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Neuronostatin: Peripheral site of action in mouse stomach

Antonella Amato, Sara Baldassano, Gaetano Caldara, Flavia Mulè*

Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), Università di Palermo, 90128 Palermo, Italy

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ABSTRACT

Neuronostatin is a 13-amino acid peptide encoded by somatostatin gene. It is distributed in different organs including gastrointestinal tract and has been involved in the control of food intake and gastrointestinal motility, likely through an action in the brain. So far, there are no reports about the occurrence of peripheral action sites in the gut. Therefore, the purpose of the present study was to examine, in the mouse, the effects of peripheral administration of neuronostatin on food intake within 24 h and on gastrointestinal motility and to analyse neuronostatin actions on the gastric and intestinal mechanical activity in isolated preparations in vitro. When compared with PBS-treated mice, intraperitoneal neuronostatin reduced food intake in doses ranging from 1 to 15 ng/g b.w. only in the first hour postinjection with a maximum effect obtained at the dose of 15 ng/g b.w. (−46.9%). The peptide (15 ng/g b.w.) significantly reduced gastric emptying rate (−31.1%) and gastrointestinal intestinal transit. Non-amidated neuronostatin failed to affect food intake, gastric emptying and intestinal transit, suggesting the specificity of action. In vitro, neuronostatin induced concentration-dependent gastric relaxation, which was abolished by tetrodotoxin. Neuronostatin failed to affect the spontaneous mechanical activity or the evoked cholinergic contractions in duodenum. These results suggest that exogenous neuronostatin is able to reduce mouse gastric motility by acting peripherally in the stomach, through intramural nervous plexuses. This indirectly action could cause reduction of food intake in the short term.

Introduction

Neuronostatin is a recently described 13-amino acid peptide hormone; which is encoded by somatostatin gene and derived from the N-terminus of pro-somatostatin. Somatostatin is a peptide hormone; originally identified in hypothalamus and subsequently found in neuroendocrine organs; gastrointestinal tract; thyroid and adrenal glands; liver; kidney; inflammatory and immune cells. As expected, neuronostatin has been found to be expressed in the same tissues as somatostatin, including brain and gastrointestinal tissues with the highest level in the pancreas followed by cerebrum and hypothalamus [19]. As somatostatin, neuronostatin is considered a brain/gut peptide due to its site of production and its ability to induce early response genes c-Fos or c-Jun in neuronal, anterior pituitary gastrointestinal tissues [19] and cardiomyocytes [13]. Despite sharing common biological effects, such as inhibitory control of cardiac activity, pancreatic and gastrointestinal functions, neuronostatin and somatostatin possess biological activities that are distinct from each other [24]. Furthermore neuronostatin has been reported not to interact with the five putative somatostatin receptors [19]. Indeed, when injected centrally in rats neuronostatin leads to a dose-related inhibition of food and water intake and an increase in mean arterial pressure [25,27]. Intracerebroventricular (i.c.v.) injections of neuronostatin in mice produce antinociceptive effect via the central melanocortin and opioid systems [22] and a depression-like effect via the central melanocortin system [23].

Neuronostatin can modify the hypothalamic neuron firing and neuronal migration [10,19] and it is able to regulate cardiac and cardiomyocyte contractile function and cardiomyocyte survival [13,21,30]. Recent studies in isolated rat pancreatic islets or in the animals in vivo suggest also a role of neuronostatin in maintaining glucose homeostasis through inhibition of glucose-stimulated insulin secretion [18].

Neuronostatin has been also involved in the control of gastrointestinal motility. In fact, i.c.v. administration of neuronostatin in mice delays the gastric emptying and the gastrointestinal transit [20]. However, to date, there are no reports about the effects of peripheral administration of neuronostatin on food intake and

Abbreviations: EFS, electrical field stimulation; GE, gastric emptying; i.c.v., intracerebroventricular; i.p., intraperitoneal; PBS, phosphate-buffered saline; TTX, tetrodotoxin.

* Corresponding author at: Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Laboratorio di Fisiologia generale, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy. Tel.: +39 91 23897515; fax: +39 91 6577501.
E-mail address: flavia.mule@unipa.it (F. Mulè).

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gastrointestinal motility. It is likely to hypothesize that there are peripheral sites of action involved in the biological effects of the neuronostatin, similarly to other peptides involved in the control of food intake and gastrointestinal functions [2,5,9,17,29]. Unlikely, the neuronostatin receptor and its distribution are still unknown, although the orphan G protein-coupled receptor, GPR107, has been proposed [26].

Therefore the purpose of the present study was to examine if intraperitoneal administration of neuronostatin influences the food intake within 24 h and the gastrointestinal motility in mouse. Moreover, to better elucidate the gastrointestinal actions of neuronostatin, the effects of the exogenous peptide on the gastric and intestinal mechanical activities in vitro were examined and the mechanism of action responsible of the observed effects was studied.

Materials and methods

Animals

The experimental procedures employed in the present study were in conformity with the Italian D.L. No. 116 of 27 January 1992 and subsequent variations and the recommendations of the European Economic Community (86/609/ECC). The studies were approved by Ministero della Sanità (Rome, Italy). Adult male C57BL/6J mice, purchased from Harlan Laboratories (San Pietro al Natisone, Udine, Italy) were singly housed under controlled environmental conditions (22 + 1 °C, 55 + 15% relative humidity, 12 h light). Tap water and standard laboratory rodent chow (Mucedola, Settimo Milanese, MI, Italy) were provided ad libitum; except as otherwise stated.

