Biochemical and Biophysical Research Communications 456 (2015) 167–172

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Prostaglandin potentiates 5-HT responses in stomach and ileum innervating visceral afferent sensory neurons

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ARTICLE INFO

Article history:
Received 7 November 2014
Available online 22 November 2014

Keywords:
Gastrointestinal disorder
Visceral afferents neuron
Prostaglandins
Serotonin
E-prostanoid receptors

ABSTRACT

Gastrointestinal disorder is a common symptom induced by diverse pathophysiological conditions that include food tolerance, chemotherapy, and irradiation for therapy. Prostaglandin E_2 (PGE_2) level increase was often reported during gastrointestinal disorder and prostaglandin synthetase inhibitors has been used for ameliorate the symptoms. Exogenous administration of PGE_2 induces gastrointestinal disorder, however, the mechanism of action is not known. Therefore, we tested PGE_2 effect on visceral afferent sensory neurons of the rat. Interestingly, PGE_2 itself did not evoked any response but enhanced serotonin (5-HT)-evoked currents up to 167% of the control level. The augmented 5-HT responses were completely inhibited by a 5-HT type 3 receptor antagonist, ondansetron. The PGE_2-induced potentiation were blocked by a selective E-prostanoid type4 (EP_4) receptors antagonist, L-161,982, but type1 and 2 receptor antagonist AH6809 has no effect. A membrane permeable protein kinase A (PKA) inhibitor, KT5720 also inhibited PGE_2 effects. PGE_2 induced 5-HT current augmentation was observed on 15% and 21% of the stomach and ileum projecting neurons, respectively. Current results suggest a synergistic signaling in visceral afferent neurons underlying gastrointestinal disorder involving PGE_2 potentiation of 5-HT currents. Our findings may open a possibility for screen a new type drugs with lower side effects than currently using steroidal prostaglandin synthetase inhibitors by selectively targeting EP_4 receptor/PKA pathway without interrupt prostaglandin synthesis.

1. Introduction

Nausea, vomiting, and diarrhea are common symptoms of gastrointestinal disorder and frequently occur during food poisoning as well as cytotoxic chemotherapy, radiation therapy, and food intolerance. There is no single etiology which can explain induction of gastrointestinal disorder in such diverse circumstance, however, positive relationship was observed between prostaglandins concentration increase and onset of the symptom [1–3]. Actually, exogenous administration of prostaglandins induces nausea, vomiting and diarrhea in mammals including human [2,4] and prostaglandin synthetase inhibitors ameliorate gastrointestinal disorders [1]. Paracrine lipid compound prostaglandins are composed with few different types and among them prostaglandin E2 (PGE_2) is most consistently reported as inducing emesis or diarrhea in human [5,6] as well as experimental animals [7]. Despite accumulated evidence, the underlying mechanism of PGE_2 mediated gastrointestinal disorder is still unknown. Visceral afferent vagus neurons innervate submucosa of the intestinal tract and transfer intestinal organs' activity to nucleus tractus solitarii in the brain stem. Electrical stimulation of vagal nerve induces emesis and diarrhea [8,9] and lesions of abdominal visceral nerve block emesis [10]. Therefore, activity of visceral afferents is closely related gastrointestinal disorder. In this experiment, PGE_2 effect was tested on acutely dispersed gastrointestinal vagal afferent neurons from rat nodose ganglia with electro physiological methods.

Abbreviations: AH6809, (6-isopropoxy-9-oxoxanthene-2-carboxylic acid); CPBG, (1-(3-chlorophenyl) biguanide hydrochloride); DiI, (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate); EC, enterochromaffin cell; EP, E-prostanoid; 5-HT, serotonin; KT5720, [(9*S*,10*R*,12*R*)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1*H*-diindolo[1,2,3-fg:3_,2_,1_-kl] pyrrolo [3,4-i] [1,6]benzodiazocine-10-carboxylicacid hexyl ester]; L-161,982, (*N*-[[4′-[[3-butyl-1,5-dihydro-5-oxo-1-[2-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazol-4-yl]methyl][1,1′-biphenyl]-2-yl]sulfonyl]-3-methyl-2-thiophenecarboxamide); Ondansetron, (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl) methyl]-4*H*-carbazol-4-one hydrochloride); PGE_2, prostaglandin E_2; PKA, protein kinase A.

