Neuropeptides 73 (2019) 71–77

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Acute restraint stress induces cholecystokinin release via enteric apelin

Mehmet Bülbül[a,*], Osman Sinen[a], Onur Bayramoğlu[a], Gökhan Akkoyunlu[b]

[a] Department of Physiology, Akdeniz University, Faculty of Medicine, Antalya, Turkey
[b] Department of Histology and Embryology, Akdeniz University, Faculty of Medicine, Antalya, Turkey

ARTICLE INFO

Keywords:
Apelin
Restraint stress
Cholecystokinin
APJ receptor
Fasting

ABSTRACT

Stress increases the apelin content in gut, while exogenous peripheral apelin has been shown to induce cholecystokinin (CCK) release. The present study was designed to elucidate (i) the effect of acute stress on enteric production of apelin and CCK, (ii) the role of APJ receptors in apelin-induced CCK release depending on the nutritional status. CCK levels were assayed in portal vein blood samples obtained from stressed (ARS) and non-stressed (NS) rats previously injected with APJ receptor antagonist F13A or vehicle. Duodenal expressions of apelin, CCK and APJ receptor were detected by immunohistochemistry. ARS increased the CCK release which was abolished by selective APJ receptor antagonist F13A. The stimulatory effect of ARS on CCK production was only observed in rats fed ad-libitum. Apelin and CCK expressions were upregulated by ARS. In addition to the duodenal I cells, APJ receptor was also detected in CCK-producing myenteric neurons. Enteric apelin appears to regulate the stress-induced changes in GI functions through CCK. Therefore, apelin/APJ receptor systems seem to be a therapeutic target for the treatment of stress-related gastrointestinal disorders.

1. Introduction

Functional gastrointestinal disorders (FGID) are defined as a group of functional abnormalities characterized by persistent pattern with no structural or biochemical abnormalities that account for the symptoms (Oshima and Miwa, 2013, 2014; Talley, 2008). In modern societies, most individuals experience mental, physical and social stressors on a daily basis. Exposure to stress is known to play a pivotal role in etiopathogenesis of FGIDs such as functional dyspepsia (FD). Chronic exposure to a stressor results in permanent changes in neurobiology of the brain-gut axis which yields autonomic dysfunction (Bhatia and Tandon, 2005; Lenz et al., 1988; Tache and Bonaz, 2007). Comprising of both physical and psychogenic components, acute restraint stress (ARS) is a commonly used model in rodents (Lenz et al., 1988) which was shown previously in rodents to induce GI motor dysfunction including delayed solid gastric emptying (GE) (Martinez et al., 2004; Nakade et al., 2005), disturbed migrating motor complex (Zheng et al., 2009) and accelerated colonic transit rate (Bulbul et al., 2012; Bulbul et al., 2016).

Apelin, the endogenous ligand for the G-protein-coupled APJ receptor, was initially isolated from bovine stomach in 1998 (Tatemoto et al., 1998). Apelin gene encodes the pre-proapelin molecule which contains 77 amino acids with a signal peptide in the N-terminal region. Among the numerous biological active products of preproapelin, apelin-13 has been shown to be the most abundant form with a greater affinity for APJ in rodents (De Mota et al., 2000; Medhurst et al., 2003). Within the GI tract, wide distribution of apelin/APJ receptor system suggests that the released apelin from gut may exhibit a regulatory role in GI functions (Han et al., 2008; Han et al., 2007; Susaki et al., 2005; Wang et al., 2004; Wang et al., 2009).

Recently, acute stress-induced increase in gastric apelin content was demonstrated in rats (Izgut-Uysal et al., 2014), furthermore, peripheral administration of apelin has been shown to induce the release of cholecystokinin (CCK), the well-defined alimentary peptide hormone which is released in the postprandial stage in response to the meal ingestion (Bulbul et al., 2017a; Flemstrom et al., 2011; Wattez et al., 2013). Therefore, using restraint stress model in male rats, the aim of the present study was to investigate whether (i) ARS alters the production of enteric apelin and CCK, (ii) increased endogenous apelin induces CCK release and through APJ receptor, (iii) the endogenous apelin-induced actions are altered upon the nutritional status. Finally, using double immunofluorescence labeling, we also investigated (iv) the putative sources of enteric CCK that responding to endogenous apelin through APJ receptor.

* Corresponding author at: Akdeniz University, Faculty of Medicine, Department of Physiology, 07070 Antalya, Turkey.
E-mail address: mehmetbulbul@akdeniz.edu.tr (M. Bülbül).

https://doi.org/10.1016/j.npep.2018.11.007
Received 30 July 2018; Received in revised form 2 October 2018; Accepted 26 November 2018
Available online 26 November 2018
0143-4179/

2. Materials and methods

2.1. Animals

Wistar rats (280–300 g) were housed at a room temperature of 22–24 °C with a 12-h light/12-h dark cycle (light on 6:30 a.m. to 6:30 p.m.). Rats were given ad libitum access to food and water. All experimental protocols used in this study were approved by the guidelines of the Animal Ethical Committee of Akdeniz University. The body weight of each rat was monitored for weight gain throughout the experimental procedures. Also, to minimize the stress, animals were acclimatized to handling for 7 days prior to the experimental procedures.

2.2. Acute restraint stress loading

The rats were placed on a wooden plate with their trunks wrapped in a confining harness for 90 min, as previously reported (Bulbul et al., 2016; Bulbul et al., 2017b). During the ARS, rats were able to move their limbs and head but not their trunks.

2.3. Experimental procedures

2.3.1. Surgery

For collection of the blood samples, the rats underwent chronic portal vein cannulation, as described previously (Strubbe et al., 1999). Briefly, under isoflurane anesthesia (5% for induction; 2.5% for maintenance in pure O_2 at a flow rate of 200–400 ml/min), the abdomen was opened through a middle incision. The superior mesenteric vein was then separated from the surrounding tissue and clamped. A 22G sterile polyurethane catheter (C30PU-RPV1435, Instech, Plymouth Meeting, PA, USA) was inserted into the mesenteric vein and advanced to the portal vein. Immediately after the insertion, the clamp was removed and the catheter was fixed onto vessel with using 5.0 silk. The catheter was exteriorized through abdominal wall and ran towards the back subcutaneously, then placed outside the neck skin. After filling with heparinized saline (500 IU/ml), the cathether was connected to a plastic port (PNP3F22, Instech) which enables chronic aseptic access to the catheter for multiple sampling. Before the experiments, all animals were allowed to recover for 7 days.

