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Age-dependent effect of obestatin on intestinal contractility in Wistar rats

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ABSTRACT

Obestatin is a 23-amino acid peptide encoded by the ghrelin gene. We have investigated the effect of obestatin on intestinal contractility in rats ranging from the suckling period till adolescence. Duodenal and middle jejunum whole-thickness preparations from neonatal and adult rats were studied in an organ bath, for isometric recording under treatment with obestatin (1 μmol L^{-1}) in the presence of acetylocholine (ACh), atropine and tetradotoxin (TTX). Both the EFS and ACh-stimulated contractile response, as well as spontaneous contractile activity is age-dependent and specific for the segment of jejunum. Except for the middle jejunum of 7 day old rats, treatment with obestatin caused a significant TTX-sensitive increase in the amplitude of EFS-stimulated off-contraction of both intestinal segments studied.

Following injection of obestatin, the amplitude of spontaneous contraction in the duodenum increased in 7 day old rats. In the middle jejunum, treatment with obestatin significantly increased both the amplitude and frequency of spontaneous contraction in rats till the 28th day of life, whereas in adult rats the observed effect of obestatin was the opposite ($P < 0.001$ and $P < 0.0001$, respectively). The effects of treatment with obestatin on stimulation with increasing doses of ACh were only observed in the preparations from suckling rats. ACh-stimulated contractility in the duodenum was decreased while in the middle jejunum the observed effect was opposite.

These results indicate the importance of peripheral obestatin in the cholinergic control of intestinal contractility in both neonatal and adult rats.

1. Introduction

Obestatin is a novel 23-amino acid peptide first identified in the rat stomach as a ghrelin-accompanying peptide, generated during the posttranslational processing of preproghrelin (polypeptide precursor of ghrelin) (Zhang et al., 2005). Studies by Zhang et al. (2005) reported that obestatin behaves as a physiological opponent to ghrelin and inhibits food intake, body weight gain, gastric emptying and jejunal contractility. With respect to gastrointestinal motility, several studies were undertaken to elucidate the effects of obestatin on stomach emptying and gastrointestinal contractility and transit in rodents. The first study by Zhang et al. (2005) reported that peripheral injection of obestatin decreases gastric emptying and contractile activity of jejunal muscular strips *in vitro*. Since then, the inhibitory effects of obestatin on gastrointestinal motility have remained controversial. For example, Gourcerol et al. (2006) reported that obestatin injected peripherally, either alone or in combination with a peripheral injection of CCK, did not have an influence on the gastric motor function of fasted rats and mice. Similarly, Bassil et al. (2007) and De Smet et al. (2007) showed that in adult rats obestatin neither inhibited nor promoted GI motility, either *in vitro* or *in vivo*. However, Ataka et al. (2008) reported the inhibitory action of obestatin given IV on the motor activity in the antrum and duodenum of conscious rats in the fed state. These contrasting results may be partially explained by the different experimental conditions, specifically the duration of monitoring, the route of administration of obestatin and the state of feeding and consciousness of the animals.

Abbreviation: EFS, electrical field stimulation.

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Moreover, studies on the effects of obestatin are complex due to the unknown functional receptor for this peptide. The initially proposed G-protein-coupled receptor GPR39, has been questioned due to a series of studies which failed to demonstrate the ability of obestatin to bind to and activate this receptor (Chartrel et al., 2007; Holst et al., 2007; Lauwers et al., 2006).

These abovementioned results on gastrointestinal motility focused on adult mice and rats. Surprisingly, there are no studies regarding the effects of obestatin on the gastrointestinal motility in neonates. Recent studies have revealed the presence of obestatin immunoreactive (IR) cells in the gastrointestinal track of newborn rats starting from the 1st day of life. Interestingly, obestatin (IR) cells in the stomach reach adults levels by 6 weeks after birth, whereas ghrelin-IR cells were not observed on the first day after birth. Although their number increased within 1 week and stayed at this plateau until the fourth week of age, the levels of ghrelin-IR cells were still lower than those of the obestatin-IR cells (Zhao et al., 2008). It is also worth mentioning that a substantial amount of obestatin has been found in human colostrum and milk which strongly supports the importance of obestatin (both endogenous and exogenous) in the regulation of gastrointestinal function in neonates. Therefore, it would be intriguing to investigate the effect of obestatin on intestinal motor function in neonates compared to adult animals.

2. Materials and methods

The experiments and treatments were conducted in compliance with the European Union regulations concerning the protection of experimental animals (EC Directive 86/609/EEC with amendments). The study protocol was approved by the 3rd Local Ethics Committee in Warsaw, according to the Polish Law for the Care and Use of Animals (Resolution no 50/2012).