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http://dx.doi.org/10.1016/j.bbrc.2014.11.053
0006-291X/

2. Materials and methods

2.1. Dissociation of nodose neuron

All animal procedures were conducted with the approval of the institutional Animal Care and Use Committee in Kyung Hee University. These procedures were in accordance with National Veterinary Research & Quarantine Service guidelines. Nodose ganglion neurons dissociated as previously described elsewhere [11]. Briefly, in deeply anesthetized (Zolazepam 0.3 ml/kg; Virbac Laboratories., Carros, France) 2–4 weeks old male Sprague–Dawley rats (Orient Bio Inc., Seongnam, Korea), vagus nerve was exposed from the jugular foramen for a length of 0.6 cm caudally. After the vagus nerve was separated from surrounding tissue, the attached ganglion was immediately placed into a Petri dish containing chilled (4 °C) artificial CSF (ACSF) solution, which was composed of the following (in mM): 125 NaCl, 3 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 glucose, and 2 $CaCl_2$, and bubbled with 95% O_2-5% CO_2. After removed excess connective tissues, the bulb of the nodose ganglion was incubated for 23–45 min at 31 °C in an ACSF solution containing type II-S trypsin (5–6 mg/ml at 1310 units/mg; Sigma–Aldrich., St. Louis, MO). Following protease treatment, the ganglion was placed in enzyme-free ACSF for 1–3 h at room temperature. The ganglia were mechanically dissociated using fire-polished Pasteur pipettes in a glass bottom perfusion chamber filled with standard external recording solution containing (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 10 glucose (pH was adjusted to 7.4 with Tris-base).

2.2. Labeling of intestinal wall innervating neurons

Studies employing stomach and ileum labeling were initiated in young Sprague–Dawley rats (3 weeks). For all surgical procedures, the rats were anesthetized with zolazepam (0.1 ml/kg) and surgeries were performed under aseptic conditions. DiI (Molecular Probes, Eugene, OR) a fluorescent lipophilic indocarbocyanine dye was used to anterogradely label vagal afferent neurons that innervating the stomach and ileum. In each tissue, DiI was injected in 10–15 sites (0.5 μl/site) using a microsyringe (Hamilton, Reno, NV) as reported elsewhere [12]. The dye was injected dorsal and ventral surfaces of the stomach and the ileum. Any spillage of dye from the injection site was immediately removed with a cotton swab. Although some labeling in the nodose ganglia was observed within 5 days after injection, we allowed 10 days after surgery for dye transport to accumulate in the nodose ganglia. Isolations were taken from at least 6 animals for both the stomach- and ileum-labeled groups. To visualize take IR-DIC and fluorescence images used a Hamamatsu ORCA-R2 CCD camera (Hamamatsu, Japan). The specific filter sets were selected from Chroma (Chroma Technology Corp., Rockingham, VT) and used with the Olympus BX51WI. For visualizing carbocyanine dyes, we used the Cy3/TRITC (Rhodamine)/DiI filter set with excitation (HQ 535/50), emitter (HQ 610/75) and dichroic (Q565lp).

