Role of prostaglandin D$_2$ in mast cell activation-induced sensitization of esophageal vagal afferents

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Zhang S, Grabauskas G, Wu X, Joo MK, Heldsinger A, Song I, Owyang C, Yu S. Role of prostaglandin D$_2$ in mast cell activation-induced sensitization of esophageal vagal afferents. Am J Physiol Gastrointest Liver Physiol 304: G908–G916, 2013. First published March 7, 2013; doi:10.1152/ajpgi.00448.2012.—Sensitization of esophageal afferents plays an important role in esophageal nociception, but the mechanism is less clear. Our previous studies demonstrated that mast cell (MC) activation releases the preformed mediators histamine and tryptase, which play important roles in sensitization of esophageal vagal nociceptive C fibers. PGD$_2$ is a lipid mediator released by activated MCs. Whether PGD$_2$ plays a role in this sensitization process has yet to be determined. Expression of the PGD$_2$ DP1 and DP2 receptors in nodose ganglion neurons was determined by immunofluorescence staining, Western blotting, and RT-PCR. Extracellular recordings were performed in ex vivo esophageal-vagal preparations. Action potentials evoked by esophageal distension were compared before and after perfusion of PGD$_2$, DP1, and DP2 receptor agonists, and MC activation, with or without pretreatment with antagonists. The effect of PGD$_2$ on 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-labeled esophageal nodose neurons was determined by patch-clamp recording. Our results demonstrate that DP1 and DP2 receptor mRNA and protein were expressed mainly in small- and medium-diameter neurons in nodose ganglia. PGD$_2$ significantly increased esophageal distension-evoked action potential discharges in esophageal nodose C fibers. The DP1 receptor agonist BW 245C mimicked this effect. PGD$_2$ directly sensitized DiI-labeled esophageal nodose neurons by decreasing the action potential threshold. Pretreatment with the DP1 receptor antagonist BW A868C significantly inhibited PGD$_2$ perfusion- or MC activation-induced increases in esophageal distension-evoked action potential discharges in esophageal nodose C fibers. In conclusion, PGD$_2$ plays an important role in MC activation-induced sensitization of esophageal nodose C fibers. This adds a novel mechanism of visceral afferent sensitization.

esophagus; mast cell; PGD$_2$; vagal afferent; C fiber

Mast cells (MCs) are found in abundance in the gastrointestinal (GI) tract. Inappropriate MC activation is a pivotal event in allergic reactions, such as food allergies, in the GI tract (30). However, the mechanism whereby inflammatory mediators induce sensitization on esophageal peripheral afferents is less clear (19).

METHODS

Male Hartley guinea pigs (Hilltop Laboratory Animals, Scottsdale, PA; 100–300 g body wt) were used in these experiments, which were approved by the University of Michigan Committee on Use and Care of Animals.

Immunofluorescence staining. Nodose ganglia (n = 4) were dissected and processed for immunofluorescence double staining of PGD$_2$ DP$_1$ and DP$_2$ receptors with isolectin B4 (IB4), as described previously (31). The primary antibodies included rabbit polyclonal anti-DP$_1$ receptor antibody (1:200 dilution; Cayman Chemical, Ann Arbor, MI) and rabbit anti-DP$_2$ receptor polyclonal antibody (1:200 dilution; Novus Biologicals, Littleton, CO). The secondary antibodies included Alexa Fluor 594-conjugated anti-rabbit antibody (1:200 dilution; Invitrogen, Carlsbad, CA). IB4 was detected by IB4-conjugated biotin (Invitrogen) and further stained by streptavidin conjugated to Alexa Fluor 488 (Invitrogen). The stained slides were briefly checked under an epifluorescence microscope, and four well-stained cross sections on each slide from each nodose ganglion were randomly selected for counting. Quantitative analysis of immunofluorescence-stained neurons on digitized images was determined by threshold intensity segmentation using iVision software (Biovision Technologies, Exton, PA); neurons were considered to be positive if fluorescence intensity was two times background fluorescence. In general, 20

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DP1 receptor-positive neurons from each section were selected. Then colocalization of DP1 or DP2 receptors with IB4 was determined. A total of 240 nodose neurons (n = 4 ganglia × 4 or 2 sections × 20 neurons = 280; 1 ganglion had only 2 sections to count) were counted for DP1 receptors and colocalization with IB4. A total of 300 nodose neurons (n = 4 ganglia × 3 or 4 sections × 20 neurons = 300; 1 ganglion had only 3 sections to count) were counted for DP2 receptors and colocalization with IB4.

