Central neuropeptide-S administration alleviates stress-induced impairment of gastric motor functions through orexin-A

Mehmet Bülbül, Osman Sinen, Onur Bayramoğlu

Department of Physiology, Akdeniz University School of Medicine, Antalya, Turkey

Cite this article as: *Bülbül M, Sinen O, Abueid L, Bayramoğlu O. Central neuropeptide-s administration alleviates stress-induced impairment of gastric motor functions through orexin-A. Turk J Gastroenterol 2020; 31(1): 65-72.*

ABSTRACT

Background/Aims: *The novel brain peptide neuropeptide-S (NPS) is produced exclusively by a small group of cells adjacent to the noradrenergic locus coeruleus. The NPSR mRNA has been detected in several brain areas involved in stress response and autonomic outflow, such as amygdala and hypothalamus, suggesting that central NPS may play a regulatory role in stress-induced changes in gastrointestinal (GI) motor functions. In rodents, exogenous central NPS was shown to inhibit stress-stimulated fecal output. Moreover, exogenous NPS was demonstrated to activate hypothalamic neurons that produce orexin-A (OXA), which has been shown to stimulate postprandial gastric motor functions via central vagal pathways. Therefore, we tested whether OXA mediates the NPS-induced alterations in gastric motor functions under stressed conditions.*

Materials and Methods: *We investigated the effect of central exogenous NPS on solid gastric emptying (GE) and gastric postprandial motility in acute restraint stress (ARS)-loaded conscious rats. The OXA receptor antagonist SB-334867 was administered centrally prior to the central NPS injection. The expression of NPSR in the hypothalamus and dorsal vagal complex was analyzed by immunofluorescence.*

Results: *Central administration of NPS restored the ARS-induced delayed GE and uncoordinated postprandial antro-pyloric contractions. The alleviative effect of NPS on GE was abolished by pretreatment of the OX1R antagonist SB-334867. In addition to hypothalamus, NPSR was detected in the dorsal motor nucleus of vagus, which suggest a direct stimulatory action of exogenous NPS on gastric motility.*

Conclusion: *NPS may be a novel candidate for the treatment of stress-related gastric disorders.*

Keywords: *Neuropeptide-S, orexin-A, gastric emptying, acute restraint stress*

INTRODUCTION

Stress exposure has been shown to play a pivotal role in developing functional gastrointestinal disorders (FGID) such as functional dyspepsia (FD) (1,2). Individuals experience multiple stressors that contain mental, physical, social components, as well as their combinations. Long-term stress exposure results in impaired brain-gut axis signaling, which plays a pivotal role in generation of FGIDs (2). In previous rodent studies, it was shown that acute restraint stress (ARS) for 90 min delayed solid gastric emptying (GE) (3) and small intestinal motility (1), while accelerating the colonic transit rate (4).

The novel brain peptide neuropeptide-S (NPS) was identified as the endogenous ligand for the NPS receptor NPSR (formerly known as GPR154) (5). Through binding NPSR, NPS has been shown to increase cellular excitability by stimulating the formation of cAMP, while increasing the intracellular calcium concentration (6). In brain, the expression of the NPS/NPSR system has been demonstrated in certain regions, including the hypothalamus, amygdala, and locus coeruleus, which are closely involved in the regulation of fear, anxiety, and stress response (5, 7). In animal studies, intranasal and central administration of NPS was reported to inhibit anxiety and stress behaviors, while increasing locomotor activity (6,8).

Accumulating findings indicate the involvement of central NPS in stress-related changes in gastrointestinal (GI) motor functions (9); in fact, only a few studies investigated the effect of central NPS on large bowel motor functions (9,10). However, to the best of our knowledge, it has not been investigated whether NPS alleviates the stress-induced alterations in the upper part of the GI tract. In rats, central injection of NPS has been shown to reduce the stress-stimulated fecal output, while no significant change was observed under basal conditions (9).

Corresponding Author: ***Mehmet Bülbül; mehmetbulbul@akdeniz.edu.tr***
Received: ***August 9, 2018*** *Accepted:* ***May 16, 2019***

DOI: ***10.5152/tjg.2020.18626***

A recent study on rats demonstrated that central administration of NPS activated the orexin-A (OXA)-producing neurons in the lateral hypothalamic area (LHA) (11). The orexin receptor type-1 (OX1R) was detected in dorsal motor nucleus of N. vagus (DMV) (12); in addition, central OXA has been demonstrated to regulate gastric motor functions through vagal pathways (12,13). Therefore, it appears possible that endogenous hypothalamic OXA may mediate the NPS-induced alterations in GI motor functions.

The present study was designed to investigate whether (i) central NPS alters gastric motor functions under stressed conditions and whether (ii) NPS-induced changes are mediated by OXA though OX1R.

