Prostaglandin regulation of gastric slow waves and peristalsis

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Forrest AS, Hennig GW, Jokela-Willis S, Park CD, Sanders KM. Prostaglandin regulation of gastric slow waves and peristalsis. Am J Physiol Gastrointest Liver Physiol 296: G1180–G1190, 2009. First published April 9, 2009; doi:10.1152/ajpgi.90724.2008.—Gastric emptying depends on functional coupling of slow waves between the corpus and antrum, to allow slow waves initiated in the gastric corpus to propagate to the pyloric sphincter and generate gastric peristalsis. Functional coupling depends on a frequency gradient where slow waves are generated at higher frequency in the corpus and drive the activity of distal pacemakers. Simultaneous intracellular recording from corpus and antrum was used to characterize the effects of PGE2 on slow waves in the murine stomach. PGE2 increased slow-wave frequency, and this effect was mimicked by EP3, but not by EP2, receptor agonists. Chronotropic effects were due to EP3 receptors expressed by intramuscular interstitial cells of Cajal because these effects were not observed in W/WV mice. Although the integrated chronotropic effects of EP3 receptor agonists were deduced from electrophysiological experiments, no clear evidence of functional uncoupling was observed with two-point electrical recording. Gastric peristalsis was also monitored by video imaging and spatiotemporal maps to study the impact of chronotropic agonists on propagating contractions. EP3 receptor agonists increased the frequency of peristaltic contractions and caused ectopic sites of origin and collisions of peristaltic waves. The impact of selective regional application of chronotropic agonists was investigated by use of a partitioned bath. Antral slow waves followed enhanced frequencies induced by stimulation of the corpus, and corpus slow waves followed when slow-wave frequency was elevated in the antrum. This demonstrated reversal of slow-wave propagation with selective antral chronotropic stimulation. These studies demonstrate the impact of chronotropic agonists on regional intrinsic pacemaker frequency and integrated gastric peristalsis.

interstitial cells of Cajal; EP receptor; gastroparesis; pacemaker; tachygastria

The interstitial cells of Cajal (ICC) generate electrical pacemaker activity (slow waves) in the gastrointestinal (GI) tract (26). Electrical coupling of ICC to smooth muscle cells facilitates coordination of phasic contractions in the stomach, small bowel, and colon (6, 7, 22, 23). Slow waves cause depolarization of smooth muscle cells and activation of L-type Ca2+ channels. Slow waves propagate actively within ICC networks, and removal of ICC causes rapid decay of slow-wave amplitude within only a few millimeters. In some regions of the gut, such as the stomach, slow waves propagate as a coherent wave front over many centimeters (2, 13, 15). This feature of ICC networks is necessary for proximal to distal slow-wave propagation that is responsible for gastric peristalsis and makes it possible for corpus pacemakers to dominate and drive the entire stomach at the corpus rate (14). Propagation of slow waves from dominant corpus pacemakers to the pyloric sphincter is a product of “functional coupling,” which we define as the ability of higher frequency pacemakers (corpus) to entrain the activity of lower frequency pacemakers (antrum and pylorus). Thus another property of gastric ICC that appears to facilitate functional coupling is the distinct frequency gradient whereby the intrinsic corpus pacemaker frequency is considerably higher than the frequency of antral and pyloric pacemakers (30). This is analogous to the dominance of the sinoatrial node pacemaker driving downstream slower pacemakers (e.g., atrioventricular node cells) in the heart.

Previous studies have shown that emergence of high-frequency pacemakers in the distal stomach (tachygastria) can disrupt functional coupling and the normal dominance of corpus pacemakers. This state is characterized by abnormal slow-wave propagation (19) and delayed gastric emptying as observed in some forms of gastroparesis (e.g., Ref. 16). Unfortunately, little is known about the pathophysiological mechanism(s) leading to the development of gastric arrhythmias, or the consequences of altering gastric pacemaker frequency on functional coupling. Several naturally occurring compounds have chronotropic effects on antral muscles, including prostaglandins (28) and cholinergic agonists (8). Chronotropic effects of these agonists appear to be mediated by EP3 receptors (17) and M3 muscarinic (18) receptors, respectively, which might couple to generation of inositol 1,4,5-trisphosphate (IP3) and acceleration of the pacemaker clock mechanism (27).

