TRANSLATIONAL PHYSIOLOGY

Host-specific differences in the physiology of acid secretion related to prostaglandins may play a role in gastric inflammation and injury

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Padol, Ireneusz T., and Richard H. Hunt. Host-specific differences in the physiology of acid secretion related to prostaglandins may play a role in gastric inflammation and injury. Am J Physiol Gastrointest Liver Physiol 288: G1110–G1117, 2005. First published January 27, 2005; doi:10.1152/ajpgi.00364.2004.—Immune mediators are involved in strain-specific manifestations of Helicobacter pylori infection, and the type of immune response is associated with production of PGE2, which in turn influences gastric acid secretion. Acid secretion plays a pivotal role, not only in the pattern of H. pylori-induced gastritis and its consequences, but also in nonsteroidal anti-inflammatory drug (NSAID)-induced gastropathies. Mice and their transgenic modifications are widely used in Helicobacter and eicosanoid research. Using [14C]aminopyrine accumulation and pylorus ligation, we aimed to study acid secretion in gastric gland preparations from the commonly used strains of BALB/c and C57BL/6 mice. We found that PGE2 does not inhibit acid secretion in gastric glands from C57BL/6 mice, in contrast to the expected antisecretory effect of PGE2 observed in BALB/c mice. In BALB/c mice the effect of histamine and carbachol was reduced by PGE2, whereas in C57BL/6 mice dose-response curves to these secretagogues were not affected. EP3 receptors are not involved in acid secretion in C57BL/6 mice, as confirmed by significantly lower expression of mRNA for the EP3 receptor. These contrary findings are important to the interpretation of the antisecretory role of eicosanoids in BALB/c and C57BL/6 mouse strains and the involvement of prostaglandins in the etiology of Helicobacter-induced inflammation and NSAID-induced gastropathies. We propose that the lack of antisecretory effect of PGE2 observed in C57BL/6 mice could reflect the extent of Helicobacter-induced inflammation and status of acid secretion in response to anti-inflammatory drugs.

ACID SECRETION PLAYS A PIVOTAL role in the etiology of many esophageal, gastric, and duodenal disorders. The secretory state of the gastric mucosa may be associated with a wide range of morphological changes, including Helicobacter-induced atrophic gastritis and nonsteroidal anti-inflammatory drug (NSAID)-induced erosions and ulcer, in animal models and in humans. Mouse models have recently been accepted into the mainstream of research into gastric pathology, inasmuch as they rely on the availability of transgenic techniques and an extensive knowledge of the immune system. Aminopyrine accumulation is a reliable method for studying acid secretion in human (21) and animal parietal cells or gastric gland preparations (3), and we recently extensively characterized the pharmacology of acid secretion in the mouse (28) to meet the demand for knowledge in this area. The mouse model has been widely used in the study of Helicobacter-induced gastritis and revealed strain-dependent patterns (20). With the use of Helicobacter felis-infected BALB/c mice, it was found that local acid production is critical for the distribution of Helicobacter species in the stomach (7, 19). In these mice, H. felis is predominantly restricted to the antrum and cardia and invades the body only with concomitant pharmacological acid suppression. In contrast, H. felis infection in C57BL/6 mice tends to colonize the secretory oxyntic mucosa of the corpus and contribute to the reduction in the number of parietal cells and subsequent development of gastric atrophy (8, 10, 29). This strain-specific difference between mice in response to Helicobacter pylori infection has been attributed to the predominant type of immune response, T helper (Th) type 2 (Th2) in BALB/c mice and type 1 (Th1) in C57BL/6 mice (24, 35). Some studies have suggested that clearance of bacteria is related to host genetic differences between strains (13).

PGE2 and its action through the EP3 receptor comprise the predominant prostanooids involved in the inhibition of acid secretion in animal models (34, 38). In rabbit parietal cells, PGE2 inhibits histamine- and carbachol-stimulated acid secretion, and preincubation with indomethacin increases aminopyrine accumulation (6). Moreover, PGE2 significantly inhibits acid secretion in human parietal cells, thus supporting its role as a local regulator of acid secretion (12). However, the role of PGE2 in the mechanisms governing gastric pathology may not be limited to its antisecretory function. It was shown in H. pylori-infected BALB/c mice that endogenous PGE2 may mediate suppression of the Th1-type immune response with a shift toward a Th2 response (5). Similar suggestions have been proposed by Kuroda and Yamashita (15), who found higher levels of PGE2 produced by macrophages from BALB/c than from C57BL/6 mice. These important findings implicate prostanoids and, thus, physiological changes in acid secretion with the type of immune response. The link between prostaglandin metabolism and H. pylori infection was suggested by showing that secretory phospholipase A2 is reduced in H. felis-infected C57BL/6 mice and leads to increased proliferation and apoptosis, in contrast to BALB/c mice (27, 37).
Because of a clear relation between acid secretion, type of immune response, mucosal prostanoids, and host-specific differences in gastric pathology, we decided to study the pharmacology of acid secretion in strains of mice that are widely used in these areas of research, namely, BALB/c and C57BL/6. We placed emphasis on the role of prostanoids as an important endogenous antisecretory mediator of acid secretion, which may play a decisive role in Helicobacter-induced inflammation as well as in NSAID-induced injury.

