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A novel simultaneous measurement method to assess the influence of intracerebroventricular obestatin on colonic motility and secretion in conscious rats

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ABSTRACT

Obestatin, a novel putative 23-amino acid peptide, is derived from mammalian preproghrelin gene via a bioinformatics approach. Although obestatin regulates thirst, sleep, memory, anxiety, activates cortical neurons in the brain and stimulate proliferation of retinal pigment epithelial cells, there is no study to explore its central impacts on the lower gut motility and secretion. We investigated the influence of intracerebroventricular (ICV) injection of obestatin on rat colonic motor and secretory functions. Colonic transit time, fecal pellet output and fecal content were assessed in freely fed, conscious rats, which were implanted with ICV and colonic catheters chronically. Human/rat corticotropin-releasing factor (h/rCRF) was applied as a stimulatory inducer of colonic motility and secretion. ICV injection of obestatin (0.1, 0.3, 1.0 nmol/rat) did not modify the colonic transit time, whereas ICV injection of h/rCRF (0.3 nmol/rat) significantly shortened colonic transit time. ICV obestatin in any dose we tested did not affect the fecal pellet output, frequency of watery diarrhea, total fecal weight, fecal dried solid weight, or fecal fluid weight in the first hour post-injection, either. In contrast, ICV injection of h/rCRF effectively stimulated fecal pellet output, as well as increased total fecal weight, fecal dried solid weight and fecal fluid weight during the first hour post-injection, compared to ICV saline controls. In conclusion, using our novel simultaneous measurement method, acutely central administration of obestatin exhibits no influence on colonic motility and secretion in conscious rats.

1. Introduction

Obestatin, a novel 23-amino acid peptide recently identified from rat stomach, is derived from C-terminal part of mammalian preproghrelin gene that also encodes ghrelin, by comparative genomic analyses [35]. It was originally projected that acyl ghrelin and obestatin exhibit opposite influence on ingestive behaviors, e.g. acyl ghrelin elicits food intake whereas obestatin inhibits it [35], although the effects of obestatin on food intake are debatable [12,13]. Acyl ghrelin binds to growth hormone secretagogue receptor 1a, and, then, signals via a $G_{q/11}\alpha$-subunit that results in the release of inositol trisphosphate and Ca^{2+} [9]. Obestatin was suggested binding to an orphan G protein-coupled receptor (GPR), termed GPR39 [7,35], and induced the increase of intracellular cAMP [35]. GPR39 expression has been detected in peripheral organs such as the duodenum and kidney but not in the pituitary or hypothalamus [22]. But recent studies reveal that obestatin is not the endogenous cognate ligand for GPR39 [8,22,23,34].

Obestatin manifested various biological functions at the central nervous system, such as inhibition of thirst [30], increase of non-rapid eye movement sleep episodes and decrease of sleep latency [29], as well as improvement of memory retention and reduction of anxiety [6] in rats. Besides, obestatin has also been reported to activate cortical neurons [15], and stimulate proliferation of retinal pigment epithelial cells [5]. In addition, a recent study indicates that mice lacking the preproghrelin gene have impaired abilities to manifest and integrate normal sleep and thermoregulatory responses to metabolic challenges [28]. When considered together, these results imply that obestatin may have some unknown but important novel roles on the central nervous system in the uninvestigated field, which deserve further exploration.

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Regarding gastrointestinal motility, many studies of obestatin focus on its effects on the upper gut [1,4,14,20]. Peripheral obestatin inhibited gastroduodenal motility in the fed state but not in the fasted state of conscious rats through activating corticotropin-releasing factor (CRF) receptor 1 and 2 in the brain, while vagal afferent pathways might be partially involved [1,16]. On the other hand, intracisternal injection of obestatin did not affect gastric phasic contraction of anesthetized fasted rats [19]. Our previous paper showed that peripheral administration of obestatin has no impact on colonic motility and secretion in rats [10]. From our PubMed search, there is no study to address the influence of centrally administered obestatin on rat colonic motor and secretory functions. As the above-mentioned multifaceted functions of obestatin in the brain, it is very interesting to investigate the potential, central effects of obestatin on colon motility and secretion. In the present study, we used our newly established animal model to simultaneously measure colonic transit time, fecal pellet output and fecal content in conscious fed rats [10]. Corticotropin-releasing factor (CRF), a well-known colonoprokinetic peptide, was applied to serve as a stimulatory agent to test the validity of this *in vivo* model.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (National Laboratory Animal Center, Taipei, Taiwan) weighing 250–320 g at the initial period of experiment were used and housed in group cages under controlled illumination (light cycle: 08:00–20:00), humidity and temperature ($22.5 \pm 1.5\,^{\circ}C$) with free access to water and laboratory chow pellets (LabDiet®, Brentwood, MO, USA). All experiments were performed since 9 a.m. in freely moving conscious fed rats, in accordance to guidelines, which have been approved by the Institutional Animal Care and Use Committee (IACUC), Taipei Veterans General Hospital.