2.3. Electrical measurements and data analysis

For voltage-clamp recording, isolated neurons were visualized using a phase contrast microscope (IX70; Olympus, Tokyo, Japan) or IR-DIC microscope (BX51; Olympus). Recordings utilized a Multiclamp 700B (Molecular Devices, Sunnyvale, California) and pClamp 9 software (Molecular Devices). To electrically record with minimal loss of intracellular constituents, perforated (nystatin) patch method was used. Recording electrodes were back-filled with a pipette solution composed of the following (in mM): 50 KCl, 100 K gluconate, and 10 HEPES; the pH of this solution was adjusted to 7.2 with Tris-OH. The final concentration of nystatin was 450 μg/ml. The nystatin-internal solution filled patch-pipettes has 6–7 MΩ resistance. Junction potential for these internal and standard external solutions was 11.9 mV and compensated throughout experiments. The neurons were voltage clamped at −50 mV, and currents were sampled at 10 kHz with 3 kHz filtering and saved to computer. Only cells with holding currents of <60 pA and peak to-peak noise levels of <15 pA were selected for detailed studies to assure the highest quality recordings. All experiments were conducted at room temperature (21–22 °C). Data were analyzed off-line using pClamp 9 software. All data are represented as mean ± standard errors (SEM). Statistical comparisons between two groups were analyzed by the Student's *t*-test. For multiple comparisons, one-way analysis of variance (ANOVA) with post hoc multiple comparisons (Bonferroni/Dunn's correction, StatView, SAS Institute Inc., Cary, NC) was used. Chi-squared test was used to compare 5-HT or PGE_2-responsive neurons distribution in stomach- or ileum-innervating neurons. Differences were considered statistically significant for p values < 0.05. The continuous curves for concentration–response relationships were constructed according to a modified Michaelis–Menten Eq. (1), using a least-square fitting routine:

$$I = I_{max}C^n/(C^n + EC_{50}) \quad (1)$$

where I is the drug-induced current amplitude, I_{max} is the maximum current amplitude and C is the corresponding drug concentration. EC_{50} and n denote the half-maximum effective concentration and the Hill coefficient, respectively. The data for the concentration-inhibition curve were plotted to the following equation using a least-square fitting routing 2:

$$I = I_{max}[1 - [C^n/(C^n + IC_{50}^n)]] \quad (2)$$

where I is the current amplitude without antagonists, C is the concentration of the antagonist, and IC_{50} is the concentration for the half-maximum inhibition.

2.4. Drugs

Serotonin (5-HT), CPBG (1-(3-chlorophenyl)biguanide hydrochloride), prostaglandin E_2 (PGE_2), and EP1 and EP2 receptor antagonist AH6809 (6-Isopropoxy-9-oxoxanthene-2-carboxylic acid), and PKA selective inhibitors KT5720 [(9*S*,10*R*,12*R*)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1*H*-diindolo[1,2,3-fg:3_,2_,1_-kl] pyrrolo [3,4-i] [1,6]benzodiazocine-10-carboxylicacid hexyl ester] were purchased from Sigma–Aldrich (St. Louis, MO). 5-HT_3 receptor selective antagonist ondansetron (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one hydrochloride) and EP4 receptor selective antagonist L-161,982 (*N*-[[4′-[[3-butyl-1,5-dihydro-5-oxo-1-[2-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazol-4-yl]methyl][1,1′-biphenyl]-2-yl]sulfonyl]-3-methyl-2-thiophenecarboxamide) were purchased from Tocris Cookson (Ballwin, MO). DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) was purchased from Molecular Probes. The drugs which were insoluble in water were first dissolved in dimethylsulfoxide (DMSO), and then the stock solution was diluted with the external solution just before use. The final concentrations of DMSO were always less than 0.1%. At these concentrations, DMSO alone had no effect on membrane potential or electrical activity. All of the drugs were applied via a rapid application Y-tube microperfusion system that provided complete solution changes surrounding the recorded neurons within 0.1 s [13].