2.1. Chemicals

Rat obestatin was synthesized in Yanaihara Institute by a solid phase methodology with Fmoc-strategy using automated peptide synthesizer (Applied Biosystem 9030 Pioneer, Foster, CA, USA). Analytical HPLC and MALDI-TOF MS confirmed the homology of the product.

The hormone was kept in powder form at −20 °C and then dissolved in 0.9% NaCl to the final concentration, just before use. Acetylcholine chloride, isoproterenol and atropine were purchased from Sigma–Aldrich (Germany). Tetrodotoxin (TTX) was purchased from Abcam (Great Britain).

2.2. Animals

At the start of the experiment 8 male and 8 female Wistar Han rats were mated. They were allowed food (commercial rat chow, Wytwórnia Pasz Morawski, Poland) and water *ad libitum* in a humidity- and temperature-controlled room on a 12-h:12-h-light:dark cycle. Eight term-born litters were assigned to each of the 4 time point groups: 7 day old (15–17 g); 14 d (33–40 g); 21 d (45–50 g) and 28 d (62–72 g), (n = 8 for each group). Until euthanasia, all pups were housed with their mothers and breast-fed *ad libitum*. The group of adult rats was obtained from the maternal animals (220–320 g; n = 8).

2.3. Experimental design

Duodenal and middle jejunum segments (15 mm long) were taken promptly from animals and immediately placed in cold Krebs–Henseleit buffer (in mM: NaCl 18, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, glucose 11). The segments were then placed vertically in 25 ml organ bath chambers (Letica Scientific Instruments, Spain) that were filled with Krebs–Henseleit solution (37 °C, pH 7.4) and continuously saturated with carbogen (95% O_2, 5% CO_2). The intestinal segments were attached to isometric transducers (Letica Scientific Instruments, Spain) under a load of 0.1 g (7 day old rats) or 0.5 g (other groups). The transducers were coupled with a PowerLab recording system (ADInstruments, Sydney, Australia). The tissues were allowed to equilibrate for 30 min (the solution in the chambers was changed once after 15 min) to regain spontaneous activity. Then the segments were subjected to a procedure that started by the addition of ACh 10^{-5} M. ACh was left in the solution for 1 min, after which the tissues were washed and allowed to equilibrate. Next, obestatin at a dose of 1 μmol L^{-1} (obestatin group) or 0.9% NaCl (control group) were added to the chambers and after 15 min of incubation, spontaneous or ACh-stimulated contractility was recorded. The dose of obestatin and its time of exposure were chosen according to previous studies (Bassil et al., 2007; De Smet et al., 2007) in order to compare our results with previous experiments. ACh-stimulated contractility was recorded as the response to growing and cumulative doses of ACh (10^{-9}–10^{-4} M). In some experiments, before obestatin was added, jejunal strips were pre-treated with atropine.

Neural contractions were evoked by electrical field stimulation (EFS) and the effect of obestatin on these responses was examined. After equilibration, the electrical field stimulation (EXP-ST-01, Experimetria, Budapest, Hungary) was performed (voltage 90 V, duration 10 s) at three frequencies: 0.5, 5 and 50 Hz with 1 min intervals between each pulse. EFS parameters were chosen based on the previous studies performed on rat whole-thickness intestinal preparations (Korczynski et al., 2006). Next, obestatin at a dose of 1 μmol L^{-1} was added to the chambers and after 10 min the EFS was repeated. In some experiments jejunal segments were pre-treated with TTX. Each experiment was completed by the administration of ACh 10^{-5} M in order to check the viability of the tissue, followed by isoproterenol 10^{-5} M in order to control its relaxation.

2.4. Statistical analysis

Results are expressed as means ± SEM with significance defined as $P < 0.05$. A one-way ANOVA followed by the Tukey post hoc test or an unpaired t test or Kruskal–Wallis test was used to assess the statistical differences between the groups. All analyses were performed using GraphPad Prism version 4.0b (GraphPad Software Inc, San Diego, CA, USA).

3. Results

3.1. Effect of obestatin on electrical field stimulation in different age groups

EFS impulses (0.5, 5, 50 Hz) resulted in a frequency-dependent increase in the amplitude of both duodenal and middle jejunum sample contractions. We also observed that the EFS stimulated contractile response was age-dependent (Table 1, Fig. 2). Among the groups tested, the duodenum samples from the 7 day old rats had the lowest responsiveness, whereas in the middle jejunum the lowest responsiveness for rising EFS frequencies was observed in rats until the second week of life (7 day old and 14 day old). No changes between 21 and 28 day old rats were observed in both the duodenum and in the middle jejunum. Only in the middle jejunum of 7 day old rats, treatment with 1 μmol L^{-1} of obestatin did not result in any changes in amplitude of contraction. In both sections from the other age groups obestatin significantly increased the amplitude of EFS stimulated contraction. Moreover, we observed