2.3.2. Blood sampling

For measurement of the CCK concentrations in plasma, the portal vein blood samples were collected before, throughout (with 30 min intervals) and 60 min after the ARS loading from the rats previously injected with vehicle or APJ receptor antagonist F13A 30 min before the initiation of stress loading. F13A (300 µg/kg, i.p.) was dissolved in sterile saline and administered by hand to the lightly restrained rats gently wrapped in a soft cloth. The dose of F13A was obtained from our previous report (Bulbul et al., 2017a). In a separate group of animals, to test whether an overnight fasting alters the ARS-induced CCK release, ad-libitum fed and fasted rats were loaded with ARS 30 min following the injection of vehicle or F13A. In our first set of experiments, the stress-induced CCK release was only detected in post-ARS samples, therefore, in the second set of experiments, the blood samples were only withdrawn 60 min after the termination of ARS. A representative flow chart of the experimental design is presented in Fig.1.

2.3.3. Quantification of CCK levels in plasma

The whole blood samples were aliquoted following a centrifugation at 10,000 *g* then kept at −80 °C prior to the analysis. Plasma CCK concentrations were quantified using commercially available enzyme immunoassay (EIA) kits (Phoenix Pharmaceuticals, CA, USA). Prior to the assay, the samples were extracted using Sep-Pak C-18 (Phoenix Pharmaceuticals) columns and assay procedures were carried out following the protocols of the manufacturer.

2.4. Histology

In a separate group of stressed (n = 3) and NS (n = 3) animals, duodenums were obtained following cardiac perfusion (with 50 ml of saline). The abdomen was opened and duodenal tissues were removed and post-fixed 4% formalin for overnight, rinse with phosphate-buffered saline (PBS) and embedded. Subsequently, 5 µm-thick duodenal sections were cut using a microtome. Serial sections were collected on SuperFrost Plus slides (Novoglas, Berne, Switzerland).

2.4.1. Immunohistochemistry

Five-micrometer-thick sections of formalin-fixed paraffin-embedded duodenal tissues were dried overnight at 56 °C. The samples were deparaffinized in xylene at room temperature for 10 min and rehydrated with a graded ethanol series and then washed in distilled water. Antigen retrieval was performed by boiling the slides in citrate buffer (pH 6.0, 0.01 M) in a microwave oven at 100 °C for 7 min. The sections were washed 3 times in PBS (pH:7.4) for 15 min each. Endogenous peroxidase activity was blocked by incubating the sections with 3% H_2O_2 for 15 min. Then, the slides were incubated with blocking serum (Lab-vision, Fremont, CA, USA) for 7 min to block non-specific immunoglobulin binding. The sections were incubated with anti-CCK mouse monoclonal primary antibody (ab37274, Abcam, Cambridge, UK) at dilution of 1:200 overnight at 4 °C. Subsequently, the sections were incubated with a biotinylated anti-mouse secondary antibody (Vector Lab. Inc., Burlingame, CA, USA) at 1:400 dilution for 1 h at room temperature. The sections were overlaid with peroxidase-labeled streptavidin (ab64629, Abcam) for 25 min and followed by rinsing in PBS for 15 min. Diaminobenzidine (DAB, Vector, Burlingame, CA, USA) was added as a substrate and the sections were observed until they simultaneously turned brown. Finally, the sections were counterstained with hematoxylin and examined with Axioplan light microscope (Zeiss, Germany) and photographed. The CCK immunostaining was quantified as the average percentage of CCK-staining area by using ImageJ software.

2.4.2. Double-labeled immunofluorescence

Double labeling was carried out in a sequential manner. Briefly, tissues were first incubated in rabbit anti-Apelin (1:200; bs-2425R, Bioss Antibodies), rabbit anti-APJ (1:100; ab84296, Abcam, Cambridge, UK), goat anti-GPR40 (1:100; SC-28416, Santa Cruz Biotechnology, CA, USA) or mouse anti-CCK (1:200; ab37274, Abcam) for overnight at 4 °C as a cocktail. Then tissues were washed in PBS three times for 5 min, and incubated in the secondary antibodies donkey anti-mouse (1:400; Alexa Fluor, A10037), donkey anti-goat (1:400; Alexa Fluor, A11057)

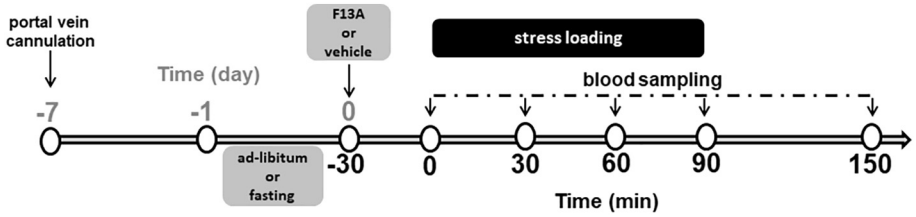

Fig. 1. Representative flow chart of the experimental design. Portal vein cannulation was performed 7 days before the experiments. Blood samples were collected before, throughout and 60 min after the ARS loading from the fasted or ad-libitum fed rats which were administered with vehicle or APJ receptor antagonist F13A (300 µg/kg, ip) 30 min prior to the stress loading.

or donkey anti-rabbit (1:400; Alexa Fluor, A21206) for 45 min at room temperature as a cocktail. Tissues were then washed in PBS for 5 min and visualized with a fluorescence microscope. The apelin immunostaining was quantified as the average percentage of apelin-staining area by using ImageJ software.

2.5. Statistics

Statistical analyses were performed using SPSS *v13.0* software. Data were expressed as mean ± SEM. The time course changes in CCK levels in plasma levels were evaluated by repeated measures one-way ANOVA, whereas two-way ANOVA followed by Tukey posthoc test were used to assess the effects of the fasting or F13A pretreatment on independent samples. The quantitative analyses of CCK and apelin immunoreactivities were performed by Kruskal Wallis test followed by Mann Whitney-*U* test. A p value < 0.05 was considered as statistically significant.