Western blotting. Equal amounts of lysates (20 μg) freshly obtained from nodose ganglia (n = 4) were separated on Ready Gel 12% Tris-HCl, transferred to nitrocellulose Hybond ECL membranes, and blotted overnight with primary antibodies and then for 1 h with secondary antibodies. The membranes were exposed to enhanced chemiluminescence buffer (Pierce, Rockford, IL) for 30 s or 5 min and then high-chemiluminescence film in darkness. The resulting bands were scanned and analyzed. The primary antibodies used for Western blotting included rabbit anti-DP1 receptor polyclonal antibody (1:1,000 dilution; Cayman Chemical) and rabbit anti-DP2 receptor polyclonal antibody (1:1,000 dilution; Novus Biologicals). The secondary antibodies included goat anti-rabbit IgG-horseradish peroxidase (1:2,000 dilution; catalog no. sc-2004, Santa Cruz Biotechnology, Santa Cruz, CA).

RT-PCR. TRIZol reagent (Invitrogen) was used to freshly extract total RNAs from nodose ganglia, the dorsal root ganglion (DRG), and the brain. Immediately thereafter, cDNAs were synthesized with reverse transcriptase (Promega, Madison, WI) using 5 μg of total RNA. The resultant cDNAs were used for PCR, which was performed with Taq DNA polymerase (Promega) through 30 cycles of denaturation (1 min at 94°C), annealing (2 min at 50°C), and extension (3 min at 72°C), with a final extension period of 10 min at 72°C. We designed two pairs of primers according to the published sequences for guinea pig DP1 receptor (accession no. XM_003471928) and DP2 receptor (accession no. NM_001172722): guanosine diphosphatase (GDP) 1 [5'-GCTGATCTCCACCCCATGTCGC-3' (forward) and 5'-GAGGTGCGGCGGTCGTC-3' (reverse)] for the DP1 receptor and GDP2 [5'-CAGCAACAATCCACGGCTGCT-3' (forward) and 5'-GTGTT-GAGCAGCAGCAGCAGC-3' (reverse)] for the DP2 receptor. A housekeeping gene, GAPDH, served as an internal control. The PCR products were loaded in a 1.2% Tris-borate-EDTA-buffered agarose gel, and the bands were visualized after gel electrophoresis by ethidium bromide staining and ultraviolet light illumination.

Extracellular single-fiber recording. The esophageal vagal preparations were set up, and extracellular recordings of action potential discharges from nodose neurons with intact nerve endings in the esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact esophagus were performed as previously described (33). Briefly, the esophagus and trachea were dissected, with care taken to leave intact

RESULTS

PGD2-induced mechanical hypersensitivity in esophageal nodose C fibers. To determine the effect of PGD2 on esophageal vagal afferent subtypes, we used ex vivo esophageal vagal preparations to perform extracellular recordings from nodose
PGD₂ SENSITIZES ESOPHAGEAL AFFERENTS

Esophageal distension-evoked action potentials were compared before and after PGD₂ perfusion. According to our previous studies (32, 33), the response to the particular stimulus was considered positive when the stimulus evoked an action potential discharge with a peak frequency of 3 Hz (in the fibers with no baseline activity) or a peak frequency at least three times the frequency of baseline activity. In esophageal nodose C fibers, 30 min of perfusion with PGD₂ (10 μl) did not evoke action potential discharges (0.6 ± 0.3 and 1.6 ± 0.5 Hz at baseline and with PGD₂, respectively) but substantially increased their responses to esophageal distension. The peaks of action potential discharges (with subtraction of baseline spontaneous activity and PGD₂-induced activity) evoked by 20-s esophageal distensions significantly increased from 3.6 ± 0.4, 7.7 ± 0.6, and 11.9 ± 0.7 Hz to 7.9 ± 0.8, 15.4 ± 1.1, and 21 ± 1.0 Hz at distension pressures of 10, 30, and 60 mmHg, respectively (P < 0.01, n = 12; Fig. 1, A and B). The increased action potential discharges were sustained after 30 min of PGD₂ washout with fresh buffer in six C fibers. In these six C fibers, the peaks of action potential discharges evoked by esophageal distensions did not significantly change before and after 30 min of PGD₂ washout (from 8.0 ± 1.3, 14.7 ± 1.5, and 21.3 ± 1.4 Hz to 7.3 ± 0.7, 15.5 ± 2.1, and 23 ± 2.3 Hz at distension pressures of 10, 30, and 60 mmHg, respectively, P > 0.05, n = 6). All nodose C fibers were confirmed to respond to the TRPA₁ agonist AITC (1 mM, peak = 10.8 ± 1.4, n = 12) at the end of each study, indicating that the recorded nerve terminals were exposed to perfused drug. In esophageal nodose Aδ fibers, 30 min of perfusion with PGD₂ (10 μl) neither evoked action potential discharges (3.8 ± 0.5 and 4.3 ± 0.5 Hz at baseline and with PGD₂, respectively, n = 4) nor changed responses to esophageal distension. The peaks of action potential discharges (with subtraction of baseline spontaneous activity) evoked by 20-s esophageal distensions were not significantly changed from 21 ± 1.8, 25 ± 3, and 27.8 ± 3.2 Hz to 20.5 ± 1.6, 27 ± 2.7, and 27 ± 1.1 Hz at distension pressures of 10, 30, and 60 mmHg, respectively (P > 0.05, n = 4; Fig. 1C). These nodose Aδ fibers were confirmed to respond to the P2X receptor agonist α,β-methylene-ATP (30 μM, n = 4) at the end of each study, indicating that the recorded nerve terminals were exposed to perfused drug.

