Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins

A. ROBERT, J. E. NEZAMIS, C. LANCASTER, J. P. DAVIS, S. O. FIELD, AND A. J. HANCCHAR

Departments of Experimental Sciences and Drug Metabolism Research, The Upjohn Company, Kalamazoo, Michigan 49001

ROBERT, A., J. E. NEZAMIS, C. LANCASTER, J. P. DAVIS, S. O. FIELD, AND A. J. HANCCHAR. Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins. Am. J. Physiol. 245 (Gastrointest. Liver Physiol. 8): G113-G121, 1983.—Several prostaglandins (PG) were found earlier to be cytoprotective for the stomach and the intestine. We now report that mild irritants, given intragastrically, are also cytoprotective by stimulating the release of PG by the stomach. Several "mild irritants," 10-20% ethanol, 0.2-0.35 M HCl, 0.05-0.075 M NaOH, 2-4% NaCl, and water at 70°C, were given orally to fasted rats. Fifteen minutes later, one of the following necrotizing agents was administered orally: 100% ethanol, 0.6 M HCl, 0.2 M NaOH, 25% NaCl solution, and boiling water. One hour later, the stomachs were removed and necrotic lesions graded. The mild irritants inhibited the necrotic lesions dose dependently. After a single treatment, protection lasted 1 h; repeated administrations maintained cytoprotection for as long as the mild irritants were being given. Indomethacin, an inhibitor of PG synthesis, abolished cytoprotection by mild irritants. After oral administration of NaOH at cytoprotective concentrations (0.01-0.1 M), the amounts of PGE2, PGF2α, and thromboxane B2 formed by the gastric mucosa increased steadily up to threefold. The protection elicited by mild irritants is called "adaptive cytoprotection." The increased synthesis of PG may represent a physiological, natural defense mechanism that may be necessary to maintain cellular integrity of the gastrointestinal mucosa, in spite of the hostile environment caused by luminal contents.

Mild irritant cytoprotection

Several prostaglandins (PG) were found earlier to prevent acute gastric necrosis produced in rats by exposure to strong irritants such as absolute ethanol (100% EtOH), 0.6 M hydrochloric acid (HCl), 0.2 M sodium hydroxide (NaOH), 25% sodium chloride (NaCl), 80 mM acidified taurocholate, and boiling water (12, 15). These same PG also prevent gastric injury due to administration of nonsteroidal anti-inflammatory compounds such as aspirin (4, 7) and indomethacin (10, 19). This property of PG was called "cytoprotection" (10, 12, 15). Since PG have been identified as normal components of gastric juice (1) and gastric tissue (3, 9), it was hypothesized (5, 12) that the stomach may synthesize and release cytoprotective PG to maintain cellular integrity of the gastric epithelium in spite of the constant presence of noxious agents in the lumen. Such endogenously produced PG would therefore play a physiological role in protecting the gastric mucosa. The present studies provide evidence that cytoprotective PG are formed endogenously by the stomach in response to irritants present in the lumen. An abstract was presented earlier (14).

METHODS

Animals

Female Upjohn rats (derived from the Sprague-Dawley strain) of 205-215 g body wt were used. Food was removed in the morning. At 3:00 P.M., water was also withheld, and the animals were placed in individual cylindrical stainless steel cages with flat bottoms and perforations to allow ventilation. These cages limited their movements and thus prevented the ingestion of hair and feces. This procedure, described earlier (17), did not appear to be stressful; actually, the animals were often observed to be asleep in such cages. On the following morning, the experiments described below were started.

Studies Performed

Three types of studies were performed: 1) oral administration of mild irritants followed by oral administration of necrotizing agents (the end point was the presence of gastric necrotic lesions); 2) same treatment as above preceded by oral or subcutaneous administration of indomethacin, an inhibitor of prostaglandin cyclooxygenase (18) (the end point was the presence of gastric necrotic lesions); and 3) measurement of prostaglandins in the gastric mucosa after oral administration of a mild irritant.

