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Long-term peripheral infusion of nociceptin/orphanin FQ promotes hyperplasia, activation and migration of mucosal mast cells in the rat gastric fundus

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ABSTRACT

The endogenous neuropeptide nociceptin/orphanin FQ (N/OFQ) modulates behavioral and gastrointestinal responses to stress. Mucosal mast cells (MMCs) are primary mediators of stress-related responses in the gastrointestinal tract. We investigated the influence of N/OFQ and of the N/OFQ peptide (NOP) receptor antagonist, UFP-101, on MMCs in the rat gastric fundus. N/OFQ was infused subcutaneously for 52 h at 0.1, 1 and 10 μg/kg/h and at 1 μg/kg/h for 4 h, 52 h, 7 days and 14 days via Alzet osmotic minipumps. Density of MMCs and connective tissue mast cells (CTMCs) was assessed histochemically and immunohistochemically. Activation and location of MMCs were assessed by transmission electron microscopy. Contacts between MMCs and nerve elements were assessed by double immunofluorescence. N/OFQ (1 μg/kg/h) and UFP-101 (10 and 30 μg/kg/h) were infused subcutaneously in the absence and presence of acute cold-restraint stress and density of MMCs was assessed. Peripheral N/OFQ dose-dependently increased the density of MMCs, while not influencing CTMCs. The increasing effect was maintained up to 14 days following continuous infusion, while after termination of the 4-h infusion, the effect declined rapidly. The peptide promoted the activation of MMCs and their migration from the lamina propria toward the epithelial layer. The association between MMCs and nerve fibers was time-dependently down-regulated following N/OFQ infusion. The stress-induced hyperplasia of MMCs was not influenced by N/OFQ and abolished by UFP-101. UFP-101 alone was ineffective. The present results suggest that endogenous N/OFQ could be considered a potential component of the circuit neuropeptides-mast cells-stress.

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1. Introduction

The heptadecapeptide nociceptin/orphanin FQ (N/OFQ) and the N/OFQ peptide (NOP) receptors are widely distributed in the central nervous system [2,30,31] and in the gastrointestinal tract [45,47]. It is well documented that the N/OFQ-NOP system participates in the processing of the behavioral response to stressful/anxiogenic stimuli. Both anxiolytic [20,15,42] and anxiogenic [14] effects are reported following the intracerebroventricular administration of N/OFQ in the rodents. N/OFQ also influences the stress-related alterations in gastrointestinal functions. Gastric ulcerogenis [16,17] and fecal pellet excretion [5] induced by cold-restraint stress in rats were inhibited by central as well as by peripheral administration of N/OFQ.

Exposure to psychological or physical stress is well established to cause disturbances in gastrointestinal epithelial, secretory and motor functions both in rodents and humans [37,40]. Stress is also associated with the initiation and reactivation of intestinal inflammatory disorders [23]. Mucosal mast cells have been identified as the primary mediators of the stress response [46]. Acute and repeated stress increases the number and promotes the activation of mucosal mast cells in the rat ileum and colon [41,34,38]. Stress-induced abnormalities in intestinal epithelial functions were absent in mast-cell deficient rats [34,38]. Acute immobilization stress increases the levels of rat mast cell protease (RMCP) II, a specific marker of rat mucosal mast cells, in mesenteric blood and in colonic explants cultured after acute stress [10]. Release of colonic mucin in response to immobilization stress was not observed in mast-cell deficient rats [11].

The present study is aimed at evaluating the ability of short- and long-term administration of N/OFQ to influence mucosal mast cells (MMCs) and connective tissue mast cells (CTMCs) in the rat gastric fundus. We also examined the effect of acute cold-restraint stress on MMCs and whether administration of N/OFQ or of the NOP receptor antagonist, [Nphe[1],Arg[14],Lys[15]]N/OFQ-

Abbreviations: CTMC, connective tissue mast cell; MMC, mucosal mast cell; N/OFQ, nociceptin/orphaninFQ; UFP-101, [Nphe[1],Arg[14],Lys[15]]N/OFQ-NH_2.

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NH_2 (UFP-101) [8,9], could alter the influence of acute stress on MMCs.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 180–200 g (Harlan, San Pietro al Natisone, UD, Italy), were housed at a constant room temperature (25 ± 1 °C) and humidity ($60 \pm 5\%$) with a 12 h light/dark cycle. All rats were individually caged. They were deprived of food but not of water for 24 h before the experiments. All the experiments were approved by the Italian Animal Care and Use Committee.

2.2. Alzet osmotic minipump insertion

Alzet osmotic minipumps (Cupertino, CA), model 2001, flow rate 1 μl/h (4 and 52 h treatment) or model 2ML2, flow rate 5 μl/h (7 and 14 day treatment) were used to deliver the peptides subcutaneously. N/OFQ was delivered at the rate of 0.1, 1 and 10 μg/kg/h. UFP-101 was delivered at the rate of 10 and 30 μg/kg/h. The doses of N/OFQ and of UFP-101 were based on our previous dose–response studies [16,17]. The peptides were dissolved in saline solution (0.9% NaCl) immediately before use and the concentration of the pump solution was prepared for each rat based on its body weight. Pumps were filled the day before the implantation and then primed in vials containing saline solution at 37 °C overnight.