3. Results

3.1. ARS increases CCK and apelin immunoreactivities in duodenum

CCK immunoreactivity was observed through the mucosa and tunica muscularis layers of duodenal coronal sections obtained from NS rats (Fig.2A). The CCK immunoreactivity was detected more intensely in ARS group (Fig. 2B). In order to elucidate the apelin expression in duodenum we applied immunoflorescence labeling (Fig.3A–F). A weak apelin expression was observed in NS group (Fig.3B), while there was an intense immunoreactivity for apelin in duodenal coronal sections obtained from ARS-loaded rats (Fig.3E). Compared with NS rats, the ARS-induced alterations in apelin and CCK immunoreactivities were observed more intensely in duodenal mucosa ($p < 0.05$ vs NS) and myenteric plexi ($p < 0.01$ vs NS), (Fig. 2C, 3G).

3.2. ARS increases CCK release through APJ receptor

Compared with the basal samples (190.7 ± 62.8 pg/ml, n = 6) collected in the pre-stress period, ARS caused a slight but non-significant increase in CCK level at the time point 90 min (392.6 ± 141.8, n = 6). However, a remarkable increase in CCK was detected in the post-ARS samples which were obtained 60 min after the termination of stress loading (1224.4 ± 155.1, n = 6, $p < 0.01$). In contrast, F13A pretreatment completely abolished the ARS-induced elevations in CCK levels in the post-ARS samples (112.5 ± 46.7 pg/ml, n = 6), (Fig. 4).

3.3. Fasting impairs the ARS-induced increases in CCK release

In post-ARS samples obtained from ad-libitum fed rats, ARS induced approximately 6-fold increase in CCK, whereas it was prevented by F13A which caused a 63.5% decline in CCK concentration. Conversely, the stimulatory effect of ARS was no longer observed in rats underwent an overnight fasting in which CCK levels exhibited a 9.2% decline. Compared with the ad-libitum fed rats, the F13A-induced decline was observed to a lesser degree (−33.4% of basal) in fasted rats (Fig.5).

3.4. APJ receptor is present in duodenal enteroendocrine I cells and myenteric neurons

GPR40 is a G-protein coupled receptor for the long-chain free fatty acids which is commonly used for identification of the CCK-producing enteroendocrine I cells. Double immunolabeling with primary antibodies for APJ receptor (APJ) (Fig.6B), and GPR40 (Fig.6C) identified individual epithelial cells in the rat duodenum, additionally, these cells were present throughout the duodenal mucosa and subset of coexpressing cells were found to be visible (Fig.6D, E). Similarly, double immunolabeling with primary antibodies for CCK (Fig.7B), and APJ (Fig.7C) identified the circular smooth muscle co-expression throughout the duodenal muscle layer, while the strongest double-positive reaction was observed in the neuronal cells of myenteric plexus (Fig.7D, E).

4. Discussion

In the present study, our findings have demonstrated that restraint stress loading for 90 min increased the expression of apelin remarkably in duodenum. The increased endogenous apelin appears to stimulate the release CCK from enteric neurons and enteroendocrine I cells in duodenum through the mediation of APJ receptors. Moreover, the apelin-induced stimulatory action of ARS on CCK seems to be dependent on the nutritional status, so that it was abolished completely in rats exposed to an overnight fasting.

The accumulating evidence provided from the recent rodent studies indicate the stimulatory action of exogenously applied apelin on the CCK release from gut (Flemstrom et al., 2011; Kapica et al., 2012; Wang et al., 2004; Wattez et al., 2013). In line with these findings, we have shown previously in rats that peripheral administration of apelin-13 caused a significant delay in solid GE through an APJ receptor-mediated pathway which was abolished by pretreatment of CCK1 receptor antagonist lorglumide suggesting the mediation of CCK in apelin-induced gastroinhibitory action. Importantly, it has been shown recently that the expression of apelin in stomach was upregulated in rats exposed to acute water immersion restraint stress (Birsen et al., 2016; Izgut-Uysal et al., 2014). Our present data demonstrated that ARS increased the CCK release significantly in samples collected 60 min after the termination of stress, however, the stimulatory effect of ARS was abolished in rats pretreated with F13A which indicates the mediation of APJ receptors in the stimulatory action of ARS on CCK release from gut.

Susaki and colleagues demonstrated the measurable levels of apelin

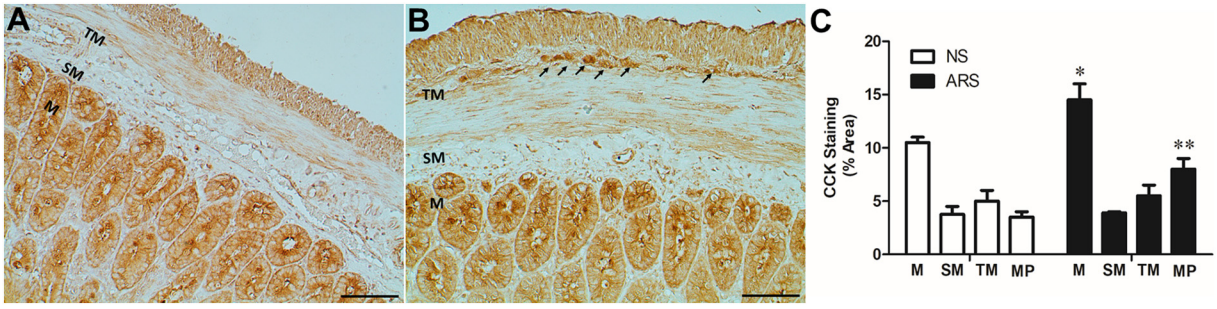

Fig. 2. Distribution of CCK-immunoreactive cells in coronal duodenal sections obtained from NS (A) and ARS-loaded (B) rats. C: the quantitative analysis of CCK immunoreactivity. TM: tunica muscularis, SM: submucosa, M: Mucosa, MP: myenteric plexus, ARS: acute restraint stress. Black arrows indicate immunoreactive cells in myenteric ganglia. Scale bars represent 100 µm. The statistical comparisons were performed by Kruskal Wallis test followed by Mann Whitney-*U* test. *$p < 0.05$, **$p < 0.01$ vs NS, n = 3 per group.