Mild irritants followed by necrotizing agents. Various agents, referred to as "mild irritants," were given orally in a volume of 1 ml through a plastic tubing attached to a 6-ml syringe. These mild irritants were EtOH at concentrations of 10-25%, HCl at concentrations of 0.05-0.35 M, NaOH at concentrations of 0.025-0.075 M, NaCl solutions at concentrations of 2 and 4%, and water at 70°C. Fifteen minutes later, one of several necrotizing agents was given orally in 1 ml. These necrotizing agents were 100% EtOH, 0.6 M HCl, 0.2 M NaOH, 25% NaCl solution, and boiling water. In the case of boiling water, the animals were first anesthetized with methoxital so-
dium (Brevital Sodium, Lilly, Indianapolis, IN), 12 mg in 0.6 ml intraperitoneally, to prevent pain. Anesthesia
was found not to interfere with the production of a gastric burn. These agents were shown earlier to produce excur-
sive gastric mucosal necrosis (15). In some experiments, the mild irritant and the necrotizing agent were the same
substance, e.g., low concentration of ethanol followed by 100% EtOH or low concentration of HCl followed by 0.6
M HCl, whereas in other experiments they were different, e.g., low concentration of EtOH followed by 0.2 M
NaOH or low concentration of HCl followed by 100% EtOH. In one study, the interval between a mild irritant
and the necrotizing agent was varied (2.5–180 min) in order to study the duration of cytoprotection afforded by
a mild irritant. The animals were killed with CO2 1 h after administration of the necrotizing agents. Their
stomachs were dissected out, opened along the greater curvature, and rinsed in cold saline. A portion of the corpus mucosa (approx 100 mg)
was stripped from the submucosa with a forceps and
weighed.

The mucosa was then processed according to a method
described earlier for ex vivo determination of PG forma-
tion (20). Briefly, the tissue was placed in plastic Microfuge tubes (Eppendorf micro test tubes of 1.5-ml capacity)
and 0.5 ml of 0.1 M phosphate buffer at pH 7.4 was added. The tissue was chipped with scissors for 1 min and
centrifuged at 15,000 rpm in a fixed-speed Eppendorf centrifuge (Brinkman Instruments, West-
bury, NY) for 30 s. The supernatant was discarded, and
0.5 ml of buffer was added. The pellet was dislodged, and
the vessel was stirred for 1 min by mixing in a Vortex at
room temperature. This procedure led to neomodlafion of PG. The reaction was stopped with 10 μg of indometh-
acin dissolved in 10 μl of 1% sodium bicarbonate. The
mixture was centrifuged at 15,000 rpm for 30 s, and the
supernatant was frozen for radioimmunoassay of PG at
a later time.

The radioimmunoassays for prostaglandin E2 (PGE2),
prostaglandin F2α (PGF2α), and thromboxane B2 (TxB2)
were performed according to standard methods (2). [3H]
PGE2, [3H]PGF2α, and [3H]TxB2 were obtained from New
England Nuclear (Boston, MA); antisera were obtained
from Seragen (Boston, MA; PGE2), from Dr. F. A. Fi-
itzpatrick (Dept. of Experimental Sciences, The Upjohn
Company, Kalamazoo, MI; PGF2α), and from Dr. C. K.
Marschke (Upjohn Diagnostics; TxB2).

Statistical Analysis

The values (avg no. of lesions/stomach, ng PG/g tis-
sue) were analyzed statistically with Student's t test.
Differences with a P value < 0.05 were considered sig-
nificant. The number of animals per group is indicated
in the figures and tables.

RESULTS

Gastric Cytoprotection by Mild Irritants

Homocytoprotection. Oral administration of 100%
EtOH, 0.6 M HCl, 0.2 M NaOH, 25% NaCl, or boiling
water regularly produced gastric mucosal necrosis in the
form of elongated bands usually parallel to the long axis
of the stomach (Fig. 1). Each stomach had 15–20 such
lesions located in the corpus (the glandular portion of
the stomach secreting acid and pepsin). Lower concen-
trations of the same agents prevented the necrosis. Thus
oral administration of EtOH at concentrations ranging
from 1.25 to 20% decreased the number of gastric necrotic
lesions produced by 100% EtOH, and oral admin-
istration of HCl at concentrations ranging from 0.05 to
0.35 M decreased the number of gastric necrotic lesions
produced by 0.6 M HCl (Figs. 1 and 2). Similar protection
was observed against 0.2 M NaOH by concentrations of
NaOH ranging from 0.025 to 0.075 M, against 25% NaCl
solution by concentrations of NaCl ranging from 2 to
4%, and against boiling water by water at 70°C (Figs. 1
and 2). In all cases, the protection by the mild irritants
was concentration dependent; the degree of protection
was linear when the concentrations are plotted on a log
scale (Fig. 2).

