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Central ghrelin increases anxiety in the Open Field test and impairs retention memory in a passive avoidance task in neonatal chicks

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ABSTRACT

Ghrelin (Grh) is an endogenous ligand for the growth hormone secretagogue receptor. Although Ghr stimulates feeding in rats, it inhibits feeding in neonatal chicks. However, little is known about other central behavioral effects of Ghr. Therefore, we investigated the Ghr effects, injected intracerebroventricularly, on anxiety and memory retention of neonatal chicks in an Open Field test and in a one-trial passive avoidance task, respectively. In the Open Field test, the administration of Ghr in a dose-dependent manner increased the latency to ambulate but decreased ambulation activity, indicating an anxiogenic effect. Furthermore, chicks trained on a passive avoidance task and injected with a dose of 30 pmol of Ghr immediately after training showed an impairment of memory retention. However, there were no significant effects on the number of pecks during the pretraining, training, retention and discrimination. In addition, different doses of Ghr produced an inhibition in food intake at different times after injection. Our results indicate that Ghr induces anxiogenesis in chicks. Moreover, we have shown for the first time that Ghr can decrease memory retention in a non-mammalian species, suggesting that Ghr may play an important role in the processes of memory retention in birds.

1. Introduction

Ghrelin (Grh) is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima et al., 1999). It is mainly produced in the rat stomach (Kojima et al., 1999) but Ghr-producing cells have also been detected in the arcuate nuclei of the rat hypothalamus, which is a feeding control center (Cowley et al., 2003). Ghr immunoreactivity was also found, in the chicken hypothalamus, although not in the arcuate nucleus, as in the case of rats (Ahmed & Harvey, 2002). Chicken Ghr was originally isolated from the proventriculus, the glandular portion of the avian stomach, indicating that this is the primary site of Ghr production (Kaiya et al., 2002). In the chicken, Ghr mRNA expression has also been detected in several parts of the brain suggesting a central action of Ghr. However, the question why ghrelin is produced in several brain areas in addition to the hypothalamus remains unanswered (Saito et al., 2005).

Central Ghr plays an important role in various physiological functions in rats; for instance, in pituitary hormone secretion, gastrointestinal function and cardiovascular systems (Date et al., 2001; Kojima et al., 1999; Nagaya et al., 2001). However, little is known about its function in birds or in any of the other non-mammalian species. It has been reported that chicken Ghr can stimulate the release of growth hormone in chicks *in vivo* and *in vitro,* as previously seen in mammals (Ahmed & Harvey, 2002; Baudet & Harvey, 2003). Furthermore, both peripherally and centrally Ghr rapidly increases food intake and body weight in rats (Nakazato et al., 2001). However, the effect of Ghr on feeding produces the opposite effect from that seen in mammals, with an intracerebroventricular (icv) injection of Ghr reported to strongly inhibit food intake in neonatal chicks (Furuse et al., 2001). The underlying mechanism related to this is still unclear, although it has been reported that the anorexic effect of Ghr could be mediated by the corticotropin-releasing factor and its receptor system (Saito et al., 2005).

In rodents, it has been shown that an icv administration of Ghr induces an anxiogenic effect (Carlini et al., 2002) and an improvement in memory retention in a step-down test (Carlini et al., 2002, 2004), in a spatial-dependent version of the novel object recognition test (Carlini, Gaydou, Schiöth, & de Barioglio, 2007; Diano et al., 2006) and in a T-maze footshock avoidance (Diano et al., 2006). These authors reported for the first time that showing Ghr promotes the formation of a hippocampal spine synapse density. Furthermore, Ghr knockout mice had a smaller number of hippocampal spine synapses than wild-type ones indicating that Ghr governs neuronal morphology of brain areas known responsible for memory (Diano et al., 2006). However, since Ghr inhibits food intake in chicks, it is possible that this peptide may have a different role to that found in rodents in the memory processes and anxiety

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behavior. Therefore, we investigated the action of distinct doses of centrally administered Ghr in neonatal chicks for different behavioral paradigms.

2. Materials and methods

2.1. Animals

Groups of 20 chicks (*Gallus gallus domesticus*) of both sexes were obtained immediately after hatching from a commercial hatchery INDACOR (Argentina) when they were only a few hours old. On arrival, this procedure was repeated three times and in all, a total of sixty chicks were housed in a white wooden box that measured 90 × 40 × 60 cm (length × width × height) before performing the Open Field test. This box was illuminated with an incandescent lamp hanging just above it and kept in a small room (3 × 3 m) at controlled temperature (30–32 °C) in a 12–12 h dark-light cycle (lights on at 7 a.m.). Tap water and food were freely available. The chicks were socially reared until they reached 3 days of age. Daily food replenishment (Cargill, broiler BB, and 20% minimum crude protein 12.34 MJ/kg) and maintenance chores were performed at 9 a.m.