Coordination of gastric pacemaker activity and the ability of the corpus pacemaker to maintain dominance during chronotropic drive are not well understood. As discussed above, the frequency gradient is important for establishing the normal proximal-to-distal spread of slow waves. A generalized increase in pacemaker frequency might result in functional uncoupling if the antrum cannot be entrained by the corpus, or if the interval between slow waves becomes so short that the antral pacemakers “escape” from the dominance of the corpus. Unequal production, metabolism, or responsiveness toward chronotropic agonists in one region vs. the other may also contribute to disruption in the frequency gradient and lead to functional uncoupling (e.g., as in antral tachygastria). In the present study we have simulated some of these possibilities in murine gastric muscles containing either the entire phasic region of the stomach (i.e., corpus and antrum) or in strips comprised of the corpus and antral regions along the greater curvature of the stomach. We tested whether the chronotropic drive of prostaglandin E2 is manifest in the proximal stomach and whether functional coupling is compromised by whole stomach stimulation by prostaglandin E2 and EP receptor agonists. We have also explored the consequences of regional application of EP3 agonists to either the antrum or corpus on gastric frequency and coordination. Our results show that although two-point electrical recording can detect changes in frequency, video imaging (which is essentially equivalent to recording activity simultaneously from a multitude

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Fig. 1. Imaging of gastric peristalsis. A: flat-sheet preparations of the gastric corpus and antrum were prepared by incisions along the lesser curvature (LC), across the stomach at the level of the esophagus, and at the duodenal junction. GC, greater curvature. A transition zone occurs in the mouse with distinctive mucosal morphological features denoting the boundary between the corpus and fundus. The entire tunica muscularis of the corpus and antrum was pinned as a flat sheet (middle). Black markers were placed in a grid on the surface of the muscle to track contractions within the preparation (see 3D cube on left in B). Gastric peristaltic contractions were detected by imaging indentations of the edges of the muscle sheets due to circumferential (circular) muscle shortening (right in A). B: degree of indentation and movement of contractions were portrayed as spatiotemporal maps (ST-EdgeMap: horizontal axis = time, vertical axis = distance and grayscale = mm of indentation). The inset shows the trajectories of the black markers (white lines) on the surface of the muscle sheet as a function of time in the vertical axis. Spatiotemporal maps of circular contraction (ST-CMap) or longitudinal contraction (ST-LMap) were also constructed from distances between the black markers and showed coordinated contractions of the circular and longitudinal muscle layers. Propagating antral contractions occurred regularly and propagated at consistent velocities under control conditions. C and D: additional ST-Maps of gastric peristalsis in flat-sheet gastric muscles. Propagation velocities of gastric peristaltic contractions were calculated from the slopes of the ST-Maps (black arrows). Note: faint higher frequency activity can also be observed in some records (e.g., C and D) and is due to contractions of the remnant of the duodenum, producing small mechanical distortions of the stomach region.
of points) is necessary to clearly detect functional uncoupling in the intact stomach.

METHODS

Balb/c mice (Charles River Laboratories, Wilmington, MA), WBB6F1/J-Kit+/Kit–/Kit– (W/W ) mice and WBB6F1/J-Kit+/Kit+ wild-type controls (Jackson Laboratories, Bar Harbor, ME) of either sex were anesthetized by isoflurane inhalation and killed by cervical dislocation. The stomachs were removed and opened along the lesser curvature. The luminal contents were washed away with Krebs-Ringer bicarbonate solution (KRB) and the mucosa was removed by sharp dissection. The animal experimental protocols were approved by the Institutional Care and Use Committee at the University of Nevada.

Intracellular recordings. For single recording experiments, the corpus muscle region was cut away and pinned, with the mucosal aspect of the circular muscle facing upward, to the bottom of a recording chamber lined with Sylgard 184 elastomer (Dow Corning, Midland, MI). For dual recording experiments, a continuous strip of corpus and antrum was cut along the greater curvature and pinned to a Sylgard-lined dish. For those experiments requiring a partitioned chamber, the tissue was first passed through an aperture made in a latex barrier before being pinned to the Sylgard. Two electrodes were used to make recordings from the tissue, one positioned in the antral end of the muscle strip and the other in the corpus.