Cross cytoprotection. In these studies, the mild irritant
was not the same substance as the necrotizing agent.
Figure 3 shows that low concentrations of EtOH (17.5
and 20%) given orally protected against gastric necrotic
lesions produced by 0.2 M NaOH also given orally 15
min later and that low concentrations of NaOH (0.05
and 0.075 M) and of HCl (0.2 and 0.3 M) protected
against gastric necrotic lesions produced by 100% EtOH.
Similarly, 20% EtOH protected against necrotic lesions
produced by boiling water. In all cases, the response was
concentration dependent and linear.

Duration of cytoprotection by mild irritants. SINGLE
ADMINISTRATION OF MILD IRRITANTS. To find out how
long cytoprotection by mild irritants lasts, three mild
irritants (1 ml of 20% EtOH, 25% EtOH, or 0.075 M
NaOH) were given orally, followed at various time inter-

ADMINISTRATION OF MILD IRRITANTS. To find out how
long cytoprotection by mild irritants lasts, three mild
irritants (1 ml of 20% EtOH, 25% EtOH, or 0.075 M
NaOH) were given orally, followed at various time inter-
Figure 1: Homocytoduction: mild irritants prevent gastric lesions produced by necrotizing agents of same type. Rat stomachs opened along greater curvature. A: control. B: 100% EtOH; multiple and severe necrotic lesions of corpus. C: 100% EtOH preceded by 20% EtOH given 15 min earlier; complete protection. D: 0.6 M HCl; multiple and severe necrotic lesions of corpus. E: 0.8 M HCl preceded by 0.35 M HCl given 15 min earlier; complete protection. F: 0.2 M NaOH; multiple and severe necrotic lesions of corpus. G: 0.2 M NaOH preceded by 0.075 M NaOH given 15 min earlier; complete protection.

Figure 4 shows that the first evidence of protection was seen at 5 min for 20% EtOH and at 2.5 min for 25% EtOH, whereas cytoprotection by 0.075 M NaOH was already maximal at 2.5 min. Protection remained maximal for about 30 min for the three agents. Thereafter, cytoprotection became progressively less. The T50 (time at which cytoprotection was still 50%) was between 60 and 90 min after administration of the mild irritants, although in the case of 25% EtOH, gastric lesions were still only 58% of control at 2 h.

Repeated Administration of a Mild Irritant. NaOH (0.075 M) was chosen to determine whether cytoprotection would persist following repeated challenge with a mild irritant. NaOH was given orally in 1 ml once, twice at 30-min intervals, or four times, also at 30-min intervals. Water instead of 0.075 M NaOH was used in the controls. Fifteen minutes after the last treatment, 1 ml of 100% EtOH was administered orally, and the animals were killed 1 h later. Twenty rats were used in each group. Repeated administrations of 0.075 M NaOH maintained cytoprotection as long as it was given, and the degree of protection was the same as after a single treatment with 0.075 M NaOH (data not shown).

Mechanism of Cytoprotection by Mild Irritants

Do mild irritants simply dilute necrotizing agents by stimulating the volume of gastric contents? Mild irritants could simply stimulate gastric secretion and thereby dilute the necrotizing agent introduced a few minutes later. If this were the case, the protection observed would not be real but only apparent. This possibility was tested in three separate studies.

