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Obestatin prevents analgesic tolerance to morphine and reverses the effects of mild morphine withdrawal in mice☆

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ABSTRACT

Obestatin is a 23-amino acid gut-derived neuropeptide, encoded by the same gene with ghrelin. The goal of this study was to examine the effects of obestatin on the acute and chronic analgesic actions of morphine and on mild morphine withdrawal. Open-field (OF) and elevated plus maze (EPM) tests were used to assess mild morphine withdrawal-induced behavior changes and the heat-radiant tail-flick assay was used to investigate analgesic actions of morphine. CFLP male mice were treated twice a day with graded doses of morphine in EPM and OF experiments and once a day in tail-flick studies. Obestatin (1.5 μg/2 μl) was administrated once a day in all experiments. Furthermore, 0.2 mg/kg naloxone or saline was administered after the final injection of morphine at a dose of 20 mg/kg in EPM and OF. These behavioral parameters were monitored in the OF: the percentage of center ambulation time and distance; whereas in the EPM: the time spent in open arms and the entries into open arms compared to the total time (%OAT) and entries (%OAE). In the OF, obestatin significantly decreased the percentage of time spent in the center in mice undergoing naloxone-precipitated mild morphine withdrawal. EPM results were similar to open field, but obestatin had no significant effect on parameters mentioned above. Besides, obestatin maintained the analgesic effect of morphine 90 and 120 min after morphine injection in mice treated with morphine receiving obestatin compared to mice treated with morphine. In tolerance studies, obestatin diminished the analgesic tolerance to morphine on the 5th day. In this study we confirmed that obestatin reversed the effect of mild morphine withdrawal and enhances the analgesic effect of morphine. These data suggest that obestatin may have a role in opioid-induced analgesia and in behavioral responses induced by opioid withdrawal.

1. Introduction

In 2005, a ghrelin-associated peptide derived from the preproghrelin was discovered by Zhang et al. [1] and named obestatin. Obestatin was purified from the rat stomach and was initially reported to reduce food intake, gastric emptying and intestinal motility [1,2]. It was also characterized as an activator of the orphan G protein-coupled GPR39 receptor and was found to be the main ligand for it. The highest levels of GPR39 mRNA were detected by in-situ hybridization in the amygdala, the hippocampus, and the auditory cortex, while lower levels were found in several other brain regions but surprisingly no expression of GPR39 was found in the hypothalamus in mice [3]. GPR39 receptor has two splice variants, GPR39-1a and GPR39-1b. GPR39-1a is expressed selectively in the gastrointestinal tract, whereas GPR39-1b has a wider expression pattern, including nuclei in the central nervous system, for example the amygdala, and hippocampus [4]. Later studies reported that GPR39 may not have obestatin as a main ligand [5–7]. After these findings, Zhang et al. confirmed that their original result was unreproducible [8] and subsequent results suggested that glucagon-like peptide-1 receptor (GLP-1R) is the receptor of obestatin [9,10]. Moreover, a few in vitro studies claimed that obestatin stimulated ERK1/2 phosphorylation, in rat tumor somatotroph cells [11]; in human pancreatic islet microendothelial cells [10]; in human β cells [9] and in human retinal pigment epithelial cells [12].

In the past few years the metabolic and body weight-regulating effect of obestatin has been investigated in detail, however, there are only a few reports, which examined the role of obestatin in exploratory behavior and its analgesic effect. A previous study on the EPM indicated that obestatin induced the elevation of the %OAT and %OAE in rat [13]. These data were later confirmed by Ishitobi et al. [14]: intracerebroventricular administration of antisense DNA for GPR39-1b caused anxiolytic-like effect in rats in two different behavioral tests. The same research group discovered that ghrelin decreased the %OAT in the EPM and increased the ambulation time in the OF test in rats and neonatal chicks [15,16], hence ghrelin exerts opposite effects on behavioral patterns. The role of ghrelin in reward (see reviews in this issue: [17,18]) and in anxiety (see review: [19]) are well-examined research areas, but the role of obestatin in these research fields has not been clarified yet.