These data demonstrate that PGD₂ does not directly activate esophageal vagal afferents but selectively sensitizes nodose C fibers to increase their responses to esophageal distension. This suggests an important role of PGD₂ in initiating mechanical hypersensitivity of esophageal vagal nociceptive afferents.

Expression of PGD₂ DP₁ and DP₂ receptors in nodose ganglion neurons. The biological effects of PGD₂ are usually mediated by two G protein-coupled receptors, designated DP₁ and DP₂. To determine which receptors participated in PGD₂-induced mechanical hypersensitivity in esophageal nodose C fibers, we first examined their expressions in nodose neurons by immunostaining. DP₁ and DP₂ receptor expression was identified in nodose neurons (Fig. 2, A–H). Among 280 nodose neurons (4 nodose ganglia from 4 guinea pigs × 3–4 sections × 20 neurons), ~80% (224 of 280) of DP₁ receptor-positive neurons were small- and medium-sized (<30 μm diameter), and 51% of DP₁ receptor-positive neurons were also positively labeled with IB4. Similarly, among 300 neurons, ~93% (280 of 300) of DP₂ receptor expression was detected in small- and medium-diameter nodose neurons, and 60% of DP₂ receptor-positive neurons were also colocalized with IB4.

Western blot analysis with anti-DP₁ or anti-DP₂ receptor antibodies also showed the presence of DP₁ and DP₂ receptors in nodose ganglia. Probing with DP₁ or DP₂ receptor antibodies revealed moderately immunoreactive bands in lysates from nodose and DRG ganglia. Strongly immunoreactive bands were also found in the brain, which is known to express high levels of DP₁ and DP₂ receptors (Fig. 2, J and K). Then, using RT-PCR, we explored DP₁ and DP₂ receptor mRNA expression in nodose ganglia. Two PCR products, 283 bp for DP₁ receptor and 420 bp for DP₂ receptor, were identified from RNA extracted from nodose ganglia and were also detected in positive (DRG and brain) controls (Fig. 2).

Our studies consistently identified the expression of DP₁ and DP₂ receptors in nodose ganglia by immunostaining, Western blotting, and RT-PCR. The high expression rate (80–90%) of PGD₂ receptors in small- and medium-diameter neurons is in agreement with the findings from our extracellular recordings showing that PGD₂ selectively sensitizes nodose C fibers (but not Aδ fibers), which usually have small- or medium-sized cell bodies (neurons) situated in the ganglia.

DP₁, but not DP₂, receptor agonist, mimicked the effect of PGD₂-induced mechanical hypersensitivity in esophageal nodose C fibers. To determine the roles of DP₁ or DP₂ receptors in PGD₂-induced mechanical hypersensitivity in nodose C fibers, we tested the hypothesis that DP₁ and/or DP₂ receptor

