Gastric Ulcers Evoke Hyperexcitability and Enhance P2X Receptor Function in Rat Gastric Sensory Neurons

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Dang, K., K. Bielfeldt, K. Lamb, and G. F. Gebhart. Gastric ulcers evoke hyperexcitability and enhance P2X receptor function in rat gastric sensory neurons. J Neurophysiol 93: 3112–3119, 2005. First published January 26, 2005; doi:10.1152/jn.01127.2004. Tissue inflammation contributes to the development of hyperalgesia, which is at least in part due to altered properties of primary afferent neurons. We hypothesized that gastric ulcers enhance the excitability of gastric sensory neurons and increase their response to purinergic agonists. The rat stomach was surgically exposed, and a retrograde tracer [1.1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine methanesulfonate (DiI)] was injected into the wall of the distal stomach. Kissing ulcers (KUs) were produced by a single injection of acetic acid (0.1 ml for 45 s; 60%) into the clamped gastric lumen. Saline injection served as control. Gastric nodose ganglion (NG) or dorsal root ganglion (DRG) cells were harvested 7 days later and acutely dissociated for whole cell recordings. Based on whole cell capacitance, gastric DRG neurons exhibited larger cell size than NG neurons. Significantly more control gastric DRG neurons compared with NG counterparts had TTX-resistant action potentials. Almost all control NG neurons (90%) compared with significantly less DRG neurons (≤38%) responded to ATP or α,β-metATP. Whereas none of the control cells exhibited spontaneous activity, about 20% of the neurons from KU animals generated spontaneous action potentials. KUs enhanced excitability as shown by a decrease in threshold for action potential generation, which was in part due to an increased input resistance. This was associated with an increase in the fraction of neurons with TTX-resistant action potentials and cells responding to capsaicin and purinergic agonists. KU doubled the current density evoked by the P2X receptor agonist α,β-metATP and slowed decay of the slowly desensitizing component of the current without affecting the concentration dependence of the response. These data show that KU sensitizes vagal and spinal gastric afferents by affecting both voltage- and ligand-gated channels, thereby potentially contributing to the development of dyspeptic symptoms.

INTRODUCTION

Abdominal pain and discomfort are common problems in western countries, with an estimated point prevalence of about 25% of the adult population (Drossman et al. 1993). In >50% of the affected individuals, no structural or biochemical abnormalities can be identified, leading to the diagnosis of functional diseases, such as nonulcer dyspepsia or irritable bowel syndrome. Many studies have shown that changes in visceral sensitivity (i.e., a visceral hypersensitivity) contribute to the development of these disorders (reviewed in Mayer and Gebhart 1994). To examine mechanisms underlying such changes in visceral sensitivity, we developed several animal models of gastric hypersensitivity characterized by enhanced nociceptive responses to mechanical or chemical stimulation of the stomach (Lamb et al. 2003a,b; Ozaki et al. 2002). Gastric hypersensitivity is also associated with changes in voltage-dependent sodium and potassium currents in primary afferent visceral neurons, suggesting that peripheral mechanisms contribute to these enhanced responses (Bieinfeldt et al. 2002; Dang et al. 2004).

Activation of purinergic receptors plays an important physiological role in the gastrointestinal tract and may contribute to nociceptive signaling (Burnstock 1996). The natural ligand ATP activates ionotropic P2X and metabotropic P2Y receptors, both of which are present in dorsal root and nodose ganglion neurons (Cook et al. 1997; Fong et al. 2002; Tominaga et al. 2001). Based on the previously shown changes in voltage-dependent ion channels in gastric sensory neurons and the potential importance of purinergic receptors in nociception, in these experiments, we examined the excitability of gastric sensory neurons following ulceration and characterized P2X receptor–mediated responses using whole cell patch-clamp techniques. A preliminary report has been presented in an abstract form (Dang et al. 2003).

METHODS

Male Sprague-Dawley rats (150–200 g; Harlan, Indianapolis, IN) were used for the experiments. Rats were housed under a 12-h light and dark cycle with free access to food and water. Animal handling adhered to the Guide for the Care and Use of Laboratory Animals; the experimental protocol was approved by the Animal Care and Use Committee of The University of Iowa.