2.3. Drugs

N/OFQ and UFP-101 were synthesized as salt derivatives at the Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, Italy. All other reagents were commercially available.

2.4. Experimental protocol

Rats were anaesthetized by intramuscular injection of tiletamine hydrochloride (200 mg/kg) and zolazepam hydrochloride (200 mg/kg), and implanted subcutaneously on the dorsal surface with osmotic minipumps. Rats recovered from anesthesia within 1 h after operation. In a first set of experiments, rats were infused with N/OFQ, 1 μg/kg/h, and sacrificed 4 h, 52 h, 7 days and 14 days after the minipump implantation ($n = 4$ rats for each time point). A control group of eight animals was implanted with a saline-filled minipump and sacrificed 4 h and 14 days after the minipump implantation ($n = 4$ rats for each time point). In a second set of experiments, rats were infused with N/OFQ, 1 μg/kg/h. After a 4 h infusion period, part of the rats ($n = 4$) was sacrificed and in the remaining animals ($n = 12$), the pumps were removed under anesthesia. After pump removal, the rats returned to their home cages and they were sacrificed 52 h, 7 days and 14 days after the minipump implantation ($n = 4$ rats for each time point). A control group of four animals was implanted with a saline-filled minipump, the pumps were removed 4 h later and the rats were sacrificed 14 days after the minipump implantation. In a third set of experiments, rats were infused with N/OFQ at different doses (0.1, 1 and 10 μg/kg/h), or saline and sacrificed 52 h after the minipump implantation ($n = 4$ rats for each time point). Experiments were performed between 9 AM and 2 PM. In a fourth set of experiments, rats were infused with either N/OFQ (1 μg/kg/h) or UFP-101 (10 μg/kg/h) or UFP-101 (30 μg/kg/h) or saline. Rats recovered from anesthesia 1 h after operation. They were then subjected to cold-restraint stress or returned to their home cages at room temperature until the sacrifice. For cold-restraint stress, rats were restrained into individual wire-mesh restraint cages and placed in a cold room at 3 °C for 3 h. The restraining period was performed between 10 AM and 2 PM. The rats were sacrificed at the end of the restraining period. Non-stressed rats were sacrificed at 2 PM. In the different groups the duration of the infusion was therefore 4 h.

2.5. Tissue preparation

On sacrifice, the abdomen was opened. The stomach was removed from the peritoneal cavity and opened along the lesser curvature. A strip (5 mm × 10 mm) of gastric tissue was excised from the fundus, 3–4 mm below and parallel to the limiting ridge, so that the greater curvature was approximately located in the middle of the strip. Tissue samples from stressed rats were taken from areas that were normal upon gross examination.

2.6. Histochemistry and immunohistochemistry

Three different tissue samples were taken from each strip of gastric fundus, fixed in 10% buffered formaldehyde and embedded in paraffin. Serial sections, 5 μm thick and perpendicular to the mucosal surface, were cut from each block. One section from each block was stained with hematoxylin and eosin and additional sections were cut for histochemistry and immunohistochemistry.

MMCs and CTMCs were identified histochemically by using a toluidine blue staining method [44]. Deparaffinized sections were incubated for 7 days in 0.5% toluidine blue in 0.5 N HCl (pH 5) at room temperature. Sections were then rinsed, dehydrated and mounted. MMCs were also identified immunohistochemically with a monoclonal antibody to rat mast cell protease (RMCP) II (MS-RM4, Moredun, Midlothian, UK). Adjacent sections were deparaffinized, rehydrated, immersed in citrate buffer and heated for 30 min at 98 °C. Sections were then incubated for 10 min in 3% H_2O_2/PBS to inhibit endogenous peroxidase activity. After washing they were incubated with normal goat serum and then with the antibody to RMCP II, diluted to 1:50 for 30 min at room temperature. The secondary antibodies were supplied with the LSAB2 staining kit used according to the manufacturer's instructions (Dako, Glostrup, Denmark). We used 3,3-diaminobenzidine (Sigma) as chromogen. Sections were counterstained with hematoxylin, dehydrated and mounted. Control experiments were performed by omission of the primary antibody from the immunostaining procedure. Well-oriented sections were examined with a light microscope (Nikon Optiphot) attached to a color camera head (DS-Fi1, Nikon).

Quantitations were performed using a color image analysis software system (NIS-Elements AR, Nikon Laboratory Imaging, Japan). Toluidine blue-positive MMCs and CTMCs as well as RMCP II-positive MMCs were counted in three different sections for each rat and at least five randomly selected visual fields were examined in each section (objective, 40×; visual field, 250 μm in length). The muscularis mucosae was established as the base. Positive MMCs were counted from the muscularis mucosae to the luminal surface for the entire thickness of the glandular mucosa. Mucosal thickness was measured from the luminal surface to the muscularis mucosa in each field. Positive CTMCs were counted from the muscularis mucosae for the entire thickness of the submucosa. For each rat the values from all the fields examined were averaged and expressed as mast cell number per millimeter length of the muscularis mucosae. These values used to calculate mean values for each experimental group.

2.7. Double immunohistochemistry of RMCP II and synaptophysin

Sections were immersed in Target Retrieval Solution (S2367, Dako) and heated for 40 min at 98 °C. All slides were then cooled

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