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Research report

The effect of obestatin on anxiety-like behaviour in mice

Júlia Szakács, Krisztina Csabafi, Nándor Lipták, Gyula Szabó*

Department of Pathophysiology, Faculty of Medicine, University of Szeged, Hungary

HIGHLIGHTS

- Obestatin induced anxiety-like effect in mice in the EPM and OF tests.
- Plasma corticosterone levels where elevated by obestatin.
- Antalarmin and [D-Lys3]-GHRP6 reversed the anxiogenic-like effects of obestatin.
- Our results suggest that obestatin acts through ghrelin receptor and HPA activation.

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ABSTRACT

Obestatin is a 23 amino acid-peptide, derived from the same preproghrelin-gene as ghrelin. Obestatin was originally reported as a ghrelin antagonist with anorexigenic activity, but later it was proven to be involved in multiple processes including sleep, memory retention, anxiety, morphine-induced analgesia and withdrawal. In the present study, in male CFLP mice, by using computerised open field (OF) and elevated plus maze (EPM) tests we have investigated the behavioural effects of the acute intracerebroventricular (icv) administration of obestatin alone, and following ghrelin receptor blockage with [D-Lys3]-Growth Hormone Releasing Peptide-6 ([D-Lys3]- GHRP6) or corticotropin-releasing hormone (CRH) receptor 1 antagonism with antalarmin. Plasma corticosterone levels were measured for each treatment group by using chemofluorescent assay. Our results in the EPM test showed that obestatin reduced the percent of time spent in the open arms. The basal locomotor activity (ambulation distance and time, rearing and jumping) was not influenced significantly neither in the obestatin-treated groups, nor in those receiving pre-treatment with antalarmin or [D-Lys3]-GHRP6. The percentage of central ambulation distance however was decreased by obestatin, while the percentage of time spent in the central zone showed a decreasing tendency. The administration of antalarmin or [D-Lys3]-GHRP6 have both reversed the effect of obestatin on central ambulation. Plasma corticosterone levels were elevated by obestatin, which effect was antagonised by the injection of antalarmin. These are the first results to indicate that obestatin exerts anxiogenic-like effect in mice, which might be mediated through ghrelin receptor and CRH activation.

1. Introduction

In the past decades two different peptides, encoded by the same preproghrelin gene were isolated from the rat gastric mucosa: ghrelin [1] and obestatin [2] Obestatin is a 23-amino-acid peptide which was found to be present in the GI tract (gastric mucosa, small and large intestine, pancreas), saliva, plasma, breast milk, Leydig cells of the testis [3] and the pituitary gland [4]. The original study by Zhang has reported that obestatin is the natural ligand of the G protein coupled receptor 39 (GPR39) and acute or chronic administration in rodents inhibits food intake, decreasing the body weight under basal and ghrelin-stimulated conditions [2]. Subsequent studies using different experimental protocols gave contradictory results [5–11] and only the acute food-intake-suppressive effects of obestatin were substantiated [12]. However, it is agreed that alterations in obestatin or ghrelin levels and the ghrelin/obestatin ratio may contribute to the pathomechanism of human obesity, anorexia nervosa, and Prader–Willi syndrome [13–15]. Furthermore, obestatin may be involved in opioid-induced analgesia and the behavioural responses induced by naloxone-precipitated morphine withdrawal [16]. The studies designed to reveal its CNS functions have demonstrated that the peptide promotes sleep, increase memory retention, and inhibit thirst and anxiety [3,17,18]. Previ-

* Corresponding author at: Department of Pathophysiology, University of Szeged, Semmelweis str. 1, 6725 Szeged, Hungary. Fax: +36 62545710.
E-mail address: szabo.gyula@med.u-szeged.hu (G. Szabó).

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ous research has shown, that the acute intracerebroventricular (icv) administration of obestatin dose-dependently exerts anxiolytic effects in elevated plus maze (EPM) test in rats, and improves learning and memory processes in behavioural tasks such as the step down and object recognition tests [11]. Ghrelin, on the other hand, as part of a complex gut-brain network, exerts multiple metabolic and neuroendocrine functions in both humans and rodents: by acting on the growth hormone secretagogue-receptor1a (GHS-R1a) stimulates GH secretion and exerts orexigenic effects on energy homeostasis [15,19–21]. Ghrelin was also shown to have a crucial role in the integration of feeding signals with those regulating emotion and mood [15,21–23]. Furthermore, it is involved in motivated and rewarding behaviours induced by food and different addictive substances (alcohol, cocaine, amphetamin) [24,25]. Considering the effects on anxiety and mood multiple studies conducted in rodents have demonstrated that ghrelin can induce not only anxiety and depression-like behaviours [22,26–28] but it also has anxiolytic- and anti-depressant-like effects [29]. As many authors have indicated these conflicting data might be due to differences in study design (dosage, route of administration, timing of injection) [23], as well as the duration and context of stress exposure [30–32]. Accordingly, in unstressed animals the administration of ghrelin would favour anxiety- and depression-like behaviour [22,26–28]. However, elevating ghrelin levels by e.g. 10 days of calorie restriction [29] or by ghrelin injection induced anxiolytic and anti-depressive behaviour in mice exposed to different types of acute stress e.g. forced swimming test, chronic social defeat stress [29] bilateral olfactory bulbectomy [33]. A possible explanation of the antidepressant effect is, as one recent publication has suggested, that ghrelin promotes hippocampal neurogenesis in mice [34]. To further complicate the issue, ghrelin was shown to enhance fear memory through GHSR signalling independently from the HPA axis in a rodent model of PTSD [35].