Cell labeling and induction of gastric ulcers

Under pentobarbital sodium anesthesia (50 mg/kg, ip), the rat stomach was surgically exposed and 1.1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine methanesulfonate (DiI; 100 mg in 2 ml DMSO; Molecular Probes, Eugene, OR) was injected to eight sites within the ventral and dorsal wall of the distal stomach (4 µl/site). The wound was closed with 4–0 silk suture, and rats were allowed to recover. Seven to 10 days later, rats were anesthetized again with pentobarbital sodium, the stomach was re-exposed, and a circular clamp (1 cm ID) was placed over the site marked by the previously injected DiI. Kissing ulcers (KUs) were induced by injecting 100 µl of 60% acetic acid into the clamped area and withdrawing it after 45 s as previously described (Lamb et al. 2003a). Control animals received saline. Nodose ganglion (NG) and dorsal root ganglion (DRG) neurons were harvested 7 days later and acutely dissociated for electrophysiology.

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physiological recordings. Only DiI-labeled gastric NG and DRG neurons were studied.

**Cell dissociation and culture**

Rats were anesthetized and decapitated, and bilateral NG or T9–T10 DRGs were rapidly removed. The ganglia were minced and incubated at 37°C, 5% CO₂, for 60 min in serum-free, supplemented Neuro-A medium (B27 supplements: 5%; l-glutamine: 0.5 mM; penicillin/streptomycin mixture: 1%; Gibco, Invitrogen, Grand Island, NY) containing collagenase (type 4; 2 mg/ml) and trypsin (1 mg/ml; Worthington Biochemical, Lakewood, NJ). Tissue fragments were gently triturated to encourage cell dissociation. Cells were collected by 5-min centrifugation at 150g and washed three times with supplemented Neuro-A medium without enzymes and resuspended in supplemented, enzyme free Neuro-A medium. The cells were plated on poly-o-lysine–coated coverslips (Becton Dickinson Labware, Bedford, MA) and incubated at 37°C, 5% CO₂, for 2–3 h before electrophysiological studies. Acutely dissociated neurons were round and devoid of any processes, thus reducing potential space-clamp errors. All recordings were performed within 10 h after plating.

**Solutions and electrophysiological recordings**

Cells were transferred to a recording chamber (1 ml) superfused continuously (2 ml/min) with external solution containing (in mM) 140 NaCl, 5 KCl, 2 MgCl₂, 2 CaCl₂, 10 HEPES, and 10 glucose. The pH was adjusted to 7.4 with NaOH (310 mOsm). Neurons that innervate the stomach were identified by content of DiI using a rhodamine filter (excitation wavelength 546 nm and barrier filter at 580 nm). Fire-polished micropipettes with tip resistances of 2–3 MΩ were used for current and voltage-clamp recordings. With such micropipettes, the uncompensated series resistance was generally about 9 MΩ or lower. The pipette was filled with internal solution containing (in mM) 130 KCl, 1 CaCl₂, 1 MgCl₂, 10 EGTA, 10 HEPES, 4 Na₂ATP, and 0.5 Tris-GTP. The pH was adjusted to 7.2 using KOH (310 mOsm). After establishing the whole cell configuration, the voltage was initially clamped at −70 mV to check for leak current and immediately switched to current- or voltage-clamp mode using an Axopatch 200B amplifier (Axon Instruments, Union City, CA), digitized at 10 kHz (Digidata 1350, Axon Instruments), and controlled by Clampex software (pclamp 9, Axon Instruments). We only studied cells that had <300 pA of leak current. In voltage-clamp experiments, cells were held at −70 mV. Series resistance was not compensated for in voltage-clamp experiments but was 100% compensated for in current-clamp mode. Cell capacitance was noted by reading the value on the Axopatch 200B amplifier. To assure stable conditions, recordings were started 2 min after gaining whole cell access. Agonists were applied using a superfusion system with the inflow pipette placed in close proximity (100 μm) to the cell (Biomedical Institutes, Prague, Czech Republic). A washout period of 4 min was allowed between agonist applications. All experiments were performed at room temperature (21–23°C).

