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Neuropeptide S inhibits stress-stimulated faecal output in the rat

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ABSTRACT

Neuropeptide S (NPS) is a recently identified bioactive peptide that activates an orphan G-protein coupled receptor, called the NPS receptor (NPSR). In rats, NPS and NPSR constitute a novel neuropeptide system expressed both in the central nervous system and in peripheral tissues, controlling visceromotor, neuroendocrine, nociceptive and behavioural responses. To improve the knowledge of the role of the NPS–NPSR system in the gastrointestinal (GI) tract, we investigated: 1- the supraspinal effect of NPS on motor functions of the upper (gastric emptying and gastrointestinal transit) and lower (distal colonic transit and faecal output) GI tract under basal conditions, 2- during pathological states (restraint stress and corticotropin releasing factor (CRF)-induced defecation) in the rat, and 3- the receptor type involved in treatment with NPS using NPS, tachykinin NK_3 and opioid receptor antagonists (([D-Cys(tBu)^5]NPS), SR142801 and naloxone, respectively).

Intracerebroventricular injection of NPS failed to modify basal gastric emptying, gastrointestinal transit and distal colon propulsion, but significantly and dose-dependently reduced faecal pellet excretion and weight stimulated by restraint stress and CRF. The inhibitory effect of NPS on stress-induced defecation was unmodified by pre-treatment with either the tachykinin or opioid receptor antagonists, but was counteracted by a NPSR antagonist.

The present study demonstrates, for the first time, that the supraspinal NPS system, which does not participate in the physiological control of GI motility, plays an inhibitory role on defecation stimulated by restraint stress and CRF. The combination of the ability of NPS to inhibit faecal output together with its known anxiolytic effect may be promising, especially in pathological conditions such as irritable bowel syndrome, where stress and the hyperactivity of the CRF system contribute to the co-morbidity of anxiety with colonic motor symptoms such as diarrhoea.

1. Introduction

Neuropeptide S (NPS) is a bioactive 20 amino acid peptide recently identified via the reverse pharmacology technique [1]. Across all species thus far examined, its primary sequence is highly conserved and its N-terminal residue is serine (S), hence the peptide is named neuropeptide S [2–4]. NPS selectively binds and activates an orphan G-protein coupled receptor, called the NPS receptor (NPSR), and induces an elevation of intracellular Ca^{2+} and cAMP, thereby acting as an excitatory neurotransmitter [4,5].

In rats, NPS and NPSR mRNA are expressed both in the central nervous system and in the peripheral tissues. The NPS precursor mRNA in the brain displays a very limited distribution with the exception of high expression in a few discrete brain areas such as a group of neurons located between the locus coeruleus and Barrington's nucleus and the lateral parabrachial nucleus of the brainstem. The highest NPSR mRNA levels are found in the cortex, thalamus, hypothalamus, amygdala, periaqueductal gray matter, and it is found in low levels in the brainstem, such as the ventral tegmental area and the substantia nigra [4,6–9]. In peripheral tissues, NPSR mRNA is expressed in gastrointestinal (GI) enteroendocrine cells and the enteric nervous system [4], suggesting a modulatory role of this system in GI motor and sensory functions. A functional polymorphism in the human NPSR gene is known and is associated with asthma [10,11], inflammatory bowel disease [12], panic disorder

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[13], celiac and allergic diseases [14]. In addition, NPS and NPSR expression is regulated by treatment with caffeine [15] and nicotine [16].

NPS and NPSR constitute a novel neuropeptide system that has been shown to play a role in controlling visceromotor, neuroendocrine, nociceptive and behavioural responses essential for survival, especially arousal, anxiety, learning and memory. In fact, NPS, administered centrally, increases locomotor activity [4,6], produces anxiolytic-like effects [17–19], potently promotes wakefulness and prevents oxidative stress damage [20,21]. NPS and NPSR are also involved in drug abuse [22,23], stress, control of fear expression [24,25] and the macrophage immune response [26]. Very recently, NPS has been reported to produce antinociception in mice through the activation of supraspinal NPSR [27] but not opioid receptors, suggesting that a central NPS–NPSR system could be a potential target for developing new analgesic drugs.

The role of the NPSR–NPS system in the GI tract is still poorly understood. Central injection of NPS was reported to regulate food intake in rats [28–33]. Recent studies have indicated that central NPS inhibits colonic motor functions in mice, while the peripheral administration of the peptide did not influence these activities [34]. NPS has also been associated with susceptibility to inflammatory bowel disease [12]. In addition, *in vitro* models have provided indirect evidence that signalling through NPSR can induce an increase in peptides and hormones also involved in the control of physiological motor and sensory functions in the GI tract [3,14]. Therefore, it is plausible that NPS–NPSR signalling may play a role in the complex gut–brain interactions modulating inflammatory responses, anxiety, nociception and gut functions [35].