Obviously ghrelin has a role in mood and anxiety-related behaviours, however, the exact mechanism of action is fairly known.

Based upon the above-mentioned conflicting and incomplete data related to the action of ghrelin and even more so, obestatin on anxiety-related behaviour, we have decided to test the effects of the acute icv administration of obestatin in male CFLP mice by using two different methods: computerised open field (OF) and EPM tests. Considering the fact that HPA axis activation is closely related to anxiety and other mood disorders [36,37], we aimed to investigate whether obestatin has an impact on HPA axis. We therefore induced CRH receptor blockage and next, we assessed the behavioural changes in the OF test. We also measured plasma corticosterone levels for each treatment group by fluorescence assay. Furthermore, to find out whether obestatin acts through GHSR signalling, we administered ghrelin receptor antagonist pretreatment.

2. Materials and methods

2.1. Experimental animals

All experiments were conducted in accordance with the instructions of the Ethical Committee for the Protection of Animals in Scientific Research of the University of Szeged. CFLP male, 6 week old mice (Animal Husbandry Services, Domaszék, Hungary), weighing 25–28 g were used for the experiments. Five animals per cage were housed in a room at controlled temperature (22–24 °C) and on a 12-h dark–light cycle (lights on at 06:00 and off at 18:00 h), with food and water available ad libitum. Each animal was used in the experiments only once.

2.2. Surgery

For icv cannulation the mice were anesthetised intraperitoneally with sodium pentobarbital (Euthasol, 35 mg/kg) and a polyethylene cannula was inserted into the lateral cerebral ventricle, at stereotaxic coordinates: 0.5 mm posterior, 0.5 mm lateral to the bregma, and 3 mm deep from the dural surface, according to the atlas of Paxinos [38], and fixed to the skull with cyanoacrylate containing instant glue. The animals were then allowed to recover for 5 days. After the end of the experiments, 2 μl of methylene blue was injected via the cannula of decapitated animals to check the permeability and the right position. Data from animals with improper cannula were excluded from statistical analysis.

2.3. Treatments

Three different treatment protocols were used.

1. One group of mice received graded doses (0.5 μg, 1 μg or 1.5 μg) of obestatin (Anaspec, Inc., USA), icv. Control groups received 2 μl artificial cerebrospinal fluid (aCSF), the vehicle for peptide treatments was also 2 μl aCSF. The behavioural tests (EPM or OF) were performed 30 min after the treatment.
2. Another group of mice was pretreated with antalarmin (Bachem, Switzerland), in a dose (0.1 μg dissolved in 2 μl of aCSF, icv) which did not influence the behavioural parameters per se in previous studies [39], followed 30 min later by the icv injection of 1.5 μg obestatin (in 2 μl aCSF), the most effective dose from the previous dose–response study. The OF test was performed 30 min after the administration of obestatin.
3. The third group of animals obtained [D-Lys3]-Growth Hormone Releasing Peptide-6 (GHRP6), a selective GHS-R1A antagonist (Sigma–Aldrich Inc., USA) in a dose of 1 μg dissolved in 2 μl aCSF (had no effect alone in behavioural testing, previously), 15 min before the administration of Obestatin (1.5 μg/2 μl aCSF), followed after 30 min by OF test.

2.4. Behavioural testing

All tests were conducted between 8.00 a.m. and 10 a.m. Mice were carried in their home cages to the experimental room and habituated for at least 30 min before testing.

2.4.1. Open field test

This is a widely used method to test the exploratory behaviour and general locomotor activity in rodents. The apparatus (conducta, Experimetria Ltd., Hungary) consisted of a wood box with an open top ($40 \times 50 \times 50\,cm^3$). A 60-W light was situated 1 m above the arena floor. The motor activity of mice was detected by high-density arrays of infrared diodes. Each animal was placed individually in the center of the open field and during the 5-min test period the following parameters were monitored: ambulation time (s) and distance (cm), number of rearings and jumpings, immobility time (in seconds), the percentage of distance traveled in the central areas compared to the distance traveled in the whole arena (central/total ambulation distance%) and the percentage of time spent in the central zone of the field compared to total time (central/total ambulation time%).

2.4.2. Elevated plus maze (EPM) test

EPM is a well-known assay to monitor anxiety-like behaviour in rodents [40]. The method is based on the natural aversion of rodents for open, illuminated spaces and heights, which conflicts with their drive to explore a new environment. The EPM apparatus consists of four arms (87-mm wide, 155-mm long) elevated 63.8 cm above the floor, with two arms enclosed by 16.3-cm-high