In current-clamp mode, resting membrane potential was determined by reading the value on the amplifier (Axopatch 200B). Only cells that had a resting membrane potential more negative than −40 mV and generated action potentials with overshoot in response to depolarizing current injections were studied. Action potentials (APs) were identified as spikes passing 0 mV. AP duration was determined at 50% of the peak amplitude. We investigated spontaneous activity by recording the baseline activity for 1 min before electrical or chemical stimulation. To identify action potential threshold, a series of 5 ms current pulses in 50-pA increments (1 s apart) was injected. The minimum current (pA) required to evoke an AP was determined and the activation threshold (rheobase) was taken as the membrane potential prior the inflection point of the AP. Input resistance was determined according to the V/I relationship by injecting a series of hyperpolarizing pulses ranging from −300 to 0 pA (5 ms) in 50-pA increments. To examine firing patterns in gastric sensory neurons, suprathreshold current (2× rheobase) was injected for 500 ms, and the number of APs was counted. Neurons generating less than three spikes during stimulus application were defined as phasias, whereas tonic neurons fired throughout the entire period of stimulation. Drugs were obtained from Sigma-Aldrich (St. Louis, MO) and prepared fresh from stock solutions on the day of the experiment.

**Statistical analyses**

All data are given as means ± SD. To compare percentage differences, Fisher’s exact test was employed. We compared EC₅₀ values and desensitization kinetics with one-way ANOVAs. Unless otherwise stated, other comparisons were made using Student’s unpaired t-test. Results were considered to be statistically significant when P < 0.05.

**RESULTS**

**Excitability of gastric sensory neurons**

To selectively examine gastric sensory neurons, we studied only NG and DRG neurons that contained DiI retrogradely transported from the sites of injection in the gastric wall. As shown in Table 1, the electrophysiological properties of gastric

**Table 1. Electrophysiological properties of gastric sensory neurons**

<table>
<thead>
<tr>
<th></th>
<th>Saline-Treated</th>
<th>DRG</th>
<th>Kissing Ulcers</th>
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<tr>
<td></td>
<td>Nodose</td>
<td>DRG</td>
<td>Nodose</td>
</tr>
<tr>
<td>Whole cell capacitance, pF</td>
<td>52 ± 3.5</td>
<td>65 ± 2.3*</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>−66 ± 1.7</td>
<td>−55 ± 1.3*</td>
<td>−61 ± 1.9</td>
</tr>
<tr>
<td>Threshold, pA</td>
<td>743 ± 63</td>
<td>565 ± 61*</td>
<td>405 ± 63†</td>
</tr>
<tr>
<td>Input resistance, mohm</td>
<td>51.0 ± 2.9</td>
<td>35.3 ± 4.1*</td>
<td>76.5 ± 5.8†</td>
</tr>
<tr>
<td>Action potential amplitude, mV</td>
<td>112.2 ± 3.7</td>
<td>98.0 ± 3</td>
<td>107.2 ± 3.1</td>
</tr>
<tr>
<td>Action potential duration, ms</td>
<td>3.3 ± 0.6</td>
<td>3.2 ± 1.1</td>
<td>2.8 ± 0.2†</td>
</tr>
<tr>
<td>Action potential frequency, 2 × rheobase</td>
<td>9.4 ± 0.7</td>
<td>10.9 ± 1.1</td>
<td>14.1 ± 1.3†</td>
</tr>
<tr>
<td>Neurons with TTX-resistant spikes, %</td>
<td>11/22 (50%)</td>
<td>24/28 (86%)*</td>
<td>22/25 (88%)†</td>
</tr>
<tr>
<td>Capsaicin-sensitive neurons, %</td>
<td>10/20 (50%)</td>
<td>20/31 (64%)</td>
<td>18/23 (78%)†</td>
</tr>
<tr>
<td>Capsaicin-evoked amplitude, mV</td>
<td>27.9 ± 4.3</td>
<td>38.1 ± 2.1*</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>No. of AP</td>
<td>8.4 ± 4.5</td>
<td>11.4 ± 2.4</td>
<td>7.7 ± 2</td>
</tr>
<tr>
<td>Capsaicin-evoked current density, pA/pF</td>
<td>105.3 ± 14.6</td>
<td>142.7 ± 10.3*</td>
<td>103.7 ± 12.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. * and †P < 0.05 compared between nodose and DRG neurons or saline-treated and KU rats, respectively. DRG, dorsal root ganglion; KU, kissing ulcer; AP, action potential.
NG and DRG neurons differed significantly. None of the cells studied under control conditions exhibited spontaneous activity or significant resting membrane oscillations (Fig. 1, studied under control conditions exhibited spontaneous activity NG and DRG neurons differed significantly. None of the cells