MATERIALS AND METHODS

Animals

Adult male Wistar rats were kept separated at room temperature with a 12 h light/12 h dark cycle. The rats were fed with standard laboratory chow and tap water. All experimental interventions were conducted with the approval of the Institutional Animal Care and Use Committee at Akdeniz University School of Medicine (with unique authorization number B.30.2.AKD.0.05.07.00/88). To minimize the effect of stress, the rats were acclimatized to handling for 7 days prior to the experimental procedures schematically summarized in Figure 1.

Intracerebroventricular cannulation

Under isoflurane (Baxter, Deerfield, IL, USA) anesthesia, the rats were placed in a stereotaxic apparatus, and a 26G injection cannula was implanted into the right lateral ventricle using the coordinates -0.8 mm to bregma, +1.4 mm lateral to midline, and -4 mm below the skull surface. The coordinates were obtained from the rat brain atlas of Paxinos (14). The cannula was then fixed with a pair of anchor screw and dental cement, and rats were allowed to recover for 7 days. At the end of the experimental protocol, proper cannula placement was also verified by injecting methylene blue (10 µL, intracerebroventricular [icv]) through each icv cannula. The brain was quickly removed, and dye in ventricles was then examined.

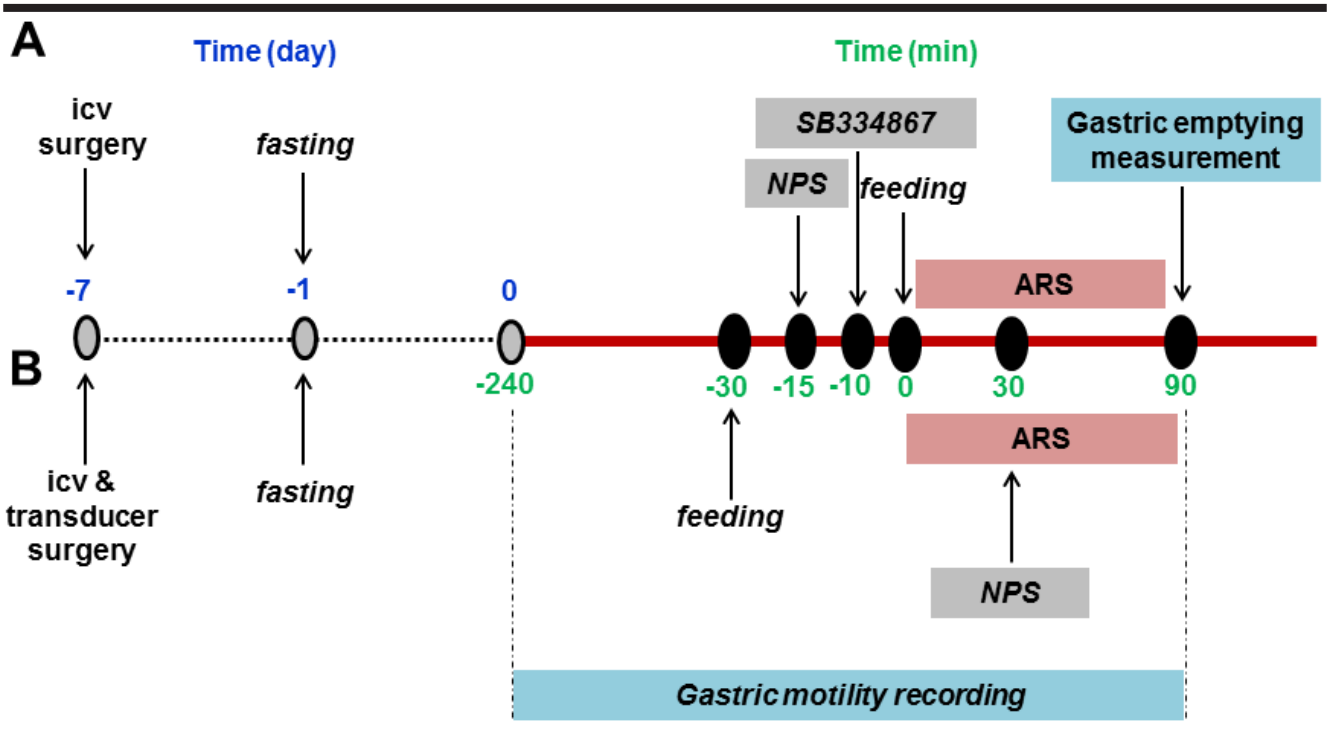

Figure 1. Representative flow chart of the experimental design. Upper and lower parts of the figure represent experimental protocols of GE (A) and gastric motility (B), respectively. The experiments were performed following a 7-day recovery period. Central NPS administration (10 nmol, icv) was performed 15 min before feeding in GE measurements, whereas, it was applied 30 min after the initiation of ARS in motility recording.

Acute restraint stress loading

The rats were fixed on a wooden plate with their trunks wrapped in a confining harness, while they were able to move their limbs and head but not their trunks, as described elsewhere (15,16).