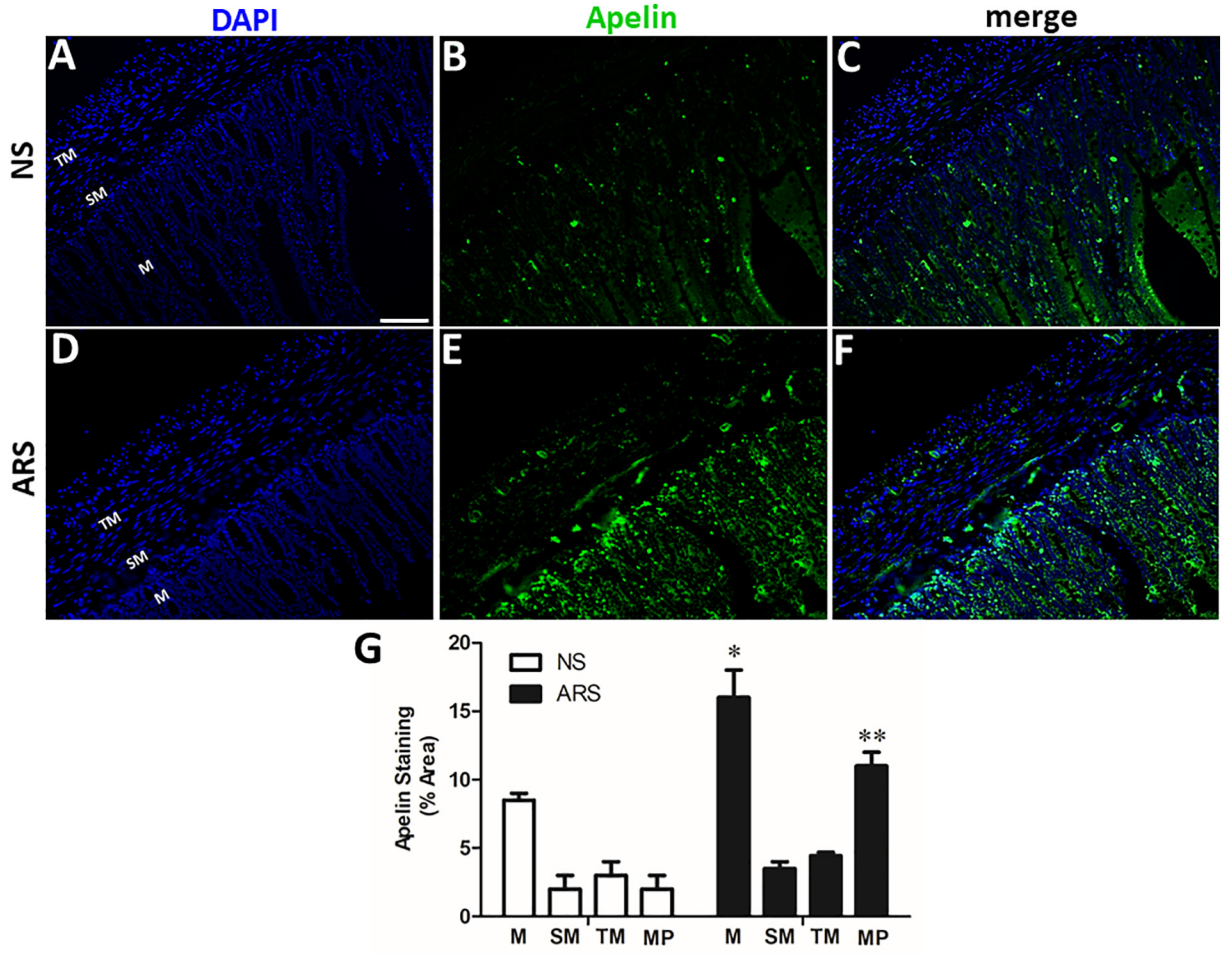

Fig. 3. Apelin immunoexpression in coronal duodenal sections obtained from NS (A-C) and ARS-loaded (D-F) rats. G: the quantitative analysis of apelin immunoreactivity. TM: tunica muscularis, SM: submucosa, M: mucosa, MP: myenteric plexus, ARS: acute restraint stress. The scale bar represents 100 µm. The Kruskal Wallis test followed by Mann Whitney-U test was carried out to statistical comparisons. **p < 0.01 vs NS, n = 3 per group.

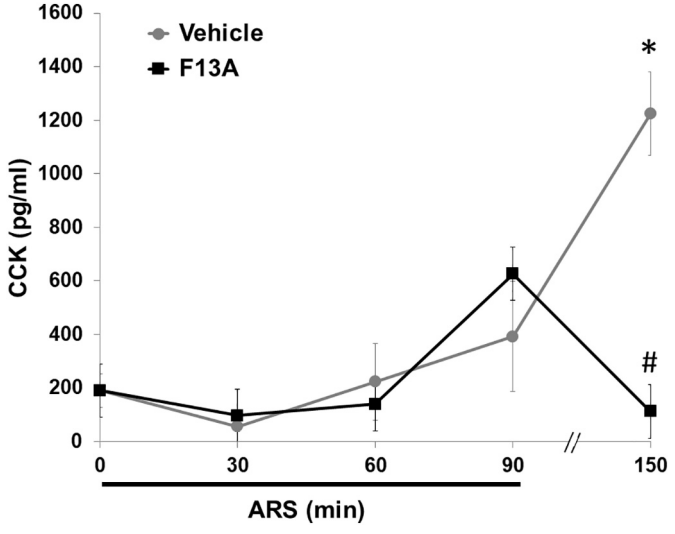

Fig. 4. Effect of F13A on ARS-induced alterations in plasma CCK levels. Data were expressed as mean ± SEM. *p < 0.05 vs basal, #p < 0.05 vs vehicle. ARS: acute restraint stress. The oblique parallel lines designate the 60 min time period spent after termination of ARS loading. The statistical analyses were performed using repeated measures one-way ANOVA, n = 6 in all groups.

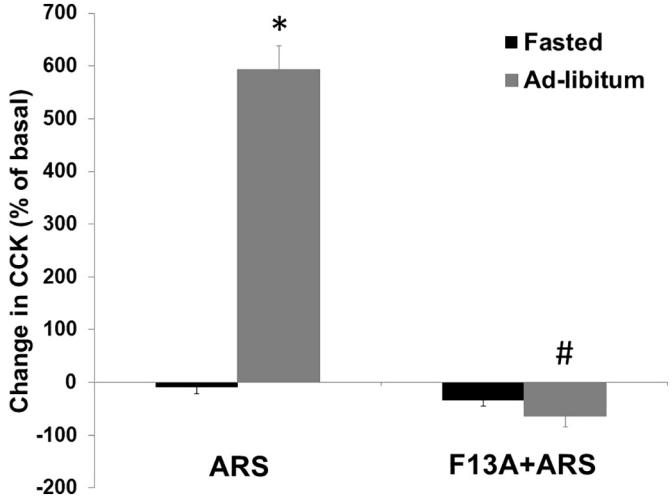

Fig. 5. The effect of fasting on ARS-induced elevations in plasma CCK levels overnight fasted or ad-libitum fed rats which were pretreated with vehicle or F13A. Data were expressed as mean ± SEM. *p < 0.05 vs fasted; #p < 0.05 vs ARS. ARS: acute restraint stress. Statistical analysis was performed using two-way ANOVA followed by Tukey posthoc test, n = 6 in all groups.