Circular muscle cells along the greater curvature were impaled with glass microelectrodes filled with a 3 M KCl solution, which had a resistance of 70–100 MΩ. Transmembrane potentials were measured with a high-impedance electrometer (WPI Intra 767, World Precision Instruments, Sarasota, FL), and outputs were displayed on a Hitachi VC-6025A oscilloscope (Hitachi Denshi, Tarrytown, NY). Electrical signals were digitized at 200 Hz and stored as computer files by use of AqKnowledge version 3.5.7. (BIOPAC Systems, Santa Barbara, CA). All experiments were performed in the presence of nifedipine (1 μM) to reduce muscle contraction and aid cell impalement.

Imaging of gastric peristalsis. Coordination of antral peristaltic contractions was evaluated by using isolated stomachs opened along the lesser curvature and pinned flat in a Sylgard-lined petri dish. For comparison, we also tested preparations cut along the greater curvature, essentially bisecting the region of the dominant pacemaker in the stomach. The fundus was removed and the preparations were pinned at the oral corpus and pylorus. The gastric sheets were constantly perfused with oxygenated KRB at 37°C. As antral peristaltic contractions propagated from the oral corpus to the pylorus, an indentation in the edges of the gastric tissue was recorded using a high-definition video camera (DMK 31AF03, ImagingSource, Charlotte, NC) and AstrolIDC software (ASC, Calgary, Alberta, Canada). The video images were converted to spatiotemporal maps and the frequency, amplitude, and propagation velocity of gastric peristaltic contractions were measured from the spatiotemporal maps (see Ref. 12). Briefly, for each frame of the movie, the edge of the tissue was thresholded and the distance of the edge of the tissue from a fixed position was calculated and converted to a line of grayscale colors. Grayscale lines from successive frames were stacked next to each other resulting in a spatiotemporal map (ST-Map) representing time (x-axis), distance (corpus—pylorus; y-axis), and amplitude of edge contraction (grayscale; lighter colors represent contraction). Frequency and amplitude of antral peristaltic contractions were measured at the level of the oral antrum and velocity was calculated by fitting a sloped line along the rising phase of the propagating contractions (50% max-min amplitude) in ST-Maps (see Fig. 1).

Solutions and drugs. The composition of the KRB used in these studies was (in mM) 118.5 NaCl, 1.2 MgCl2, 23.8 NaHCO3, 1.2 KH2PO4, 11.0 dextrose, and 2.4 CaCl2. The pH of the buffer was 7.3–7.4 when bubbled with 97% O2-3% CO2 at 37 ± 0.5°C. Prostaglandin E2, butaprost, sulprostone, and nifedipine were purchased from Sigma. GR63799 was a gift from GlaxoSmithKline, and ONO-248 was a gift from ONO Pharmaceutical. ONO-AE-248 and GR63799 were dissolved in water. Nifedipine, butaprost, and PGE2 were dissolved in ethanol, and sulprostone was dissolved in DMSO. The final bath concentration of both DMSO and ethanol did not exceed 0.1% (vol/vol), and neither solvent had any effect at this concentration. All drugs tested were applied via bath perfusion for 10–20 min.

Analysis of data. Data are expressed as means ± SE. The n values reported in the text refer to the number of animals from which muscle strips were taken. SigmaStat Statistical Software for Windows version 2.03 (SPSS Science, Chicago, IL) was used for statistical analyses. Before tests of significance were performed, data were examined for normality and equal variance to determine whether parametric or nonparametric tests should be employed. Paired t-tests and one-way ANOVA (repeated measures) followed by multiple comparisons against the control (Tukey’s test) were used for statistical comparisons. A probability of P < 0.05 was used as a cutoff for statistical significance in all statistical procedures. P values quoted for ANOVA are the values for the individual post hoc test.

