Autonomic Neuroscience: Basic and Clinical 212 (2018) 17–22

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Dual autonomic inhibitory action of central Apelin on gastric motor functions in rats☆

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ARTICLE INFO

Keywords:
Apelin
Gastric emptying
Gastric motility
Autonomic nervous system

ABSTRACT

Centrally administered apelin has been shown to inhibit gastric emptying (GE) in rodents, however, the relevant mechanism has been investigated incompletely. Using male Wistar rats, we investigated the efferent pathways involved in gastroinhibitory action of central apelin. Stereotaxic intracerebroventricular (icv) cannulation, subdiaphragmatic vagotomy (VGX) and/or celiac ganglionectomy (CGX) were performed 7 days prior to the experiments. Apelin-13 was administered (30 nmol, icv) 90 min prior to GE measurement. Nitric oxide synthase inhibitor L-NAME (100 mg/kg), sympatholytic agent guanethidine (5 mg/kg) and/or muscarinic receptor agonist bethanechol (1 mg/kg) were administered intraperitoneally 30 min prior to the central apelin-13 injection. Two strain gages were implanted serosally onto antrum and pylorus to monitor gastric postprandial motility. Heart rate variability (HRV) analysis was performed before and after central vehicle or apelin-13 administration. Apelin-13 delayed solid GE significantly by disturbing coordinated antral and pyloric postprandial contractions. The apelin-induced delayed GE was attenuated partially by CGX or VGX, whereas it was restored completely in rats underwent both CGX and VGX. L-NAME did not change the apelin-induced alterations. Guanethidine or bethanechol restored the apelin-induced gastroinhibition partially, while it was abolished completely in rats received both agents. Apelin-13 decreased the HRV spectral activity in high-frequency range by increasing low-frequency component and the ratio of LF:HF. The present data suggest that (1) both vagal parasympathetic and sympathetic pathways play a role in apelin-induced gastroinhibition, (2) central apelin attenuates vagal cholinergic pathway rather than activating nonadrenergic-noncholinergic pathway. Apelin/APJ receptor system might be candidate for the treatment of autonomic dysfunction and gastrointestinal motor disorders.

1. Introduction

Apelin was first isolated from bovine stomach extracts as the endogenous ligand for the G-protein-coupled APJ receptor (Tatemoto et al., 1998). Within central nervous system (CNS), both apelin and APJ receptor mRNA are expressed widely in forebrain and brainstem structures including the paraventricular nucleus of the hypothalamus (PVN) (O'Carroll et al., 2003; Pope et al., 2012), subventricular organs (Dai et al., 2013) and rostral ventrolateral medulla (RVLM) (Zhang et al., 2009), nucleus tractus solitarius (NTS), dorsal motor nucleus of N.vagus (DMV) and hypoglossal nucleus (HGN) (Bulbul et al., 2018).

Although the role of apelin and APJ receptor in cardiovascular functions has been well-elucidated, little is known for the effect of the central apelinergic system on gastrointestinal (GI) functions. In mice, it has been found previously that central exogenous apelin-13 decreased gastric emptying (GE), GI transit rate (Lv et al., 2011) and distal colonic transit (Yang et al., 2010). In addition, we have found previously that APJ receptor is expressed in stomach-projecting DMV neurons, furthermore, apelin-induced gastroinhibitory action was abolished by truncal vagotomy suggesting that central apelin may regulate parasympathetic outflow by altering vagal efferent signaling (Birsen et al., 2016; Bulbul et al., 2018).

On the other hand, intra-RVLM application of apelin-13 was shown to increase heart rate, arterial pressure and renal sympathetic nerve activity in rats (Masaki et al., 2012; Seyedabadi et al., 2002; Yao et al., 2011; Zhang et al., 2009) raising the possibility that central apelin inhibits gastric motor functions via activating sympathetic outflow in addition to its suppressor action on vagal parasympathetic signaling. However, the relevant mechanism has not been investigated. Neurons within the RVLM directly project to the sympathetic preganglionic neurons within the intermediolateral (IML) column of the spinal cord to regulate sympathetic outflow to the viscera (Badoer, 2001; Browning and Travagli, 2014). In rats, it was demonstrated previously that central administration of corticotropin-releasing factor (CRF) as well as acute

☆ No conflicts of interest.

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https://doi.org/10.1016/j.autneu.2018.03.005
Received 11 February 2018; Received in revised form 15 March 2018; Accepted 27 March 2018

restraint stress loading inhibited solid GE by disturbing the coordination of postprandial antro-pyloric contractions through peripheral α-2 adrenergic receptor-mediated sympathetic pathway (Nakade et al., 2005; Nakade et al., 2006).

Thus, delayed GE could be a consequence of the withdrawn vagal parasympathetic signaling or activated sympathetic outflow. The effect mechanisms of central apelin in autonomic pathways are elucidated incompletely, therefore, in the present study we sought to investigate the potential involvement of autonomic pathways in gastroinhibitory action of centrally administered apelin.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 280–300 g were housed under conditions of controlled temperature (22–24 °C) and illumination (12-h light cycle starting at 6:30 AM). Rats were allowed ad libitum access to food and water. This study was approved by the Animal Ethical Committee of Akdeniz University and performed with standard guidelines for care and use of laboratory animals. All efforts were made to mitigate animal suffering and to reduce the number of animal in experiments. The animals were allowed to get acclimatized to the laboratory conditions 7 days prior to the experiments. Following surgical interventions, postoperative analgesia was provided by intramuscular injection of tramadol hydrochloride on postoperative day-1 (40 mg/kg, twice daily).