Recording of the gastric motility

In a separate group of rats, through a midline laparotomy, the stomach was exposed, and two miniature strain gage transducers (Kyowa Electronic Instruments, Tokyo, Japan) were implanted onto the serosal surface of the antrum and pylorus. The wires of the transducers were exteriorized from the back and covered by a jacket. Then, the rats were allowed to recover for 7 days. Following an overnight fasting, the spontaneous antral and pyloric contractions were monitored in conscious, freely moving rats. After recording the basal fasting motor pattern through a bridge amplifier and data acquisition system (PowerLab 8/35, ADI instruments, Colorado Springs, CO, USA) for 60-90 min, the rats were given pre-weighed pellets (1.6 g). ARS was initiated after the coordinated postprandial antro-pyloric contractions were observed. The central administration of NPS was performed through an icv cannula, while the antro-pyloric contractions were being recorded under stress. The antral and pyloric motility indices (MI) were calculated as the area under curve (AUC) in the 30 min period before and after the central NPS injection. The changes in MI were expressed as the percentage of pre-injection period.

Drugs

All drugs were prepared freshly in artificial cerebrospinal fluid (aCSF) on the experimental days, except SB-334867, which was dissolved in dimethyl sulfoxide (DMSO). NPS was administered (10 nmol, icv) 15 min prior to the ARS loading. The pretreatment of selective OX1R antagonist SB-334867 (40 µg, icv) was performed 10 min before the NPS injection. The present doses of NPS (17) and SB-334867 (18) were used in our previous rat studies. All icv injections were performed in restrained rats by gently wrapping them in a soft cloth. The drugs were applied in a volume of 5 µl, using a glass gastight Hamilton syringe over the course of 60 sec. Following the injection, the needle was kept in place for at least 30 sec.

Measurement of solid GE

As previously reported (16,19), prior to experiments, rats underwent an overnight fasting. On the day of experiment, animals were transferred to the plexiglass cages, and pre-weighed pellets (1.6 g) were introduced. Immediately after the completion of feeding, the rats were loaded ARS for 90 min. The rats were euthanized under isoflurane anesthesia, and their stomachs were removed. The gastric content was collected and dried at room temperature. After weighing, GE was calculated according to the following formula:

%GE = 1-(weight of dried content / weight of pellet)x100

Immunohistochemistry

The brain sections were obtained from a separate group of rats (n=3) following a cardiac perfusion with 4% paraformaldehyde (PFA). After, the brain tissues were post-fixed in 4% PFA +20% sucrose for at least 24h. Then, 50-µm-thick brain sections were cut using a freezing microtome. The slices were labeled using rabbit anti-NPSR (1:200 dilution; ABN12, *Merck Millipore*, Darmstadt, Germany). The antibody was diluted in 0.1 M phosphate buffered saline (PBS) (pH 7.4) containing 10% normal horse serum, 0.3% Triton X-100, and 0.01 M TRIS. The sections then were incubated with primary antibody for 24 h at room temperature and washed in PBS containing 0.3% Triton X and 0.01 M TRIS and incubated with secondary antibody (donkey anti-rabbit conjugated with Alexa Fluor 568 for NPSR; 1:400; Invitrogen, Carlsbad, CA, USA) at room temperature for 24 h. After, the slices were mounted on glass slides, and the images of paraventricular nucleus (PVN), LHA, and DMV were captured using a fluorescent microscope equipped with appropriate filters.

Statistical analysis

Statistical analyses were performed using the Graphpad Prism software v.5. Data were expressed as mean±SEM. Statistical analysis was performed according to one-way or two way ANOVA followed by Tukey's post-hoc or Bonferroni's post-hoc analysis, as appropriate. A p-value<0.05 was considered as statistically significant.

RESULTS

Gastric postprandial motility

To investigate the mechanism underlying the exogenous NPS-induced restoration of ARS-induced changes in GE, the GI-fed motor pattern was monitored in conscious and freely moving rats. Following an overnight fasting, the basal motor pattern was recorded, and the rats were then given 1.6 g of pellet. Approximately 30 min after the completion of feeding, the coordinated antro-pyloric contractions were observed (Figure 2B). Following ARS, the coordination of the antro-pyloric