RESULTS

Propagation of gastric slow waves and peristaltic contractions. Visualization of gastric peristaltic contractions in flat-sheet preparations consisting of the entire gastric corpus and antrum (see Fig. 1A) showed that gastric contractions originated spontaneously at a frequency of 6.3 ± 0.9 cycles per min (cpm) in the upper region of the corpus and propagated to the pyloric terminus at an average velocity of 1.4 ± 0.2 mm/sec (n = 10; Fig. 1, B–D). Propagation of repetitive peristaltic sweeps could be better displayed and analyzed by creating ST-Maps that illustrate the directionality and rate of propagation in a condensed format (Fig. 1, B–D, and see Supplementary Movie S1 in the online version of this article). Digital imaging showed that the occurrence and propagation of peristaltic contractions was highly regular in flat-sheet preparations of the stomach and comparable to the peristalsis observed in whole, ex vivo stomachs before the flat-sheet preparations were created (Fig. 1 and Supplementary Movie S1). This was not the case when flat-sheet preparations were cut along the greater curvature, bisecting the area of highest slow-wave frequency (K. M. Sanders, unpublished observations). These preparations generated uncoordinated contractile patterns (not shown), most likely because of separation of the high-fre-
Changes in slow-wave frequency in response to PGE2 were not accompanied by significant changes in resting membrane potential (i.e., $-65.8 \pm 1.3$ mV and $-65.2 \pm 1.8$ mV in control and PGE2-treated tissue, respectively; $P > 0.05$, paired $t$-test). Once it was established that PGE2 caused chronotropic effects in both the corpus and antrum, we tested the effects of this agonist on strips of Balb/c gastric muscles, prepared from the greater curvature of the stomach and extending from the oral corpus through the distal antrum. Simultaneous recordings were made from cells in corpus and antrum. PGE2 (100 nM) increased slow-wave frequency significantly in the antrum and corpus (e.g., antrum: $9.6 \pm 0.7$ min$^{-1}$ and $11.4 \pm 0.6$ min$^{-1}$ in control and PGE2, respectively; and corpus: $10.0 \pm 0.6$ min$^{-1}$ and $11.5 \pm 0.4$ min$^{-1}$; $P < 0.05$, paired $t$-test, $n = 4$; Fig. 3). PGE2 caused no significant change in membrane potential or slow-wave amplitude. These data demonstrate that simultaneous exposure of corpus and antrum to PGE2 produces generalized positive chronotropic effects and that both regions are capable of keeping pace with the increased slow-wave frequency. Thus functional coupling between regions did not appear to be adversely affected by a generalized increase in slow-wave frequency of ~15%.

PGE2 affects several EP receptors (EP$_1$–EP$_4$), which PCR analysis showed were all expressed in the tunica muscularis of the murine stomach (data not shown). Previous studies suggested that EP$_3$ receptors mediate the chronotropic effects of PGE$_2$ in the murine antrum (17). Therefore, we tested three EP$_3$-receptor agonists [GR63799 (100 nM), ONO-AE-248 (100 nM; $n = 5$), and sulprostone (100 nM; $n = 4$)] to determine whether functional coupling is preserved under conditions of more specific chronotropic stimulation. Sulprostone is also an EP$_1$ agonist, so in the case of this drug we also added SC19220 (10 μM), an EP$_1$ antagonist. All of the EP$_3$ agonists caused significant increases in slow-wave frequency (GR63799; $n = 4$; $P < 0.05$), ONO-AE-248 (100 nM; $n = 5$; $P < 0.05$), and sulprostone (100 nM; $n = 4$; $P < 0.05$), and the increases were equivalent in the corpus and antrum (Table 1). EP$_3$ agonists also had a tendency to depolarize the antral and corpus regions; however, this effect did not reach a level of significance. Figure 4 shows an example of the chronotropic effects of sulprostone. From the two points of recording, these data also suggested that functional coupling was preserved during EP$_3$ agonist stimulation. For comparison, we also tested a specific agonist for EP$_2$ receptors that are abundant in gastric muscles (not shown).

**Table 1.**

<table>
<thead>
<tr>
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<th>Antrum</th>
<th>Corpus</th>
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<tr>
<td>Chronotropic effect</td>
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<tr>
<td>ONO-AE-248</td>
<td>118.67±5.62</td>
<td>119.45±5.58</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>137.72±13.28</td>
<td>138.16±13.57</td>
</tr>
<tr>
<td>GR63799</td>
<td>146.86±17.62</td>
<td>145.75±16.30</td>
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<tr>
<td>Membrane potential</td>
<td></td>
<td></td>
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<tr>
<td>ONO-AE-248</td>
<td>97.05±1.62</td>
<td>99.51±4.00</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>95.79±4.31</td>
<td>90.03±8.09</td>
</tr>
<tr>
<td>GR63799</td>
<td>90.66±4.16</td>
<td>88.49±9.11</td>
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Both resting membrane potential and slow-wave frequency recorded in the presence of ONO-AE-248 (100 nM), GR63799 (100 nM), or sulprostone (100 nM) were expressed as a percentage of the control values obtained. Student’s $t$-test for unpaired data was performed between the corpus and antrum values for each treatment group and no significant differences were observed.