3. Results

3.1. 5-HT evoked inward currents through 5-HT$_3$ receptor activation

In artificial CSF solution, acutely dispersed nodose neurons have resting membrane potential −53 ± 3 mV (n = 5). Thus, throughout experiments holding potential is clamped at −50 mV. To examine prostaglandin effect on visceral afferent signaling, 1–50 μM PGE_2 was tested on nodose ganglion neurons. Surprisingly, in all tested concentrations, two minute continuous application of PGE_2 failed to induce any detectable current (n = 15, Fig. 1A(a)). Given this unexpected result, we considered the possibility that prostaglandin was acted by augmenting other emetogenic stimulant evoked response. It is reported that PGE_2 induced emesis is inhibited by 5-HT$_3$ receptor antagonist [7] and co-treatment of 5-HT$_3$ receptor antagonist with prostaglandins synthesis inhibitor exert an additive effect to inhibit emesis [14]. Aforementioned reports suggest that 5-HT$_3$ receptor activation underlie PGE_2-mediated emesis. In further experiments, we tested whether that PGE_2 raise visceral afferent neurons activity by augmenting the 5-HT evoked response. For that we first characterized 5-HT responses in nodose ganglion neurons. Among 92 tested neurons 35% (n = 32) of them responded to 5-HT by evoking inward current. From 1 to 1000 μM concentration, 5-HT current was elicited in a concentration dependent manner (Fig. 1A(b)). To configure ion selectivity of the 5-HT currents, we performed steady-state current–voltage (I–V) relationships. The constructed I–V plot has an average reversal potential 7.5 ± 2.5 mV (n = 5, Fig. 1B) and suggests its close relation with nonselective cation channel 5-HT$_3$ receptor. The I–V plot also exhibited strong inward rectification. In further experiments selective agonist and antagonist of the 5-HT$_3$ receptors are used to configure subtype of the 5-HT receptor. A 5-HT$_3$ selective agonist, CPBG, induced currents similar to the 5-HT responses (Fig. 1A(c)). The maximum currents induced by 1 mM 5-HT and 0.1 mM CPBG were 380 ± 60.9 and 376 ± 60.8 pA, respectively. The current amplitudes of 5-HT and CPBG responses are plotted in Fig. 1C, in which half-maximum effective concentration and the Hill coefficients were 6.72 μM and 1.23 for 5-HT (n = 17), and 1.67 μM and 0.87 for CPBG (n = 17), respectively. A 5-HT$_3$ receptor antagonist, ondansetron (10 nM), inhibited the 4 μM 5-HT responses concentration dependent manner in all tested neurons (n = 7) (Fig. 1C). Half-maximum inhibition concentration for ondansetron on 5-HT evoked currents is 2.2×10^{-10} M. However, 5-HT$_4$ receptor selective antagonist, GR113808 (100 nM) failed to suppress the 5-HT induced currents (p > 0.5, paired t-test, n = 4). These results show that nodose ganglion neurons express functioning 5-HT$_3$ receptors, which induce inward currents in response to >0.1 μM 5-HT.

3.2. ProstaglandinE_2 potentiate 5-HT response via PKA pathway

In the following experiments, from 0.001 to 10 μM of the PGE_2 effect was tested on the 5-HT-evoked currents. Each concentration of the PGE_2 were pre-applied for 5 min before simultaneous application with 3 μM 5-HT. PGE_2 augmented the 5-HT responses in half of 5-HT responsive neurons (n = 7/15). The 5-HT responses were significantly potentiated (p < 0.001, repeated measure ANOVA) by ⩾0.01 μM PGE_2 and the potentiation peaked at 1 μM. The potentiation rate by 0.01, 0.1, 1 and 10 μM the PGE_2 were 130 ± 13.1, 151 ± 13.4, 167 ± 27 and 160 ± 130% of the control response, respectively (n = 5, Fig. 2A). Ondansetron (10 nM) completely blocked the augmented 5-HT response in all tested neurons (n = 4). In the consecutive experiments, PGE_2 induced 5-HT response potentiation was measured by increasing PGE_2 pretreatment time from 0.5 to 5 min. As shown in Fig. 2B, the augmented current grew with PGE_2 pretreatments of up to 2 min before plateauing (p < 0.02, ANOVA). The potentiation rate after 0.5, 2 and 5 min pre-treatment with PGE_2 were 125 ± 22.2, 167 ± 27.4, and 168 ± 22% of the control response, respectively. Thus, in subsequent experiments, PGE_2 pretreatments were fixed at 2 min. This time-dependent slow augmentation suggests that intracellular second messenger system may underlie PGE_2-mediated 5-HT