Fig. 1. PGD₂ on esophageal nodose afferent subtypes. A: perfusion with PGD₂ increased mechanoeexcitability of esophageal nodose C fibers. Action potential discharges evoked by esophageal distensions significantly increased at distension pressures of 10, 30, and 60 mmHg. Values are means ± SE (n = 12), *P < 0.01. B: typical traces of action potential discharges from esophageal nodose C fibers at distension pressure of 60 mmHg for 20 s before and after 30 min of perfusion with PGD₂ (10 μM). C: perfusion with PGD₂ did not change mechanoeexcitability of nodose Aδ fibers. Values are means ± SE (n = 4), P > 0.05.
agonists mimicked the PGD₂-induced sensitization effect. We chose the selective DP₁ receptor agonist BW 245C and the DP₂ receptor agonist 15(R)-PGD₂ for our experiments. Perfusion with the DP₁ receptor agonist BW 245C (10 μM) for 30 min did not evoke action potential discharges (1.2 ± 0.3 Hz at baseline vs. 1.2 ± 0.3 Hz with BW 245C, n = 8) in esophageal nodose C fibers. However, this was associated with a significant increase in their responses to esophageal distension. The peaks of action potential discharges (with subtraction of baseline spontaneous activity and DP₁ receptor agonist-induced activity) evoked by 20-s esophageal distensions significantly increased from 6.4 ± 1.8, 12 ± 2.3, and 17 ± 3.4 Hz to 12.1 ± 3.5, 18.1 ± 3.6, and 26 ± 4.8 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively (P < 0.05, n = 8; Fig. 3A). In another set of experiments, 30 min of perfusion with the DP₂ receptor agonist 15(R)-PGD₂ (2–6 μM) did not significantly evoke action potential discharges [1.6 ± 0.4 Hz at baseline vs. 2.6 ± 0.8 Hz with 15(R)-PGD₂, n = 5]. The action potentials evoked by esophageal distension slightly increased but did not reach significance before and after perfusion with 15(R)-PGD₂ (1.8 ± 0.3, 5.8 ± 1.1, and 10.5 ± 1 Hz vs. 2 ± 0.4, 5.5 ± 0.9, and 11 ± 1 Hz at distension pressures of 10, 30, and 60 mmHg, respectively, P > 0.05, n = 5; Fig. 3B). These nodose C fibers were confirmed to respond to the TRPA1 agonist AITC (1 mM) and/or the TRPV1 agonist capsaicin (1 μM) at the end of each study. The finding that the DP₁ receptor agonist mimicked the effect of PGD₂-induced mechanical hypersensitivity suggests that the DP₁ receptor has a role in the PGD₂-induced sensitization effect in esophageal vagal nodose C fibers.

**DP₁ receptor antagonist, but not DP₂ receptor antagonist, prevented PGD₂-induced mechanical hypersensitivity in esophageal nodose C fibers.** To further confirm the involvement of DP₁ and/or DP₂ receptors in PGD₂-induced sensitization of esophageal nodose C fibers, we tested the hypothesis that pretreatment with DP₁ or DP₂ receptor antagonists prevented PGD₂-induced mechanical hypersensitivity in esophageal vagal nodose C fibers. First, we determined whether perfusion with the DP₁ receptor antagonist BW A868C changed the mechanoexcitability of esophageal nodose C fibers. Before and after 30 min of perfusion with BW A868C (10 μM), action potentials evoked by esophageal distension did not change significantly.

**Fig. 2. DP₁ and DP₂ receptor expression in nodose neurons.** A–C and E–G: immunofluorescence staining of DP₁ and DP₂ receptors and their colocalizations with isolectin B4 (IB4) in nodose ganglion neurons. About 80% (224 of 280) of DP₁ receptor-positive neurons were small- and medium-sized (<30 μm diameter) (D), and 51% of DP₁ receptor-positive neurons were also positively labeled with IB4 (C). About 93% (280 of 300) of DP₂ receptor-positive neurons were small- and medium-sized (<30 μm diameter) (H), and 60% of DP₂-positive neurons were also positively labeled with IB4 (G). DP₁ and DP₂ receptor proteins were revealed by Western blotting (J and K), and DP₁ and DP₂ receptor mRNA were detected by RT-PCR (I) in guinea pig nodose ganglia (NG) compared with positive controls [dorsal root ganglion (DRG) and brain].
(2.5 ± 0.3, 4.8 ± 0.5, and 8.1 ± 0.8 Hz vs. 2.9 ± 0.4, 5.0 ± 0.4, and 9.8 ± 0.9 Hz at distension pressures of 10, 30, and 60 mmHg, respectively, P > 0.05, n = 8). Next, we studied the inhibitory effect of BW A868C on PGD2-induced mechanical hypersensitivity. After 30 min of perfusion with and in the presence of BW A868C (10 μM), 30 min of PGD2 perfusion (10 μM) failed to induce mechanical hypersensitivity in esophageal nodose C fibers. Action potentials evoked by esophageal distensions did not significantly change from 2.9 ± 0.3, 5.0 ± 0.4, and 9.8 ± 0.9 Hz (with BW A868C) to 2.9 ± 0.4, 5.5 ± 0.6, and 9.5 ± 0.9 Hz (with BW A868C + PGD2) at distension pressures of 10, 30, and 60 mmHg, respectively (P > 0.05, n = 8 in each paired value; Fig. 3C).