☆ The authors have no conflicts of interest to declare.

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Thus, the aim of the present study was to investigate the actions of obestatin on morphine-induced analgesia and on mild morphine withdrawal in mice using three behavioral methods.

2. Materials and methods

2.1. Animals

Male CFLP white mice (30 ± 5 g of weight) of an outbred strain (Domaszék, Hungary) were used. They were kept under a standard light–dark cycle (lights on between 07.00 and 19.00 h) with food and water available ad libitum. The animals were kept and treated according to the rules of the Ethical Committee for the Protection of Animals in Research (Faculty of Medicine, University of Szeged, Hungary).

2.2. Surgery

For intracerebroventricular (i.c.v.) cannulation, the mice were anesthetized with an intraperitoneal (i.p.) injection of Sodium Pentobarbital (Nembutal®, Phylaxia-Sanofi, Budapest, Hungary; 50 mg/kg), and a polyethylene cannula was inserted into the right lateral cerebral ventricle and cemented to the skull with cyanoacrylate-containing instant glue. The experiments were started 4 days after i.c.v. cannulation. Upon conclusion of the experiments, 10 μl of methylene blue were injected into the cerebral ventricle of the decapitated animals and the position of the cannula was inspected visually. The spread of methylene blue throughout the ventricular space indicated that the whole amount of obestatin got into the ventricles. Mice with improper cannula placement were excluded from the final statistical analysis.

2.3. Drugs

For intracerebroventricular (i.c.v.) treatments obestatin (Anaspec, Inc.) was dissolved in artificial cerebrospinal fluid (aCSF) and injected in a volume of 2 μl. For testing the morphine effects, subcutan (s.c.) morphine–HCl (Sigma-Aldrich) and naloxone–HCl (Sigma-Aldrich) injections were used. Control mice received saline s.c. and aCSF i.c.v.

2.4. Elevated plus maze (EPM)

The elevated plus maze (EPM) is an accepted model for studying anxiety-like behavior in mice [20]. Conditions that decrease time spent in the open arms are associated with anxiety-like behavior, whereas increased time spent in the open arms is associated with an anxiolytic effect. The EPM apparatus (Columbus Instruments, Columbus, Ohio, USA) consists of four arms (87-mm wide, 155-mm long) elevated 63.8 cm above the ground, with two arms enclosed by 16.3-cm-high opaque walls and illuminated with a 60 W light situated 1 m above the maze. The combination of height, luminosity and open space is assumed to induce anxiety-like behavior in mice. Behavioral testing was conducted between 10.00 and 12.00 h. Mice were carried to the experimental room in their home cages and habituated to the laboratory for at least 30 min before testing. Only one EPM apparatus per testing room was present. The apparatus was thoroughly cleaned with ethanol (96%) and water between mice. Mice were placed in the center of the maze facing toward an enclosed arm and behavioral activities were recorded for 10 min [21]. The following behavioral parameters were monitored: the time spent in open arms and the entries into open arms compared to the total time (%OAT) and entries (%OAE) and the total activity which was defined as the total number of crosses between any two arms.

2.4.1. The effect of naloxone and obestatin on EPM behavior in mice treated with morphine

We used twice daily injections of ascending doses of morphine (08.00 and 16.00 h.) as follows: day 1: 10 mg/kg, day 2: 20 mg/kg, day 3: 40 mg/kg or saline [22]. Mice were also treated once a day with either obestatin (1.5 μg/2 μl, i.c.v.) or aCSF (i.c.v.) at 08.15 h. On the test day (day 4) animals received a single dose of morphine (20 mg/kg, s.c.) or saline (s.c.) at 08.00 h and either aCSF or obestatin (i.c.v.) was given at 09.45 h. Naloxone treatment in a dose of 0.2 mg/kg, s.c. preceded behavioral assessment by 5 min. The behavioral changes were measured for 10 min 2 h after the final morphine treatment with EPM [21,23]. The treatment of specific groups is described below (Figs. 1A, B, 2 and Table 2).