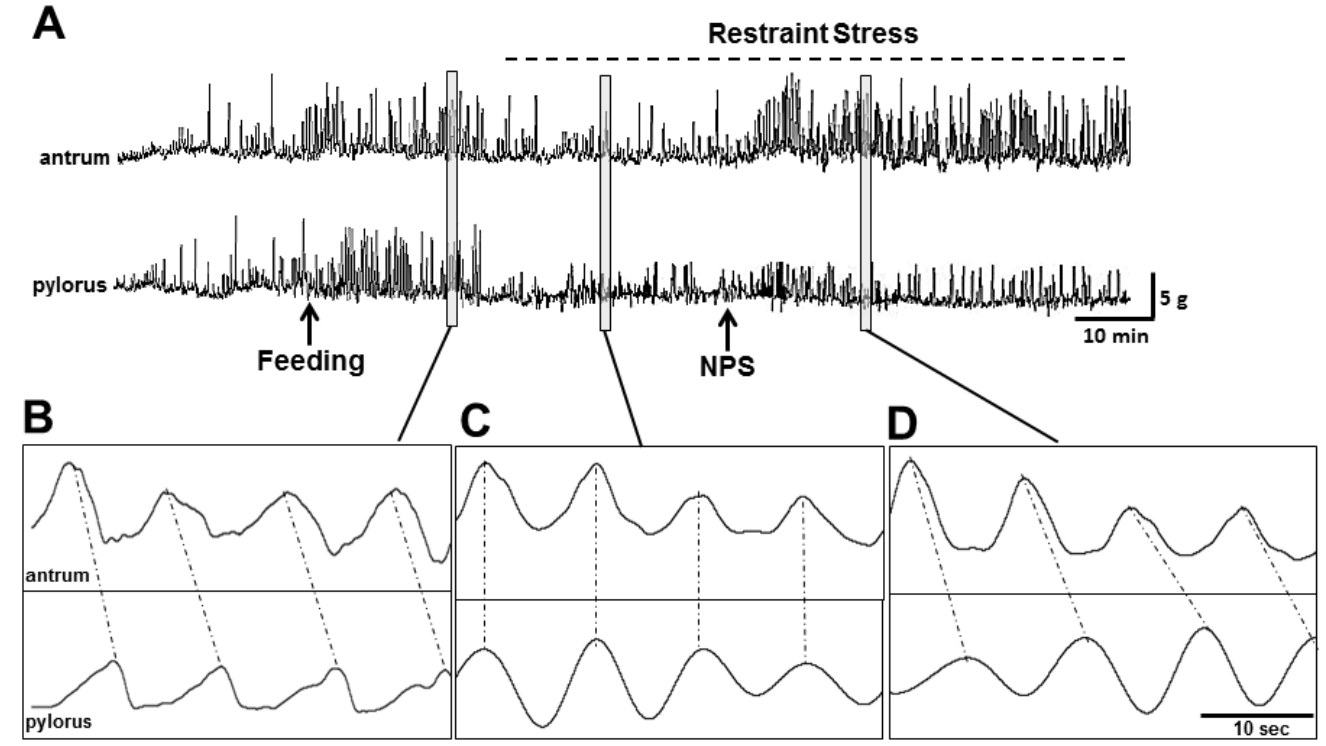

Figure 2. Effect of central NPS administration on the GI-fed motor pattern in conscious freely moving rats. Coordinated spontaneous antro-pyloric contractions were observed following feeding (A). The coordination of the antro-pyloric contractions was disturbed by ARS, and in addition, the amplitudes were reduced especially in antrum (B). Vehicle (n=6) did not cause any significant effect (not shown in trace), whereas the central administration of NPS (10 nmol, icv, n=6) restored the GI-fed motor pattern remarkably (C). For simplicity and better description, the recording trace acquired from vehicle-injected rats was not included in A; the amplitudes were not scaled in B-D.

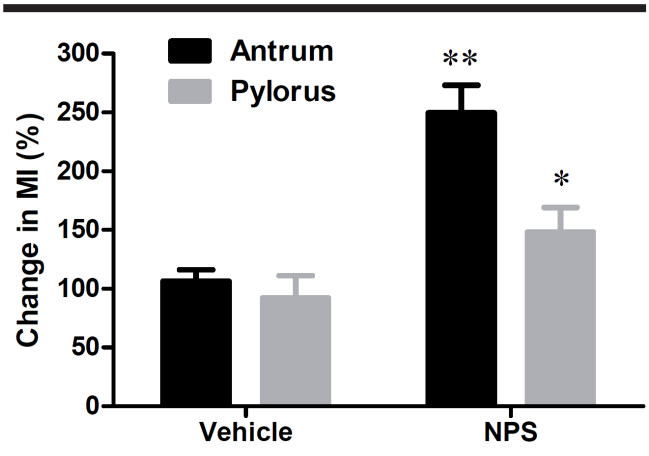

Figure 3. The effect of central NPS administration on the antral and pyloric MI. The MI was calculated as AUC in the 30-min period before and after the central NPS injection. The changes in MI were expressed as the percentage of the pre-injection period. Statistical analysis was performed according to two-way ANOVA followed by Bonferroni's post-hoc analysis. All values are means±SEM. **p<0.01; *p<0.05 vs vehicle.

contractions was disturbed (Figure 2C, n=6), while their amplitudes were decreased remarkably, which was observed more prominently in antrum (Figure 2A). Vehicle injection did not have any effect on ARS-induced alterations of antro-pyloric contractions. However, the central NPS administration restored the fed motor pattern completely (n=6) by recovering the coordination of the antro-pyloric contractions (Figure 2D), while increasing their amplitudes (Figure 2A). Compared with vehicle (106.3%±13.7, n=5) and in antrum (92.1%±26.8, n=5 in corpus), central NPS administration significantly increased the MI both in antrum (249.5%±33.2, p<0.01, n=6) and pylorus (146.8%±29.6, p<0.05, n=6); however, the NPS-induced changes were more remarkable in antrum (Figure 3).

