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New satiety hormone nesfatin-1 protects gastric mucosa against stress-induced injury: Mechanistic roles of prostaglandins, nitric oxide, sensory nerves and vanilloid receptors

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ABSTRACT

Nesfatin-1 belongs to a family of anorexigenic peptides, which are responsible for satiety and are identified in the neurons and endocrine cells within the gut. These peptides have been implicated in the control of food intake; however, very little is known concerning its contribution to gastric secretion and gastric mucosal integrity. In this study the effects of nesfatin-1 on gastric secretion and gastric lesions induced in rats by 3.5 h of water immersion and restraint stress (WRS) were determined. Exogenous nesfatin-1 (5–40 μg/kg i.p.) significantly decreased gastric acid secretion and attenuated gastric lesions induced by WRS, and this was accompanied by a significant rise in plasma NUCB2/nefatin-1 levels, the gastric mucosal blood flow (GBF), luminal NO concentration, generation of PGE_2 in the gastric mucosa, an overexpression of mRNA for NUBC2 and cNOS, as well as a suppression of iNOS and proinflammatory cytokine IL-1β and TNF-α mRNAs. Nesfatin-1-induced protection was attenuated by suppression of COX-1 and COX-2 activity, the inhibition of NOS with L-NNA, the deactivation of afferent nerves with neurotoxic doses of capsaicin, and the pretreatment with capsazepine to inhibit vanilloid VR1 receptors. This study shows for the first time that nesfatin-1 exerts a potent protective action in the stomach of rats exposed to WRS and these effects depend upon decrease in gastric secretion, hyperemia mediated by COX-PG and NOS-NO systems, the activation of vagal and sensory nerves and vanilloid receptors.

1. Introduction

In 1992 a new peptide that binds to Ca^{2+} and DNA was identified and named nucleobindin or NEFA (DNA binding/EF-hand/acidic amino acid rich region) in both human and mouse cells [26]. Later on two types of nucleobindin: nucleobindin1 (NUCB1) and nucleobindin2 (NUCB2) were discovered [1,36]. At its N-terminus a NUCB2 molecule contains a signal peptide of 24-amino acids and a chain of 396 amino acids with a sequence homology in rodents and humans, thus suggesting its physiological similarity and significance in both species [36,38]. As a result of post-translational modifications of a NUCB2 molecule in a presence of prohormone convertase, (PC)-1/3, three compounds are formed: nesfatin-1 (1-82), nesfatin-2 (85-163) and nesfatin-3 (166-396) [38,41]. Nesfatin-1 described for the first time by Oh-I et al. [38] belongs to the family of anorexigenic peptides responsible for satiety.

Initially, mRNA expression for NUCB2 was only detected in a group of neurones in the hypothalamus and the brain stem, which includes: the paraventricular nucleus (PVN), the supraoptic nucleus, arcuate nucleus, lateral hypothalamic area, zona incerta and nuclei of the solitary tract [2]. These findings have been confirmed immunohistochemically [19,41]. Cells which are immunopositive for NUCB2/nesfatin-1 are also present in other areas of the hypothalamus, the midbrain and the hindbrain including the dorsomedial hypothalamic nucleus, tuberal hypothalamic area, PVN, Edinger–Westphal nucleus, locus coeruleus, medullary raphe nuclei and dorsal motor nucleus of the vagus nerve [2,20]. Goebel et al. [20] found neurons immunoreactive for nesfatin-1 in rat insular cortex, central amygdaloidal nucleus, ventrolateral medulla and cerebellum, and the presence of nesfatin-1 was documented in sympathetic and parasympathetic preganglionic neurones of the spinal cord within the thoracic, lumbar and sacral sections. Among the nesfatins, only nesfatin-1 exhibited biological activity so far, because this peptide inhibits food intake and gastric emptying in rats when administered either peripherally or

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centrally [21,45]. Furthermore, nesfatin-1 immunoreactivity has been identified and characterized in various endocrine cell types of the rat gastric oxyntic mucosa [45]. Studies in rats revealed that some physiological effect of nesfatin-1 are mediated by the vagal nerve [55]; however, little is known of the contribution of this peptide to the mechanism of gastric mucosal defense and gastroprotection against damage induced by stress or topical necrotizing irritants such as ethanol.

This study was designed to determine the effects of intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) administration of nesfatin-1 on gastric acid secretion in rats equipped with chronic gastric fistula (GF) and gastric lesions induced by 3.5 h of water immersion and restraint stress (WRS) [4]. For comparison, the effects of nesfatin-1 on the acute gastric lesions induced by intragastric application of 75% ethanol and the accompanying changes in the gastric blood flow (GBF) were assessed [10]. We examined the involvement of prostaglandins (PG), nitric oxide (NO), as well as vagal using vagotomized animals and sensory innervations using capsaicin-denervated animals in nesfatin-1-induced gastroprotection against gastric mucosal damage induced by WRS. The gastric mucosal expression of mRNA for cNOS, iNOS, NUCB2 and proinflammatory cytokines IL-1β and TNF-α as well as plasma levels of these cytokines were determined in rats pretreated with nesfatin-1 and exposed to WRS.