**MATERIALS AND METHODS**

**Animals**

Six- to eight-week-old BALB/c and C57BL/6 mice (Charles River, St. Constant, PQ, Canada) were kept under standard housing conditions (21–23°C, 40–50% humidity, and 12:12-h light-dark cycle) and fed Purina Lab Rodent Chow for up to 12 wk. Five mice from each strain were used in each experiment. Mice were fasted for 24 h (water ad libitum) and killed by cervical dislocation. The stomachs were quickly removed, opened along the lesser curvature, and placed in oxygenated 37°C PBS buffer at pH 7.3. Use of animals was approved by the Animal Research Ethics Board at McMaster University.

**Preparation of Gastric Glands From Mice**

The gastric gland preparation was performed as we previously described (28). Briefly, the gastric mucosa was scraped off the underlying muscle with a scalpel blade, pooled separately from each mouse strain, and washed twice (~200 g for 5 min) in PBS. The mouse gastric mucosa was enzymatically digested at 37°C for 45 min, and the conditions were identical for both mouse strains. After enzymatic digestion, the gastric glands were passed through a nylon mesh to separate the debris and the undisgested remains of the gastric mucosa. The preparation was then washed three times (~200 g for 5 min) in enzymatic buffer that contained neither collagenase nor trypsin inhibitor. Finally, the preparations were resuspended in 25 ml of incubation medium containing 2 mg/ml of BSA, 2 mg/ml of glucose, 1 mM MgSO4, and a total of 2 mM CaCl2. Therefore, the yield of the preparation from each mouse strain was 25 ml with ~10 μl of packed gastric glands per milliliter.

**Measurement of Acid Secretion in Mouse Gastric Glands**

Acid secretion was measured by accumulation of weak base [14C]aminopyrine as described by Berglind (3), with some modifications (28). Briefly, the experiment was carried out in closed 1.5-ml Eppendorf tubes containing 0.5 ml of resuspended gastric glands with added secretagogue (e.g., 0.01 mM carbachol or 0.1 mM histamine) and antisecretory compound (e.g., PGE2, somatostatin, omeprazole, or ranitidine). The tubes representing basal acid secretion did not contain a secretagogue (e.g., 0.01 mM carbachol or 0.1 mM histamine). Also, 20 μl, equal to 0.25 μCi of [14C]aminopyrine, were added to the tubes, and the incubation was carried out at 37°C for 60 min with rotation. Therefore, all reagents tested, including [14C]aminopyrine, were added at the same time and were incubated with the gastric glands. The tubes were spun, the supernatant was aspirated, and the pellets were counted in a scintillation counter (model LS 5801, Beckman). Each sample was tested in triplicate within each individual experiment. Each experiment was repeated in different gland preparations from each mouse strain, and this replication is represented by the number of individual experiments (n). Basal and carbachol- and histamine-stimulated aminopyrine uptake was 2,056 ± 784, 12,316 ± 4,419, and 38,772 ± 16,193 disintegrations/min, respectively. Data are presented as percentages of maximal response for each secretagogue.

**Measurement of Acid Secretion in the Mouse In Vivo**

Acid secretion was measured in vivo by means of pylorus ligation (31). Briefly, mice were fasted for 24 h (with water ad libitum), and, under anesthesia, a midline incision was made in the abdomen. A pyloric ligature of silk was placed, and the wound was closed with sutures. Drugs such as carbachol and misoprostol were administered subcutaneously at the time of ligation; then the animals were allowed to awaken from anesthesia, were placed in metabolic cages, and received nothing for the next 3 h. After that time, the mice were anesthetized again, a ligature was placed, this time at the esophageal junction, and the stomachs were removed. A small incision was made, through which the contents of the stomach were collected and centrifuged, and the volume of gastric juice was recorded and sampled for acidity by titration of 50-μl samples to pH 7.0 with 0.1 M NaOH. Total acid output was calculated and expressed as microequivalents of HCl per 100 g body wt.