Similarly, we studied the inhibitory effect of the DP2 receptor antagonist BAY-u3405 on PGD2-induced mechanical hypersensitivity. Perfusion with BAY-u3405 (1 μM) for 30 min neither evoked action potentials nor changed the mechanoeexcitability of esophageal nodose C fibers (action potentials: 3.3 ± 0.7, 8.6 ± 1.7, and 12.3 ± 2.2 Hz before BAY-u3405 vs. 4.9 ± 1.0, 10.7 ± 2.5, and 15.9 ± 3.2 Hz after BAY-u3405 at distension pressures of 10, 30, and 60 mmHg, respectively, P > 0.05, n = 7). After 30 min of perfusion with and in the presence of BAY-u3405 (1 μM), 30 min of perfusion with PGD2 (10 μM) still enhanced the mechanoeexcitability of esophageal nodose C fibers. Action potentials evoked by esophageal distensions significantly increased from 4.9 ± 1.0, 10.7 ± 2.5, and 15.9 ± 3.5 Hz (with BAY-u3405) to 7.3 ± 1.0, 16.3 ± 2.9, and 28 ± 4.5 Hz (with BAY-u3405 + PGD2) at distension pressures of 10, 30, and 60 mmHg, respectively (P < 0.05, n = 7 in each paired value; Fig. 3D). Only nodose C fibers that were confirmed to respond to the TRPA1 agonist AITC (1 mM) and/or the TRPV1 agonist capsaicin (1 μM) at the end of each study were included in the analysis. These data support an important role of the DP1 receptor in PGD2-induced mechanical hypersensitivity in esophageal nodose C fibers.

15-Deoxy-Δ12,14-PGJ2, a PGD2 metabolite, did not change the mechanoeexcitability of esophageal nodose C fibers. PGD2 is known to metabolize to 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) (20), which has been shown to induce nociception (6, 18) via direct interaction with TRPA1 on sensory neurons (26). Our previous studies demonstrated that the majority of esophageal nodose C fibers expressed TRPA1 and responded to the TRPA1 agonist AITC (31). Whether 15d-PGJ2 displays activation/sensitization effects on esophageal nodose C fibers is unknown. Perfusion with 15d-PGJ2 (30 μM) for 30 min slightly evoked action potential discharges over baseline activity (2.1 ± 0.4 and 4.6 ± 1.2 Hz at baseline and with 15d-PGJ2, respectively, P < 0.05, n = 10) in esophageal nodose C fibers (Fig. 4A). However, this was not associated with a significant increase in their responses to esophageal distension. The peaks of action potential discharges (with subtraction of baseline spontaneous activity and 15d-PGJ2-induced activity) evoked by 20-s esophageal distensions did not significantly change from 6.0 ± 1.4, 12.8 ± 3.8, and 15 ± 3.4 Hz to 6.8 ± 1.9, 10.2 ± 1.9, and 13 ± 2.8 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively (P > 0.05, n = 10; Fig. 4B). The TRPA1 agonist AITC activated all these C fibers at the end of recording (2.1 ± 0.4 and 15 ± 5.1 Hz at baseline and with AITC, respectively, P < 0.01, n = 10; Fig. 4A), indicating the presence of TRPA1 in the nerve terminals. This result suggests that the PGD2 metabolite 15d-PGJ2 is unlikely to have participated in PGD2-induced sensitization of esophageal nodose C fibers under the conditions in our studies.