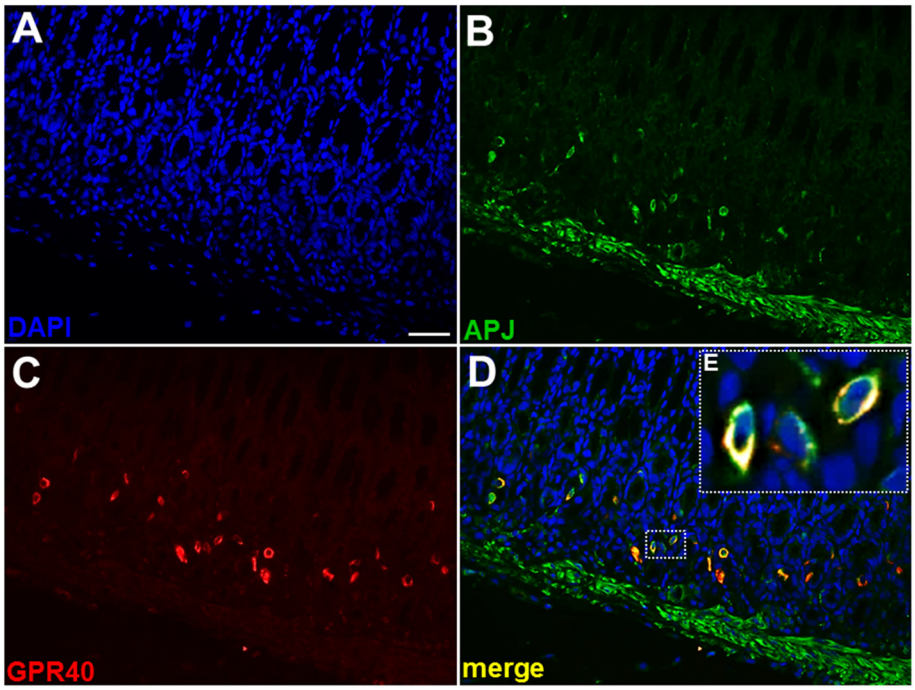

Fig. 6. Immunofluorescence staining in coronal duodenal section obtained from ad-libitum fed NS rats. A: DAPI (blue), B: APJ receptor (green), C: GPR40 (red) and D: colocalization of APJ with GPR40 (yellow). The scale bar represents 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

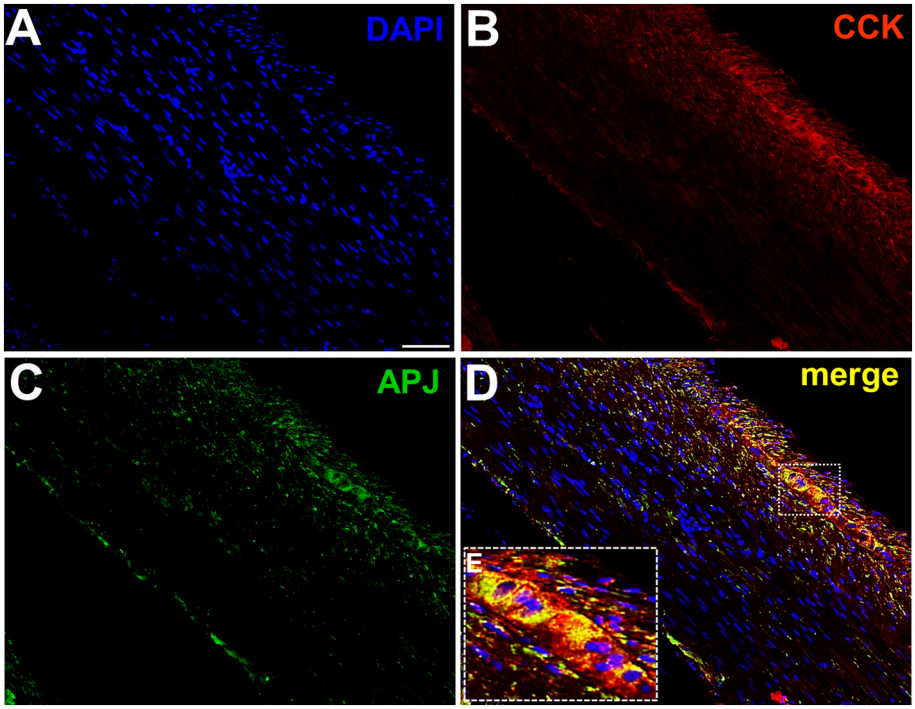

Fig. 7. Immunofluorescence staining in coronal duodenal section obtained from ad-libitum fed NS rats. A: DAPI (blue), B: CCK (red), C: APJ receptor (green) and D: colocalization of APJ with CCK (yellow). E: high magnification of boxed region depicting double-positive cells in myenteric plexus. The scale bar represents 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels both in the gut lumen and the systemic circulation suggesting that endogenous apelin acts on mediation of the GI functions in an endocrine and/or paracrine manner (Susaki et al., 2005). Within GI tract, the expression of apelin/APJ receptor system expression has been demonstrated in mucosal enteroendocrine cells, mucous-producing epithelial cells (Susaki et al., 2005) and parietal cells in oxyntic mucosa (Susaki et al., 2005; Wang et al., 2004), while the immunoreactivity for APJ receptor was also shown in neuronal cells in myenteric plexus (Fournel et al., 2015), duodenal goblet cells (Wang et al., 2009), pyloric mucosal cells (Pope et al., 2012) and smooth muscle layers of the GI tract (Wang et al., 2009). Despite the previous findings suggesting that the released apelin from gut may play a role in regulation of postprandial alterations in GI functions through CCK-mediated pathway, the apelin-responding source of CCK has not been identified. However, our immunohistochemical analyses demonstrated the expression of APJ receptor in CCK-producing cells in myenteric plexus and GPR40-positive enteroendocrine cells in duodenal mucosa. Therefore, these findings suggest that in response to stress-induced or exogenous administered apelin, CCK is released possibly from distinct sources which appear to be I cells and neuronal cells in myenteric plexi.

