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Effects of Endotoxin on Neurally-mediated Gastric Acid Secretion in the Rat

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Abstract

The effects of a peripheral administration of *E. coli* endotoxin on neurally-mediated gastric acid secretion and the role of endogenous opioids or PAF receptors in endotoxin effects have been evaluated in the continuously perfused stomach of the anaesthetized rat.

Gastric acid secretion stimulated by distension (20 cm H_2O) was reduced dose-dependently by single intravenous bolus injection of endotoxin (0·1–10 $\mu g\ kg^{-1}$). Doses of 5 $\mu g\ kg^{-1}$ induced a peak reduction of distension-stimulated acid output and significantly reduced the secretory response induced by an intravenous bolus of 2-deoxy-D-glucose (150 mg kg^{-1}). This dose of endotoxin did not significantly modify mean systemic arterial blood pressure throughout the experimental period. Pretreatment with the opioid receptor antagonist naloxone (1 mg kg^{-1}, i.v.) or the platelet-activating factor (PAF) receptor antagonist WEB 2086 (2 mg kg^{-1}, i.v.) did not reverse the inhibitory effects of endotoxin (5 $\mu g\ kg^{-1}$, i.v.) on acid secretion stimulated by both distension and 2-deoxy-D-glucose.

These findings suggest that endotoxin-induced acute inhibition of neurally-mediated acid responses, stimulated by gastric distension or administration of 2-deoxy-D-glucose, do not involve the activation of endogenous opioids or PAF receptors.

Acute infections trigger a generalized host reaction which is accompanied by inhibition of gastric acid secretion; exogenous administration of endotoxin has been shown to reproduce this phenomenon in experimental models (Uehara et al 1990; Martínez-Cuesta et al 1992). Although little is known about the mechanism of the anti-secretory action of endotoxin, the secondary release of endogenous mediators seems to play an important role (Wallace et al 1987). Thus, acute release or action of nitric oxide is involved in endotoxin-induced inhibition of pentagastrin- and distension-stimulated gastric acid secretion in rats (Martínez-Cuesta et al 1992; Barrachina et al 1995) and the blockade of PAF receptors induced a partial reversal of the inhibitory effects of endotoxin on pentagastrin-stimulated acid output (Martínez-Cuesta et al 1992). In addition, Tsuji et al (1992) reported that an intact prostaglandin system is required by endotoxin to inhibit gastric acid secretion in pylorus-ligated rats. The release of endogenous opioids by endotoxin has been shown in in-vivo and in-vitro models (Bone et al 1981; Harbour et al 1985; Johansen et al 1995). Opioid receptors are distributed along the gastrointestinal tract (Bueno & Fioramonti 1988), and exogenous administration of opioids has been shown to inhibit neurally mediated gastric acid secretion in rats (Watanabe et al 1987; Kromer 1988; Esplugues et al 1992). However, it has not yet been determined whether endogenous release of opioids is involved in the anti-secretory actions of endotoxin. By using the opioid receptor antagonist naloxone we have evaluated the role of endogenous opioids in the anti-secretory mechanism triggered by endotoxin. Furthermore, the involvement of endogenous release of PAF in the acute inhibitory effects of endotoxin on neurally mediated gastric acid secretion has been assessed by use of the PAF receptor antagonist WEB 2086.

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Materials and Methods

Drugs

Endotoxin (lipopolysaccharide from *E. coli*, serotype 0111: B4), 2-deoxy-D-glucose and naloxone were purchased from Sigma (USA). WEB 2086 was a gift from Drs Hoefke and Heuer (Department of Pharmacology, Boehringer Ingelheim K.G., Germany). All drugs were dissolved in saline immediately before use and administered in a volume of 1 mL kg^{-1}.

General

Wistar rats, 180–250 g, of either sex were fasted for 24 h before the experiments but drinking water was freely available. Under urethane anaesthesia (1·5 g kg^{-1}, i.p.) the trachea was intubated, a jugular vein cannulated and in some experiments, systemic arterial blood pressure measured by means of a cannula inserted into a carotid artery (Spectramed Stathan P-23XL pressure transducer). Two soft catheters were inserted into the stomach through incisions made in the cervical oesophagus and the duodenum. When surgical preparation had been completed the gastric lumen was flushed with 50–100 mL saline to remove any solid content. After a 1-h stabilization period the stomach lumen was continuously perfused (0·9 mL min^{-1}) with isotonic saline at room temperature via the oesophageal catheter. The gastric effluent was collected every 30 min and H^+ output determined by automatic titration (Radiometer Copenhagen, Denmark) of 8 mL portions of the perfusate to pH 7 with 0·01 N NaOH. When gastric acid output had remained constant for 60 min it was considered as the basal acid secretion. Thereafter, distension of the stomach was achieved by placing the tip of the antroduodenal cannula