Effect of a Mild Irritant on the Volume of Gastric Contents. One milliliter of 0.075 M NaOH, a
Cytoprotective mild irritant, was given orally once. Other animals received two treatments with 0.075 M NaOH, 30 min apart, and still other animals were given 0.075 M NaOH four times, also 30 min apart. There were five animals per group. In all cases, they were killed 1 h later. Two milliliters of 100% EtOH, instead of the usual 1 ml, were given to counteract possible dilution caused by water (in controls) or the mild irritants given intragastrically 15 min earlier. Preliminary experiments had showed that 1 ml of 100% EtOH, under these conditions of pylorus ligation, did not produce maximal necrotic lesions. Table 2 shows that in a pylorus-ligated animal the gastric necrotic lesions produced by 2 ml of 100% EtOH were equally inhibited by 20% EtOH and 0.075 M NaOH.

The pylorus was ligated under ether anesthesia. Immediately after, 1 ml of water (control), 20% EtOH, or 0.075 M NaOH (2 mild irritants) was administered orally. Fifteen minutes later, 2 ml of 100% EtOH (the necrotizing agent) were given orally, and the animals were killed 1 h later. Two milliliters of 100% EtOH, instead of the usual 1 ml, were given to counteract possible dilution caused by water (in controls) or the mild irritants given intragastrically 15 min earlier. Preliminary experiments had showed that 1 ml of 100% EtOH, under these conditions of pylorus ligation, did not produce maximal necrotic lesions. Table 2 shows that in a pylorus-ligated animal the gastric necrotic lesions produced by 2 ml of 100% EtOH were equally inhibited by 20% EtOH and 0.075 M NaOH.

The volume of gastric contents was measured after water or the two mild irritants in five additional pylorus-ligated rats in each group. These animals were killed after 15 min, i.e., at the time when 100% EtOH would normally have been administered, as in the animals whose values are shown in Table 2. The gastric contents were as follows—water (group I): 1.14 ± 0.10 ml; 20% EtOH (group II): 1.39 ± 0.11 ml; 0.075 M NaOH (group V): 1.54 ± 0.05 ml. The increase in contents after 0.075 M NaOH (from 1.14 to 1.54 ml), although statistically significant, is too small to account for any appreciable dilution of the necrotizing agent.

Role of endogenous prostaglandins. Three studies were performed to explore the role of endogenous prostaglandins in cytoprotection produced by mild irritants.

**REVERSAL OF CYTOPROTECTION BY INDOMETHACIN.** Indomethacin, given orally at doses ranging from 0.25 to 2.5 mg/kg 60 min before oral administration of 20% EtOH, inhibited cytoprotection produced by 20% EtOH against mucosal damage produced by 100% EtOH. The degree of reversal was dependent on the dose of indomethacin (Fig. 5). A 50% reversal was obtained by a dose of about 0.3 mg/kg of indomethacin. Figure 6 illustrates this reversal of cytoprotection by indomethacin. Similar reversal by indomethacin of mild irritant-induced cyto-
CYTOPROTECTION BY MILD IRRITANTS

FIG. 4. Duration of cytoprotection by mild irritants. Mild irritants (1 ml of 20% EtOH, 25% EtOH, or 0.075 M NaOH) were given orally at various time intervals (from 2.5 to 180 min) before giving 1 ml of 100% EtOH, also orally. Animals were killed 1 h after 100% EtOH. Ordinate shows average number of gastric necrotic lesions per stomach ± SE. This figure shows that a mild irritant of a certain type protects against a necrotizing agent of a different type. Thus low concentrations of EtOH prevented gastric necrotic lesions produced by 0.2 M NaOH or boiling water, and low concentrations of NaOH or of HCl prevented necrotic gastric lesions produced by 100% EtOH. Degree of protection was concentration dependent and linear. Ten to 20 animals/point. *P < 0.05; **P < 0.01.

protection was seen after other combinations of mild irritants and necrotizing agents (Fig. 7). In Fig. 7, indomethacin (given orally or subcutaneously) reversed cytoprotection by 20% EtOH and by 0.35 M HCl against 100% EtOH as well as cytoprotection by 0.05 M NaOH against 0.2 M NaOH (homocytoprotection and cross cytoprotection). Animals receiving only indomethacin had normal stomachs, since a dose of 5 mg/kg is much too low to produce gastric erosions.