Next we performed intracellular microelectrode recordings to investigate the events underlying gastric peristaltic contractions and the effects of positive chronotrophic agents on gastric slow waves. Kim et al. (17) showed that PGE2 and EP$_3$-receptor agonists increased the frequency of slow waves in the mouse antrum. We tested whether slow-wave frequency is also enhanced in mouse corpus by these agonists. PGE2 (100 nM) caused significant chronotropic effects on Balb/c corpus muscle preparations ($8.1 \pm 0.6$ and $9.9 \pm 0.8$ min$^{-1}$ in control and PGE2-treated tissue, respectively; $P < 0.05$, paired $t$-test, $n = 6$; Fig. 2).
Butaprost (100 nM) had no effect on the slow-wave frequency in recordings from either corpus or antrum cells (n = 5; Fig. 5).

**Effects of EP3 agonist on gastric peristalsis.** Impalements of two cells in different regions of the stomach can demonstrate integrated frequency effects of chronotropic agonists, but it is difficult from two-point electrical recordings to determine the point of initiation of slow waves and whether increasing slow-wave frequency disrupts the normal proximal-to-distal direction of slow-wave propagation. Therefore, we also performed imaging studies of gastric peristalsis using intact corpus and antrum muscle sheets (Fig. 6A) to determine the effects of the EP3 agonist on the propagation of contractions. Muscles were imaged for 5 min under control conditions (Fig. 6B) and then exposed to sulprostone (100 nM) or to sulprostone (100 nM) and SC19220 (10 μM) in combination. As in electrophysiological experiments, sulprostone increased the frequency of peristaltic contractions from 5.83 ± 1 cpm to 7.8 ± 1 cpm; P < 0.01. Although not apparent in electrophysiological recordings, these studies revealed that the chronotropic effects of sulprostone resulted in aberrant propagation patterns. For example, instances of contractions being initiated in the antrum or failure to propagate as a coherent wave front were observed (Fig. 6C). Addition of SC19220 did not affect the chronotropic effects of sulprostone (i.e., 8.1 ± 1 cpm after addition of sulprostone) and aberrant propagation patterns persisted after SC19220 (Fig. 6D). These data demonstrate the tendency for a...
breakdown in functional coupling with whole organ stimulation of EP3 receptors.

_Dual recordings (_+/+ and W/W'_v)_). Dual electrical recordings were also made in intact corpus and antrum strips from _W/W'_v (WBB6F1/J-Kit^+/^-) mice and strain-matched (WBB6F1/J-Kit^+/+/W/W'_v) controls. _W/W'_v mice lack intramuscular interstitial cells of Cajal (ICC-IM; Ref. 4), and these mice were used to determine whether the chronotropic effects of EP3 agonists might be mediated via ICC-IM. As in muscles of Balb/c mice, PGE2 produced significant chronotropic effects in the corpus and antrum of wild-type muscles (e.g., antrum: 7.4 ± 0.9 min^{-1} and 9.1 ± 1.4 min^{-1} in control and PGE2, respectively; corpus: 7.3 ± 0.7 min^{-1} and 9.5 ± 0.9 min^{-1}; _P_ < 0.05, paired _t_-test, _n_ = 3; Fig. 7, A, C, and E), but we observed no significant effect of PGE2 on slow-wave frequency in muscles of _W/W'_v mice (e.g., antrum: 11.3 ± 0.2 min^{-1} and 10.9 ± 0.6 min^{-1} in control and PGE2, respectively; corpus: 11.5 ± 0.1 min^{-1} and 11.3 ± 0.9 min^{-1}; _P_ < 0.05, paired _t_-test, _n_ = 3; Fig. 7, B, D, and E). No effects were noted on slow-wave amplitude or resting membrane potential either wild-type or _W/W'_v in response to PGE2.