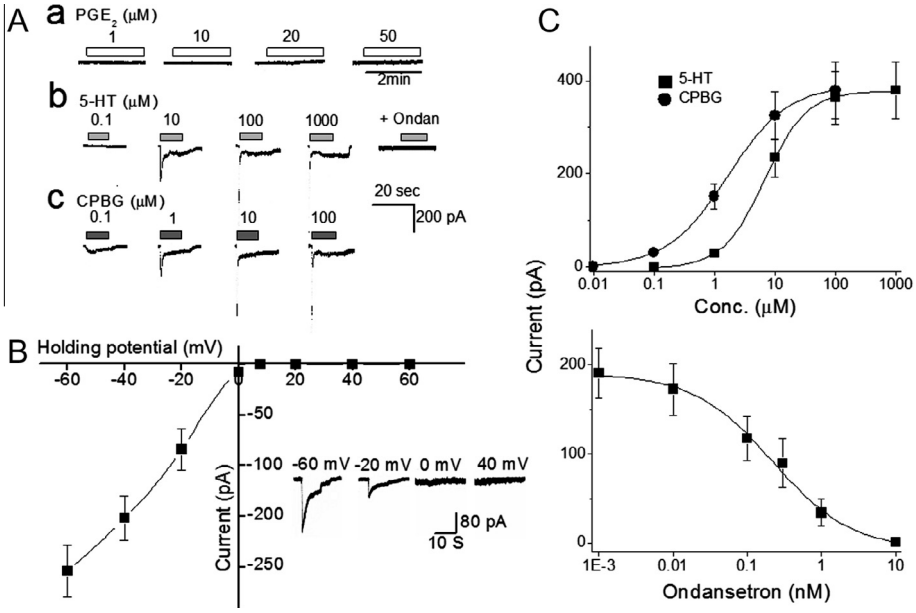

Fig. 1. 5-HT-induced inward rectifying current in acutely dispersed afferent vagus neurons. (A) (a) PGE2 failed to induce any current responses in nodose neurons. 5-HT-(b) and CPBG (c)-evoked currents at selected concentrations. Ondansetron (10 nM, Ondan) blocked 10 μM 5-HT-evoked currents. (B) The I–V relationships for the 3 μM 5-HT-induced currents. 5-HT-induced currents (insets) at various holding potentials in the same neuron. Each point and vertical line represents the mean and SEM from 5 different neurons. (C) The upper graphs shows concentration–response relationships for 5-HT (■) and CPBG (●). The bottom graph shows concentration-inhibition relationships for the ondansetron, on 5-HT currents. Each point and the vertical lines represent the mean + s.e.mean from 17 neurons. The current recordings were made at a holding potential of −50 mV.