**RT-PCR of the EP3 Receptor From Mouse Gastric Mucosa**

RNA isolation from mouse gastric mucosa. Samples of oxyntic mucosa scraped from the stomachs of each strain of mice were placed in tubes containing 1 ml of RNALater (Ambion), designated samples A and B, and the following procedures were carried out in a blinded fashion. Tissue samples were separated from RNALater using a tissue sieve and weighed: 68.8 mg (sample A) and 28.8 mg (sample B). Then samples were disrupted and homogenized for 45 s using a Polytron at setting 6. Total RNA was isolated using the Qiagen RNeasy Midi system according to the manufacturer’s instructions. Total RNA was eluted using nuclelease-free sterile water, and the final concentration was determined by spectrophotometric analysis: 706.8 ng/μl (sample A) and 326.8 ng/μl (sample B). RNA samples were stored overnight at −80°C.

**RT reaction and PCR amplification of EP3.** cDNA was obtained from the RNA samples using RT Omniscript in the presence of oligo(dt) primers to select for mRNA. Five sets of PCR primers were designed using Primer3 software (Table 1). The primer pairs were screened using mouse genomic DNA amplification was carried out as follows: 30 cycles of 92°C for 1 min, denaturation at 52°C for 1 min, annealing, and extension at 72°C for 1 min.

**Chemicals**

Histamine, carbachol, and indomethacin were purchased from Sigma; PGE2, butaprost, sulprostone, and misoprostol from Cayman Chemical; and somatostatin from Cambridge Research Chemicals. All chemicals were dissolved in water, with the exception of PGE2, butaprost, and sulprostone, which were dissolved in DMSO. Indomethacin and omeprazole were dissolved in water with addition of

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<td><strong>EP3-1</strong></td>
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NaHCO3 (0.6 mg/ml). [14C]aminopyrine had a specific activity of 115 mCi/mmol and was purchased from Amersham Pharmacia Biotech. All other chemicals were purchased from Sigma.

Statistical Analysis

Data were calculated as percentage of maximal response of aminopyrine uptake to various stimulants, and n represents the number of gland preparations for which each data point was tested in triplicate. Values are means ± SE. The significance of differences was tested by ANOVA with Bonferroni’s post hoc examination of means and considered statistically significant if P < 0.05.

RESULTS

The enzymatic digestion of gastric mucosa from BALB/c and C57BL/6 mice resulted in the preparation of functional gastric glands with the ability to secrete acid for up to 5 h. In preparations from both strains, nearly all (>95%) cells in the glands were viable as measured by trypan blue exclusion. The yield from gastric glands was the same for both strains, and all data are expressed as percentage of maximal response to each specified secretagogue: histamine or carbachol.

Construction of dose-response curves revealed that the histamine response curve in gastric glands from C57BL/6 mice is shifted to the left compared with that from BALB/c mice (ED50 = 2.2 ± 1.6 and 7.4 ± 1.2 μM, respectively, P < 0.05), with 0.1 mM giving the maximal response for both strains (Fig. 1A). For carbachol, there was no statistically significant shift in dose-response curves in glands from C57BL/6 mice; however, there was a distinct difference in the maximal response to carbachol between the strains (Fig. 1B). In C57BL/6 and BALB/c mice, the maximal response to carbachol was observed at 1.0 and 10.0 μM, respectively.

The experiments with each secretagogue were repeated in the presence of 10.0 μM indomethacin, which resulted in overlapping dose-response curves (data not shown). These experiments excluded the possibility that endogenous prostaglandins are involved in the inhibition of acid secretion in our strain-specific gastric gland preparations.

PGE2 inhibited histamine-stimulated acid secretion to a maximal effect of 60% at 1.0 μM in glands from BALB/c mice but had no effect at any concentration tested in glands from C57BL/6 mice (Fig. 2A). Similarly, for the carbachol-stimulated response, 10 μM PGE2 inhibited acid secretion up to 36% in glands from BALB/c mice but not at any concentration in glands from C57BL/6 mice (Fig. 2B). Carbachol dose-response curves were not affected by the presence of 10 μM ranitidine, excluding the possibility of involvement of histamine in these preparations (data not shown). The differences were consistent in all experiments with PGE2 in both strains of mice, and the inhibitory effects were always present in glands from BALB/c mice and absent in glands from C57BL/6 mice. To eliminate any possibility of artifact, we have cross-switched the preparations, digestion flasks, sequence of tubes, and sequence of procedures, including performing experiments in a blinded fashion. The ability of PGE2 to inhibit secretagogue-stimulated acid secretion always followed the mouse strain origin of the gastric mucosal samples and was found only in BALB/c mice.