2.2. Intracerebroventricular (icv) cannulation

Under isoflurane (Baxter, Deerfield, IL, USA) anesthesia (5% for induction; 2.5% for maintenance in pure O_2 at a flow rate of 200–400 ml/min), the rats were placed on a stereotaxic apparatus and a 26G injection cannula was implanted into the right or left lateral ventricle according to the coordinates (−0.8 mm RC, 1.4 mm ML, lateral from the Bregma; 4 mm ventral from the skull surface) obtained from a rat brain atlas (Paxinos, 1997). The cannula was fixed with an anchor screw and dental cement onto skull surface. Following the surgery, rats were allowed to recover for 7 days. At the end of the experiments, proper cannula placement was validated by injecting of 5 μl of 5% methylene blue solution. After brains were removed and cut sagittally, the spread of the dye in ventricles was examined macroscopically.

2.3. Subdiaphragmatic vagotomy and celiac ganglionectomy

Subdiaphragmatic vagotomy (VGX) and/or celiac ganglionectomy (CGX) were performed in a separate group, following icv cannulation. For VGX, rats were exposed to laparotomy and their stomach was exposed gently and placed on a humidified gauze pad. Under a surgical microscope, anterior and posterior branches of the vagal nerves were transected. CGX was carried out by stripping of the celiac plexus and other visible nerves in the vicinity, as described previously (Nakade et al., 2005). The sham-operated rats underwent only laparotomy and their abdomen was closed without any further surgical intervention. After the surgery, all rats were allowed to recover for 7 days.

2.4. Solid GE measurement

Pre-weighed dry pellets (1.6 g) were given to the rats fasted overnight, as reported previously (Bulbul et al., 2012; Zheng et al., 2010). After the pellets were given, rats were allowed to finish eating within 10 min. Central administration of apelin-13 (30 nmol, icv) was performed immediately after the completion of feeding. Then, rats were sacrificed by exsanguination under isoflurane anesthesia 90 min after completion of feeding. The stomach was removed and gastric content was recovered, dried, and weighed. The data obtained from rats that did not consume 1.6 g of food within 10 min were excluded from the study. The solid GE was calculated as follows:

$$\%GE = 1 - (\text{weight of dried content}/\text{weight of pellet}) \times 100$$

2.5. In-vivo gastric motility

In a separate group of animals, following the icv cannulation, stomach was exposed through a midline incision under isoflurane anesthesia. Two miniature strain gages (Kyowa Electronic Instruments, Tokyo, Japan) were implanted onto the serosal surface of antrum and pylorus. The wires from transducers were exteriorized from the back and protected by a jacket. Animals were allowed to recover for 7 days. On the experiment day, motility experiments were carried out in rats fasted for 18 h. The transducers were connected to a data acquisition system (PowerLab 8/35, ADinstruments, New South Wales, Australia) through a custom made Wheatstone bridge amplifier. After recording the basal fasting motor pattern, 1.6 g pellet was introduced and gastric motility was recorded during feeding and post-prandial period. Central apelin-13 was administered in dose of 30 nmol through the icv cannula 30–45 min after feeding. The fed pattern of antro-pyloric motility was assessed by propagation velocity of coordinated contractions. The area under the curve (AUC) was calculated as motility index (MI) for pre- and post-injection periods. The drug-induced alterations in antral and pyloric postprandial contractions were expressed as % change in MI.

2.6. Chemicals

All drugs were prepared freshly in phosphate buffered saline (PBS) on the experimental days. Nitric oxide synthase (NOS) inhibitor L-NAME, sympatholytic agent guanethidine and muscarinic receptor agonist bethanechol (Sigma-Aldrich, St Louis, MN, USA) were pre-administered intraperitoneally in a volume of 0.5 ml 30 min prior to the central injection apelin-13 (Biotechnology, Dallas, TX, USA). The icv injections were performed using a 33G infusion needle linked to a glass gastight Hamilton syringe with polyethylene tubing while rats were restrained lightly by gently wrapping in a soft cloth. Apelin-13 was delivered in volume of 5 μl over the course of 60 s. Following the injection the needle was kept in place at least for 30 s to prevent backflow of the drug solution.

2.7. Heart rate variability (HRV) analysis

Under isoflurane anesthesia, rats were placed on a stereotaxic frame and arterial pulse recording data were performed using a custom made pulse transducer attached to the tail. Recordings were performed using a digital data acquisition system (PowerLab 8/35, ADinstruments) and interfaced to a personal computer. HRV was assessed within the frequency domains 30 min before (basal) and 30 min after vehicle or apelin-13 administration. The oscillatory components were quantified into low frequency (LF; 0.05–0.75 Hz) and high frequency (HF; 0.75–2.5 Hz) bands.

2.8. Statistical analysis

Data were expressed as mean ± SEM. One-way ANOVA or Kruskal Wallis test followed by Student's *t*-test or Mann Whitney-*U* test were used to determine the significance among groups, as appropriate. For evaluation of the HRV analyses, the spectral analyses were performed using fast Fourier transformation and power of LF and HF components were expressed in normalized units (nu). Repeated measures one-way ANOVA followed by Tukey post hoc were carried out to the statistical comparisons. Repeated-measures ANOVA was performed to compare the apelin- or vehicle-induced changes. All statistical analyses were performed using Graphpad Prism software v.5. A p value < 0.05 was considered to be significant.