Neuropeptides 47 (2013) 19–23

Contents lists available at SciVerse ScienceDirect

Neuropeptides

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

Pancreatic polypeptide and peptide YY_{3-36} induce Ca^{2+} signaling in nodose ganglion neurons

Yusaku Iwasaki [a], Masafumi Kakei [b], Hajime Nakabayashi [c], Enkh-Amar Ayush [a], Misato Hirano-Kodaira [a], Yuko Maejima [a], Toshihiko Yada [a,d,*]

[a] Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Shimotsuke, Tochigi, 320-0498, Japan
[b] First Department of Medicine, Saitama Medical Center, Jichi Medical University School of Medicine, Omiya 1-847, Saitama 337-8503, Japan
[c] Health Science Service Center, Kanazawa University, Kanazawa, Ishikawa, 920-1192, Japan
[d] Department of Developmental Physiology, Division of Adaptation Development, National Institute for Physiological Sciences, Okazaki, Aichi 444-8585, Japan

ARTICLE INFO

Article history:
Received 16 February 2012
Accepted 9 July 2012
Available online 1 September 2012

Keywords:
Pancreatic polypeptide
Peptide YY
Nodose ganglion
Cytosolic Ca^{2+}
Vagal afferent nerves
Feeding

ABSTRACT

Peripheral injection of pancreatic polypeptide (PP) and peptide YY_{3-36} (PYY_{3-36}), the hormones released in response to meals, reduce food intake, in which the rank order of the potency is PP > PYY_{3-36}. These anorectic effects are abolished in abdominal vagotomized rats, suggesting that PP and PYY_{3-36} induce anorexia via vagal afferent nerves. However, it is not clear whether PP and PYY_{3-36} directly act on vagal afferent neurons. In this study, we examined the effects of PP and PYY_{3-36} on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated nodose ganglion neurons of the mouse vagal afferent nerves. At 10^{-11} M, PP but not PYY_{3-36} recruited a significant population of nodose ganglion neurons into $[Ca^{2+}]_i$ increases. PP at 10^{-11} to 10^{-7} and PYY_{3-36} at 10^{-10} to 10^{-7} M increased $[Ca^{2+}]_i$ in a concentration-dependent manner. At submaximal to maximal concentrations of 10^{-10} and 10^{-8} M, PP increased $[Ca^{2+}]_i$ in approximately twice greater population of nodose ganglion neurons than PYY_{3-36}. Furthermore, the majority of PP-responsive neurons also exhibited $[Ca^{2+}]_i$ responses to cholecystokinin-8, a hormone known to induce satiety through activating nodose ganglion neurons. The results demonstrate that PP and PYY_{3-36} directly activate nodose ganglion neurons and suggest that the marked effect of PP on cholecystokinin-8-responsive nodose ganglion neurons could be linked to the regulation of feeding.

Introduction

Vagal afferent nerves sense the peripheral metabolic signals including hormones, nutrients and metabolites, and convert them to neural signals. The neural signals are conveyed to the central nerves systems, thereby regulating feeding and energy metabolisms. The gastrointestinal hormones cholecystokinin-8 (CCK-8) and glucagon-like peptide 1 (GLP-1) are released in response to meals and thought to suppress food intake via their action on vagal afferent nerves (Berthoud, 2008). In fact, CCK-8 and GLP-1 directly interact with and increase cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in the nodose ganglion neurons of vagal afferent nerves (Kakei et al., 2002; Simasko et al., 2002). Recently, we demonstrated that nesfatin-1, a novel anorexigenic peptide expressed in the peripheral organs as well as the brain (Stengel and Tache, 2010), increases $[Ca^{2+}]_i$ in nodose ganglion neurons via Ca^{2+} influx through N-type Ca^{2+} channels (Iwasaki et al., 2009), and that nesfatin-1 reduces food intake via the vagal afferent nerves (Shimizu et al., 2009a,b).