In the present study, our findings have revealed that apelin expression is up-regulated upon ARS, moreover, the stimulatory action of ARS on CCK release was abolished completely in rats exposed to an overnight fasting suggesting the effect of nutritional status on APJ

receptor signaling. The accumulating evidence indicates that CCK-mediated effects of exogenously administered apelin appear to be dependent on the nutritional status. It has been demonstrated in mice that fasting caused a decrease in apelin mRNA expression in adipocytes, while it was recovered by feeding (Boucher et al., 2005). More importantly, it has been demonstrated previously in rats by Flemström that an overnight fasting caused an 8-fold decrease in APJ receptor expression in duodenal mucosa which resulted in ineffectiveness of 100-fold greater dose of exogenous apelin on bicarbonate secretion compared with the ad-libitum fed rats (Flemstrom et al., 2011). In line with the latter finding, we have found recently in rats that exposure to an 18 h of fasting decreased the expression of APJ receptor mRNA remarkably in both gastric and duodenal samples (Bulbul et al., 2018). In our experiments, we have found that ARS caused a 2-fold increase in plasma CCK level. In contrast, the ARS-induced stimulatory action was abolished and no longer observed in rats fasted overnight. CCK is released in response to the dietary fatty acids from upper GI tract (Liou et al., 2011; Sykaras et al., 2014; Wang et al., 2001). The physiological effects of CCK on GI motor functions in the postprandial period mainly include retarding of GE (Grider, 1994; Yamagishi and Debas, 1978) and CT (Varga et al., 2004) and the disruption of MMC (Deloose et al., 2012; Romanski, 2009). It has been demonstrated that the inhibitory actions of CCK on GI motor functions are mediated mainly by CCK1 receptors expressed on the peripheral endings of vagal sensory fibers, however, the enteric neurons have been also demonstrated to produce CCK in rodents (Lay et al., 1999). Therefore, in addition to acting in endocrine fashion, CCK seems to exert inhibitory actions on GI motor functions in neurocrine manner (Monnikes et al., 1997; Zittel et al., 1999).

Along with the previous findings, our present data suggest that during the postprandial period, endogenous apelin reinforces and regulates the actions of CCK on GI functions (Bulbul et al., 2017a; Flemstrom et al., 2011; Wang et al., 2004). In physiological perspective, in the postprandial stage, the released CCK from upper small intestine exhibits a strong inhibitory effect on GE (Yamagishi and Debas, 1978). During distension of the stomach, lipids are major triggers of dyspeptic symptoms such as nausea, bloating, pain, and fullness so that they modulate upper gastrointestinal sensations and symptoms, so that CCK is a major mediator of the sensitization of gastric perception by lipids in patients with functional dyspepsia (FD) as the CCK-A receptor antagonists markedly diminish this effect (Fried and Feinle, 2002). Individuals with FD, the increased peripheral tonus of CCK is believed to contribute to the development of the dyspeptic symptoms following the meal ingestion (Lal et al., 2004; Mayer et al., 2006; Saad and Chey, 2006). In rodent studies, CCK-induced sensitization of distension-related sensory and motor responses have been demonstrated (Cremonini et al., 2005; Lal et al., 2004). In fact, CCK1 receptor antagonists are being tested and under development for the treatment of the patients suffering from FD and constipation-predominant form of irritable bowel syndrome (Cann et al., 1994; Dobrek and Thor, 2009; Varga, 2002; Varga et al., 2004). Our immunohistochemical analyses revealed that ARS for 90 min increased the CCK immunoreactivity in duodenum, whereas the most prominent changes were observed in myenteric plexus suggesting the possibility that the upregulated CCK contributes to the stress-induced alterations in small intestinal motility through a local neurocrine mechanism.

Interestingly, double immunofluorescence analyses demonstrated the presence of APJ-receptor in the CCK-producing myenteric neurons in duodenum which indicates the mediation of APJ receptors in neuronal CCK production. Thus, it appears to be convincible that the antagonism of enteric APJ receptors may improve the treatment strategies designed with CCK receptor antagonists. Although recent rodent studies have demonstrated that endogenous apelin was upregulated in brain (Bulbul et al., 2016) and gut (Izgut-Uysal et al., 2014) upon stress, in fact, it has not been investigated whether endogenous apelin in gut alters in pathophysiological conditions such as functional GI disorders. However, it was proposed previously in humans that apelin levels in serum could be a potential predictor for such diseases including myocardial infarction (Liu et al., 2015), neonatal sepsis (Gad et al., 2014), chronic hepatitis (El-Mesallamy et al., 2011), type 2 diabetes mellitus (Karakoc et al., 2016). Importantly, it has been demonstrated recently in mice that apelin inhibited the response of gastric tension sensitive afferents to circular stretch in SLD mice fed with control diet while this effect was not observed in mice fed chronically with high fat diet. These findings suggest the modulatory role of apelin in gastric vagal afferent signaling in a nutritional status dependent manner (Li et al., 2018). Therefore, it could be speculated that stress exposure increases the endogenous apelin which in turn contributes to the stress-induced alterations in GI functions through the mediation of CCK.

5. Conclusion

Taken together, the present findings highlight that stress exposure increases the apelin production in gut which in turn stimulates the release of CCK from enteric sources through an APJ receptor mediated pathway. Endogenous enteric apelin might be a regulator of both stress-induced and meal-related alterations in GI functions through CCK.

Funding

The present study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) within Research Program 1001, unique project number: 214S349.

Conflict of interests

The authors declare that they have no conflict of interest.