2.2. Surgery

2.2.1. Implantation of intracerebroventricular (ICV) catheter

Rats were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg, Nembutal; Abbott Laboratories, Abbott Park, IL, USA), placed in a stereotaxic apparatus, and implanted with a guide cannula (25-gauge; Eicom, Kyoto, Japan), which reached the right lateral ventricle. Stereotaxic coordinates were 0.8 mm posterior to bregma, 1.4 mm right lateral to the midline, and 4.5 mm below the outer surface of the skull using a stereotaxic frame (Benchmark™, myNeuroLab, St. Louis, MO, USA) with the incisor bar set at the horizontal plane passing through bregma and lambda. The guide cannule was secured with dental cement anchored by two stainless steel screws fixed on the dorsal surface of the skull. After surgery, a dummy cannula (Eicom) was inserted into each guide cannula, and a screw cap (Eicom) was put on the guide cannula to prevent blockade [11]. The correctness of ICV cannula placement was verified by injection of 100 μl dye (0.05% cresyl violet, Sigma) into the right lateral ventricle by the brain sections at the end of experiments after euthanasia [11]. Before beginning all colonic motor and secretory function tests, those animals receiving implantation of colonic tubes with ICV catheters were allowed 7 days for recovery. All ICV injections were performed over 60 s in 5 μl using the AMI-5 (Eicom).

2.2.2. Implantation of colonic catheter

Rats were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg, Nembutal; Abbott). After the lower abdomen laparatomy, a catheter (3 Fr, 1-mm diameter; ATOM) was implanted into the proximal colon 2 cm distal from the ceco-colonic junction chronically. The catheter was fixed at the colonic wall by a purse-string suture and routed subcutaneously to the interscapular region, where it was exteriorized through the skin using for intracolonic administration of dye marker [10]. Before simultaneous measurement of colonic motor and secretory functions, rats were allowed 7 days to recovery.

2.3. Preparation of drugs

Rat obestatin (Peptides International, Inc., Louisville, KY, USA) and human/rat CRF (h/r CRF) (American Peptide Company, Sunnyvale, CA, USA) were kept in powder form at $-20\,^{\circ}C$, and dissolved in sterile, pyrogen-free 0.9% saline (Otsuka, Tokyo, Japan) immediately before use. The doses of obestatin, 0.1, 0.3, and 1.0 nmol/rat, were selected similar to those effective in elevated plus maze test [6] and thirst study [30], while the dose of human/rat CRF (0.3 nmol/rat) was chosen mimicking the effective one in manometric recording [2].

2.4. Colonic motor and secretory function tests

2.4.1. Measurement of colonic transit time

Colonic transit time was calculated using an enteral dye marker, trypan blue (Sigma Chemical Co, St. Louis, MO, USA), a non-absorbable dye. The dye was injected in 0.2 ml volume through the catheter positioned in the proximal colon, and followed by a 0.2 ml saline flush 10 min after the ICV injection of either saline, obestatin, or h/rCRF [10]. Colonic transit time was defined as the time interval between the dye injection and the discharge of the first blue pellet.

2.4.2. Measurement of fecal pellet output

Rats were accustomed to single housing cage individually for 7 days before the experiment. The total number of pellets was recorded every hour after an intracolonic injection of trypan blue for 2 h.

2.4.3. Measurements of characteristics of fecal content

The frequency of watery diarrhea was assessed by the number of rats expelling loose watery stool. The total fecal material was collected every hour after the intracolonic injection of trypan blue for 2 h, and its content was weighed then desiccated overnight at $50\,^{\circ}C$, and the fecal fluid and solid output were calculated from the total and dry weights [10,26].

2.5. Statistical analysis

All results are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls *post hoc* test was used to analyze difference among groups. Differences were considered statistically significant with $p < 0.05$.

3. Results

3.1. Colonic transit time

ICV injection of obestatin at 0.1, 0.3, and 1.0 nmol/rat did not change the colonic transit time (290.7 ± 29.9, 265.6 ± 29.4, and 292.9 ± 19.8 min vs. 300.3 ± 21.0 min, $p > 0.05$), compared to ICV saline controls (Fig. 1). Contrary, ICV injection of h/rCRF at 0.3 nmol/rat significantly shortened colonic transit time (190.3 ± 23.5 vs. 300.3 ± 21.0 min, $p < 0.05$) in conscious fed rats.

3.2. Fecal pellet output

ICV obestatin (0.1, 0.3, and 1.0 nmol/rat) did not affect the number of fecal pellet output (1.17 ± 1.08, 0.92 ± 0.47, and 0.58 ± 0.29 vs. 1.00 ± 0.48 per hour), whereas ICV injection of h/rCRF at 0.3