![Diagram](image.png)

FIG. 1. Changes in baseline activity and increased excitability in gastric sensory neurons after gastric ulceration. Representative voltage tracings from nodose ganglion (NG; A) and dorsal root ganglion (DRG; B) neurons show a stable resting membrane potential without spontaneous activity in control, saline-treated rats, whereas gastric ulceration [kissing ulcer (KU)] produced fluctuations in baseline activity and spontaneous firing in both NG (C) and DRG (D) neurons (arrows indicate oscillations of resting membrane potential). Current injection (2× rheobase, 500 ms) depolarized and evoked firing in NG (E) and DRG (F) neurons from control animals. KU significantly increased the number of action potentials in response to current injection in NG (G) and DRG (H) neurons.

NG and DRG neurons differed significantly. None of the cells studied under control conditions exhibited spontaneous activity or significant resting membrane potential oscillations (Fig. 1, A and B, arrows). Gastric NG neurons had more negative resting membrane potentials and required stronger depolarizing current injections to evoke an AP than gastric DRG neurons (P < 0.05), although the threshold for AP generation was similar in NG and DRG neurons. As shown in Fig. 1, E and F, most gastric NG and DRG neurons gave tonic responses to current injection, with only 3 of 28 NG neurons and 4 of 35 DRG neurons generating only one AP at the onset of 500-ms suprathreshold stimulation. Interestingly, 1 μM TTX abolished APs in 50% (11/22) of NG neurons, but abolished APs in only 14% (4/28) of DRG neurons (P < 0.05).

**Responses to purinergic agonists**

Application of 100 μM ATP rapidly depolarized 12 of 31 DRG and 18 of 20 NG neurons (P < 0.01), with a peak amplitude of 13.5 ± 3.8 and 27 ± 2.3 mV, respectively (P < 0.01; Table 2; Fig. 2). The depolarization exceeded AP threshold in 10 DRG and 13 NG neurons, triggering about five APs in both DRG and NG neurons. We next examined whether the depolarization was due to activation of ligand-gated P2X channels in the same cells, using αβ-metATP, a P2X1 and/or 3 receptor agonist. As shown in Fig. 2, 100 μM αβ-metATP depolarized 9 of 31 DRG neurons and 18 of 20 NG neurons (P < 0.01; Table 2), with an average amplitude of 15.9 ± 4.5 and 25.9 ± 1.5 mV for DRG and NG neurons, respectively (P < 0.01). In 6 DRG and 13 NG neurons, αβ-metATP triggered 5.5 ± 3.2 and 9 ± 2.1 APs, respectively. ATP depolarized all NG neurons responsive to αβ-metATP, whereas 3 of 12 DRG neurons responded to ATP but not to αβ-metATP, suggesting the presence of homomeric P2X2 receptors or other purinergic receptors in these neurons.

**P2X current characteristics in gastric sensory neurons**

To examine the effects of P2X receptor stimulation in more detail, we performed voltage-clamp experiments. Consistent with the current-clamp data described above, 100 μM αβ-metATP triggered inward currents in virtually all NG neurons (26 of 27), whereas only 12 of 35 DRG neurons responded to the agonist (P < 0.01). Based on desensitization kinetics, fast and slow components could be distinguished (Fig. 3). Most (20/27) NG neurons exhibited a mixture of fast and slow currents (Fig. 3A), with six having only a slow component. In contrast, αβ-metATP activated only either fast (n = 9) or slow (n = 3) desensitizing currents in DRG neurons (Fig. 3, C and D). To examine the concentration dependence of P2X-evoked currents, we studied neurons predominantly expressing one of the kinetically distinct currents using 1–300 μM αβ-metATP. The fast desensitizing component was more sensitive to the agonist. Estimated EC50s for NG and DRG neurons, respectively, were 13.9 ± 2 (n = 6) and 15.2 ± 3 μM (n = 5) for the fast desensitizing component compared with 23.6 ± 3 (n = 6) and 42 ± 17 μM (n = 3) for the slow desensitizing component (P < 0.01; Fig. 4). The rapid current decay could be fitted with a single exponential, yielding desensitization time constants of 87.8 ± 24 and 65.2 ± 11 ms for NG and DRG neurons.

