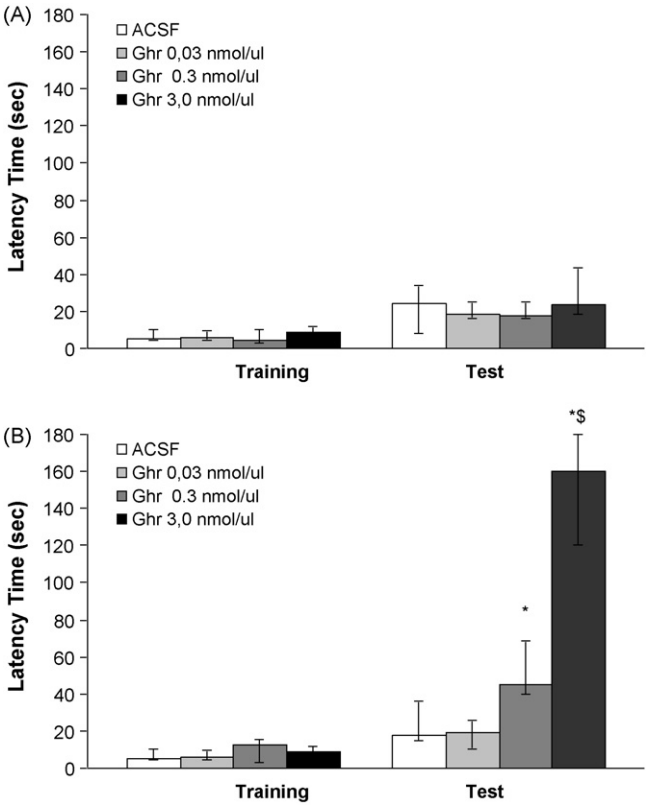

Fig. 1. Effect of Intra-hippocampal Ghr administration (previous the training session) upon memory performance in step down test: (A) short-term memory and (B) long-term memory. Animals were administrated with Ghr (0.03, 0.3 and 3.0 nmol/μl) previous the training session and evaluated 1 h (A) or 24 h (B) after training. The results are expressed as medians with the respective interquartile range. $n = 8–10$ animals/group. *Significant differences related to control animals (ACSF), $p \leq 0.05$. $Significant differences related to treated animals with Ghr 3.0 nmol/μl, $p \leq 0.05$.

2.7. Statistics

Variables analyzed from data find avoidance being did not follow a normal distribution and its variance did not fulfil the assumption of homoscedasticity, these results were expressed as medians (inter-quartile range) and analyzed by non parametric tests (Mann–Whitney or Kruskal–Wallis).

Statistical inference of the data from plus-maze were expressed as mean ± standard error (SEM) and analyzed by one-way multiple analysis of variance (MANOVA) followed by the post-hoc Hotelling T2 ($p \leq 0.05$).

3. Results

3.1. Effect Ghr infused previous the training session

Fig. 1 shows the effect of Ghr administered before the training session into the hippocampus on memory performance in a step down test. The Kruskal–Wallis ANOVA analysis revealed a significant Ghr effect only in LTM (chi-square = 23.01, df = 3, $p = 0.00$). As it can be seen in the figure Ghr enhanced LTM in a dose-dependent manner. (ACSF vs Ghr 0.03 nmol/μl, $p = 0.38$; ACSF vs Ghr 0.3 nmol/μl, $p = 0.00$; ACSF vs Ghr 3.0 nmol/μl, $p = 0.00$; Ghr 0.03 vs Ghr 0.3 nmol/μl, $p = 0.00$; Ghr 0.3 vs Ghr 3.0 nmol/μl, $p = 0.00$; Ghr 0.03 vs Ghr 3.0 nmol/μl, $p = 0.00$; $n = 8–10$