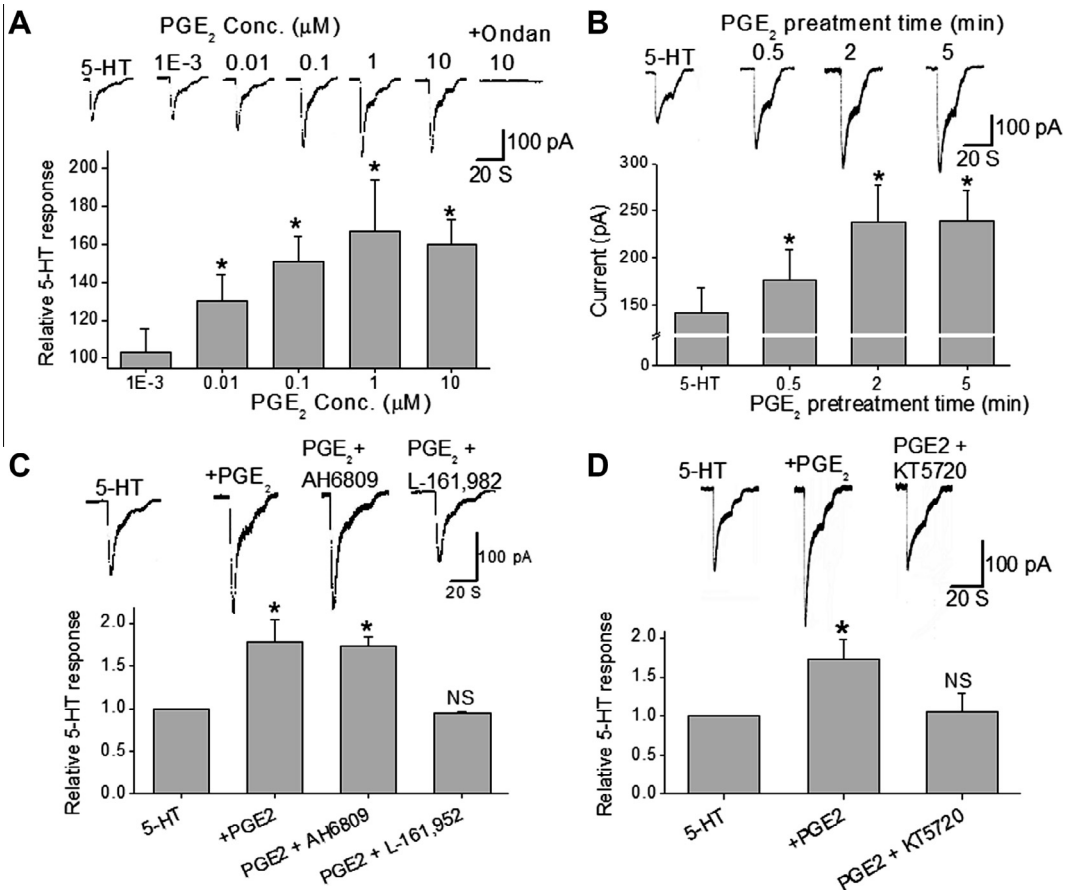

Fig. 2. EP receptors antagonists and PKA inhibitors effects on PGE2-induced potentiation. (A) The traces show PGE2 concentration dependent potentiation of 5-HT currents. The augmented 5-HT responses were completely blocked by ondansetron (Ondan). The pretreatment time for PGE2 was 5 min and successively 3 μM 5-HT was applied simultaneously. The traces were taken form same cell. The graph summarized various concentration of PGE2 effects on 5-HT currents ($n = 5$). $^*p < 0.05$. (B) 1 μM PGE2-induced augmentation of 5-HT-evoked currents varied depends on pretreatment time. The traces were taken form same cell. The graph summarized PGE2 effects on 5-HT currents at different pretreatment time ($n = 6$). $^*p < 0.05$. (C) The representative traces shown that EP 4 receptor selective antagonist L-161,982 (10 μM), but not EP1 and EP2 receptor antagonist AH6809 (3.5 μM), blocked PGE2-induced potentiation in same tested cell. EP receptor antagonists were co-applied with 1 μM PGE2. The graph summarized EP receptors antagonists' effect on PGE2-induced potentiation ($p > 0.06$). (D) The traces show that PKA selective inhibitors KT5720 blocked PGE2-induced potentiation of the 5-HT response. PGE2 (1 μM) failed to potentiate 5-HT current in the presence of KT5720 ($n = 9$). The current responses were normalized by own 5-HT evoked control response. All 5-HT control responses were taken by 3μ 5-HT.

response potentiation. To identify the specific E-prostanoid (EP) receptor responsible for potentiation of 5-HT currents, selective EP_1 and EP_2 receptor antagonist AH6809 and EP_4 receptor selective antagonist L-161,982 were tested. PGE_2-induced potentiation was completely blocked ($p < 0.001$, $n = 5$) by L-161,982 (10 μM), but AH6809 (3.5 μM) had no effect ($p > 0.66$, $n = 8$) (Fig. 2C). This result shows that EP_4 receptor mediate facilitation of 5-HT current. It is known that the cloned human EP_4 receptor signals through increased cAMP [15]. Hence, we further tested whether PGE_2 potentiate 5-HT response in a cAMP dependent manner or not in visceral afferent neurons. As shown in Fig. 2D, PGE_2 failed to augment 5-HT response after 4 min continuous treatment with a membrane permeable selective PKA inhibitor, KT5720. The above results show that PGE_2 augmented 5-HT currents via activation of EP_4 receptor/PKA pathway in the vagal afferent neurons.