**Table 2. Responses of gastric sensory neurons to purinergic stimulation**

<table>
<thead>
<tr>
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<th>Saline-Treated</th>
<th>DRG</th>
<th>Kissing Ulcers</th>
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<tbody>
<tr>
<td></td>
<td>Nodose</td>
<td>DRG</td>
<td>Nodose</td>
</tr>
<tr>
<td>ATP-sensitive cells</td>
<td>18/20 (90%)</td>
<td>12/31 (38%)*</td>
<td>17/23 (74%)</td>
</tr>
<tr>
<td>Peak depolarization</td>
<td>27 ± 2.3</td>
<td>13.5 ± 3.8*</td>
<td>39.6 ± 3†</td>
</tr>
<tr>
<td>αβ-met ATP-sensitive cells</td>
<td>5 ± 1</td>
<td>4.8 ± 1.8</td>
<td>18.5 ± 3†</td>
</tr>
<tr>
<td>Peak depolarization</td>
<td>25.9 ± 1.5</td>
<td>15.9 ± 4.5*</td>
<td>42.2 ± 3†</td>
</tr>
<tr>
<td>Number of AP</td>
<td>9 ± 2.1</td>
<td>5.5 ± 3.2</td>
<td>24.6 ± 3†</td>
</tr>
</tbody>
</table>

Values are means ± SD or n (%). * and †P < 0.05 compared between nodose and DRG neurons or saline-treated and KU rats, respectively. See Table 1 for abbreviations.
respectively (Fig. 5). The kinetics of the slowly desensitizing current were concentration-dependent with time constants of 3.5 \( \pm \) 0.2 and 2.3 \( \pm \) 0.3 s for NG and DRG neurons, respectively (Fig. 5).

We used the nonselective P2X antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) to confirm that inward currents observed were indeed due to activation of P2X receptors. In the presence of 50 \( \mu \)M PPADS, responses to 100 \( \mu \)M \( \alpha,\beta\)-metATP were completely inhibited (\( P < 0.001 \)). We observed only partial recovery (38.7 \( \pm \) 1.2% of control amplitude; \( n = 5 \)) 4 min after PPADS exposure, consistent with a previous report (Zhong et al. 2001).

To further characterize the properties of neurons responding to \( \alpha,\beta\)-metATP, we applied capsaicin. Superfusion with 1 \( \mu \)M capsaicin triggered a depolarization in 20 of 31 DRG and 10 of 20 NG neurons (\( P < 0.05 \)). Only 6 of 20 capsaicin-sensitive neurons depolarized from control levels in response to 1 \( \mu \)M capsaicin. 4 min after PPADS exposure, consistent with a previous report (Zhong et al. 2001).

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DRG neurons also responded to α,β-metATP, whereas the P2X agonist depolarized 9 of the 10 capsaicin-sensitive NG neurons (P < 0.01). Three of 11 DRG and 9 of 10 NG neurons that were capsaicin-insensitive responded to α,β-metATP.