To further clarify the poorly understood physio-pathological GI profile of NPS in the rat, in the present study, we sought to first investigate the effects of NPS at the supraspinal level on some motor functions of the upper (gastric emptying and GI transit) and lower (distal colonic transit and faecal output) GI tract under basal conditions, and on faecal pellet output during pathological states (restraint stress and CRF-induced defecation), and, second, to define the receptor type involved by using treatment with NPS [20], tachykinin NK_3 and opioid receptor antagonists [36–39].

2. Materials and methods

2.1. Animals

Adult male Wistar rats (180–200 g), were housed individually in plastic boxes under standard controlled environmental conditions with 12 h light/dark cycles and food and water *ad libitum*. For intracerebroventricular (i.c.v.) injections, rats were anaesthetised with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg), and implanted with a permanent Akulon cannula (Linca, Tel Aviv, Israel) stereotaxically inserted through a skull hole drilled over the left lateral ventricle (AP = −0.5 mm and $L = \pm 1.8$ mm relative to the bregma; $V = -1.0$ mm relative to the skull surface, calculated from the rat brain atlas of Paxinos and Watson [40]) and secured to the bone with dental cement. Accurate placement of the guide cannula was verified, at the end of the experimental period, by injecting the rats with 5 μl of methyl ethylene blue dye solution and checking post mortem for ventricular system perfusion. Data from injection sites not within the ventricle were discarded. Rats were euthanised by 70% CO_2 and each study was conducted according to the guidelines of the Italian Ministry of University and Research (D.L.116, 27/01/92) and the European Communities Council Directive (86/609/EEC). The experimental protocol was authorised by the Italian Ministry of Health. All possible efforts were made to minimise the number of animals used (about six to eight for each experimental group) and their discomfort.

2.2. Drugs and administration

Neuropeptide S (NPS) kindly made available by the Chemical Laboratories at the Department of Pharmaceutical Sciences and Biotechnology Centre, University of Ferrara, Italy, and corticotrophin-releasing factor (CRF) purchased from Sigma Chemicals (St. Louis, MO), were dissolved in distilled water. The agonists were i.c.v. injected at various doses and times as indicated in each assay, whereas the NPSR ([D-Cys(tBu)^5]NPS, 60 nmol/rat) and tachykinin NK_3 antagonist (SR142801, 16 nmol/rat) were centrally administered at the same time of NPS and the opioid antagonist (naloxone, 1 mg/kg i.p.) was peripherally injected 15 min before NPS.

2.3. Gastric emptying assay

In the gastric emptying assay [41], a test meal (1.5 ml per rat) consisting of a 50 mg phenol red solution in 100 ml aqueous methylcellulose (1.5%) was administered by gavage through an oro-gastric polyethylene catheter to 24 h fasted rats, but with free access to water. Rats were administered either saline (control) or test compounds in a constant volume (5 μl per rat) by the i.c.v. route immediately before the liquid and acaloric meal and euthanised 10 min after the test meal, as at this time point the maximal differences between the saline and peptide groups were observed [42–44]. The stomach was then exposed by laparotomy, quickly ligated at the pylorus and the cardia and then removed. The stomach and its contents were homogenised with 100 ml of NaOH 0.1 N. Phenol red was assayed according to a previously described procedure [42]. The suspension was allowed to settle for 1 h at room temperature and 5 ml of the supernatant were added to 0.5 ml of 20% trichloroacetic acid (w/v) and then centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 ml of 0.5 N NaOH and the absorbance of the sample was read at a wavelength of 560 nm with a spectrophotometer (LKB-Biochrom). Phenol red recovered from animals euthanised immediately after the administration of the test meal was used as the standard (0% emptying). Percent gastric emptying in the 10 min period was calculated according to the following formula: gastric emptying (%) (GE%) = (1 − A560 sample/A560 reference) × 100, where the A560 sample was the absorption at 560 nm of the gastric content at 10 min and A560 reference was the absorption at 560 nm of the gastric content at zero emptying time. Each value is expressed as percent changes with respect to percentage of gastric emptying in saline treated rats ($68.6 \pm 2.3 = 100\%$).

2.4. Upper gastrointestinal transit assay

Upper gastrointestinal transit was measured with the charcoal meal test [45]. Briefly, rats received 1 ml of a 20% (w/v) charcoal suspension in a 5% (w/v) gum Arabic solution via a stomach tube. Immediately afterwards, the rats were injected i.c.v. with NPS at doses of 1 and 4 nmol/rat and were killed (70% CO_2) 10 min [42] after receiving the charcoal meal; the small intestine was removed en bloc. Small bowel propulsion was determined by calculating the ratio between the distance travelled by the charcoal meal and the total length of the small bowel for each rat. The data are presented as the percent gastrointestinal transit measured as a quotient of the propulsion value in drug-treated rats and that in saline-treated rats ($42.5 \pm 3.2 = 100\%$).

2.5. Distal colonic propulsion assay

Distal colonic propulsion was measured according to the method of Raffa et al. [46]. In brief, immediately after rats were injected i.c.v. with NPS or saline, a single 5 mm diameter glass bead was inserted into the distal colon to a distance of about 2 cm from