3.3. PGE_2 unequally activate vagal afferent neurons from ileum and stomach

Activation of the gastrointestinal tract innervating vagal afferent neuron is integral for induce emesis [8]. To investigate PGE_2 sensitivity of specific regions of the gastrointestinal tract projecting vagus neurons, a carbocyanine dye DiI was implanted on stomach and ileum (see methods). Ten days after surgery, the stomach or ileum innervating neurons are identified by their fluorescent cell bodies among our dispersed neurons (Fig. 3A). Stomach-innervating neurons (33 cells from 6 different animals) were divided into 3 different groups: where 15.1% of tested cells responded to both PGE_2 and 5-HT but 20.9% of the neurons responded to only 5-HT. The remaining 64% of cells responded to neither drugs (Fig. 3B). Meanwhile, Ileum-innervating neurons (38 cells from 5 different animals) were also divided into same groups: where 21.2% of tested cells responded to both PGE_2 and 5-HT but 25.8% of the neurons responded to only 5-HT. The remaining 53% of cells responded to neither drugs. Thus, the ileum innervating vagus neurons have higher response rate for PGE_2 and 5-HT than stomach-innervating neurons (Chi-squared test, $p < 0.038$). However, PGE_2-induced potentiation rate were not differ between stomach- and ileum-innervating neurons ($p > 0.05$).

4. Discussion

In current experiment, PGE_2 alone did not induce any response but augmented 5-HT_3 receptor-mediated currents via activation of EP_4 receptor/PKA pathway in the gastrointestinal tract innervating vagal afferent neurons. Therefore, PGE_2-mediated gastrointestinal disorder necessitates concurrent concentration increase of 5-HT

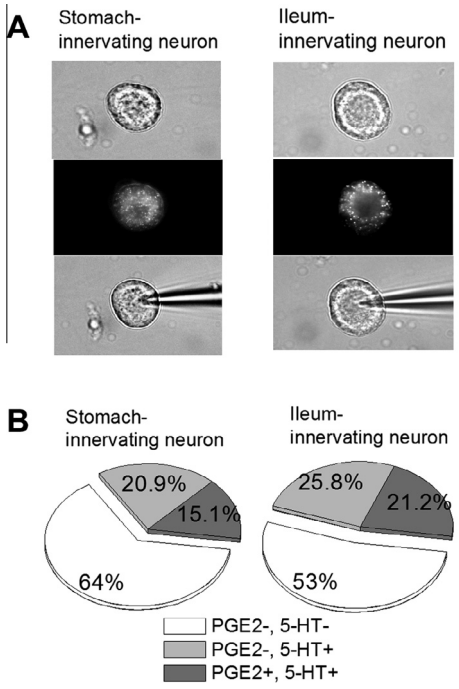

Fig. 3. Stomach and ileum projecting neurons have different sensitivity for PGE2 and 5-HT. (A) IR-DIC image (top) of representative nodose ganglion neuron for which we found co-localized fluorescence from stomach or ileum labeling. Second micrographs display images with typical pattern of fluorescent captured in the same neurons. Overlay of fluorescence images with an IR-DIC image of nodose ganglion neuron cell body (bottom), which used for recording currents. (B) A schematic summary representing the distribution of PGE2 and 5-HT neurons in stomach or ileum-innervating group neurons. Both of the PGE2 and 5-HT responsive group (PGE2 +, 5-HT +), PGE2 non-responsive and 5-HT responsive group (PGE2 −, 5-HT +), and non-responsive group for both of the PGE2 and 5-HT. The numbers represent the ratio of the neurons in each group.