RESTORATION OF CYTOPROTECTION BY A PROSTAGLANDIN IN INDOMETHACIN-TREATED ANIMALS. Figure 8 shows that 16,16-dimethyl-PGE2 (3 μg/kg) given orally in a volume of 0.5 ml 5 min before the necrotizing agent (in this case 100% EtOH) abolished the reversal by indomethacin of cytoprotection produced by 20% EtOH. This prostaglandin was also cytoprotective in animals receiving 100% EtOH alone and in animals receiving indomethacin plus 100% EtOH.

MEASUREMENT OF PROSTAGLANDINS IN THE GASTRIC MUCOSA AFTER A MILD IRRITANT GIVEN ORALLY. Formation of PGE2, PGF2α, and TxB2 by the gastric mucosa (corpus) was markedly increased by oral treatment with a mild irritant such as NaOH, given at concentrations up to 0.1 M (Fig. 9). For instance, after administration of 0.075 M NaOH, values for PGE2 rose from 149 ± 31 (control) to 388 ± 69 μg/g fresh tissue (+160%); values for PGF2α rose from 61 ± 5 to 158 ± 21 μg/g (+159%); and TxB2 rose from 51 ± 7 to 77 ± 8 μg/g (+51%) (P < 0.01 in all cases).
TABLE 1. Cytoprotection by a mild irritant is not due to gastric dilution of necrotizing agent

<table>
<thead>
<tr>
<th>Group No.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Treatment</td>
<td>Water: 1 ml</td>
<td>Water: 1 ml</td>
<td>0.075 M NaOH: 1 ml</td>
<td>Water: 1 ml</td>
<td>0.075 M NaOH: 1 ml</td>
<td>Water: 1 ml</td>
</tr>
<tr>
<td>Time 0</td>
<td>Water: 0.6 ml</td>
<td>100% EtOH: 1 ml</td>
<td>100% EtOH: 1 ml</td>
<td>100% EtOH: 2 ml</td>
<td>100% EtOH: 2 ml</td>
<td>100% EtOH: 2 ml</td>
</tr>
<tr>
<td>+60 min: kill</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gastric lesions

| No./stomach & SE | 14.4 ± 1.2 | 10.4 ± 1.8 | 2.3 ± 0.7 | 19.3 ± 1.3 | 16.0 ± 1.6 | 4.1 ± 0.9 |

% Inhibition

* Not significantly different from group I. †P < 0.01 compared with group II. ‡P < 0.01 compared with group V.

TABLE 2. Mild irritants are cytoprotective in pylorus-ligated rats

<table>
<thead>
<tr>
<th>Group No.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Treatment</td>
<td>Water</td>
<td>Water</td>
<td>20% EtOH</td>
<td>20% EtOH</td>
<td>0.075 M NaOH</td>
<td>0.075 M NaOH</td>
</tr>
<tr>
<td>Time 0</td>
<td>Kill</td>
<td>Kill</td>
<td>Kill</td>
<td>Kill</td>
<td>Kill</td>
<td>Kill</td>
</tr>
<tr>
<td>+60 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gastric lesions

| No./stomach & SE | 0 | 12.9 ± 1.7 | 0 | 4.1 ± 1.0* | 0 | 4.3 ± 1.0* |

% Inhibition

*P < 0.01 compared with group II. †P < 0.05 compared with group I.

Fifteen minutes later, rats of groups I, III, and V were killed and gastric contents measured. Other rats (groups II, IV, and VI) received 2 ml of 100% EtOH, they were killed 1 h later, and gastric lesions were counted.

FIG. 5. Indomethacin (INDOM) reverses cytoprotection by a mild irritant, dose dependently. Indomethacin was given orally 60 min prior to oral administration of 20% EtOH, and 100% EtOH was given 15 min after 20% EtOH. Ordinate shows average number of gastric necrotic lesions per stomach ± SE. Indomethacin alone (2nd bar) did not affect significantly gastric lesions produced by 100% EtOH. Protection by 20% EtOH (3rd bar) was reversed by indomethacin, and degree of reversal was dependent on dose of indomethacin. Ten animals/group. *P < 0.05 and **P < 0.01 by comparison with either 100% EtOH alone (1st bar) or indomethacin + 100% EtOH (2nd bar).

