Release of Protective Products, Different from Prostaglandins, by Rat Stomachs Exposed to Mild Irritant

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The factors responsible for the mediation of mild irritant-induced (adaptive) cytoprotection to the rat stomach are not fully understood. The existence of cytoprotective products that are released by the gastric mucosa in response to its exposure to a mild irritant is assessed in this work. Gastric contents of rats exposed to a mild irritant (0.3 N HCl) or to 0.3 N NaCl (control) were collected, titrated to neutrality, and administered orally to prefasted animals followed by 100% ethanol. Ethanol-induced gross hemorrhagic injury in rats pretreated with the 0.3 N HCl gastric contents were significantly less than in control treated rats (P < 0.01). Pretreating the donor or recipient rats with indomethacin did not interfere with the generation or protective action of the 0.3 N HCl gastric contents. These findings demonstrate that the exposure of the gastric mucosa to a mild irritant causes the release of protective products, which are different from prostaglandins, into the gastric lumen.

KEY WORDS: adaptive cytoprotection; mucosal protection; prostaglandins; ethanol; mucosal injury; rat stomach; gastric mucosa.

Exposure of the gastric mucosa to a mild irritant confers to that tissue an increased ability to withstand the damage caused by subsequent exposure to a number of injurious agents (adaptive cytoprotection) (1). This protection has so far been attributed to enhanced mucosal prostaglandin synthesis associated with the exposure to the mild irritant (2). More recently, however, the involvement of locally generated prostaglandins in the phenomenon of adaptive cytoprotection has been questioned. Some mild irritants have been shown to protect the gastric mucosa without necessarily stimulating mucosal prostaglandin synthesis (3). Along the same lines, treatment of animals with indomethacin preceding the exposure of their gastric mucosa to mild irritants does not consistently reverse the protective action of these irritants (4, 5).

The existence of endogenous "protective products" other than prostaglandins that are released in response to exposure of the gastric mucosa to a mild irritant has so far not been appreciated. In this work the postulate posed is that adaptive cytoprotection is mediated by endogenous products other than prostaglandin that are secreted into the gastric lumen. The gastric contents collected from rats exposed to 0.3 N HCl, a mild irritant (1), were assessed for protective ability against ethanol-induced hemorrhagic injury. Parts of this work have already been presented in abstract form (6, 7).

MATERIALS AND METHODS

Male F344 rats weighing 175–225 g, obtained from Charles River Laboratories, were fasted for 16 hr and
deprived of water for 2 hr preceding the experiment. The animal protocol for this work has been reviewed and approved by the IACUC.

Under general anesthesia with sodium pentobarbital (60 mg/kg intraperitoneal) rat stomachs were cannulated through the pylorus from an incision in the duodenum, and the gastric lumen washed with warm isotonic saline.

**Effect of Concentration of HCl on Protectiveiveness of Gastric Contents Generated:** One milliliter of HCl at concentrations of 0.05, 0.1, 0.2, or 0.3 N or 1 ml of 0.3 NaCl (control) was instilled into the gastric lumen for 5 min, following which the gastric contents were aspirated back. The volume, pH, and osmolarity of the gastric contents obtained from donor rats exposed to 0.3 N HCl or 0.3 N NaCl were determined. The osmolarity was quantitated by freezing-point depression using an Osmomette S apparatus (Precision Systems Inc. Sudbury, Massachusetts). Five samples from each of the concentration groups was saved for prostaglandin E₂ determination. The rest of the samples were titrated to pH 7.0 with 0.1 N NaOH and 1 ml of each type of the gastric contents given orogastrically to prefasted rats (eight per type), followed 20 min later by 1 ml of 100% ethanol.

As a second control group, eight rats were treated orogastrically with a 1-ml solution of 0.3 N HCl titrated to pH 7.0 with NaOH. Twenty minutes later 1 ml of 100% ethanol was administered orogastrically.

**Time Effect for Generation of Protective Gastric Contents.** Donor rats operated on in the same fashion stated above had their gastric mucosa exposed to 0.3 N HCl or 0.3 N NaCl for 5, 10, 20, or 30 min. The gastric contents were aspirated back and titrated to pH 7.0 with NaOH. One milliliter of each type of the titrated gastric contents was administered orogastrically to prefasted rats (eight per type), followed 20 min later by 1 ml of 100% ethanol.

