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DECREASED MEMORY FOR NOVEL OBJECT RECOGNITION IN CHRONICALLY FOOD-RESTRICTED MICE IS REVERSED BY ACUTE GHRELIN ADMINISTRATION

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Abstract—It has been demonstrated, in normal and aged rats and mice, that acute i.c.v. ghrelin (Ghr) administration increases memory retention. In order to evaluate if this treatment, restores memory retention in animals exhibiting impaired memory, in the present work we selected a chronic food restriction mouse model (since undernutrition prejudices higher nervous functions).

We employed adult female mice with 28 days of 50% food restriction and evaluated: a) behavioral performance using novel object recognition test for memory, and plus maze for anxiety-like behavior, b) some morphometric parameters as body and hepatic weights and c) plasma Ghr levels.

The animals with 50% food restriction showed an increase in plasma Ghr levels and a decrease in morphometric parameters and in the percentage of novel object recognition time. When the peptide was i.c.v. injected in food-restricted animals (0.03, 0.3 or 3.0 nmol/μl), memory increases in relation to food-restricted mice injected with vehicle, reaching a performance similar to controls.

Key words: ghrelin, memory retention, food restriction, spontaneous object recognition test, plus maze test.

Ghrelin (Ghr) is an orexigenic peptide (28-amino acid) that is mainly produced in the stomach (Ariyasu et al., 2001). Plasma Ghr concentrations are increased in fasted conditions and are reduced after feeding (Cummings et al., 2001; Tshop et al., 2001). It has been postulated that this peptide participates in the modulation of several processes related to nutrient homeostasis, including appetite regulation, gastrointestinal functions and growth hormone–releasing activity (Kojima and Kangawa, 2005). The mRNA for Ghr and its receptor (GHS-R1a) has been detected in various tissues, including the reproductive, cardiovascular and nervous systems (Barreiro and Tena-Sempere, 2004; Kojima and Kangawa, 2005). In the CNS, the Ghr receptors are mainly expressed in the hypothalamus mediating the orexigenic effects of Ghr (Nakasato et al., 2001; Tschop et al., 2000). However, receptors for Ghr are also expressed in extrahypothalamic structures such as hippocampus (Bennett et al., 1997; Guan et al., 1997), a region that is associated with learning and memory, suggesting a possible role of the peptide in cognitive processes. We have recently shown that Ghr increases memory retention in a dose-related manner when injected i.c.v., into the hippocampus, amygdala and dorsal raphe nucleus (Carlini et al., 2002, 2004) in rats. In addition, Diano et al. (2006) published that peripheral acute injection of Ghr (into the left jugular vein) enters to the hippocampus where it promotes dendritic spine synapse formation and generation of long-term potentiation. Moreover, Ghr-null mice exhibit memory impairment and Ghr administration restores this functional deficiency.

It is well known that chronic food restriction (FR) induces impairment of higher nervous functions such as learning and memory in mammals (Bedi, 1991; Georgieff, 2007). In an attempt to mimic the conditions of undernourishment and/or anorexia in the human species, in a first set of experiments we set up a mouse model of chronic FR. In this model we evaluated morphometric parameters, such as body and hepatic weights, plasma Ghr levels and we also applied two behavioral paradigms, an elevated plus maze (EPM) for anxiety-like behavior and a novel object recognition test for memory. In a second set of experiments, we tested if acute i.c.v. Ghr administration modifies the performance in the mentioned memory task in chronically food-restricted mice.

EXPERIMENTAL PROCEDURES

All procedures performed in the present work were conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and approved by the Animal Care and Use Committee, School of Chemical Sciences, National University of Cordoba. The experiments described were made to minimize the number of animals used and their suffering.

Animals

The experiments were performed on intact adult female mice (Albino Swiss-SWR/J (q)) with an initial body weight of ≅25 g. The colony room was maintained under controlled temperature

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Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; EPM, elevated plus maze; FR, food restriction; Ghr, Ghrelin; GHS-R, growth hormone secretagogues receptor sub-type 1a; MANOVA, multiple analysis of variance; S.E.M., standard error mean.

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(20±2 °C) and light (12-h light/dark). Each animal was used in only one experiment. The number of animals ranged between 6 and 15 per treatment.

Estrous cycles were daily evaluated by vaginal smears to females of all the experimental groups (control (Ctrl), treatment). The measures of body weight and plasma Ghr levels were performed in different groups of animals than that of memory and anxiety tasks (then the number animals are different).

Experimental procedure: Two sets of experiments were performed

First set of experiments. In order to evaluate the effects of chronic FR on memory and anxiety behavior, the animals were divided in three groups; each of one received a different treatment for 28 days. Body weight was measured daily and at the end of each treatment. Hepatic weight, plasma Ghr levels, memory performance and anxiety-like behavior were evaluated at the end of the treatment.

Control group (without treatment): mice had free access to standard pelleted food (Cargill, Córdoba, Argentina); 20% food restricted group: animals received 80% of the amount of food consumed by the control animals; 50% food restricted group: animals received 50% of the food consumed by the control animals.

Ghr quantification. A commercial rat mouse radio immunoassay kit (Phoenix Pharmaceutical Co.) for total Ghr was employed, with a sensitivity of ≈6 to ≈25 pg/tube and a range of 10–1280 pg/ml. Blood samples were obtained by decapitation (in heparinized tubes) after 28 days of treatment (in the diestrous or the anestrous phase for controls and food restricted animals respectively). Blood samples were centrifuged, supernatant removed and plasma samples were stored at −20 °C until Ghr quantification.