2. Methods

2.1. Compounds

Nesfatin-1 was purchased from Bachem AG, Bubendorf, Switzerland, indomethacin, N^G-nitro-L-arginine (L-NNA), L-arginine and capsazepine were purchased from Sigma Co., St. Louis, MO, USA), SC-560 was purchased from Cayman Chemical Co., Ann Arbor, MI, USA) and rofecoxib was a gift from Merck Sharp and Dhome, Warsaw, Poland.

The study was approved by the Institutional Animal Care and Use Committee of Jagiellonian University Medical College in Cracow and was conducted in accordance with the statements of Helsinki Declaration regarding handling of experimental animals (animal protocol number N402 457 139/3).

2.2. Intracerebroventricular administration and gastric secretory studies

The effects of nesfatin-1 on gastric acid secretion were examined in 35 conscious rats weighing 200–230 g and equipped about 1 month earlier with a Thomas-type GF as described previously [10,32]. The animals were fasted overnight but had free access to water 24 h before the experiment and they were placed in individual Bollman-type cages to maintain the minimum restraint necessary. For the i.c.v. injection of vehicle (saline) or nesfatin-1, the GF rats had undergone surgery 48 h before the secretory studies according to the method published elsewhere [10,16,53]. Briefly, under pentobarbital anesthesia, rats were placed in a stereotactic frame (Stoelting, WoodDale, IL, USA) and an incision was made along with the mid-line of the skull, the skull bones were cleaned of connective tissue and the point of intersection between the sagittal and coronary sutures was visualized. The hole 1.3 mm lateral to the midline and 1.5 mm caudal to bregma according to the stereotaxic atlas by Paxinos and Watson [39] was made by rotary movement of the needle and the wound of the head was closed by a clip. At the day of secretory experiment, fasted rats were anesthetized with isoflurane vaporizer (Double-Output Anesthesia System, Ugo Basile, Varese, Italy) and a specially modified Hamilton syringe (Hamilton Co., Reno, NV, USA) was used to inject the lateral ventricle to a depth of 3.5 mm as reported before [10,16,53]. Vehicle (saline in a volume of 5 μl i.c.v. or 1 ml i.p.) or nesfatin-1 was freehand injected in gradually increasing doses ranging from 5 to 40 μg/kg and 5 to 1000 ng/rat, respectively, each dose being administered i.c.v. on a separate test day. Thirty minutes later after animals were awaken due to fast recovery from isoflurane anesthesia, the GF was opened and the stomach rinsed gently with about 5 ml of tap water at 37 °C and the basal gastric secretion was collected for 60 min. The reliability and consistency of i.c.v. injections was routinely verified during actual experiments by injecting 10 μl of dye (0.1% toluidine blue) in parallel blank animals and macroscopically observing proper coloration of the ventricle [10,16,43,53]. The collection of gastric juice was continued for the final 2 h after i.p. or i.c.v. injections of nesfatin-1 or vehicle (control). The volume and acid concentration of each collected sample of gastric juice were measured and acid outputs (expressed in term of micromoles of acid per 30 min) and pepsin outputs (expressed in term of mg of pepsin per 30 min) were determined as described previously [10,14].

2.3. Studies on gastroprotection by nesfatin-1 and measurement of gastric blood flow

To determine nesfatin-1 contribution to the mechanism of gastric protection, the acute gastric lesions, the animals were exposed to 3.5 h of WRS according to the procedure described by Takagi et al. [51], which was modified by our group before [6,8]. In a separate group of animals, the gastric lesions were induced by 75% ethanol applied i.g. by a volume of 1 ml in order to check whether this peptide could also be effective against the necrotizing type of gastric injury [10]. At 3.5 h at the end of WRS or following 1 h of ethanol application, the animals were lightly anesthetized with Phenobarbital (60 mg/kg i.p.), their abdomen was opened by a midline incision and the stomach exposed for a measurement of the GBF by means of a H_2-gas clearance technique as described previously [3,5]. For this purpose double electrodes of an electrolytic regional blood flow meter (Biotechnical Science, Model RBF-2, Osaka, Japan) were inserted into the gastric mucosa. The measurements were made in three areas of the oxyntic mucosa and the mean values of the measurements were calculated and expressed as a percent change of those recorded in the vehicle (saline) treated animals. After the GBF measurement, the stomach was removed, rinsed with saline and pinned open for macroscopic examination. The number of gastric bleeding erosions was determined by computerized planimetry (Morphomat, Carl Zeiss, FRG) on photographed stomachs [7,9,10,32] by the person blinded to which experimental group the animals belonged to.

In some experiments with i.p. injection of nesfatin-1 standardized specimens from the corpus of the stomachs were fixed in 10% buffered formalin, and the paraffin sections were stained with hematoxylin-eosin for histology evaluation [11]. A Nikon microscope equipped with Microplan II digital image system was used for the quantitative histology examination (morphometry) of the sections. Coded specimens of mucosa stained with hematoxylin-eosin were evaluated quantitatively at 500× magnification under blinded conditions.

2.4. Determination of plasma NUCB2/nesfatin-1, gastrin and TNF-α and IL-1β levels in the rats exposed to WRS with or without nesfatin-1 pretreatment

Immediately after GBF measurement, a venous blood sample (about 3 ml) was withdrawn from the *vena cava* and placed into EDTA-containing vials and used for the determination of plasma NUCB2/nesfatin-1 and gastrin levels by specific radioimmunoassays (RIAs) and plasma proinflammatory cytokine TNF-α and IL-1β