To investigate further this distinct difference in PGE2 results, we tested the effect of another potent endogenous antisecretory mediator, somatostatin. The dose responses to somatostatin were almost identical for both mouse strains (Fig. 3A). In addition, we tested the proton pump inhibitor omeprazole and the histamine H2 receptor antagonist ranitidine on histamine-stimulated acid secretion and observed distinct antisecretory dose-response curves in both mouse strains, with a trend for both drugs to be more potent in the BALB/c preparation (Fig. 3B). However, the shift to the left observed in BALB/c mice reached statistical significance only for ranitidine (ED50 = 6.1 ± 1.3 and 21.3 ± 1.6 μM, respectively, P < 0.05). Experiments with somatostatin and antisecretory drugs were done with the inclusion of control tubes containing PGE2, which as already described, again strain specifically inhibited acid secretion. Thus we further excluded the possibility of preparation artifact.

In the next set of experiments, we tested the effect of 1.0 μM PGE2 (maximal effect) on dose-response curves to histamine and carbachol in preparations from both mouse strains. Con-
PGE₂ had an effect on histamine-stimulated acid secretion (Fig. 5B) in glands from C57BL/6 mice, indicating that, in this setting, the EP₁ receptor is not involved in this mouse strain.

To verify further these dichotomous findings, which we observed in the in vitro mouse model of the effect of prostaglandins on acid secretion, we tested the effect of the PGE₁ analog misoprostol and a selective EP₃ receptor agonist sulprostone in vivo. Misoprostol and sulprostone (50 μg/kg sc) administered at the time of pylorus ligation almost completely inhibited carbachol-stimulated acid secretion in BALB/c mice but had no significant effect in C57BL/6 mice (Fig. 6).

To identify EP₃ receptors in gastric mucosa from both mouse strains, we first screened the primers. Primer pairs EP₃-1 and EP₃-2 resulted in a single PCR product of the correct size using genomic DNA (Fig. 7). The EP₃-2 primer pair was more efficient for PCR and was used for RT-PCR. Primer pairs EP₃-3 to EP₃-5 did not result in amplification of genomic DNA.

In RT-PCR, equal amounts of RNA from samples A and B were used for the RT reaction (6 μg). Primer pair EP₃-2 was...
used for the subsequent PCR step. EP3 was amplified to 6.91 ng/μl by PCR of sample A and to 1.52 ng/μl by PCR of sample B. Decoding of the samples revealed that expression of message for the EP3 receptor was about five times lower in oxyntic mucosa from C57BL/6 than from BALB/c mice (Fig. 8).

DISCUSSION

Our experiments indicate host-specific differences in acid secretion in gastric glands and the pylorus-ligated mouse model. BALB/c mice have a prostaglandin-mediated antisecretory feedback loop, and C57BL/6 are “prostaglandin blind” in terms of acid secretion. These findings are important not only to understanding the role of prostaglandins in the heterogeneity of gastric acid physiology in a given population of animals but also for the unique possibility to study prostaglandin involvement in H. pylori infection and/or NSAID-induced gastropa-
ties. Until now, investigators have not been aware of these strain-specific differences and may have drawn inaccurate conclusions from animal models or their possible clinical relevance.