Food intake

Fasted (16 h) mice were injected with intraperitoneal (i.p.) 100 μl of either vehicle (phosphate-buffered saline, PBS) or neuronostatin (1, 6, 10, 15, 30 ng/g b.w.), in the early light phase (0800–0900 h). Prior to the initial study, mice received a daily i.p. injection of 100 μl PBS for 7 days to habituate them to the procedure. Doses of neuronostatin were determined on the basis of the literature [30] and from preliminary experiments in our laboratory. A minimum of 72 h was allowed between each trial in the same mouse. Following injection, each mouse was returned to its home cage with a pre-weighed amount of chow. The food intake was determined at 1, 2, 4, 8, and 24 h following peptide or vehicle administration, by measuring the difference between the pre-weighed chow and the weight of chow at the end of each time interval. Any spillage was collected and weighed.

Gastric emptying and intestinal transit

To examine gastric emptying and small intestinal transit, the animals were deprived of food for 24 h before the experiments began. Then, mice were injected with i.p. 100 μl of either PBS or neuronostatin (15 ng/g b.w.) 10 min before gastric load. This dose was chosen because it induced maximum effect in the food intake experiments. The experiments to assess gastric emptying and transit were conducted as previously reported [16]. Briefly, mice received by gavage 0.3 ml of test meal (a non-nutrient meal of 50 mg phenol red in 100 ml 1.5% carboxymethylcellulose) and were euthanized by cervical dislocation immediately ($t = 0$) or 20 min after gavaging. Under laparatomy, the stomach and the small intestine were excised after legation of the pylorus and the cardias. The stomach and its contents were homogenized in 25 ml of 0.1 N NaOH. The mixture was then kept for 1 h at room temperature. Then, 8 ml of the supernatant was added to 1 ml of 33% of trichloroacetic acid to precipitate proteins. After centrifugation (3000 rpm for 30 min at 4 °C) the supernatant was added to 2 ml of 2 N NaOH to develop the maximum color intensity. The amount of phenol red was determined from the absorbency at 560 nm. This correlates with the concentration of phenol red in the stomach, which in turn depends on the gastric emptying. The gastric emptying (GE) rate was calculated as $GE = (1 - X/Y)100$, where X is absorbance of phenol red recovered from the stomach of animals sacrificed 20 min after test meal. Y is mean ($n = 4$) absorbance of phenol red recovered from the stomachs of animals killed at 0 min following test meal.

Immediately after the excision of the stomach, the whole small intestine was grossly freed from its mesenteric attachments and its length (from the pyloric sphincter to the ileocecal junction) was measured. The intestine was opened at the level of the front of the test meal, which was revealed by few drops of 0.1 N NaOH. The rate of intestinal transit was expressed as the ratio between the distance traveled by the test meal and the total length of intestine, as previously described [16].

Functional studies in vitro

After animal sacrifice, the abdomen was immediately opened, the esophagus was tied just below the lower oesophageal sphincter, and stomach and duodenum were excised.

Isolated stomach

As previously described [17], whole stomach was used in order to examine the muscle function under conditions where the influence of external factors is removed, but the muscle performs in a manner analogous to its in vivo capacity. The entire stomach was mounted in a custom designed organ bath, which was continuously perfused with oxygenated (95% O_2 and 5% CO_2) and heated (37 °C) Krebs solution with the following composition (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $CaCl_2$ 2.5; and glucose 11.1. The pyloric end was tied around the mouth of a J-tube, which was connected to a standard pressure transducer (Statham Mod. P23XL; Grass Medical Instruments, Quincy, MA, USA) and the changes of endoluminal pressure were recorded on ink-writer polygraph (Grass model 7D). Preparations were allowed to equilibrate for about 60 min before starting the experiment. Under these conditions, mouse stomach exhibits spontaneous small rhythmic contractions and basal tone, allowing testing the relaxant activity directly without the use of a contractile agent. At the beginning of each experiment, the preparation was challenged with isoproterenol (1 μM), until reproducible responses were obtained, to ensure that a stable and acceptable level of sensitivity had been reached before the experimental procedure was begun. Isoproterenol was tested also at the end of the experiment to assess the relaxant ability of the preparations. Isoproterenol was added into the bath after switching off the perfusion and left in contact with the preparation for 2 min. The responses to non-cumulative concentrations of neuronostatin (0.01–3 nM) were examined on the gastric basal tone. Neuronostatin was added into the bath at increasing concentrations in volumes of 50 μl at 45-min intervals. Each concentration was left in contact with the tissue for 7 min. In some experiments, to confirm the specificity of the observed effect, non-amidated neuronostatin (0.01–3 nM) was tested. The response to neuronostatin was also tested in presence of tetrodotoxin (TTX) (1 μM), a voltage-dependent Na^+-channel blocker, which was added to the perfusing solution at least 30 min before testing the peptide. TTX concentration was proven to be effective at blocking response induced by electrical field stimulation [15].

Duodenal segment

The mechanical activity of duodenal preparations was recorded as previously described [2]. In brief, the distal end of each segment