PGD2 directly sensitized Dil-labeled esophageal nodose neurons. The effects of PGD2 on the electrophysiological properties of Dil-labeled esophageal nodose ganglion neurons were studied by whole cell patch-clamp recording. Extracellular
lar application of PGD2 (1 μM) sensitized 6 of 11 (55%) recorded Dil-labeled neurons, which were also positively labeled with DP1 receptors (Fig. 5, A–C). The current required to evoke an action potential (rheobase) decreased significantly in Dil-labeled esophageal nodose neurons before and after superfusion of PGD2 (1 μM): 78 ± 11 vs. 55 ± 8 pA (P < 0.05, n = 6; Fig. 5D). The number of spikes elicited by depolarizing current stimulation (double rheobase, 500 ms) increased significantly from esophageal retrogradely labeled nodose neurons treated with PGD2 (1 μM): 2.5 ± 0.4 vs. 4.6 ± 0.9 pA (P < 0.05, n = 6; Fig. 5E). This result not only supports our findings from extracellular recordings that PGD2 increased neuronal excitability but also suggests that such a sensitization effect is directly evoked by PGD2, rather than by other mediators/factors released after PGD2 perfusion in the esophagus. The current-voltage (I-V) relationship generated from the current-clamp recording demonstrated that PGD2 increased the slope of the I-V relationship, and the effect reversed at −105 mV, which is close to theoretical K+ reversal potential in the conditions recorded.

Fig. 5. Effect of PGD2 on excitability of 1,1'-dioctadecyl-3,3',3',tetramethylindocarbocyanine perchlorate (DiI)-labeled esophageal nodose neurons. PGD2 superfusion directly sensitized 6 of 11 Dil-labeled esophageal nodose neurons. A: photomicrograph of an isolated esophageal nodose neuron retrogradely labeled with Dil (DiI+) and a DP1 receptor-positive neuron (DP1+). B and C: representative current-clamp traces in response to hyperpolarizing (−100 pA) and depolarizing (double rheobase, 500 ms) current pulses in a labeled neuron before and after superfusion with PGD2 (1 mM). D: summary data of current required to evoke action potential (rheobase) in nodose neurons before and after superfusion of PGD2. Data show significant decrease in action potential threshold (rheobase). Values are means ± SE (n = 6). *P < 0.05. E: summary data of number of spikes elicited by depolarizing current stimulation (double rheobase, 500 ms). Number of spikes from labeled neurons treated with PGD2 increased significantly (*P < 0.05). F: current-voltage (I-Vm) relationship before and after application of PGD2. Neuronal conductance decrease had a reversal potential of −105 mV, which is close to theoretical K+ reversal potential in the conditions recorded.
mV, which is close to theoretical K\(^+\) reversal potential (−107 mV) in the conditions recorded (Fig. 5F). This may indicate that the effect of PGD\(_2\) is mediated by closure of K\(^+\) channel(s).

**DP\(_1\)** receptor antagonist attenuated esophageal mast cell activation-induced mechanical hypersensitivity in esophageal nodose C fibers. Our previous study showed that 30 min of perfusion with OVA induces MC activation, as indicated by increased release of histamine in the tissue and decreased staining of tryptase-positive MCs in the esophagus (32). To clarify the role of the DP\(_1\) receptor in mediating esophageal MC activation-induced sensitization of esophageal nodose C fibers, we tested the hypothesis that pretreatment with a DP\(_1\) receptor antagonist prevented esophageal MC activation-induced mechanical hypersensitivity in esophageal vagal nodose C fibers. First, we determined whether 30 min of perfusion with the DP\(_1\) receptor antagonist BW A868C changed the mechanoexcitability in esophageal nodose C fibers from OVA-sensitized guinea pigs. Before and after 30 min of perfusion with BW A868C (10 \(\mu\)M), action potentials evoked by esophageal distension did not significantly change (4.4 ± 0.8, 13.5 ± 2.2, and 21.3 ± 3.9 Hz vs. 5.5 ± 1.0, 16.8 ± 2.9, and 24.4 ± 5.3 Hz at distension pressures of 10, 30, and 60 mmHg, respectively, \(P > 0.05, n = 7\)). Next, we studied the inhibitory effect of BW A868C on MC activation-induced mechanical hypersensitivity. After 30 min of perfusion with and in the presence of BW A868C (10 \(\mu\)M), MC activation induced by 30 min of OVA challenge (10 \(\mu\)g/ml) failed to induce mechanical hypersensitivity in esophageal nodose C fibers from OVA-sensitized guinea pigs (action potentials: 5.5 ± 1.0, 16.8 ± 2.9, and 24.4 ± 5.3 Hz vs. 6.4 ± 1.6, 16.5 ± 2.7, and 30.7 ± 4.3 Hz before and after MC activation at distension pressures of 10, 30, and 60 mmHg, respectively, \(P > 0.05, n = 7\); Fig. 6A). In contrast, pretreatment with the DP\(_2\) receptor antagonist did not prevent mechanical hypersensitivity induced by MC activation in esophageal vagal nodose C fibers. Before and after 30 min of perfusion with BAY-u3405 (1 \(\mu\)M), action potentials evoked by esophageal distension did not significantly change (4.0 ± 1.0, 11.3 ± 2.0, and 19.9 ± 3.2 Hz vs. 4.9 ± 1.4, 12.0 ± 1.1, and 18.0 ± 2.6 Hz at distension pressures of 10, 30, and 60 mmHg, respectively, \(P > 0.05, n = 7\)). After 30 min of perfusion with and in the presence of BAY-u3405 (10 \(\mu\)M), MC activation induced by 30 min of OVA challenge (10 \(\mu\)g/ml) significantly increased mechanical excitability of esophageal nodose C fibers from OVA-sensitized guinea pigs (action potentials: 4.9 ± 1.4, 12.0 ± 1.1, and 18.0 ± 2.6 Hz vs. 7.6 ± 1.4, 19.0 ± 3.2, and 28.0 ± 4.0 Hz in the presence of BAY-u3405 before and after MC activation at distension pressures of 10, 30, and 60 mmHg, respectively, \(P < 0.05, n = 7\); Fig. 6B). These data suggest an important role for the DP\(_1\) receptor in MC activation-induced mechanical hypersensitivity in esophageal nodose C fibers.