References

Bhatia, V., Tandon, R.K., 2005. Stress and the gastrointestinal tract. J. Gastroenterol. Hepatol. 20, 332–339.
Birsen, I., Gemici, B., Acar, N., Ustunel, I., Izgut-Uysal, V.N., 2016. The role of apelin in the healing of water-immersion and restraint stress-induced gastric damage. J. Physiol. Sci. 67, 373–385.
Boucher, J., Masri, B., Daviaud, D., Gesta, S., Guigne, C., Mazzucotelli, A., Castan-Laurell, I., Tack, I., Knibiehler, B., Carpene, C., Audigier, Y., Saulnier-Blache, J.S., Valet, P., 2005. Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology 146, 1764–1771.
Bulbul, M., Babygirija, R., Cerjak, D., Yoshimoto, S., Ludwig, K., Takahashi, T., 2012. Impaired adaptation of gastrointestinal motility following chronic stress in maternally separated rats. Am. J. Physiol. Gastrointest. Liver Physiol. 302, G702–G711.
Bulbul, M., Izgut-Uysal, V.N., Sinen, O., Birsen, I., Tanriover, G., 2016. Central apelin mediates stress-induced gastrointestinal motor dysfunction in rats. Am. J. Physiol. Gastrointest. Liver Physiol. 310, G249–G261.
Bulbul, M., Sinen, O., Birsen, I., Nimet Izgut-Uysal, V., 2017a. Peripheral apelin-13 administration inhibits gastrointestinal motor functions in rats: the role of cholecystokinin through CCK1 receptor-mediated pathway. Neuropeptides 63, 91–97.
Bulbul, M., Sinen, O., Gemici, B., Izgut-Uysal, V.N., 2017b. Opposite effects of central oxytocin and arginine vasopressin on changes in gastric motor function induced by chronic stress. Peptides 87, 1–11.
Bulbul, M., Sinen, O., Izgut-Uysal, V.N., Akkoyunlu, G., Ozturk, S., Uysal, F., 2018. Peripheral apelin mediates stress-induced alterations in gastrointestinal motor functions depending on the nutritional status. Clin. Exp. Pharmacol. Physiol. 1–11.
Cann, P.A., Rovati, L.C., Smart, H.L., Spiller, R.C., Whorwell, P.J., 1994. Loxiglumide, a CCK-A antagonist, in irritable bowel syndrome. A pilot multicenter clinical study. Ann. N. Y. Acad. Sci. 713, 449–450.
Cremonini, F., Camilleri, M., McKinzie, S., Carlson, P., Camilleri, C.E., Burton, D., Thomforde, G., Urrutia, R., Zinsmeister, A.R., 2005. Effect of CCK-1 antagonist, dexloxiglumide, in female patients with irritable bowel syndrome: a pharmacodynamic and pharmacogenomic study. Am. J. Gastroenterol. 100, 652–663.
De Mota, N., Lenkei, Z., Llorens-Cortes, C., 2000. Cloning, pharmacological characterization and brain distribution of the rat apelin receptor. Neuroendocrinology 72, 400–407.
Deloose, E., Janssen, P., Depoortere, I., Tack, J., 2012. The migrating motor complex: control mechanisms and its role in health and disease. Nat. Rev. Gastroenterol. Hepatol. 9, 271–285.
Dobrek, L., Thor, P.J., 2009. Pathophysiological concepts of functional dyspepsia and irritable bowel syndrome future pharmacotherapy. Acta Pol. Pharm. 66, 447–460.
El-Mesallamy, H.O., Hamdy, N.M., Rizk, H.H., El-Zayadi, A.R., 2011. Apelin serum level in Egyptian patients with chronic hepatitis C. Mediat. Inflamm. 2011, 703031.
Flemstrom, G., Makela, K., Purhonen, A.K., Sjoblom, M., Jedstedt, G., Walkowiak, J.,