DISCUSSION

These results show that a variety of mild irritants, given orally, protect the stomach against mucosal necrosis produced by strong irritants. Cytoprotection by mild irritants is not specific to any particular type of chemicals; agents that are very different from one another, such as 20% EtOH, 0.35 M HCl, 0.075 M NaOH, or water at 70°C, were equally active. Mild irritants protected against necrotizing agents either of the same nature, e.g., 0.35 M HCl protected against 0.6 M HCl (homocytoprotection), or of a different nature, e.g., 0.075 M NaOH protected against absolute ethanol (cross cytoprotection). We conclude that the protection is not related to any particular chemical structure but rather to the gastric irritating property of the agent used.

The fact that prior treatment with indomethacin abolished the protection by mild irritants suggested that the effect was mediated by endogenous release of cytoprotective prostaglandins. Further support for this conclusion is provided by the data of Fig. 8, in which the reversal of cytoprotection by indomethacin was overcome by exogenous administration of a prostaglandin. This conclusion, however, was based on indirect evidence, i.e., reversal by indomethacin and restoration by a prostaglandin. Definite proof is provided by the data of Fig. 9, which shows that the amounts of PGE2, PGE2a, and TXB2 formed by the gastric mucosa were markedly increased by exposure to a mild irritant such as NaOH, given at cytoprotective concentrations (0.05–0.1 M). A 10% NaCl solution was reported to increase PGE content of gastric juice and tissue (6).
The phenomenon by which the stomach is protected in response to mild irritants placed in its lumen has been called "adaptive cytoprotection" (5) in contrast to "direct cytoprotection" obtained by administration of prostaglandins, since it can be viewed as an adaptation of the stomach to the presence of damaging agents that results in rendering such agents innocuous. As long as cytoprotection was observed only after exogenous treatment with prostaglandins, such effect could have been considered pharmacological, in the sense that PG were given as drugs in order to produce a beneficial response. The present studies suggest that cytoprotection is also a physiological phenomenon, in the sense that the local synthesis and release of prostaglandins may represent a natural defense mechanism that protects the gastric mucosa against noxious agents to which it is constantly exposed. When one considers the composition of gastric contents (foodstuffs at various temperatures and at pH ranging from 1 to > pH 10, the acid-pepsin complex, spices, and sometimes alcohol and irritating drugs), it is surprising that the mucosa remains intact. Any of these ingredients introduced into other internal cavities (e.g., abdominal, pleural, synovial) would cause severe inflammation and necrosis. One reason for the unique resistance of the stomach may be that the very irritants it harbors stimulate the continuous production of prostaglandins. When, on the other hand, the ability of the gastric mucosa to synthesize prostaglandins is impaired, such as after administration of indomethacin, these mild irritants cease to be protective and the mucosa becomes necrotic if exposed to stronger irritants. This unique ruggedness of the stomach may represent an evolutionary development allowing that part of the body, as well as the rest of the gastrointestinal tract, to coexist with a hostile environment. The fact that prostaglandins can be extracted from the gastrointestinal mucosa in large quantities (1, 3, 9) suggests indeed that they are formed continuously, since prostaglandins have a very short half-life, from a few seconds to about 2 min. Such continuous formation may be essential to maintain cellular integrity of the gastric mucosa. The same conclusions can be drawn for the small and the large intestine, since
FIG. 7. Indomethacin (INDOM) reverses cytoprotection by various mild irritants. Indomethacin was given at a dose of 5 mg/kg I subcutaneously, 90 min before 0.05 M NaOH (1 ml orally), or 2) orally, 60 min before 20% EtOH or 0.35 M HCl (1 ml orally). Fifteen minutes after these mild irritants, necrotizing agents (0.2 M NaOH or 100% EtOH) were given orally in 1 ml. “Vehicle” substances were saline with Tween 80 (instead of indomethacin) and water (instead of mild irritants). Animals were killed 1 h after necrotizing agents. Ordinate shows average number of gastric necrotic lesions per stomach ± SE. In every case, indomethacin reversed cytoprotection produced by mild irritants. Twenty-five animals/group. On top bars: *P < 0.05 and **P < 0.01 by comparison with group receiving indomethacin + necrotizing agent (2nd bar in each set). Next to curved arrows: **P < 0.01 comparing 2 connected groups.