The effects of ONO-AE-248 were also tested on wild-type and _W/W'_v gastric muscles. Positive chronotropic effects were observed in wild-type muscles (e.g., antrum: 9.1 ± 1.4 min^{-1} and 10.0 ± 1.4 min^{-1} in control and ONO-AE-248, respectively; corpus: 9.1 ± 1.4 min^{-1} and 10.0 ± 1.4 min^{-1}; _P_ < 0.05, paired _t_-test, _n_ = 3). In stomachs of _W/W'_v mice ONO-AE-248 failed to elicit a chronotropic effect (e.g., antrum: 9.9 ± 0.5 min^{-1} and 10.2 ± 0.5 min^{-1} in control and ONO-AE-248, respectively; corpus: 9.9 ± 0.5 min^{-1} and 10.2 ± 0.5 min^{-1}; _P_ < 0.05, paired _t_-test, _n_ = 3; Fig. 7).

_Corpus and antral slow waves recorded using a partitioned bath_. Experiments above demonstrate that simultaneous stimulation of the corpus and antrum with EP3 agonists results in chronotropic effects in both regions, and motility studies showed that whole stomach stimulation with an EP3 agonist can cause aberrant motility patterns. Responses to prostaglandins may be more complicated in vivo since it is possible that regional induction of prostaglandin synthesis may occur (e.g., in localized inflammation). To simulate this situation, we utilized a partitioned bath where EP3 agonists could be added to either the corpus or antrum selectively.

Longitudinal strips of muscle from the corpus to the antrum were pulled through a latex membrane that created two chambers in which the muscles could be perfused individually.
Propagating slow-wave activity was recorded by impalements of circular layer smooth muscle cells in the corpus and antrum. PGE₂ added to the corpus chamber of the partitioned bath caused a significant increase in slow-wave frequency in both corpus and antrum (e.g., corpus: 9.1 ± 0.7 min⁻¹ and 10.7 ± 0.3 min⁻¹ in control and PGE₂, respectively; antrum: 9.1 ± 0.7 min⁻¹ and 10.7 ± 0.3 min⁻¹; P < 0.05, paired t-test, n = 6; Fig. 8, A and B). No differences were noted in membrane potential or slow-wave amplitude in either chamber in response to the drug. Similarly, when PGE₂ was added to the antral chamber of the bath, a significant increase in slow-wave frequency was observed in corpus and antrum (antrum: 8.0 ± 0.7 min⁻¹ and 10.1 ± 1.1 min⁻¹ in control and PGE₂, respectively; corpus: 8.0 ± 0.5 min⁻¹ and 10.1 ± 0.8 min⁻¹; P < 0.05, paired t-test, n = 6; Fig. 8, A and B).

After responses to PGE₂ were recorded, the antrum and corpus regions were separated by a scalpel cut close to the partition. When PGE₂ was added to the chamber housing the antrum, the chronotrophic effects were maintained in the antral region (i.e., 10.1 ± 0.8 min⁻¹) but were reduced to pre-PGE₂ levels in the corpus (i.e., 7.9 ± 0.2 min⁻¹; Fig. 9, C and D). No differences were noted in the membrane potential or amplitude of slow waves after the drug was added or after the antral and corpus regions were separated. These data indicate that when the antral region is selectively exposed to elevated PGE₂, the positive chronotropic drive on antral pacemakers can cause this region to become dominant.

Similar experiments were performed using sulprostone (100 nM). Addition of this compound to the corpus chamber caused significant chronotropic effects on slow waves recorded in both regions of the stomach (e.g., antrum: 7.6 ± 0.7 min⁻¹ and 9.1 ± 0.6 min⁻¹ in control and sulprostone, respectively; corpus: 7.5 ± 0.7 min⁻¹ and 9.1 ± 0.5 min⁻¹; P < 0.05, paired t-test, n = 5; Fig. 8D). The chronotropic effects on antral muscles were completely abolished in the antral portion of the muscle strip when the antrum was separated by transection (i.e., slow-wave frequency decreased to 4.6 ± 0.8 min⁻¹ after loss of drive from corpus pacemakers). Slow-wave fre-
Summary of the effects of sulprostone (100 nM) in 6 experiments. *Significantly different from control (P < 0.05 by ANOVA, n = 5; Fig. 9E). After transection of the muscle strip, the antral frequency remained high (i.e., 8.0 ± 0.6 min^{-1}), whereas the corpus frequency returned to the control frequency (i.e., 6.9 ± 1.2 min^{-1}). These data indicate that localized stimulation of antral EP3 receptors causes chronotropic effects that drive more proximal pacemakers.