Herzig, K.H., 2011. Apelin stimulation of duodenal bicarbonate secretion: feeding-dependent and mediated via apelin-induced release of enteric cholecystokinin. Acta Physiol (Oxford) 201, 141–150.
Fournel, A., Drougard, A., Duparc, T., Marlin, A., Brierley, S.M., Castro, J., Le-Gonidec, S., Masri, B., Colom, A., Lucas, A., Rousset, P., Cenac, N., Vergnolle, N., Valet, P., Cani, P.D., Knauf, C., 2015. Apelin targets gut contraction to control glucose metabolism via the brain. Gut. 66 (2), 258–269.
Fried, M., Feinle, C., 2002. The role of fat and cholecystokinin in functional dyspepsia. Gut 51 (Suppl. 1), i54–i57.
Gad, G.I., Ismail, R.I., El-Masry, S.A., Gouda, H.R., 2014. Serum apelin in early-onset neonatal sepsis: is it diagnostic? J. Neonatal-Perinatal Med. 7, 207–212.
Grider, J.R., 1994. Role of cholecystokinin in the regulation of gastrointestinal motility. J. Nutr. 124, S1334–S1339.
Han, S., Wang, G., Qiu, S., de la Motte, C., Wang, H.Q., Gomez, G., Englander, E.W., Greeley Jr., G.H., 2007. Increased colonic apelin production in rodents with experimental colitis and in humans with IBD. Regul. Pept. 142, 131–137.
Han, S., Wang, G., Qi, X., Lee, H.M., Englander, E.W., Greeley Jr., G.H., 2008. A possible role for hypoxia-induced apelin expression in enteric cell proliferation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1832–R1839.
Izgut-Uysal, V.N., Gemici, B., Birsen, I., Acar, N., Ustunel, I., 2014. The protective effect of apelin against water-immersion and restraint stress-induced gastric damage. J. Physiol. Sci. 64, 279–289.
Kapica, M., Jankowska, A., Antushevich, H., Pietrzak, P., Bierla, J.B., Dembinski, A., Zabielski, R., 2012. The effect of exogenous apelin on the secretion of pancreatic juice in anaesthetized rats. J. Physiol. Pharmacol. 63, 53–60.
Karakoc, A., Sahin, A., Polat, E.S., Aliyev, E., Yildirim, A., Bakan, N., Dokumacioglu, E., 2016. Serum apelin and ADMA levels in type 2 diabetics with and without vascular complications. Diabetes Metab Syndr. 10, 106–109.
Lal, S., McLaughlin, J., Barlow, J., D'Amato, M., Giacovelli, G., Varro, A., Dockray, G.J., Thompson, D.G., 2004. Cholecystokinin pathways modulate sensations induced by gastric distension in humans. Am. J. Physiol. Gastrointest. Liver Physiol. 287, G72–G79.
Lay, J.M., Gillespie, P.J., Samuelson, L.C., 1999. Murine prenatal expression of cholecystokinin in neural crest, enteric neurons, and enteroendocrine cells. Dev. Dyn. 216, 190–200.
Lenz, H.J., Raedler, A., Greten, H., Vale, W.W., Rivier, J.E., 1988. Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor. Gastroenterology 95, 1510–1517.
Li, H., Kentish, S.J., Wittert, G.A., Page, A.J., 2018. Apelin modulates murine gastric vagal afferent mechanosensitivity. Physiol. Behav. 194, 466–473.
Liou, A.P., Lu, X., Sei, Y., Zhao, X., Pechhold, S., Carrero, R.J., Raybould, H.E., Wank, S., 2011. The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. Gastroenterology 140, 903–912.
Liu, H.T., Chen, M., Yu, J., Li, W.J., Tao, L., Li, Y., Guo, W.Y., Wang, H.C., 2015. Serum apelin level predicts the major adverse cardiac events in patients with ST elevation myocardial infarction receiving percutaneous coronary intervention. Medicine (Baltimore) 94, e449.
Martinez, V., Wang, L., Rivier, J., Grigoriadis, D., Tache, Y., 2004. Central CRF, urocortins and stress increase colonic transit via CRF1 receptors while activation of CRF2 receptors delays gastric transit in mice. J. Physiol. 556, 221–234.
Mayer, E.A., Tillisch, K., Bradesi, S., 2006. Review article: modulation of the brain-gut axis as a therapeutic approach in gastrointestinal disease. Aliment. Pharmacol. Ther. 24, 919–933.
Medhurst, A.D., Jennings, C.A., Robbins, M.J., Davis, R.P., Ellis, C., Winborn, K.Y., Lawrie, K.W., Hervieu, G., Riley, G., Bolaky, J.E., Herrity, N.C., Murdock, P., Darker, J.G., 2003. Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. J. Neurochem. 84, 1162–1172.
Monnikes, H., Lauer, G., Arnold, R., 1997. Peripheral administration of cholecystokinin activates c-fos expression in the locus coeruleus/subcoeruleus nucleus, dorsal vagal complex and paraventricular nucleus via capsaicin-sensitive vagal afferents and CCK-A receptors in the rat. Brain Res. 770, 277–288.
Nakade, Y., Tsuchida, D., Fukuda, H., Iwa, M., Pappas, T.N., Takahashi, T., 2005. Restraint stress delays solid gastric emptying via a central CRF and peripheral sympathetic neuron in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R427–R432.
Oshima, T., Miwa, H., 2013. Functional gastrointestinal disorders (FGID): progress in diagnosis and treatments. Topic I. basic knowledge of functional gastrointestinal disorders (FGID); 1. Epidemiology of FGID. Nihon Naika Gakkai zasshi 102, 4–10.
Oshima, T., Miwa, H., 2014. Gastrointestinal motility disorders of functional dyspepsia. Nihon Shokakibyo Gakkai zasshi 111, 1071–1078.
Pope, G.R., Roberts, E.M., Lolait, S.J., O'Carroll, A.M., 2012. Central and peripheral apelin receptor distribution in the mouse: species differences with rat. Peptides 33, 139–148.
Romanski, K.W., 2009. Dose-related effects of cholecystokinin octapeptide administered during various phases of the migrating motor complex on small-intestinal motility in sheep. Acta Vet. Hung. 57, 495–508.
Saad, R.J., Chey, W.D., 2006. Review article: current and emerging therapies for functional dyspepsia. Aliment. Pharmacol. Ther. 24, 475–492.
Strubbe, J.H., Bruggink, J.E., Steffens, A.B., 1999. Hepatic portal vein cannulation for infusion and blood sampling in freely moving rats. Physiol. Behav. 65, 885–887.
Susaki, E., Wang, G., Cao, G., Wang, H.Q., Englander, E.W., Greeley Jr., G.H., 2005. Apelin cells in the rat stomach. Regul. Pept. 129, 37–41.
Sykaras, A.G., Demenis, C., Cheng, L., Pisitkun, T., McLaughlin, J.T., Fenton, R.A., Smith, C.P., 2014. Duodenal CCK cells from male mice express multiple hormones including ghrelin. Endocrinology 155, 3339–3351.
Tache, Y., Bonaz, B., 2007. Corticotropin-releasing factor receptors and stress-related alterations of gut motor function. J. Clin. Invest. 117, 33–40.
Talley, N.J., 2008. Functional gastrointestinal disorders as a public health problem. Neurogastroenterol. Motil. 20 (Suppl. 1), 121–129.
Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M.X., Kawamata, Y., Fukusumi, S., Hinuma, S., Kitada, C., Kurokawa, T., Onda, H., Fujino, M., 1998. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem. Biophys. Res. Commun. 251, 471–476.
Varga, G., 2002. Dexloxiglumide rotta research lab. Curr. Opin. Investig. Drugs 3, 621–626.
Varga, G., Balint, A., Burghardt, B., D'Amato, M., 2004. Involvement of endogenous CCK and CCK1 receptors in colonic motor function. Br. J. Pharmacol. 141, 1275–1284.
Wang, L., Martinez, V., Rivier, J.E., Tache, Y., 2001. Peripheral urocortin inhibits gastric emptying and food intake in mice: differential role of CRF receptor 2. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281, R1401–R1410.
Wang, G., Anini, Y., Wei, W., Qi, X., Am, O.C., Mochizuki, T., Wang, H.Q., Hellmich, M.R., Englander, E.W., Greeley Jr., G.H., 2004. Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. Endocrinology 145, 1342–1348.
Wang, G., Kundu, R., Han, S., Qi, X., Englander, E.W., Quertermous, T., Greeley Jr., G.H., 2009. Ontogeny of apelin and its receptor in the rodent gastrointestinal tract. Regul. Pept. 158, 32–39.
Wattez, J.S., Ravallec, R., Cudennec, B., Knauf, C., Dhulster, P., Valet, P., Breton, C., Vieau, D., Lesage, J., 2013. Apelin stimulates both cholecystokinin and glucagon-like peptide 1 secretions in vitro and in vivo in rodents. Peptides 48, 134–136.
Yamagishi, T., Debas, H.T., 1978. Cholecystokinin inhibits gastric-emptying by acting on both proximal stomach and pylorus. Am. J. Phys. 234, E375–E378.
Zheng, J., Dobner, A., Babygirija, R., Ludwig, K., Takahashi, T., 2009. Effects of repeated restraint stress on gastric motility in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R1358–R1365.
Zittel, T.T., Glatzle, J., Kreis, M.E., Starlinger, M., Eichner, M., Raybould, H.E., Becker, H.D., Jehle, E.C., 1999. C-fos protein expression in the nucleus of the solitary tract correlates with cholecystokinin dose injected and food intake in rats. Brain Res. 846, 1–11.