Effects of gastric ulcers on excitability of gastric sensory neurons

Consistent with previous reports (Lamb et al. 2003b), injection of acetic acid into the gastric lumen produced ulcers with disruption of mucosal integrity, heaped up margins, and granulation tissue in the center in all KU rats. While there was a trend toward a lower resting membrane potential in neurons from KU rats, the resting membrane potential for both types of neurons from KU rats did not differ significantly from neurons taken from saline-treated rats (Table 1). All neurons obtained from KU animals exhibited fluctuations in resting membrane potential (>3.5 mV above baseline), which were associated with spontaneous action potential discharges in 6 of 23 DRG and 6 of 25 NG neurons from KU animals studied (Fig. 1, C and D). In contrast, only four DRG and five NG neurons obtained from control animals showed some membrane fluctuations. A comparison of neurons with and without significant oscillation showed a lower resting membrane potential for DRG (−48.1 ± 1.6 vs. −54.3 ± 1.8 mV, respectively; P < 0.05), but not NG (−60.4 ± 1.9 vs. −62.9 ± 1.9 mV, respectively; not significant) neurons. While none of the control cells with resting membrane potentials negative of −55 mV showed such oscillations, 3 DRG and 19 NG neurons from KU rats with resting membrane potentials as negative as −70 mV exhibited such fluctuations. Similarly, there was a significant increase in excitability as shown by a lower AP threshold and an increase in AP frequencies during prolonged depolarization, which was in part due to an increase in input resistance (Fig. 1, G and H; Table 1). The duration of APs in NG, but not in DRG neurons, was significantly longer after gastric ulceration, which was associated with a significant increase in NG neurons with TTX-resistant APs (Table 1). While the number of capsaicin-responsive NG and DRG neurons rose significantly after KU induction (Fisher’s exact test, P < 0.05), we did not observe any changes in capsaicin-evoked depolarization or current density in neurons from KU animals compared with those from saline-treated rats (Table 1).

Effect of gastric ulcers on response to purinergic agonists

In contrast to control conditions, ATP depolarized nearly all gastric DRG neurons from KU rats (Table 2). In addition, the response magnitude was increased in both NG and DRG neurons associated with an increase in AP generation. Consistent with the results obtained with ATP, KUs also significantly increased the proportion of α,β-metATP–sensitive gastric DRG neurons. Although the majority of ATP-sensitive neurons was also responsive to application of α,β-metATP, 4 of 19 ATP-sensitive DRG neurons did not respond to α,β-metATP. As was seen in response to ATP, the depolarization evoked by α,β-metATP was significantly greater (Fig. 2) after gastric ulceration. In 12 of 15 NG and 15 of 19 DRG neurons that responded to α,β-metATP, depolarization by α,β-metATP triggered a burst of APs with a significant increase in AP frequency compared with controls (Table 2).

Consistent with current-clamp data, under voltage-clamp mode the fraction of α,β-metATP–sensitive DRG neurons in KU rats (18/21) was significantly increased relative to the control neurons from saline-treated rats (P < 0.01). All (23/23) gastric NG neurons responded to the P2X agonist. In addition, α,β-metATP–evoked inward currents were larger in NG and DRG neurons from KU compared with saline-treated rats. While there was a significant increase in current density, gastric ulceration did not affect the concentration dependence of α,β-metATP–evoked inward currents. The EC_{50} values for NG and DRG neurons, respectively, were 11.8 ± 3 (n = 7) and 15.8 ± 2 μM (n = 6) for the fast desensitizing component and 22.9 ± 3 (n = 8) and 26.3 ± 5 μM (n = 3) for the slowly desensitizing component (Fig. 4). The slow component desensitized significantly more slowly in KU animals; in contrast, the kinetics of the fast component remained unchanged (Fig. 5).

DISCUSSION

We have previously reported behavioral changes in several animal models of gastritis or gastric ulceration consistent with the development of hypersensitivity (Lamb et al. 2003a,b; Ozaki et al. 2002). Such changes arise from both peripheral and central contributions, and this study focused on peripheral mechanisms contributing to these behavioral effects. The importance of peripheral contributions to gastric hypersensitivity is supported by the key findings reported here. First electrical properties differ significantly for gastric NG and DRG neurons. Second, only a small number of DRG neurons compared with a greater percentage of NG neurons innervating the stomach respond to P2X agonists, pointing at differential expression of P2X receptors for gastric vagal and splanchnic afferent nerves. Third, gastric ulceration and inflammation 1) cause slow oscillations of the resting membrane potential associated with spontaneous action potential generation and an increase in excitability in both DRG and NG neurons that innervate the stomach; 2) increase the fraction of neurons responsive to purinergic agonists; and 3) increase the peak density of current activated by the P2X agonist α,β-metATP.