FIG. 8. 16,16-Dimethyl prostaglandin E₂ (16,16-DiMe E₂) restores adaptive cytoprotection blocked by indomethacin (INDOM). Ordinate shows average number of mucosal necrotic lesions per stomach ± SE produced by oral administration of 1 ml of 100% EtOH. Animals were killed 1 h after 100% EtOH. Necrotic lesions were inhibited either by 3 μg/kg 16,16-dimethyl-PGE₂ (3rd bar) or by 1 ml of 20% EtOH (4th bar), given 5 and 15 min before 100% EtOH, respectively. Indomethacin (5 mg/kg subcutaneously), administered 60 min before 20% EtOH, reversed the protection afforded by 20% EtOH (5th bar). 16,16-Dimethyl-PGE₂ restored cytoprotection that had been blocked by indomethacin (6th bar). No. of animals/group are given in parentheses. On top bars: *P < 0.01 by comparison with group receiving indomethacin + 100% EtOH (2nd bar). Next to curved arrow: *P < 0.01 comparing 9 connected groups.

FIG. 9. Prostaglandin (PG) and thromboxane B₂ (TXB₂) content of gastric mucosa in response to a mild irritant. Gastric (corpus) mucosa was removed 15 min after oral administration of 1 ml of NaOH at various concentrations. Production of PGE₂, PGF₂α, and TXB₂ increased proportionately to concentration of NaOH. Six rats/group. *P < 0.05 and **P < 0.01 by comparison with controls.

prostaglandins are cytoprotective for these organs as well (8, 11, 13, 16).

The duration of adaptive cytoprotection by mild irritants was approximately 1 h. The reason a single administration of a mild irritant cytoprotecst the stomach for not much longer than 1 h may be that by that time the
CYTOPROTECTION BY MILD IRRITANTS

irritant has left the stomach through gastric emptying. However, by giving a mild irritant repeatedly, one can extend the protection much longer, probably indefinitely. In our studies, cytoprotection persisted as long as a mild irritant, such as 0.075 M NaOH, continued to be administered. Under normal conditions, exogenous mild irritants do not need to be administered purposely. The continuous presence of "physiological" irritants in the stomach and intestine may play the same role.

The fact that repeated treatment with a mild irritant maintains cytoprotection shows that the capacity for gastric cytoprotection is not exhausted by repeated exposure. If, as indicated by the present studies, the effect is due to increased formation of prostaglandins by the stomach, such production may go on indefinitely as long as the mucosa is in contact with mild irritants.

It was important to ascertain whether mild irritants cytoprotected the stomach by a specific mechanism or whether they simply stimulated gastric secretion and/or retarded gastric emptying. If either of the latter events was taking place, the necrotizing agent given subsequently might have been diluted by the gastric contents. This would have led to less damage than in the control stomachs receiving water instead of the mild irritants. Our results answer this question. Since mild irritants 1) did not alter the volume of gastric contents, 2) were cytoprotective even when the volume of necrotizing agent was doubled, and 3) were cytoprotective even after pylorus ligation, the protection is not due to dilution of the necrotizing agents or an alteration in gastric emptying but to another mechanism. The evidence presented here suggests that this mechanism involves formation of cytoprotective prostaglandins by the gastric mucosa when the latter is exposed to mild irritants.

It had been recognized for many years that tissues traumatized in vitro, such as by chopping or homogenization, release within a few seconds large amounts of prostaglandins, because of activation of the cyclooxygenase whenever cells are damaged. This rapid synthesis of prostaglandins in fact invalidates many published results in which the tissue or organ content of prostaglandins was measured, because no inhibitor, such as indomethacin, had been added to the extracting fluids to prevent neof ormation of prostaglandins during the extraction process. The present studies suggest that tissues exposed to mild irritants in vivo can also produce increased amounts of prostaglandins. Apparently, such mild irritants, although too innocuous to cause morphological injury, are "strong" enough to stimulate the prostaglandin synthetic machinery of the cells. One can view this response as a cellular autodefense mechanism.

Received 9 June 1982; accepted in final form 28 January 1983.

REFERENCES


