Central Autonomic Pathways Contribute to Delayed Colonic Transit Induced by Peripheral Apelin-13

Osman Sinen, Mehmet Bülbül

Akdeniz University, Faculty of Medicine, Department of Physiology
Antalya, Turkey
e-mail: osmansinen@akdeniz.edu.tr

Introduction

- Apelin is the endogenous ligand for the apelin receptor (APJ). The apelin/APJ system is expressed in both central nervous system and gut [1-3].
- The vascular organ of the lamina terminalis (OVLT), subfornical organ (SFO) and area postrema (AP) comprise the sensory circumventricular organs (CVO) which are central structures that lie outside the blood brain barrier and are thought to provide an interface between peripherally circulating signals and the brain through their projections to central autonomic structures [4].
- The moderate level of apelin-positive nerve fibers was detected in the OVLT. In contrast, numerous and intensely stained fibers are distributed within the SFO and the AP. Furthermore, APJ is expressed in the subfornical organ. These findings suggest that circulating apelin could indirectly regulate GI motility through acting on CVOs [5-6].
- Despite our recent study indicates that in rats that intraperitoneally administered apelin-13 inhibited colonic transit (CT) through a cholecystokinin (CCK)-dependent and capsaicin-sensitive vagally-mediated pathway [7], the relevant mechanism is incompletely understood.

Aim

- This study aimed to investigate (i) whether APJ is expressed in circumventricular structures involved in autonomic functions and (ii) whether they are activated by peripherally administered apelin, (iii) investigating the role of autonomic pathways in peripheral exogenous apelin-induced colonic dysmotility together with (iv) the changes in apelin levels in the extracellular environment of brain following its peripheral application.

Methods

- ***Animals:*** Adult male Wistar rats (250-300 g) were housed at a room temperature of 22-24◦C with a 12-h light/12-h dark cycle (light on 6:00 a.m. to 6:00 p.m.). All the protocols were approved by the guidelines of the Animal Ethical Committee of Akdeniz University (with user protocol number B.30.2.AKD.0.05.07.00/101).
- ***Experimental Design:*** Parasympathectomy was induced by subdiaphragmatic vagotomy (VGX) alone or combined with pelvic nerve denervation (PD); whereas 6-hydroxydopamine (6-OHDA) injection were performed for sympathectomy. For CT measurements, a silicon catheter was inserted via the cecum into the proximal colon and fixed with sutures in rats. Following a 7-day recovery, CT was spectrophotometrically quantified as the geometric center of distribution of phenol red solution applied through the catheter. The relevant surgical procedures are represented in Fig.2. Ninety minutes after the administration of vehicle or apelin-13 (300 µg·kg-1, ip) plasma and cerebrospinal fluid (CSF) samples were collected for measurement of apelin. Additionally, apelin-induced c-Fos expression in the CVOs was examined by immunohistochemistry. The experimental procedures are summarized in Fig.1.