Gastric emptying

Compared with NS rats (61.2±3.4%, n=6), ARS loading for 90 min caused a significant delay (24.4±3.2%, p<0.01, n=7) in solid GE. The ARS-induced delayed GE was re-

stored completely (62.5±5.7%, p<0.01, n=7) by central administration of NPS, which was abolished significantly by pretreatment with selective OX1R antagonist SB-334867 (38.9±2.7%, p<0.05, n=7), (Figure 4). The icv administration of DMSO itself did not exert any noticeable change in solid GE (*data not shown*).

Immunohistochemistry

The NPSR immunoreactivity was detected in PVN, LHA, and DMV, where the corticotrophin releasing factor (CRF)-producing, orexinergic, and excitatory vagal preganglionic cells reside, respectively (Figure 5).

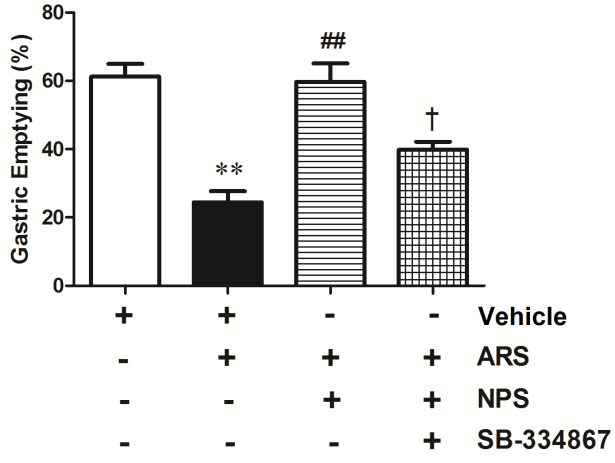

Figure 4. Effect of central NPS administration on ARS-induced delayed solid GE in rats. Vehicle (5 µL of aCSF; n=6) or NPS (10 nmol in 5 µL; n=7) was administered 15 min prior to the ARS loading, whereas SB-334867 (40 µg in 5 µL; n=7) was pretreated 15 min before the central NPS injection. Statistical analysis was performed according to one-way ANOVA followed by a Tukey post-hoc test. Data are means±SE. **p<0.01 vs aCSF; ##p<0.01 vs ARS; †p<0.05 vs ARS+NPS.

DISCUSSION

Our present findings demonstrated that central NPS administration restored the ARS-induced delayed solid GE, which appears to be mediated through the OXA/OX1R system. Moreover, central exogenous NPS recovered the impaired GI-fed motor pattern, which is characterized by uncoordinated antro-pyloric contractions. Our immunohistochemical analyses revealed that NPSR is present in DMV, in addition to the hypothalamic regions such as PVN and LHA. These findings suggest that central exogenous NPS may exert its alleviative actions through the mediation of OXA or acting directly on the stomach-projecting preganglionic cells in DMV.

In rats, ARS stimulates the release of CRF in the amygdala and hypothalamic PVN, which in turn plays a pivotal role in impairment of motor functions in the upper part of GI tract via central CRF2 receptors and peripheral α-2 adrenergic receptor-mediated sympathetic pathways by impairing the coordination of postprandial antro-pyloric contractions (3,20). Along with a group of cells in rostral ventrolateral medulla, the preautonomic neurons in PVN project directly to the sympathetic preganglionic neurons in the spinal cord to maintain the sympathetic innervation of the viscera (21). In our GE and motility measurements, we found that ARS delayed the solid GE by impairing the antra-pyloric coordination, which was alleviated by single administration of central exogenous NPS. In contrast to the study by Nakade and colleagues in which ARS was found to enhance the postprandial gastric motility via a vagal cholinergic pathway (20), we observed the inhibitory effect of ARS both on antral and pyloric postprandial contractions. It seems to be quite possible that the amount of food and the duration of the pre-stress period may play a role. Nakade and colleagues fed the rats with 3 g of chow and recorded gastric motor activities for 90