**Length of Treatment of Recipient Rat Needed for Protection.** The titrated gastric contents of 0.3 N HCl- or 0.3 N NaCl-exposed donor animals was administered to prefasted rats, followed 5, 10, 20, or 30 min later by 1 ml 100% ethanol.

All recipient rats were sacrificed 60 min following ethanol, their stomachs dissected out, opened along the greater curvature, and the exposed mucosa photographed. The images of the mucosal surfaces were projected on a digitizer board (Houston Instrument, Houston, Texas) and the extent of gross hemorrhagic damage to the gastric mucosa determined blindly by planimetry.

**Effects of Indomethacin Pretreatment on Generation of Protective Gastric Contents.** Donor rats were pretreated with indomethacin (10 mg/kg) subcutaneously or 0.5 ml of the vehicle (5% NaHCO₃) for 1 hr preceding surgery. Following cannulation of the stomach, 1 ml of 0.3 N HCl was instilled intragastrically for 5 min. The gastric contents were collected, and aliquots saved for prostaglandin E₂ determination. The samples were titrated to pH 7.0 and 1 ml administered orogastrically to groups of eight rats each, followed 20 min later by 1 ml of 100% ethanol. The rats were sacrificed 60 min later and the extent of hemorrhagic damage to the mucosal surface quantitated by planimetry.

**Effect of Indomethacin Pretreatment on Action of Protective Gastric Contents.** A group of eight rats was pretreated with indomethacin (10 mg/kg) given subcutaneously, followed 60 min later by 1 ml of the titrated gastric contents of 0.3 N HCl-treated animals, given orogastrically. Control rats were pretreated with 0.5 ml NaHCO₃ (vehicle) given subcutaneously for 60 min before receiving 1 ml of the titrated gastric contents of 0.3 N HCl-treated animals (eight rats), or with an injection of 0.5 ml NaHCO₃, followed 60 min later by 1 ml orogastrically of the titrated gastric contents derived from 0.3 N NaCl-exposed animals (eight rats). All rats were next given 1 ml of 100% ethanol orogastrically, sacrificed 60 min later, and the extent of hemorrhagic damage to their stomachs determined by planimetry.

**Effect of the Predamaging Gastric Mucosa on Generation of Protective Factors.** Donor rats were pretreated with 1 ml 1 N HCl or 1 N NaCl given orogastrically 20 min preceding surgery. The stomach was then cannulated, washed with warm isotonic saline, and 1 ml of 0.3 N HCl instilled intragastrically for 5 min. The gastric contents were collected, titrated to neutrality, and 1 ml of the solutions administered orogastrically to two groups of eight rats each, followed 20 min later by 1 ml of 100% ethanol. The extent of hemorrhagic damage to the gastric mucosa of the recipient rats was determined by planimetry.

**Determination of Prostaglandin E₂ in Gastric Contents.** The aliquots of the gastric contents were saved on ice (five per group), titrated to pH 3.0, and prostaglandins extracted using a C₁₈ cartridge (Fisher Scientific) on the same day the gastric contents were derived (9). In brief the C₁₈ cartridges were prewashed with 5 ml methanol followed by 10 ml water. The samples were applied to the columns, washed with 10 ml water, and the prostaglandins extracted with 5 ml diethyl ether. The ether was evaporated under a gentle stream of nitrogen at room temperature, and the dried samples kept at −20°C until assay time. Recoveries of prostaglandins were better than 75%. Prostaglandin E₂ standards and antisera were purchased from Advanced Magnetics Inc. (Cambridge, Massachusetts); radioactive prostaglandin E₂ was obtained from NEN (Boston, Massachusetts).

**Effect of Treatment with Gastric Contents on Gastric Secretion and Emptying.** Two groups of five prefasted rats each, had their stomachs cannulated under general anesthesia. The first group of rats received intragastrically 1 ml of titrated gastric contents of 0.3 N HCl-treated animals, while the second group received the gastric contents of 0.3 N NaCl-treated animals. The pyloric were tied off and the gastric contents collected 20 min later. The volumes and pHs of these samples were determined. To study the effect of the donor gastric contents on gastric emptying, two groups of five rats each received 1 ml of gastric contents of 0.3 N HCl- or 0.3 N NaCl-treated rats. The recipient animals were sacrificed 20 min later and the weight of their gastric contents measured.

All data are expressed as mean ± standard error of the mean. Difference in the extent of gross mucosal hemorrhagic damage was analyzed using the Kruskal-Wallis statistic. Significance was considered at associated probability (P value) of <0.05.