Mice with targeted gene disruptions have entered the mainstream of research in the field of eicosanoids and their role in gastric physiology. A thorough review lists 18 different gene knockout models that are relevant to eicosanoid biology (1). However, this report does not list the wild-type or background mouse strains and lacks any suggestion that strain-specific differences in prostaglandin biology might exist. Similarly, another review focusing on the regulation of acid secretion through analysis of genetically engineered mice makes no comment on antisecretory prostaglandin pathways (30). Lack of information about acid secretion in wild-type mice may have led to the conclusion that acid secretion is maintained without involvement of cyclooxygenase-1 in mice (4). Consequently, the same study that used the C57BL/6 strain showed no effect of indomethacin and naproxen on acid secretion. Similarly, reports have concluded that prostaglandins from the cyclooxygenase-1 pathway are not involved in gastric homeostasis (17, 18). In another study with C57BL/6 mice, the authors concluded that EP1, not EP3, receptors are involved in PGE2 inhibition in indomethacin-induced gastric lesions (32). These conclusions could be misleading, because C57BL/6 mice lack a response to PGE2, as demonstrated in our study. Mapping of genes encoding for the mouse PGE receptor comes from studies in C3H/HeJ mice and cellular localization of mRNAs for receptor subtypes from ddY mice (11, 25, 33). We should be cautious, therefore, in extrapolating these findings to other mouse strains and should consider taking a multistrain approach in mouse prostanoid research. Furthermore, some of the reports on the role of prostaglandins in gastric acid secretion have been published without mention of the mouse strain used (22, 23). Our RT-PCR revealed that the strain difference we have characterized pharmacologically is related to lower expression of message for the EP3 receptor in gastric mucosa from C57BL/6 than from BALB/c mice. This may be sufficient for compromised EP3 receptor status, receptor number, and/or function on parietal cells of C57BL/6 mice and subsequent lack of PGE2 inhibitory effect on acid secretion in this strain. However, the actual status of EP3 receptors on parietal cells can only be explored by appropriate binding studies using pure populations of these cells, and this may be technically challenging. Furthermore, arachidonic acid is released from esterified precursors by the action of phospholipase A2 (16), and it is possible that levels of prostanoids are influenced by a lack of phospholipase A2 in C57BL/6 mice (14). In our BALB/c in vivo studies, misoprostol inhibited acid secretion by 97% compared with 36% in vitro, suggesting that in the intact animals central EP receptors may be involved, resulting in such an effective inhibition. This, combined with the lack of an in vivo effect of misoprostol on acid secretion in C57BL/6 mice, further suggests that a genetic component that extends beyond the gastric mucosa may be involved in this strain difference. The in vivo experiment with sulprostone, a PGE2 analog and selective agonist for EP3 receptor, confirmed that EP3 is not involved in acid secretion in the C57BL/6 mouse, in contrast to the BALB/c mouse.

Besides the already established differences in Th1 vs. Th2, our observations identify a new dichotomy of host-specific differences between BALB/c and C57BL/6 strains that may explain different patterns of H. pylori colonization and gastritis in this model. The association of Th2 with PGE2 may not be the only explanation for the reported strain differences in Helicobacter-induced gastritis. It has been shown in mice that the relatively alkaline epithelial surface pH of the stomach is acidified by indomethacin, and this was reversed by a PGE2 analog (2). This prostaglandin-mediated alkalization of the luminal surface plays a critical role in the stimulation of gastrin and subsequent increase in acid. We propose that, in C57BL/6
mice, such a mechanism does not exist and that Helicobacter-induced prostaglandins do not increase surface pH, preventing this physiological acid increase. This compromised secretory ability of the oxyntic mucosa facilitates colonization by the bacterium, with spread of subsequent inflammation, resulting in a reduction of parietal cells, leading to atrophic gastritis. We believe that local acid production, related to the genetically determined difference in prostaglandin response described here, is responsible for the impairment of secretory function in animal models as well as, perhaps, clinically. In addition, strain-specific differences between BALB/c and C57BL/6 mice, irrespective of Th cell responses and attributed to the genetic background, have been shown in antigen-induced pulmonary eosinophilia (26) and the immune responses to pulmonary Mycobacterium bovis infection (36).

The mechanisms by which NSAIDs might cause different effects depending on the host could also be explained by our present observations. In BALB/c mice, as prostaglandin levels are inhibited by NSAIDs, this might result in the loss of a natural antisecretory mechanism and, subsequently, an increase in acid secretion, leading to mucosal injury. In C57BL/6 mice, fluctuations of prostaglandin levels do not result in modulation of net acid output, because in our experiments PGE2 does not play a role in the inhibition of acid in this strain. Hence, BALB/c mice should be more prone to NSAID-induced, acid-driven mucosal damage than “prostaglandin-blind” C57BL/6 mice. We previously performed experiments with NSAIDs in mice and observed strain-specific differences in gastric and, especially, duodenal damage (unpublished data). Contrasting differences in basal acid output have been reported among healthy volunteers after indomethacin administration, suggesting that endogenous prostaglandins play an antisecretory role in some more than others (9). Thus a similar mechanism, which relies on the differences in antisecretory response to prostaglandins described here, may also exist in the clinical setting and may be responsible for the observed variability in NSAID-related gastric adverse effects. Therefore, we suggest that identification of genetic markers that are linked to eicosanoid biology might predict individuals at risk of NSAID injury, which would be of clinical importance for our aging population.

In summary, we have shown that strain-specific differences in the physiology of acid secretion related to prostaglandins exist and that these differences can be studied in the mouse model, leading to a better understanding of the role of eicosanoids in H. pylori infection and NSAID-induced gastropathies.

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REFERENCES


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