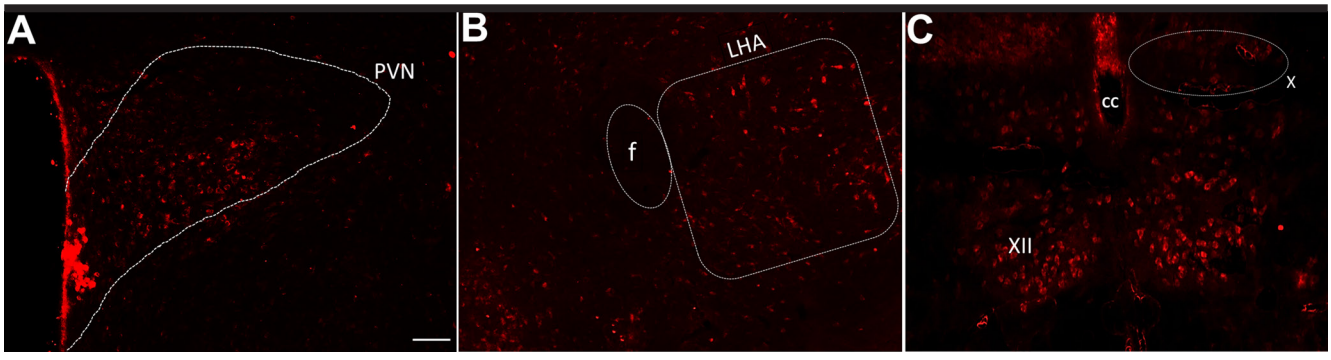

Figure 5. NPS immunoreactivity in coronal hypothalamic (A: PVN, B: LHA) and brainstem (C: DMV) sections (n=3). PVN, paraventricular nucleus; LHA, lateral hypothalamic area; f, fornix; DMV, dorsal motor nucleus N. vagus; XII, hypoglossal nucleus; CC, canalis centralis. The scale bar represents 100 µm.

min before the restraint stress loading. However, in our study, the rats were introduced a 1.6 g of pellet, moreover, the ARS loading was initiated immediately after the acquisition of coordinated postprandial antro-pyloric contractions. In the postprandial state, the gastric vagal afferents detect the variety of mechanical stimuli including distension and stretch, which ignite the vagally mediated signals to the brain stem. Therefore, the contrary data of these studies could be potentially related to the different patterns of meal-related signals that trigger the vagovagal pathways.

It has been demonstrated previously that central administration of NPS increased the Fos-like immunoreactivity in the OXA-positive cells in LHA (11). Moreover, in rats, intra-LHA application of NPS-induced ethanol-seeking behavior, which was blocked by the pretreatment of the OX1R antagonist SB-334867 (22). These morphological and functional findings suggest the mediation of that central orexingergic system in NPS-induced changes in GI motor functions. In parallel, Kobashi and colleagues showed that intra-DMV injection of OXA increased the distal stomach motility, while relaxing the proximal part via vagal pathway (23). In addition, central exogenous OXA-induced stimulation of gastric acid secretion and motility was demonstrated in rats (24). Indeed, the expression of OX1R has been demonstrated in stomach-projecting DMV neurons (12). Our immunofluorescence experiments indicated that the NPSR receptor is expressed in the DMV neurons. Thus, although not confirmed in the present study, it is highly convincing that the reparative effect of NPS on stress-induced impairment of gastric contractions might be mediated by direct stimulation of stomach-projecting vagal cholinergic excitatory neurons.

Although the effect of central exogenous NPS on stress-induced changes in GI motor functions has not been understood completely, there are findings suggesting that NPS-induced alleviative actions could be mediated by decreased the tonus of central CRF. Petrella and colleagues demonstrated that the central administration of NPS significantly and depending on the dose reduced the fecal pellet excretion and weight stimulated by restraint stress and central exogenous CRF (9). The amigdaloid synaptic circuits process fear and expression of fear memories. NPS induces the release of glutamate in the synaptic contacts to a subset of GABAergic interneurons, which are axonally connected to central part of the amygdala (CeA) (25). The dense expression of CRF has been demonstrated in the CeA neurons, which represents the main output station of the amygdala to the brain stem and hypothalamus (26).

On the other hand, in a recent rat study, central exogenous NPS has been shown to induce somatodendritic release of oxytocin (OXT) in PVN, which harbors the majority of CRF-producing neurons in the brain. Activation of the OXT receptor in PVN causes a delay in the transcription of the CRF gene (27). It has been previously shown in rats that central OXT restored the stress-induced delayed solid GE (28) and the ARS-induced disturbed gastric postprandial motility (29). Therefore, either via direct or OXT-induced indirect actions, it appears to the be convincing that exogenous NPS may inhibit the CRF signaling on the brain centers that regulate visceromotor functions, such as PVN and dorsal vagal complex.

Anatomically, the NPS-producing cells are restricted located directly adjacent to the noradrenergic and CRF-producing cells in LC and Barrington's nucleus, respectively (30). In addition, NPS is also expressed to a lesser degree by the cells in lateral parabrachial nucleus and principle trigeminal nucleus (7). Although the expression of NPS is restricted to the brainstem, the NPSR is distributed widely in numerous brain regions, including the hypothalamus and amygdala, the key modulator centers of the central stress circuitry in the brain (7,31), suggesting the putative regulatory role of central NPS in stress response. Indeed, the NPS/NPSR system has been receiving an increasing attention as a potential therapeutic option for the treatment of anxiety- and stress-related pathologies. In rodents, central administration of exogenous NPS has been demonstrated to attenuate the anxiety and stress-related behaviors (5,32). Interestingly, similar anxiolytic actions have been demonstrated in rats receiving nasal application exogenous NPS (8,33).