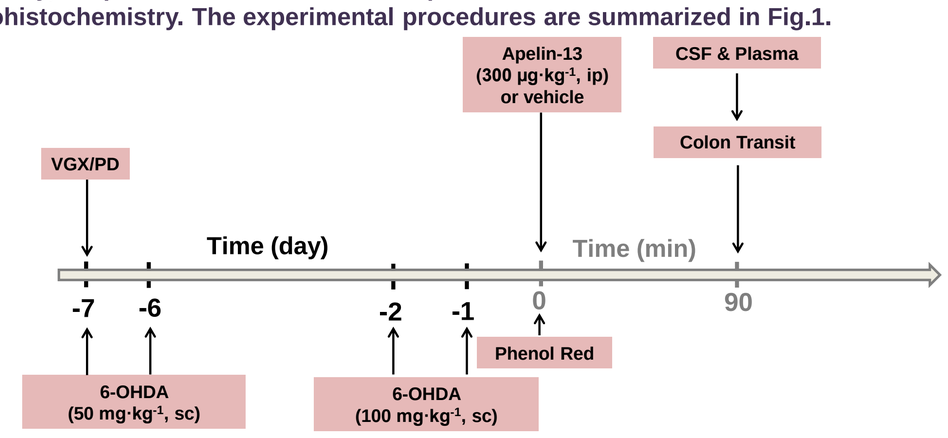

Fig.1 Flow chart of the experimental design. For colon transit experiments, parasympathectomy was performed by the combination of vagotomy (VGX) and pelvic nerve denervation (PD), where chemical sympathectomy was applied by selective destruction of adrenergic nerve with chronic 6-OHDA injection. Chronic catheterization was performed by implantation of a silicone tubing into the proximal colon 7 days prior to the CT measurements. All rats were allowed to recover for 7 days after the surgical interventions. For measurement of apelin, plasma and CSF samples were collected 90 min after vehicle or peripheral apelin-13 administration (300 µg·kg-1, ip). Similarly, colon transit was measured 90 min after the apelin-13 injection.

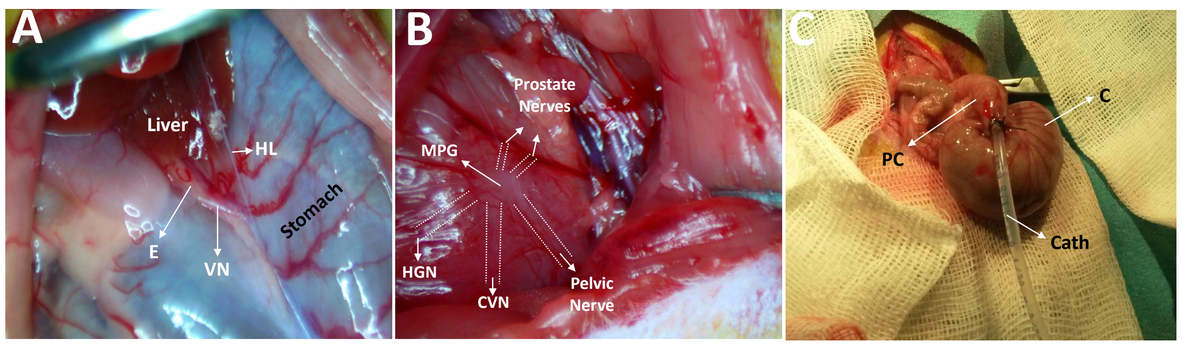

Fig.2 Representative images of surgical procedures. Macroscopic organization of the subdiaphragmatic vagus nerve (A) and major pelvic ganglion (B). C: placement of colonic catheter into the proximal colon for the CT measurements. The dashed lines depict the structure of the nerve fibers (B) that run from MPG. HL: hepatogastric ligament, E: esophagus, VN: vagus nerve, MPG: major pelvic ganglion, CVN: cavernous nerve, HGN: hypogastric nerve, C: cecum, PC: proximal colon, Cath: catheter.

- ***Statistics:*** Statistical analyses were performed using Graphpad Prism software v.5. The data of CT measurements were compared with one-way ANOVA followed by Tukey posthoc test. Student's t-test was performed to compare the apelin concentrations in plasma and CSF. Quantitative analysis of c-Fos-positive neurons was analyzed by Kruskal−Wallis test followed by Dunn's test. A p value<0.05 was considered to be significant.

Results

Peripheral apelin-13 injection elevates apelin levels in plasma and CSF

- Plasma apelin level was 49.4±5.5 ng/ml in vehicle-injected control rats (n=8). Similar to our previous study, apelin-13 administration (300 µg·kg-1, ip) significantly increased the plasma apelin concentration (67±3.3 ng/ml, p<0.05, n=8), (Fig.3A). Likewise, compared with vehicle-treated rats (81.0±6.7 ng/ml, n=9), the apelin concentration in CSF was significantly increased following apelin-13 administration (133.1±25.3 ng/ml, p<0.01, n=9), (Fig.3B).