**DISCUSSION**

For two decades, PGD\(_2\) has been recognized to be present in the esophagus (2). However, its physiological function and participation in esophageal disorders are largely unknown. PGD\(_2\) is mainly synthesized and released from activated MCs. It participates in MC activation-induced type I hypersensitivity, including smooth muscle contraction, vascular leak, and vasodilation. It also displays potent chemotactic effects on eosinophils, basophils, and Th2 cells. In addition, it may potentiate the inflammatory response induced by other relevant mediators. The biological effects of PGD\(_2\) are mediated by two G protein-coupled receptors, DP\(_1\) and DP\(_2\) (23). Expression of DP\(_1\) and DP\(_2\) receptors has been identified in central and peripheral neurons (7, 17, 22). DP\(_1\) receptor activation leads to Gi protein-mediated decreases in intracellular cAMP, while DP\(_2\) receptor activation leads to G\(_s\) protein-mediated increases in intracellular cAMP. These intracellular signaling changes are likely to regulate the excitability of sensory afferent. Our data in the present study demonstrate that PGD\(_2\) has different effects on esophageal vagal afferent nerve subtypes. In esophageal nodose C fibers, perfusion with PGD\(_2\) does not evoke action potential discharges but sensitizes their response to esophageal distension. This sensitization effect can be mimicked by a PGD\(_2\) DP\(_1\) receptor agonist and further prevented by pretreatment with a DP\(_1\) receptor antagonist. In contrast, in esophageal nodose A\(_\beta\) fibers, perfusion with PGD\(_2\) neither evokes action potential discharges nor sensitizes their response to esophageal distension. We also observed that PGD\(_2\) directly sensitized dissociated Dil-labeled esophageal nodose neurons. These patch-clamp data support the observation by extracellular recording that the sensitization effect in the nerve terminals...
is directly induced by PGD2, rather than by a secondary released mediator after PGD2 perfusion in the esophageal tissue. Our findings provide the first direct evidence for a sensitization effect of PGD2 on esophageal vagal afferent subtypes. The observation that PGD2 failed to evoke action potential discharges in esophageal nodose C fibers but substantially increased their excitability to esophageal distension is similar to our previous observation of a MC activation-induced effect on esophageal vagal afferents (32). Pretreatment with the DP1 receptor antagonist BW A868C did not totally abolish, but significantly attenuated, MC activation-induced sensitization of esophageal nodose C fibers. This suggests that, in addition to histamine, PGD2 also plays an important role in MC activation-induced sensitization of esophageal afferents.