Treatment protocol was the same in the open-field.

2.5. Open-field (OF) test

Obestatin effects on mild morphine withdrawal were also tested by the Conducta System (Experimetria Ltd., Budapest, Hungary). The apparatus consists of five black-painted testing boxes (40 cm × 50 cm × 50 cm each) set in an isolated room; the movements of mice were detected by high-density arrays of infrared diodes. One animal was placed in one box, the apparatus is able to test 5 mice at the same time and there is no connection between them. The floor of the box was washed with ethanol (96%), water and dried prior to the next animal testing. On the test day, mice were transported to the testing room and the percentage of time spent in the center and ambulation distances in the center were recorded individually for each animal and separately for each box.

2.5.1. The effect of graded doses of acute obestatin on OF behavior in mice

Obestatin was administrated i.c.v. at graded doses: 0.5–2 μg. Mice were tested 15 min after the obestatin treatment for 10 min.

2.5.2. The effect of naloxone on OF behaviors in mice treated with obestatin

We used twice daily injections of saline. Mice were also treated once a day with either obestatin (1.5 μg/2 μl, i.c.v., respectively) or aCSF (i.c.v.) at 08.15 h. On the test day (day 4) animals received saline (s.c.) at 08.00 h and either aCSF or obestatin (i.c.v.) was given at 09.45 h. Naloxone treatment in a dose of 0.2 mg/kg, s.c. preceded behavioral assessment by 5 min. The behavioral changes were measured 2 h after the final saline treatment in the OF. See the specific treatments under Fig. 3A, B and Table 1.

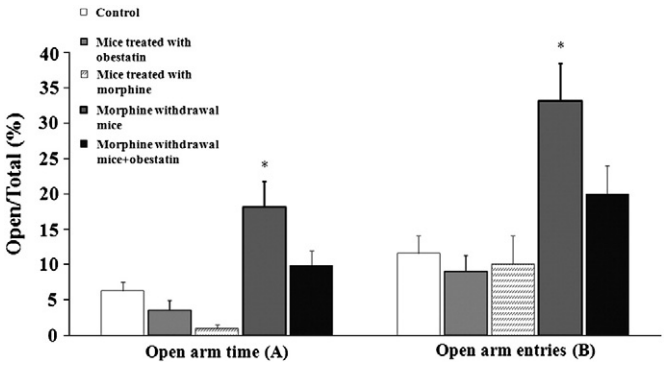

Fig. 1. A, B: The effect of naloxone and obestatin on EPM behavior (%OAT and %OAE) in mice treated with morphine. The graded doses of morphine (mg/kg, s.c. per injection) or saline were given twice daily for 3 days (day 1, 10; day 2, 20; day 3, 40). On day 4, naloxone (0.2 mg/kg) or saline was administered 2 h after the final injection of morphine at a dose of 20 mg/kg, and the EPM behavior was measured 5 min after naloxone injection. Mice were also treated once a day with either obestatin (1.5 μg/2 μl) or aCSF i.c.v 15 min after morphine injection for 3 days. On day 4, obestatin or aCSF was administrated 15 min prior to test. Numbers of mice: control: 8, mice treated with morphine: 9, morphine withdrawal mice: 8, morphine withdrawal mice + obestatin: 7, mice treated with obestatin: 8. Bars represent the %OAT (Fig. 1A) and the %OAE (Fig. 1B), vertical lines on the top of the bars denote S.E. M. A *: $P < 0.05$ vs. control mice, mice treated with morphine and mice treated with obestatin. B *: $P < 0.05$ vs. control mice, mice treated with morphine and mice treated with obestatin.