Treatment of central nervous system (CNS) diseases is considered a challenge as the blood-brain barrier (BBB) restricts the entry of small, non-polar compounds. Intranasal administration of drugs provides a direct access to the therapeutic substances into the CNS; therefore, it appears to be a useful alternative strategy to treat a variety of diseases/disorders of the CNS, including stroke, Parkinson's disease, multiple sclerosis, Alzheimer's disease, epilepsy, and psychiatric disorders (34). Indeed, a functional NPSR gene polymorphism has been found to be involved in the regulation of the neuroendocrine stress response, while the carriers showed higher salivary cortisol levels and larger stress responses in humans (35). In line with the latter findings, Domschke and colleagues

demonstrated a female-dominant role of the NPSR gene variation in individuals having panic disorder with heightened autonomic arousal and distorted processing of anxiety-relevant emotional stimuli (36). Therefore, intranasal application of NPS appears to be a therapeutic approach for treatment of patients with psychiatric illnesses such as anxiety or panic disorders (8).

Taken together, central administration of NPS restored the stress-induced gastric dysmotility, which was abolished by an OXA antagonist. Although exogenous NPS-induced restoration appears to be partly OXA dependent, further studies are warranted to elucidate the putative roles of other peptidergic systems, such as OXT and/or CRF. Central NPSR might be a therapeutic target for the treatment of stress-related functional disorders such as FD.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the Institutional Animal Care and Use Committee at Akdeniz University School of Medicine (with unique authorization number B.30.2.AKD.0.05.07.00/88)

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.B.; Design - M.B.; Supervision - M.B.; Resources - M.B.; Data Collection and/or Processing - M.B., O.S., O.B.; Analysis and/or Interpretation - M.B., O.S., O.B.; Literature Search - M.B.; Writing Manuscript - M.B., O.S., O.B.; Critical Review - M.B.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The present study was supported by The Research Foundation of Akdeniz University (project number: TAY-2018-3186).

REFERENCES

1. Lenz HJ, Raedler A, Greten H, Vale WW, Rivier JE. Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor. Gastroenterology 1988; 95: 1510-7. [CrossRef]
2. Tache Y, Bonaz B. Corticotropin-releasing factor receptors and stress-related alterations of gut motor function. J Clin Invest 2007; 117: 33-40. [CrossRef]
3. Nakade Y, Tsuchida D, Fukuda H, Iwa M, Pappas TN, Takahashi T. Restraint stress delays solid gastric emptying via a central CRF and peripheral sympathetic neuron in rats. Am J Physiol Regul Integr Comp Physiol 2005; 288: R427-32. [CrossRef]
4. Nakade Y, Fukuda H, Iwa M, et al. Restraint stress stimulates colonic motility via central corticotropin-releasing factor and peripheral 5-HT3 receptors in conscious rats. Am J Physiol Regul Integr Comp Physiol 2007; 292: G1037-44. [CrossRef]
5. Xu YL, Reinscheid RK, Huitron-Resendiz S, et al. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004; 43: 487-97. [CrossRef]
6. Reinscheid RK, Xu YL. Neuropeptide S as a novel arousal promoting peptide transmitter. FEBS J 2005; 272: 5689-93. [CrossRef]
7. Xu YL, Gall CM, Jackson VR, Civelli O, Reinscheid RK. Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. J Comp Neurol 2007; 500: 84-102. [CrossRef]
8. Lukas M, Neumann ID. Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: social versus non-social effects. Neuropharmacology 2012; 62: 398-405. [CrossRef]
9. Petrella C, Agostini S, Guerrini R, et al. Neuropeptide S inhibits stress-stimulated faecal output in the rat. Pharmacol Res 2011; 64: 471-7. [CrossRef]
10. Wan Saudi WS, Halim MA, Rudholm-Feldreich T, et al. Neuropeptide S inhibits gastrointestinal motility and increases mucosal permeability through nitric oxide. Am J Physiol Gastrointest Liver Physiol 2015; 309: G625-34. [CrossRef]
11. Niimi M. Centrally administered neuropeptide S activates orexin-containing neurons in the hypothalamus and stimulates feeding in rats. Endocrine 2006; 30: 75-9. [CrossRef]
12. Krowicki ZK, Burmeister MA, Berthoud HR, Scullion RT, Fuchs K, Hornby PJ. Orexins in rat dorsal motor nucleus of the vagus potently stimulate gastric motor function. Am J Physiol Gastrointest Liver Physiol 2002; 283: G465-72. [CrossRef]
13. Bulbul M, Babygirija R, Ludwig K, Takahashi T. Central orexin-A increases gastric motility in rats. Peptides 2010; 31: 2118-22. [CrossRef]
14. Paxinos CWaG. The Rat Brain in Stereotaxic Coordinates, 3rd edition. Academic Press, San Francisco 1997.
15. Bulbul M, Babygirija R, Cerjak D, Yoshimoto S, Ludwig K, Takahashi T. Impaired adaptation of gastrointestinal motility following chronic stress in maternally separated rats. Am J Physiol Gastrointest Liver Physiol 2012; 302: G702-11. [CrossRef]
16. Bulbul M, Izgut-Uysal VN, Sinen O, Birsen I, Tanriover G. Central apelin mediates stress-induced gastrointestinal motor dysfunction in rats. Am J Physiol Gastrointest Liver Physiol 20122016; 310: G249-61. [CrossRef]
17. Bulbul M, Sinen O, Izgut-Uysal VN. Central Exogenous Neuropeptide-S Ameliorates Stress-induced Impaired Gastric Emptying through Orexin-mediated Pathway in Rats. Faseb J 2016; 30.
18. Sinen O, Bulbul M, Izgut-Uysal VN. The Role of Endogenous Central Orexin-A in Gastric Emptying in Rats. Acta Physiol 2015; 215: 25.
19. Babygirija R, Yoshimoto S, Gribovskaja-Rupp I, Bulbul M, Ludwig K, Takahashi T. Social interaction attenuates stress responses following chronic stress in maternally separated rats. Brain Res 2012; 1469: 54-62. [CrossRef]
20. Nakade Y, Tsuchida D, Fukuda H, Iwa M, Pappas TN, Takahashi T. Restraint stress augments postprandial gastric contractions but impairs antropyloric coordination in conscious rats. Am J Physiol Gastrointest Liver Physiol 2006; 290: R616-24. [CrossRef]
21. Browning KN, Babic T, Toti L, Holmes GM, Coleman FH, Travagli RA. Plasticity in the brainstem vagal circuits controlling gastric motor function triggered by corticotropin releasing factor. J Physiol 2014; 592: 4591-605. [CrossRef]
22. Kallupi M, Cannella N, Economidou D, Ubaldi M, Ruggeri B, Weiss F, et al. Neuropeptide S facilitates cue-induced relapse to cocaine seeking through activation of the hypothalamic hypocretin system. Proc Natl Acad Sci U S A 2010; 107: 19567-72. [CrossRef]