**DISCUSSION**

The corpus hosts the normal dominant pacemaker in the murine stomach, as in other animal models (21, 30). Dominance of corpus pacemakers has been established by experiments showing that the corpus intrinsically produces six to eight slow waves per minute. When the corpus and antrum are left attached, the antrum is entrained by corpus slow waves, and a one-to-one relationship exists between slow waves in the two regions. When the antrum is detached from the corpus, the intrinsic slow-wave frequency is found to be 2–4 cycles/min (8, 21). Functional coupling in the intact stomach allows coordination of slow-wave activity between regions, facilitating the propagation of gastric peristalsis, as we observed in motility studies and displayed in ST-Maps (Fig. 1) and movies (Supplemental Movie S1). Here we have shown that chronotropic agonists can alter slow-wave frequencies, potentially leading to the emergence of ectopic pacemakers, collisions of peristaltic waves, and instances when antral pacemaking is dominant (antiperistalsis; Ref. 5).

Prostaglandins are local regulatory agents or paracrine substances that are synthesized by a variety of cells. Many prostaglandins (e.g., PGE2, PGD2, PGF2α, PGA2) are synthesized in mammalian GI tunica muscularis, and PGE2 was found to be a prominent species produced in gastric muscles (28). Both cyclooxygenase (COX) 1 and 2 are constitutively expressed in murine gastric muscles, and COX-2 was specifically localized in neurons containing nitric oxide synthase and intramuscular ICC (24). Previous studies have shown that endogenous prostaglandins tonically suppress spontaneous electrical and mechanical activities in canine antrum (29) and that exogenously applied PGE2 produces relaxation and reduction in slow-wave amplitude and duration in dog, guinea pig, and murine antrum (17, 20). The frequency of electrical slow waves and corresponding contractions is reduced when basal prostaglandin synthesis is inhibited (24, 29), suggesting that ongoing synthesis contributes to the regulation of slow-wave frequency. Endogenous prostaglandins have been linked to symptoms of gastric dysrhythmias, nausea, and vomiting in human patients, and clinical studies have demonstrated that blocking prostaglandin synthesis prevents these symptoms in some patients (10).

The present study suggests that dysrhythmias displayed in gastric muscles in response to prostaglandin synthesis (which may be associated with the nausea and vomiting) depend on the receptor population expressed by intramuscular ICC. Other responses to prostaglandins, such as membrane potential effects and relaxation, may be more dependent on specific receptors, transduction mechanisms, ion channels, and contractile protein regulatory pathways expressed by smooth muscle cells. Inflammation upregulates COX-2 in GI muscles (1), so elevated prostaglandin levels may underlie gastric dysrhythmias in some clinical conditions. Little is known about pro-

![Image](http://ajpgi.physiology.org/Downloaded_from/http://ajpgi.physiology.org/2009/05/29/june2009www.ajpgi.org)
duction of prostaglandins in diabetic gastric muscles, but it has been reported that hyperglycemia can induce gastric arrhythmias and indomethacin, a nonspecific inhibitor of COX-1 and 2, blunts these effects (10).

Both the corpus and antral regions of the murine stomach express EP receptors, and our results show that both regions display equivalent chronotropic responses to PGE2 (and see Ref. 17). EP3 agonists appear to mediate the chronotropic effects as 3 EP3 agonists also enhanced slow-wave frequency, but an agonist of EP2 receptors, which are also prominently expressed in gastric muscles, was without effect. EP2 and EP4 agonists couple through Gαs so it is likely that stimulation of EP4 receptors would have similar effects to EP2 receptor binding. The effects of sulprostone, an EP1 and 3 receptor agonist, were undiminished by SC19220, an EP1 receptor antagonist, suggesting that the chronotropic effects of PGE2 are mainly due to EP3 receptors. The EP3 receptors involved in chronotropic effects appear to be localized to intramuscular ICC, because chronotropic effects to PGE2 and sulprostone were not detected in W/Wv mice which lack this class of ICC in the murine stomach (4). We attempted immunohistochemical localization of EP3 receptors, but with the antibodies available, we failed to obtain meaningful images.