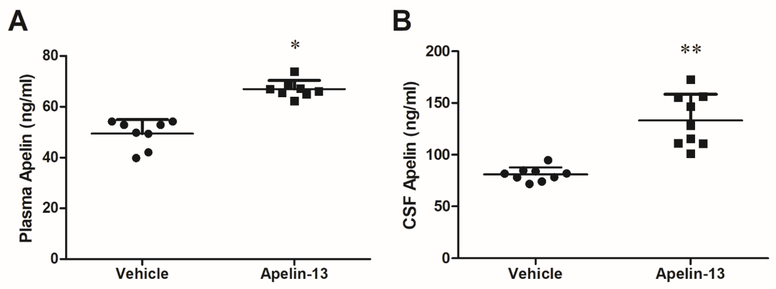

Fig.3 Effect of peripheral apelin-13 administration (300 µg·kg-1, ip) on plasma (A) and CSF (B) apelin levels. All samples were collected 90 min following the injection. Apelin-13 administration significantly increased the apelin levels both in plasma and CSF. Saline (250 µL, ip) was used as vehicle. All values are means ± SD. *p<0.05; **p<0.01 vs vehicle.

Contribution of autonomic pathways in apelin-induced colonic motor dysfunction

- Compared with vehicle-treated rats (n=10), apelin-13 significantly inhibited CT (p<0.001, n=10). The apelin-induced inhibition in CT was partially abolished by parasympathectomy (p<0.05, n=9); whereas, a complete recovery was observed in rats underwent sympathectomy alone (p<0.01, n=9) and the combination of sympathectomy and parasympathectomy (p<0.01, n=10), (Fig.4).

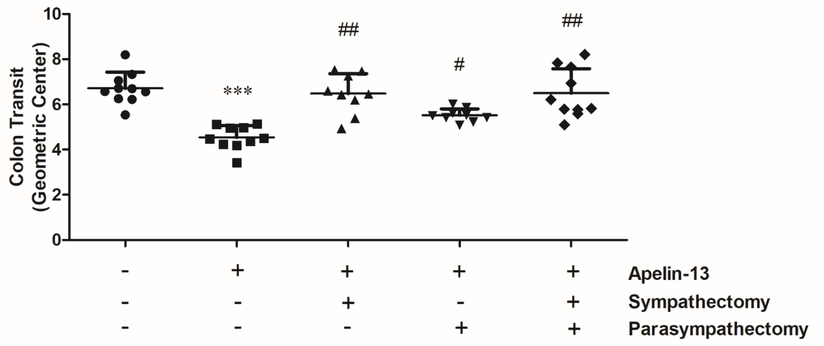

Fig.4 Effect of peripherally administered apelin-13 (300 µg·kg-1, ip) on CT. The inhibitory effect of apelin-13 on CT is predominantly mediated by sympathetic pathway. All values are means ± SD. ***p<0.001 vs vehicle, #p<0.05; ##p<0.01 vs apelin-13.

Neurons in CVOs express APJ

- Using immunohistochemistry, we have detected the expression of APJs in the CVO structures. As observed in Fig.5, we detected prominent APJ immunoreactivity in AP and OVLT, while a weak staining was observed in SFO (Fig.5D-F)

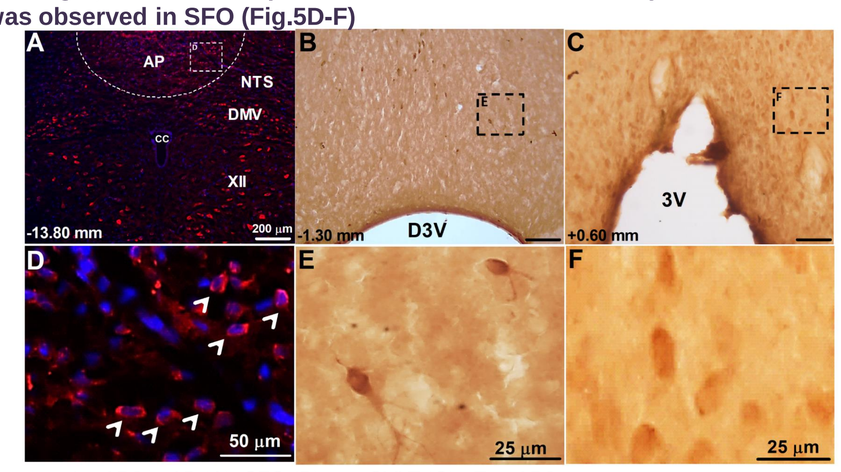

Fig.5 Immunoreactivity for APJ in AP (A), SFO (B) and OVLT (C). The APJ immunoreactive signals were observed mainly in cell bodies (D, E and F). Changes in nuclear morphology were observed by DAPI (DAPI: blue, APJ: red, A and D). AP: area postrema, XII: hypoglossal nucleus, DMV: dorsal motor nucleus of the vagus, NTS: nucleus tractus solitarius, CC: canalis centralis, D3V: dorsal part of the third ventricle, 3V: third ventricle. The scale bar represents 100 µm, unless otherwise indicated.