and PGE_2. In mammals, over 90% of the 5-HT in the body is stored in enterochromaffin cells (EC) in the epithelia lining of the digestive tracts and released by physical or chemical stimuli [16–18]. Unlike 5-HT, there is no single origin of the prostaglandins but it's concentration in the intestinal tract increases by chemotherapy drug [19], food intolerance, and radiation therapy [2,6]. Thus, previous reports shown that gastrointestinal disorder inducing physical and chemical stimuli also elevate 5-HT and PGE_2 level in the intestinal tract.

In this experiment, visceral afferent neuron of the rat was employed to investigate neuronal mechanism of PGE_2-mediated gastrointestinal disorder. Emesis is one of the major symptoms of the gastrointestinal disorder and the rat did not vomit. Therefore, most *in vivo* studies has been used ferret, mink, dog, or even monkey to test emetic or antiemetic efficacy of drugs. However, recent discovery of emetogenic drug-induced kaolin-consuming behavior, pica, in the rat make possible to use the rat to test emetic or antiemetic efficacy of diverse compounds. De Jonghe et al., [20] inferred that kaolin consuming may help to ameliorate cisplatin-induced gastrointestinal discomfort. Now this pica model is used in many other research groups to test anti-emetic drugs' efficacy with the rat [21]. Aforementioned reports imply that the rat is an appropriate subject for test gastrointestinal disorder including emesis.

It is clear that abdominal vagal afferent neurons from gastrointestinal tract exert major role on gastrointestinal disorder but it is still obscure that whether vagal afferents from different regions of the gastrointestinal tract were differently contribute for gastrointestinal signaling [8–10]. To answer those questions, ascending afferent vagus neurons from stomach and ileum were identified using anterograde labeling technique. Among the small intestine ileum is most frequently studied in relation with cytotoxic drug induced gastrointestinal disorder [17,22]. In that reason we also selected ileum for this experiment. For first time, we found that visceral afferent sensory neurons from the stomach and ileum of the small intestine did not have equal sensitivity for PGE_2 and 5-HT. These results suggest that targeting delivery of drugs for more sensitive regions of the gastrointestinal tract may improve efficacy of the treatment.

A steroidal anti-inflammatory drug dexamethasone has been used for suppress gastrointestinal disorder during chemotherapy and radiation therapy [23,24]. In clinical trials dexamethasone alone effective to prevent chemotherapy-induced side effects, especially for nausea and vomiting, but combined use with 5-HT_3 antagonist was more effective than either dexamethasone or 5-HT_3 antagonist alone [25]. Anti-emetic efficacy of corticosteroids increases in proportion to their anti-inflammatory potency [24]. Likewise, nonsteroidal anti-inflammatory drugs, indomethacin and meloxicam, also inhibit cisplatin-induced emesis in experimental animals [26]. Thus, it has been speculated that anti-inflammatory drugs exert their antiemetic activity via inhibiting prostaglandins synthesis [27]. Despite wide use of dexamethasone, steroidal anti-inflammatory drugs have many side effects including gastrointestinal disturbance and immunosuppressant action [28]. Consequently there has been a necessity for develop new anti-emetics for replace steroidal anti-inflammatory drugs. In this experiment, PGE_2-induced 5-HT response potentiation was completely blocked by EP_4 receptor antagonist and PKA inhibitor. Therefore, our new findings open a possibility for screen a new type anti-emetic and anti-diarrhea drug with lower side effects than steroidal anti-inflammatory drug by selectively targeting single receptors in the downstream of prostaglandin signaling pathway without interrupt prostaglandin synthesis.

Conflict of interest

None of the authors have any conflicts of interests.

Acknowledgments

This work was supported by grant from National Research Foundation of Korea (2010-0005509).

We are grateful to Dr. M.C. Andresen for helpful criticisms in preparing the manuscript.

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