Intracellular electrical recording provided important information about the general impact of chronotropic agonists on slow-wave frequency, but the natural frequency and duration of gastric slow waves in the mouse complicated our attempt to understand how these agonists affected functional coupling between the corpus and antrum. Our electrophysiological experiments, using simultaneous intracellular recording from cells in the corpus and antrum, failed to detect clear evidence of functional uncoupling, even in conditions of significant and specific elevation of antral pacemaker frequency. This is because the regular occurrence and frequency of gastric slow waves makes it difficult to clearly establish the temporal lag between antral and corpus events and to determine where events are initiated and the directionality of slow-wave propagation. We also had trouble precisely determining slow-wave conduction velocity because it is not clear, from dual recordings of spontaneous activity, which antral slow wave represented the propagated event from a given corpus slow wave. A technique that would allow determination of direction and velocity of propagation would be to elicit a premature slow wave in the corpus, identify the phase-advanced antral slow wave that follows, and measure its time of propagation. However, we found it unreliable to elicit clearly premature slow waves in murine corpus muscles, possibly because of the post-slow-wave refractory properties of ICC and the relatively short durations between events. It should be noted that with the same techniques it was possible to phase advance slow waves in isolated antral muscles, either utilizing field stimulation of intrinsic cholinergic nerves or by directly activating ICC with longer pulse stimulation (3, 9). A recent report describes recording of gastric slow waves with large-scale extracellular electrode arrays (19), and this technique offers substantially improved resolution of speed and...
mapping of propagation. The short distances and latencies between electrodes and occurrence of slow waves with the array recording, leaves little doubt about the directionality and velocity of propagation. Computation of ST-Maps from the data provided very important information about how naturally occurring tachygastrias affect slow-wave propagation. Studies of the impact of chronotropic agonists will be enhanced by application of this approach.

Ultimately motility studies are required to determine the impact of electrical arrhythmias on gastric motor function. Difficulties in understanding coordination via two-point electrical recording were offset in the present study by inclusion of motility studies and transformation of peristaltic contractions into ST-Maps. These studies clearly showed that gastric peristaltic contractions are normally initiated in the proximal corpus and spread with nearly constant velocity toward the pyloric sphincter. Similar measurements were made on intact mouse stomachs (Fig. 1), as previously described for guinea pig (11), to validate our flat-sheet preparation and confirm that dissecting the stomach in this manner did not introduce artifacts that might unduly affect frequency and propagation velocity of spontaneous gastric peristaltic activity. It should be noted that there were small differences in mean slow-wave frequency measured by electrophysiological techniques (7.5 ± 0.3 cpm) and the frequency of peristaltic contractions (i.e., 6.3 ± 0.9 cpm) under control conditions. This difference might be explained by the fact that, to maintain intracellular impalements, it is necessary to impose stretch on muscle strips. Previous studies of the murine stomach have demonstrated an elevation in gastric slow waves in response to stretch (31). To image gastric peristaltic contractions it was essential not to restrict muscle shortening. Thus, of necessity, the two preparations differed in terms of the imposed tension during experiments.

We utilized specific application of EP3 receptor agonists to either the corpus or antrum utilizing a partitioned bath and independent perfusion of each area. When chronotropic agonists were applied to the corpus, frequency increased throughout the antral muscle in intact strips, but when the corpus was transected from the antrum, the antral frequency fell to control levels. Analogous results were obtained when the agents were applied selectively to the antrum: slow-wave frequency increased equally throughout the antrum and corpus but fell to control values in the corpus when the antrum was transected from the corpus. The latter indicates that the corpus follows the elevated frequency of the antrum when there is selective application of chronotropic agonist to the antral pacemakers. Region-specific exposure to chronotropic agonists could occur in a localized inflammatory response, in which inducible cyclooxygenase increased in a specific area, driving up the frequency of local pacemakers and creating an ectopic pacemaker site that might begin to drive abnormal gastric contractions. Such a localized response may interfere with gastric peristalsis and emptying without widespread inflammation. It is also possible that unbalanced cholinergic input or response, which also has dramatic chronotropic effects on gastric pacemakers (8, 18), could initiate ectopic pacemaking. Our study shows that enhancing the intrinsic frequency of a localized population of pacemakers can have widespread impact on integrated responses of the intact stomach.

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