The pancreatic polypeptide (PP) family of peptides, also known as the neuropeptide Y family, consists of PP, peptide YY (PYY), and neuropeptide Y (NPY) (Schwartz, 1983; Gehlert, 1998; Cerda-Reverter and Larhammar, 2000). NPY is a potent orexigenic peptide, which is distributed in and acts on the central nerves system (Morton and Schwartz, 2001). In contrast, PP and PYY_{3-36} are anorexigenic peptides that are released primarily from PP-cells of pancreatic islets and from L-cells within the intestinal mucosa of the ileum and large intestine, respectively (Ekblad and Sundler, 2002). PP and PYY are 36-amino-acid peptides with high homology, and PYY_{3-36} is a peptide cleaved from PYY by dipeptidyl peptidase IV in the circulation (Eberlein et al., 1989). PP and PYY_{3-36} are released in the postprandial period (Adrian et al., 1976, 1985) and, when peripherally injected, strongly reduce food intake in both rodents and humans (Malaisse-Lagae et al., 1977; Batterham et al., 2002, 2003a,b). The postprandial release and plasma levels

Abbreviations: CAP, capsaicin; CCK, cholecystokinin; $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; GLP-1, glucagon-like peptide 1; HKRB, HEPES-buffered Krebs–Ringer bicarbonate buffer; MEM, hypothalamic minimal essential medium; NPY, neuropeptide Y; PYY, peptide YY; PP, pancreatic polypeptide.

* Corresponding author at: Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, 3311-1, Yakushiji, Shimotsuke, Tochigi, 329-0498, Japan. Tel.: +81 285 58 7319; fax: +81 285 44 9962.
E-mail address: tyada@jichi.ac.jp (T. Yada).

0143-4179/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.npep.2012.07.006

and PP and/or PYY_{3-36} are reduced or blunted in humans with morbid obesity (Lassmann et al., 1980; Marco et al., 1980), which could be causally implicated in the pathophysiology of obesity and eating disorders. The reduction of food intake by intraperitoneal injection of PP and that of PYY_{3-36} are impaired in the abdominal vagotomized animals (Asakawa et al., 2003; Koda et al., 2005), suggesting that PP and PYY_{3-36} exert the anorexigenic effects through their action on the vagal afferent nerves. However, it is not clear whether PP and PYY_{3-36} directly act on vagal afferent nerves.

In this study, to investigate the direct effect of PP and PYY_{3-36} on the vagal afferent nerves, we isolated single neurons from the mouse nodose ganglion, monitored their activity by measuring $[Ca^{2+}]_i$, and investigated direct effects of the hormones on $[Ca^{2+}]_i$. CCK-8 is an well established peripheral peptide that acts on the vagal afferent nerves to produce anorexia (Berthoud, 2008). Capsaicin (CAP) is also known to activate vagal afferent nerves (Gallego and Eyzaguirre, 1978). We examined whether the nodose ganglion neuron that responds to PP is distinct from or overlapping with the CCK-8- or CAP-responsive neuron.

Materials and methods

Materials

Rat PP was purchased from GeneScript (Piscataway, NJ, USA). Human PYY_{3-36} and CCK-8 (sulfated form) were purchased from Peptide Institute, Inc. (Osaka, Japan). CAP was obtained from Sigma (MO, USA).

Animals

Adult male ICR mice (Japan SLC, Shizuoka, Japan) were housed for at least 1 week under conditions of controlled temperature (23 ± 1 °C), humidity (55% ± 5%), and lighting (light on at 07:30 and off at 19:30). Food and water were available ad libitum. Procedures of animal experiments were approved by the Animal Care and Use Committee of the Jichi Medical University.