PGD2, which is involved in sleep regulation and pain perception, has been well known as the major prostanoid produced in the central nervous system (8, 29). Its effect on peripheral afferents is less clear. In cultured DRG neurons, PGD2 evoked an accumulation of cAMP (25) and increased the amplitude of TTX-resistant Na+ currents via the DP1 receptor (7). In nodose neurons, PGD2 inhibited postspike hyperpolarization, resulting in an increase in the firing frequency (5). Extracellular recording of direct-current potentials indicated that PGD2 perfusion caused a depolarization response of vagal C fibers (25). However, in another study using in vivo electrophysiological single-unit recording from rat trigeminal ganglion, PGD2 failed to sensitize meningeal nociceptors (34). In contrast, responses of spinal afferent neurons to PGD2 are different from responses of nodose and DRG neurons. In one study using a spinal-tail preparation, PGD2 had no or less effect on sensitization of spinal afferents than other prosanoid products such as PGE2 and PGI2 (21). Another studies demonstrated that PGD2 and DP1 receptor agonist application to the spinal cord did not change mechanoeexcitability of a normal knee joint but attenuated mechanohypexcitability in an inflamed knee joint by in vivo extracellular recording from dorsal horn neurons with a peripheral nerve ending in the knee joint (27). We thought that several factors, such as the location of recorded afferent neurons, the presence of other mediators in the system, and the outcome measurements, might contribute to these different observations. Interestingly, the different responses (DRG and vagal afferents vs. spinal neuron afferent) may also suggest that the PGD2-induced sensitization effect more likely occurred in the peripheral afferent, which is strategically located near the sites of tissue injury and inflammation. Our study provides new evidence for the important role of PGD2 in sensitizing sensory afferents in the GI tract. This expands our knowledge of the mechanism of MC activation-induced esophageal afferent sensitization.

The intracellular signaling pathway and downstream ion channel involvement in PGD2-induced sensitization of sensory afferents are less unclear. Previous studies showed that PGD2 blocked a slow Ca2+-activated K+-dependent afterhyperpolarization in visceral afferent neurons (9). A recent study demonstrated that PGD2, via mainly the DP1 receptor, hyperpolarized DRG neurons, depending on action, at Na+1.9 and influenced Na+,1.8 channel functions (7). In the present study, perfusion with PGD2 increased the number of action potentials evoked by the positive current pulse. The I-V relationship generated from the current-clamp recording demonstrated that PGD2 increased the slope of the I-V relationship, and the effect reversed at −105 mV, which is close to the K+ reversal potential (~107 mV). This may suggest that the effect of PGD2 is mediated by closure of the K+ channel(s). The exact role of the K+ channel and the intracellular mechanism of PGD2-induced sensitization of esophageal vagal afferent deserves further exploration.

MC activation not only releases PGD2, but also histamine, and our previous study (32) demonstrated that histamine could sensitize esophageal afferents to induce mechanical hyperexcitability. This may explain why, in the present study, the PGD2 DP1 receptor antagonist does not totally abolish the PGD2-induced sensitization effect but only attenuates MC activation-induced sensitization on esophageal nodose C fibers. It is unlikely, in our ex vivo model, that the effect of PGD2 on the response of the esophageal nodose C fiber to intraluminal pressure (esophageal distension) is secondary to the effects on the stress-strain relationships caused by smooth muscle contraction. First, our previous study using the same preparation showed that methacholine, which contracts esophageal smooth muscle, did not increase the response of the esophageal nodose C fiber to esophageal distension (32). Second, PGD2 did not participate in regulation of smooth muscle contraction (intestinal peristalsis) in guinea pig GI tract (24). Our data also indicate that PGD2-induced sensitization of esophageal afferents is not secondary to the effect of the PGD2 metabolite 15d-PGJ2, which may interact with TRPA1 in nodose neurons (26, 31).

Clinical studies suggest that increased eosinophil and MC infiltration into the esophagus might contribute to abnormal esophageal sensation, such as heartburn, in patients with eosinophilic esophagitis (11, 16). The present study provides the first evidence that PGD2, one of the important MC mediators, displays distinctive sensitization effects on esophageal sensory afferents, which contributes to MC activation-induced sensitization of esophageal afferents. This adds to our knowledge of the mechanisms of esophageal nociception, which may lead to development of a novel treatment approach for these common, but difficult to manage, clinical problems.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
S.Z., G.G., X.W., M.K.I., A.H., I.S., and S.Y. performed the experiments; S.Z., G.G., X.W., M.K.I., A.H., I.S., and S.Y. analyzed the data; S.Z., G.G., X.W., and S.Y. interpreted the results of the experiments; C.O. and S.Y. are responsible for conception and design of the research; S.Y. prepared the figures; S.Y. drafted the manuscript; S.Y. edited and revised the manuscript; S.Y. approved the final version of the manuscript.

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