Spontaneous object recognition test. This task was performed in agreement with the procedure described by Ennaceur and Aggleton (1997). Behavioral testing: All animals were given one habituation session in which they were allowed to explore the apparatus (without objects) for 5 min. On the object trial, the mouse was placed into the box with two identical objects (1 and 2) and allowed to explore for 3 min (training). The time spent by the animal exploring each object and also the time spent by the animal exploring both objects and the box were measured. The mouse was then removed to its home cage and 1 h after the training it was placed in the box for the retention test and allowed to explore the objects for 3 min, in order to measures short term memory: one of the objects was the same as used for training (1, familiar object) and the other one was a novel object (object number 3, novel). Twenty-four hours later, the mouse was tested in the box, but object 3 was changed for another novel object (object number 4, novel) that the mouse had never seen before, in order to measures long term memory. The time that the animals spent exploring the novel and the familiar objects were measured; results are expressed as percentage of novel object recognition time (time percentage=tnovel/[tnovel+tfamiliar]×100).

The object exploration was defined as directing the nose to the object at a distance lesser than 2 cm. Turning around or sitting on the object was not considered as exploratory behavior. The parameters analyzed were 1) percentage of time that the animals explore identical objects during training and 2) the percentage of time that the animals explore the novel object at 1 and 24 h after training. We considered this last parameter as an index of memory retention.

EPM. The apparatus was made of wood and consisted of two open arms opposite to each other (measuring 30 long×5 width cm), crossed by two enclosed arms (measuring 30 long×5 width cm×15 cm high walls) with an open roof. The arms extended from a central platform (5 cm×5 cm) and the whole apparatus was elevated 40 cm above the floor. To avoid having the mice fall down, the open arms were bordered by transparent plastic walls (1 cm high). The test was performed under red light (Lister, 1987; Cruz et al., 1994).

Individual mice were placed onto the central platform and observed for 5 min. The behavioral parameters recorded were the number of entries in the open arms, the number of entries into closed arms, time spent in open arms, rearing, grooming and risk-assessment. The test was performed as a single trial per animal.

Second set of experiments. Since it has been demonstrated that acute i.c.v. Ghr administration increases memory retention in control animals (Carlini et al., 2002; Diano et al., 2006) we designed this set of experiments in order to evaluate the effects of acute i.c.v. Ghr administration on memory and anxiety-like behavior in a model of impaired memory retention, as that of chronic FR of female mice (that exhibit chronic elevated plasma Ghr levels). We injected 0.03, 0.3 or 3.0 nmol/μl of Ghr i.c.v. in control and 28 days 50% food-restricted animals. Different animals were employed for each dose of the drug.

Surgery. The animals were anesthetized with 55 mg/kg ketamine HCl (Vetanarcol König: Laboratorios König S.A., Argentina) and 11 mg/kg xylazine (Kensol König: Laboratorios König S.A.) at day 21 of treatment and placed in a stereotaxic apparatus. Mice were operated i.c.v. with help of a steel guide cannula, according to the atlas of Paxinos and Watson (1998). The coordinates relative to bregma were anterior: 0.2 mm, lateral: 1.0 mm and vertical: 2.8 mm. Cannulae were fixed to the skull surface with dental acrylic cement. After 7 days of recovery, animals were injected with Ghr or artificial cerebrospinal fluid (ACSF) using a 10 μl Hamilton syringe connected by Pe-10 polyethylene tubing to a 30-gauge needle extending it 0.75 mm beyond the guide cannula. Each infusion of 1 μl was delivered over a 1 min period.

Drug administration. Rat Ghr was purchased from Neosystem, France. The peptide was dissolved in ACSF, divided into aliquots and kept at −20 °C until the day of the experiment. The drug was injected i.c.v. through the cannula immediately after training for the novel object recognition test or 10 min before the EPM.

Histology. The position of the cannula was assessed histologically on frozen brain slices (−20 °C) at the end of the behavioral testing. Only results obtained from those animals that had the tips of the cannulas correctly placed into cerebral ventricles were included in the study.

Statistic

The data from the object recognition test are expressed as mean percentage of time in novel object recognition (tnovel/[tnovel+tfamiliar]×100)± standard error mean (S.E.M.) and analyzed by one-way analysis of variance (ANOVA). The data from the EPM test, morphometric parameters and plasma Ghr levels are expressed as mean±S.E.M. EPM was analyzed by multiple analysis of variance (MANOVA) and the two last parameters were analyzed by one-way ANOVA.

The behavioral results were statistically treated in different ways, according to the experiments. First set of experiments: the data from the object recognition test were evaluated with repeated measures analysis of variance, with two treatment factors: FR treatment and time. The data from the EPM test were analyzed with one-way MANOVA. Second set of experiments: the data from the object recognition test were evaluated with repeated measures analysis of variance, with two treatment factors, Ghr and time (training time and test time), and two blocks: control and chronic food restricted. The data from the plus maze test were analyzed with one-way MANOVA, with two blocks: control–chronic food restricted and ACSF–Ghr.

When ANOVA was used, Tukey's post hoc test were applied to determine the source detected significant and P values ≤0.05