Preparation of nodose ganglion neurons from mice

Single neurons were isolated from mouse nodose ganglia, as previously described (Iwasaki et al., 2009). Briefly, male ICR mice were anesthetized with α-chloralose and urethane (0.1 g/kg and 1 g/kg, i.p., respectively), and nodose ganglia were excised. These ganglia were rapidly teased using 27G needle in ice-cold HEPES-buffered Krebs–Ringer bicarbonate buffer (HKRB) composed of 4.7 mM KCl, 1.2 mM KH_2PO_4, 129 mM NaCl, 5 mM $KaHCO_3$, 1.2 mM $MgSO_4$, 1.8 mM $CaCl_2$, and 10 mM HEPES with pH adjusted at 7.4 using NaOH supplemented with 5.6 mM glucose. The tissue pieces were dissociated by incubation for 20 min at 37 °C in the HKRB containing 0.5 mg/ml collagenase Ia (Sigma), 0.5 mg/ml dispase II (Roche, Basel, Swiss), 15 μg/ml DNase II type IV (Sigma), and 0.75 mg/ml bovine serum albumin. Cells were gently triturated using fire-polished pasteur pipettes and centrifuged two times in Eagle's minimal essential medium (MEM) containing 5.6 mM glucose supplemented with 10% feral bovine serum, 100 μg/ml streptomycin, and 100 units/ml penicillin. Cells were resuspended in MEM and plated onto coverslips coated with poly-L-lysine. Cells were cultured in MEM for overnight.

Measurements of $[Ca^{2+}]_i$ in nodose ganglion neurons

Measurements of $[Ca^{2+}]_i$ in primary cultured nodose ganglion neurons were carried out according to the previous report (Iwasaki et al., 2009). Briefly, following incubating with 2 μM fura-2 AM (DOJINDO, Kumamoto, Japan) for 30 min at 37 °C, the cells were mounted in a chamber and superfused with HKRB at 1.3 ml/min at 30 °C. Fluorescence images at 510 nm due to excitation at 340 and 380 nm were detected every 10 s with an intensified charge-coupled device camera, and ratio images were produced by an Aquacosmos ver. 2.5 (Hamamatsu Photonics, Shizuoka, Japan). Nodose ganglion neurons were selected by their large and round cell bodies, while non-neuronal cells had spindle or filamentous shapes. When $[Ca^{2+}]_i$ changed within 5 min after addition of agents and their amplitudes were at least twice larger than the fluctuations of the baseline, they were considered responses. Only the cells that responded to 55 mM KCl at the end of recordings were analyzed.

Data analysis

All data were shown as means ± SEM. Statistical analysis was performed chi-square test using Prism 5 software. $P < 0.05$ was considered significant.

Results

PP increased $[Ca^{2+}]_i$ in nodose ganglion neurons

To evaluate the direct effect of PP on the vagal afferent nerves, we measured the effect of PP on $[Ca^{2+}]_i$ in fura-2 loaded nodose

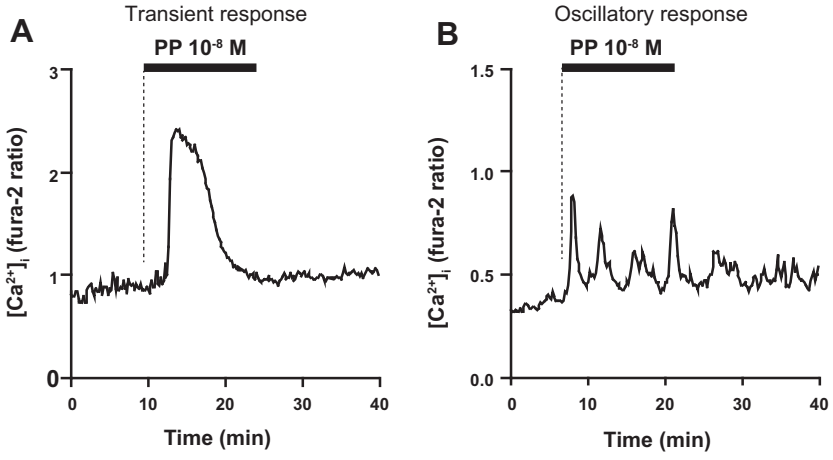

Fig. 1. Pancreatic polypeptide (PP) increases $[Ca^{2+}]_i$ in nodose ganglion neurons. (A and B): Administration of 10^{-8} M PP for 15 min induced $[Ca^{2+}]_i$ in transient (A) or oscillatory (B) patterns, and the $[Ca^{2+}]_i$ returned to the basal level after washing out PP in single nodose ganglion neurons. $[Ca^{2+}]_i$ is expressed by fura-2 ratio and bars above the tracings indicate the periods of administration of agents specified. The data are representative of six neurons in A and 20 neurons in B.