Peripheral apelin-13 administration activates c-Fos expression in CVOs.

- Compared with vehicle-treated rats, the number of c-Fos-positive neurons in the SFO (n=3) and OVLT (n=3) significantly increased after injection of apelin-13 (p<0.05, n=3), (Data could not be represented as there was not enough space).
- Similarly, apelin-13 significantly increased the number of c-Fos positive neurons in the AP (p<0.01, n=3) compared with vehicle-treated rats (n=3). The apelin-induced c-Fos immunoreactivity positive was remarkably decreased by pretreatment of CCK1 receptor antagonist lorglumide (p<0.05, n=3); whereas, it was completely abolished in VGX rats (p<0.01, n=3), (Fig.6).

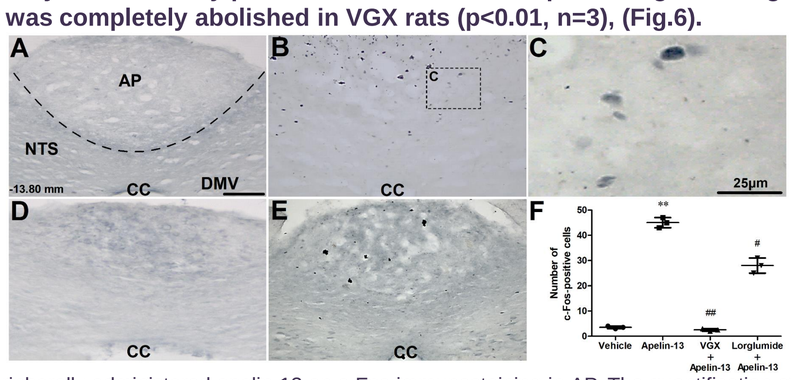

Fig.6 Effect of peripherally administered apelin-13 on c-Fos immunostaining in AP. The quantification of c-Fos-positive neurons in rats injected with vehicle (A) and apelin-13 (B and C). The effects of vagotomy and pretreatment of CCK1 receptor antagonist lorglumide on apelin-induced c-Fos expression are represented in D and E, respectively. In (C) a high magnification of boxed region in (B) is presented. The quantitative analysis of c-Fos-positive neurons in AP (F). All values are means ± SD. **p<0.01 vs vehicle, #p<0.05; ##p<0.01 vs apelin-13. The scale bars represent 100 µm, unless otherwise indicated.

Conclusion

- Taken together, it is intriguing to consider that APJ in CVOs could be potential candidate for the treatment of autonomic dysfunction, particularly gut motor disorders.

References

1. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, 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.
2.Bulbul M, Sinen O, Gok M & Travagli RA (2018). Apelin-13 inhibits gastric motility through vagal cholinergic pathway in rats. Am J Physiol Gastr. Liver Phy. 314, G201-G210.
3. Pope GR, Roberts EM, Lolait SJ & O'Carroll AM (2012). Central and peripheral apelin receptor distribution in the mouse: species differences with rat. Peptides 33, 139-148.
4. Shapiro RE & Miselis RR (1985). The central neural connections of the area postrema of the rat. J Comp Neurol 234, 344-364.
5. Dai L, Smith PM, Kuksis M & Ferguson AV (2013). Apelin acts in the subfornical organ to influence neuronal excitability and cardiovascular function. J Physiol 591, 3421-32
6. Griffiths PR, Lolait SJ, Bijabhai A, O'Carroll-Lolait A, Paton JFR & O'Carroll AM (2020). Increased apelin receptor gene expression in the subfornical organ of spontaneously hypertensive rats. PLoS One 15, e0231844.
7. Bulbul M, Sinen O, Birsen I & Izgut-Uysal V (2017). Peripheral apelin-13 administration inhibits gastrointestinal motor functions in rats: The role of cholecystokinin through CCK1 receptor-mediated pathway. Neuropeptides 63, 91-97.