23. Kobashi M, Mizutani S, Fujita M, Mitoh Y, Shimatani Y, Matsuo R. Central orexin inhibits reflex swallowing elicited by the superior laryngeal nerve via caudal brainstem in the rat. Physiol Behav 2014; 130: 6-12. [CrossRef]
24. Grabauskas G, Moises HC. Gastrointestinal-projecting neurones in the dorsal motor nucleus of the vagus exhibit direct and viscerotopically organized sensitivity to orexin. J Physiol 2003; 549: 37-56. [CrossRef]
25. Pape HC, Jungling K, Seidenbecher T, Lesting J, Reinscheid RK. Neuropeptide S: a transmitter system in the brain regulating fear and anxiety. Neuropharmacology 2010; 58: 29-34. [CrossRef]
26. Sanford CA, Soden ME, Baird MA, Miller SM, Schulkin J, Palmiter RD, et al. A Central Amygdala CRF Circuit Facilitates Learning about Weak Threats. Neuron 2017; 93: 164-78. [CrossRef]
27. Jurek B, Slattery DA, Hiraoka Y, Liu Y, Nishimori K, Aguilera G, et al. Oxytocin Regulates Stress-Induced Crf Gene Transcription through CREB-Regulated Transcription Coactivator 3. J Neurosci 2015; 35: 12248-60. [CrossRef]
28. Bulbul M, Babygirija R, Cerjak D, Yoshimoto S, Ludwig K, Takahashi T. Hypothalamic oxytocin attenuates CRF expression via GABA(A) receptors in rats. Brain Res 2011; 1387: 39-45. [CrossRef]
29. Bulbul M, Babygirija R, Ludwig K, Takahashi T. Central oxytocin attenuates augmented gastric postprandial motility induced by restraint stress in rats. Neurosci Letters 2010; 479: 302-6. [CrossRef]
30. Reinscheid RK, Xu YL. Neuropeptide S and its receptor: a newly deorphanized G protein-coupled receptor system. Neuroscientist 2005; 11: 532-8. [CrossRef]
31. Leonard SK, Ring RH. Immunohistochemical localization of the neuropeptide S receptor in the rat central nervous system. Neuroscience 2011; 172: 153-63. [CrossRef]
32. Grund T, Goyon S, Li Y, et al. Neuropeptide S Activates Paraventricular Oxytocin Neurons to Induce Anxiolysis. J Neruosci 2017; 37: 12214-25. [CrossRef]
33. Medina G, Ji G, Gregoire S, Neugebauer V. Nasal application of neuropeptide S inhibits arthritis pain-related behaviors through an action in the amygdala. Mol Pain 2014; 10: 32. [CrossRef]
34. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Adv Drug Deliv Rev 2012; 64: 614-28. [CrossRef]
35. Kumsta R, Chen FS, Pape HC, Heinrichs M. Neuropeptide S receptor gene is associated with cortisol responses to social stress in humans. Biol Psychol 2013; 93: 304-7. [CrossRef]
36. Domschke K, Reif A, Weber H, et al. Neuropeptide S receptor gene -- converging evidence for a role in panic disorder. Mol Psychiatry 2011; 16: 938-48. [CrossRef]