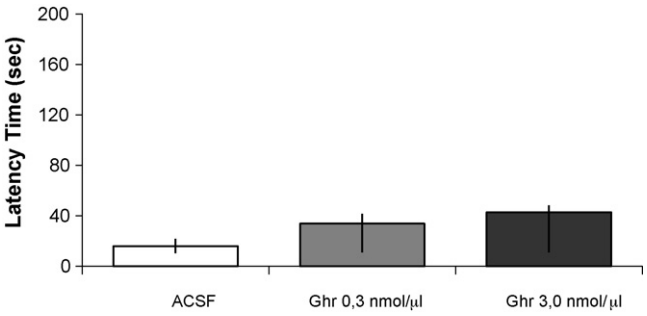

Fig. 2. Effect of Intra-hippocampal Ghr administration (previous the test session) upon memory performance in step down test. Animals were administrated with Ghr (0.3 and 3.0 nmol/μl) previous the test session (24 h after training). The results are expressed as medians with the respective inter-quartile range. $n = 8–10$ animals/group.

animals/group).). However Ghr did not induce changes in STM (chi-square = 4.92, df = 3, $p = 0.18$; $n = 8–10$ animals/group).

Oppositely, when Ghr was administrated icv both, STM and LTM were enhanced (chi-square = 13.54, df = 3, $p = 0.00$) (ACSF = 33.97 (18.53–44.38); Ghr 0.03 nmol/μl = 180 (95–180); Ghr 0.3 nmol/μl = 180 (130–180); Ghr 3.0 nmol/μl = 180 (142.36–180) for STM and ACSF = 20.57 (10.53–42.54); Ghr 0.03 nmol/μl = 115.38 (90–165); Ghr 0.3 nmol/μl = 165 (128–180); Ghr 3.0 nmol/μl = 180 (120–180) for LTM acquisition; $n = 9–11$ animals/group).

3.2. Effect Ghr infused previous the test session

Fig. 2 shows the effect of Ghr administered before the test session into the hippocampus on memory performance in a step down test. As it can be seen retrieval was not affected by the intra-hippocampal peptide administration (chi-square = 2.57, df = 2, $p = 0.28$; $n = 8–10$ animals/group).

The icv Ghr administration did not affect the LTM retrieval (chi-square = 5.21, df = 2, $p = 0.65$) (ACSF = 40.56 (20.18–45.21); Ghr 0.3 nmol/μl = 32.58 (15.42–41.52); Ghr 3.0 nmol/μl = 27.56 (11.52–33.85); $n = 10–13$ animals/group).

3.3. Ghr effect on anxiety-like behavior

Table 1 shows the effects of different Ghr doses in the relative indexes of anxiety and number of total entries 24 h after administration. Intra-hippocampal treated rats did not display changes in any of the parameters measured. These results could suggest that their effects on LTM acquisition are not due to interferences with locomotion activity or anxiety (or prior anti-conflict behavior) levels. The MANOVA test showed that Ghr administration did not have a significant effect in the parameters related to the anxiety, as the number of entries into open arms, time spent in open arms, the percentage of open arms entries and percentage of time spent in open arms (Wilks = 0.02, df = 3, $p > 0.05$). In addition, Ghr did not have significant effects on the total number of entries, a measure of overall locomotor activity. Similar results were observed in icv treated rats (data not shown).

4. Discussion

The main finding of this paper was that Ghr administration into the hippocampus of rats previous the training session in a step-down test improved LTM, without altering the STM. Nevertheless, when the Ghr was administrated previous the test session, not changes were seen in the memory performance.

It has been demonstrated that during the memory acquisition the animal associates the context (step-down box) and the shock. During consolidation, which can last from minutes to days, this memory is moved from a labile to a more persistent state. During retrieval, the animal is returned to the context where memory for the context–shock association was assessed [1].

Our results showing that the intra-hippocampal Ghr administration previous the training session enhanced LTM, but did not modify STM, could probably be attributed to the fact that the hippocampal mechanisms of STM and LTM in the step-down test can operate separately [19]; thus, STM can be selectively altered by many treatments that do not affect LTM [16–20].