For the passive avoidance task, another 20 chicks on a total of 58 ones were obtained immediately after hatching from a commercial hatchery and then were housed in pairs in 24 × 20 cm cages and kept under quiet conditions under a dim red light with water and food freely available for 24 h. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee of the Universidad Nacional de Córdoba, and efforts were made to minimize animal suffering and to keep the number of animals used to a minimum.

2.2. Peptide and icv injection

The Ghr peptide (purchased from Neostystem, France) was dissolved in 0.85% saline containing 0.1% Evans Blue solution, and then administered in doses of 20, 30, 40, 80 and 200 pmol. Intracerebroventricular injections were given freehand at a volume of 10 μl using a Hamilton syringe (Andrew, 1991; Johnston, Clements, & Rose, 1999). The depth of the injection was 3 mm, controlled by using a plastic sleeve on a 27-gauge needle. As the chicks have soft unossified skulls, this procedure does not require an anesthetic and is routinely performed without administration of analgesics (Andrew, 1991). The control group was given saline containing Evans Blue solution. The regional targets are the forebrain hemispheres, such as telencephalic structures, neurochemically and functionally comparable to the mammalian neocortex, claustrum, and pallial amygdale, in addition to other pallial areas such as hippocampus (Reiner et al., 2004).

2.3. Open Field test

Sixty chicks (3 days old) were individually gently captured, injected with the different doses of Ghr indicated above or with saline, and immediately after placed in a cardboard box before being carried to a separate room where the chick was placed in the center of a 60 × 60 cm Open Field (OF) apparatus with sides 30 cm high. This OF was made of white wood and the floor was marked off into 25 squares of 12 cm × 12 cm each, illuminated by a 100 W overhead bulb (Gallup & Suarez, 1980). The following types of behaviors were analyzed for 10 min: latency to ambulate, locomotor activity (number of squares crossed), latency for defecation, number of defecations and number of escapes. After testing, the floor of the OF apparatus was cleaned with towels wetted with 70% ethanol. Spontaneous activity was recorded by a digital camera suspended 1.5 m above the center of the apparatus (Day 1 of the experiment). The monitoring system was set up in a separate room to avoid disturbing the birds.

Twenty four hours later (Day 2 of the experiment), each chick was again placed in the center of the OF and its behavior was analyzed as described above. The birds were immediately decapitated after the experiment. The brains were then removed and inspected in order to control the accuracy of the placement of the injection.

2.4. One-trial passive avoidance task

Fifty-eight chicks were trained in a one-trial passive avoidance task at the age of 24 h as described by Lössner and Rose (1983) according to a model first introduced by Cherkin (1969). Chicks were pretrained by three 10 s presentations of a small (2.5 mm) white bead. This bead was positioned directly in front of and between 0.5 and 1 cm from the tip of each chicks beak. Chicks that did no peck at the bead at least two times out of the three pretraining trials were excluded from further testing (approximately 11%). After 30 min, half of the birds were trained by a presentation of a 4 mm chrome bead coated with 100% solution of methylantranilate (MeA) for 30 s. The other half of the chicks was trained by the presentation of the same bead coated with water. Chicks that did not peck at the chrome bead during training were also excluded from further experimentation (approximately 4%). Immediately after training, chicks from two groups (water or MeA-trained chicks) were injected with saline or 30 pmol of Ghr, in order to measure memory retention. Retention was tested 24 h after training to avoid the confounding effects of residual short-term memory, pro-active performance deficits, and circadian variations (Cherkin, 1969). Testing involved the presentation of a dry chrome bead to each chick for 30 s followed at least 5 min later by a 10-s presentation of a white bead. Chicks were considered to be amnesic if they responded to the test by pecking at both the previously aversive dry chrome and white beads. However, chicks which pecked exclusively at the white bead and avoided the chrome bead were considered to show recall of the training experience. Finally, the number of pecks directed towards the bead by each chick was recorded during the pretraining, training, retention and discrimination in order to check for any non-specific effects which Ghr may have had on pecking. All birds were immediately decapitated after the discrimination test. The brains were then removed and inspected in order to control the accuracy of the placement of the injection.

2.5. Control of the food intake

The quantity of food intake was determined 30, 60 and 120 min after the injection by measuring the disappearance of diet from the pre-weighed feeder with a digital balance of a precision of 0.01 g. In most cases, no spillage was observed due to the fact that a limited amount of food was available in the feeder. However, if spillage was observed, this was taken into account.

2.6. Statistical analysis

Data from OF behavior assumed a non-normal distribution and were analyzed using Kruskal–Wallis nonparametric tests. Whenever the test indicated significant effects ($p < 0.05$), a pairwise comparison Dunn test was carried out. A p value < 0.05 was considered to represent a significant difference. An avoidance score for each experimental group was calculated by dividing the number of chicks in that group which did not peck at the chrome bead in the test by the total number of chicks allocated to the group, expressed as a percentage. Avoidance scores of Ghr treatment were