The stomach receives dual innervation from vagal and spinal (splanchnic) afferent fibers. It is generally held that vagal fibers mediate information about innocuous stimuli, whereas noxious stimuli activate spinal pathways (Cervero 1994). A previous report from this laboratory documented that splanchnectomy, but not vagotomy, abolishes gastric mechanical hypersensitivity (Ozaki et al. 2002). In contrast, vagotomy but not splanchnic nerve resection blocks gastric chemoanociception (Lamb et al. 2003a). Consistent with such functional specialization, we noted significant differences between these two pathways when examining the biophysical properties of gastric NG and DRG neurons and their responses to capsaicin and purinergic agonists. All gastric NG neurons studied had more negative resting membrane potentials than gastric DRG neurons. Although the action potential threshold was similar for both groups of neurons, gastric NG neurons required significantly stronger current injection to evoke an action potential than did DRG neurons. Nearly 90% of DRG neurons, compared with significantly fewer NG neurons (50%), had TTX-resistant action potentials. Furthermore, the majority of gastric sensory neurons responded to capsaicin. These characteristics are generally associated with small diameter DRG neurons that presumably
function as nociceptors (Akopian et al. 1996; Caterina et al. 1997). Based on whole cell capacitance measurements, gastric sensory NG and DRG neurons with nociceptor-like properties are medium to large in size, suggesting that cell diameter is not a reliable indicator of presumed function and further emphasizing that visceral sensory neurons have unique properties.

Essentially all gastric NG neurons responded to both ATP and α,β-metATP, which selectively activates homomeric P2X1, P2X3, or heteromeric P2X2/3 receptors, whereas these P2X agonists only depolarized approximately one-third of gastric spinal DRG neurons. Furthermore, P2X receptor agonists evoked significantly greater amplitude depolarizations and greater current density in gastric NG compared with DRG neurons. Our findings confirm prior studies on unlabeled nociceptive neurons and are consistent with prior reports showing that essentially all NG neurons respond to P2X receptor agonists (Khakh et al. 1995; Zhong et al. 2001). This is in contrast to the lower number of P2X-responsive gastric DRG neurons and differs significantly from previous reports showing response rates between 70 and 90% in unlabeled DRG neurons (Burgard et al. 1999; Grubb and Evans 1999; Petruska et al. 2000; Zhong et al. 2001). Considering the relatively small number of visceral sensory neurons within the DRG, it is likely that >90% of unlabeled neurons innervate somatic structures. These findings highlight differences between gastric vagal and splanchnic afferent neurons and somatic sensory neurons and further support the notion that visceral and somatic sensory neurons are functionally distinct (McMahon 1997). However, studies of mouse bladder and colon sensory neurons showed that >90% of neurons innervating these viscera respond to P2X agonists (Wynn et al. 2004; Zhong et al. 2003). While α,β-metATP triggered currents in a similar percentage of bladder and cutaneous neurons, the kinetic properties pointed at differences in subunit composition between the groups (Zhong et al. 2003). Additional experiments are needed to determine whether this is due to species differences or differences between the innervation of stomach and pelvic organs.

A more detailed analysis of P2X currents reveals additional distinction between spinal and vagal neurons innervating the stomach. Consistent with prior reports on the properties of native and heterologously expressed P2X receptors, α,β-metATP triggered fast and slow desensitizing currents in NG and DRG neurons (Burgard et al. 1999; Grubb and Evans 1999; Lewis et al. 1995; Liu et al. 2001; Zhong et al. 2001). However, NG neurons exhibited mixed or—less commonly—slow kinetics, whereas gastric DRG neurons showed either a predominantly fast or a slow current decay in response to α,β-metATP. These results indicate that some DRG neurons express homomeric P2X3 receptors, whereas heteromeric P2X2/3 receptors predominate in NG neurons. Our data are consistent with previous reports showing the presence of P2X2 and 3 receptors in NG and DRG neurons (Vulchanova et al. 1997).