It has been demonstrated that fear conditioning often results in a robust memory for the context–shock association after a single shock presentation, which allows isolation of the stages of memory along time. The pharmacological approaches offer a highest temporal specificity because they can be applied and removed from the system within a relatively short time window. Nevertheless, it is important to take in mind that manipulations before acquisition will affect early stages of consolidation, and manipulations before retrieval may affect late stages of consolidation or retention [1].

Previous results of our laboratory showed that the peptide administrated immediately after training session [11] increased both STM and LTM (for all doses tested) and the increments were higher than those when Ghr was administered previous the training session. Thus, these findings and those from Fig. 1 in this study are in accordance with the hypotheses that suggest that Ghr is more effective in the modulation of the memory consolidation rather than in the acquisition.

The present results also demonstrated that the icv Ghr administration induced enhancement of both STM and LTM. The difference found between the Ghr effects after icv or intra-hippocampal administration previous the training session on STM could be attributed to the participation of the other structure, such as the amygdala when Ghr was administrated icv. It has been demonstrated that the amygdala presents a specific role in memory acquisition but not in memory consolidation [1]. Furthermore, it is relevant for the formation of motivated memories [24,25] playing

Table 1
Ghr effect 24 h after intra-hippocampal administration on elevated plus maze test. The results are expressed as mean ± SEM. The numbers between parentheses indicates the numbers of animals.

Parameter	Control (12)	Ghr Hi 0,3 (9)	Ghr Hi 3,0 (9)
Number of entries to open arms	3.0 ± 0.2	3.9 ± 0.4	2.9 ± 0.8
Number of entries to closed arms	5.0 ± 0.5	5.4 ± 0.9	6.0 ± 0.5
Number of total entries (OA + CA)	8.0 ± 0.7	9.3 ± 0.3	8.9 ± 0.5
Percent of entries to open arms	38.2 ± 2.1	43.7 ± 5.5	28.2 ± 7.7
Number of rearings	8.1 ± 1.1	7.7 ± 1.1	8.4 ± 0.7
Time spent on open arms (s)	60.8 ± 3.6	70.6 ± 12.8	56.9 ± 16.8
Time spent on closed arms (s)	194.2 ± 5.7	182.5 ± 13.2	216.3 ± 24.2
Percent of time spent on open arms	23.8 ± 1.0	27.8 ± 4.8	22.6 ± 6.9
Risk-assessment	3.5 ± 1.1	3.3 ± 0.6	2.5 ± 0.9
Grooming	4.3 ± 0.7	3.4 ± 0.4	3.2 ± 0.4

a key role in the learning and memory processes occurring during emotional events [3,4,21,25–28].

Another interesting finding of the present study is that the increase in the latency time observed in the step-down test was no attributed to the anxiogenic effect of the peptide. It is well known that freezing is a common behavior observed in anxious rats exposed to a novel environment [23]. Our results showed that Ghr did not induce any anxiety-like behavior 24 h after administration. In addition, the intra-hippocampal Ghr administration induced anxiogenesis only at the highest dose tested (3.0 nmol/μl) 15 min after administration [10].

Our results also showed that Ghr could affect either the memory acquisition or consolidation but certainly did not affect the retrieval. The pharmacological findings have shown that NMDA receptors, which are critical for memory acquisition, are not involved in retrieval of previously established memories [34,35]. It has been suggested that the activation of hippocampal NMDA receptors are primarily related to the contextual aspects (formation of a representation of the training environment) whereas NMDA receptors in the amygdale are more related to the aversive aspects (footshock) of the task [13,33]. Similarly, protein-kinase A (PKA) plays an important role on acquisition, and it is critically involved in consolidation, but, appears to be not necessary for retrieval [8,35].

Taking into account these results and previous findings from our laboratory, we could hypothesize that Ghr may modulate specific molecular intermediates involved in the memory acquisition/consolidation processes but not in those related to retrieval.

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