The increase in neuron excitability observed in neurons from KU animals is consistent with previous reports showing changes in action potential threshold, input resistance, and firing patterns after experimental induction of visceral inflammation (Moore et al. 2002; Stewart et al. 2003; Yoshimura and de Groat 1999). Unlike others, we also noted spontaneous activity in one-fifth of the neurons obtained from KU rats, whereas none of the neurons from control rats generated APs in the absence of electrical or chemical stimulation. This was associated with the occurrence of membrane potential oscillations. Regularly occurring oscillations of the resting membrane potential have also been described in demyelinated axons and after chronic constriction injury of spinal nerves (Amir et al. 1999; Kapoor et al. 1997; Xing et al. 2003). The oscillations in these studies have been attributed to changes in the properties of voltage-sensitive sodium currents and/or potassium channels and may play an important role in the pathogenesis of neuropathic pain (Amir et al. 2002; Kapoor et al. 1997). While not the focus of this study, we noted an increase in the fraction of NG neurons with TTX-resistant action potentials, consistent with previous reports showing inflammation-induced changes in the expression and properties of voltage-sensitive sodium currents (Moore et al. 2002; Stewart et al. 2003; Yoshimura and de Groat 1999). We have previously shown a decrease in the density of transient potassium currents after induction of gastric ulcers (Dang et al. 2004). In conjunction with changes in leak conductances, these alterations may account for the spontaneous fluctuations of the resting membrane potential described. However, additional studies are necessary to directly investigate the ionic mechanisms underlying membrane potential oscillations after KU induction. In addition, KUs also significantly increased the percentage of gastric sensory neurons sensitive to capsaicin. Considering the importance of TRPV1 channels in nociception (Caterina et al. 1997; Tominaga et al. 1998) and their potential role in integrating different signal modalities, our results clearly show several important changes in the properties of peripheral sensory neurons that may contribute to the development of gastric hypersensitivity.

Gastric ulcers increased the fraction of gastric DRG, but not NG, neurons responding to α,β-metATP. In addition, desensitizing kinetics of the slow, but not fast, current significantly slowed. While the sample size in this study was small and possibly misleading for the fast component, there were significant changes in desensitizing kinetics for the slow current in both groups of neurons after KU induction. Furthermore, KUs significantly enhanced DRG neuron response magnitude to purinergic stimulation. Consistent with these findings, others recently showed increased expression of P2X2 and P2X3 receptors and enhanced responses to purinergic agonist in primary afferent fibers/neurons during inflammation of the foot pad or colon (Hamilton et al. 2001; Wynn et al. 2004; Xu and Huang 2002). Our approach only allowed us to examine changes in the property of the cell body. However, immunohistochemical studies have shown the presence of P2X receptors in peripheral and central terminations of sensory neurons (Brouns et al. 2000; Xiang et al. 1998). Activation of presynaptically located P2X receptors increases glutamate release, thereby enhancing synaptic transmission onto second-order neurons within the spinal cord (Gu and MacDermott 1997). Thus a central effect may add to the changes in activation of peripheral terminals due to ATP release in the inflamed tissue, further contributing to sensitization.

Mounting evidence suggests an important role for purinergic signaling in visceral sensation. Distension of the bladder or colon triggers ATP release from the urothelium or the colonic epithelium, which in turn activates primary afferent neurons, in part, via P2X3 receptors (Vlaskovska et al. 2001; Wynn et al. 2003). Genetic deletion of P2X3 receptors in knockout mice significantly impairs the micturition reflex and...
attenuates formalin-induced pain behavior (Cockayne et al. 2000). Others recently reported that ATP and α,β-metATP increase action potential firing in response to colorectal distension, which was enhanced in animals with colitis. This change in response characteristics ofafferent fibers was associated with an increase in colon sensory neurons responsive to ATP (Wynn et al. 2004). More importantly, the nonselective P2X receptor antagonist PPADS blunted responses to colorectal distension in control animals and even more significantly in animals with colitis. Taken together, these data show that both gastric vagal and spinal sensory neurons sensitize in response to gastric ulceration/inflammation. Purinergic receptors may play an important role in this peripheral sensitization, as peripherally released purinergic agonists can activate more neurons. Moreover, the increased amplitude of P2X currents directly affects action potential generation and may indirectly enhance signaling within the spinal cord. This peripheral sensitization may contribute to dyspeptic symptoms and the development of a visceral hyperalgesia.

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GRANTS

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