20 cm above the perfused stomach, where it was maintained for the remainder (180 min) of the experimental period. Endotoxin (*E. coli* lipopolysaccharide, 0·1, 1, 5 or 10 $\mu g\ kg^{-1}$) or saline was administered intravenously 30 min before the beginning of gastric distension. In another group of experiments rats received a single intravenous injection of endotoxin (5 $\mu g\ kg^{-1}$) or saline and 30 min later, gastric acid secretion was stimulated by an intravenous bolus injection of 2-deoxy-D-glucose (150 mg kg^{-1}).

To analyse the role of endogenous opioids or PAF in the acid inhibitory effects of endotoxin, further experiments were performed with rats treated with naloxone (1 mg kg^{-1}, i.v.) or WEB 2086 (2 mg kg^{-1}, i.v.) 15 min before endotoxin and acid secretion was stimulated as mentioned above.

Statistical analysis

The amount of acid produced during stimulation was calculated for each animal as the difference between the total production of acid in each 30-min period after stimulation and the basal levels of acid secretion over a similar period of time and expressed as $\Delta\mu Eq\ H^+\ (100\ g)^{-1}\ (30\ min)^{-1}$ when referring to the acid output of a particular 30-min period or $\Delta\mu Eq\ H^+\ (100\ g)^{-1}\ (180\ min)^{-1}$ when referring to total acid production over the 180-min period. Doses of 2-deoxy-D-glucose, naloxone or WEB 2086 were obtained from preliminary dose-response studies. Results are shown as mean ± s.e.m. Comparisons between groups were made by Student's *t*-test for unpaired data, except for blood-pressure studies in which Student's *t*-test for paired data was used. A probability of $P < 0.05$ or less was considered as indicative of statistical significance.

Results

Effects of endotoxin on gastric acid secretion

In urethane-anaesthetized rats, basal levels of gastric acid secretion were $3.6 \pm 0.8\ \mu Eq\ (100\ g)^{-1}\ (30\ min)^{-1}$. Distension of the rat stomach in-vivo induced a progressive increase in acid secretion which peaked at 120 min ($35.2 \pm 7.9\ \Delta\mu Eq\ H^+\ (100\ g)^{-1}\ (30\ min)^{-1}$, n = 6) after intragastric pressure application (20 cm H_2O) with a net H^+ production of $96.1 \pm 32.5\ \Delta\mu Eq\ H^+\ (100\ g)^{-1}\ (180\ min)^{-1}$ (n = 6) (Fig. 1a). Intravenous pre-treatment (15 min) with endotoxin (0·1, 1, 5, 10 $\mu g\ kg^{-1}$) dose-dependently reduced the acid responses to distension (79.7 ± 35.4, n = 4; 55.0 ± 21.1, n = 5; 3.8 ± 2.1, n = 3, $P < 0.05$; and 8.5 ± 1.3, n = 4, $P < 0.05$, $\Delta\mu Eq\ H^+\ (100\ g)^{-1}\ (180\ min)^{-1}$, respectively) compared with rats treated with an intravenous injection of saline (1 mL kg^{-1}). Administration of 2-deoxy-D-glucose (150 mg kg^{-1}, i.v.) induced a progressive increase in gastric acid secretion which peaked 120–150 min later with H^+ production of $41.1 \pm 9.7\ \Delta\mu Eq\ H^+\ (100\ g)^{-1}\ (30\ min)^{-1}$ (n = 6). Acid responses to 2-deoxy-D-glucose were also reduced by 89·4% by a single intravenous injection of endotoxin (5 $\mu g\ kg^{-1}$) (Fig. 1b).

Effects of opioid receptor antagonist on endotoxin-induced inhibition of gastric acid secretion

Endotoxin- (5 $\mu g\ kg^{-1}$, i.v.) induced inhibition of either distension- (96%, n = 14) or 2-deoxy-D-glucose- (89·4%, n = 9) stimulated gastric acid secretion was not significantly modified by pretreatment with the opioid receptor antagonist, naloxone (72·3%, n = 10, inhibition; and 80·1%, n = 10, inhibition, respectively) (Figs 1a and 1b).

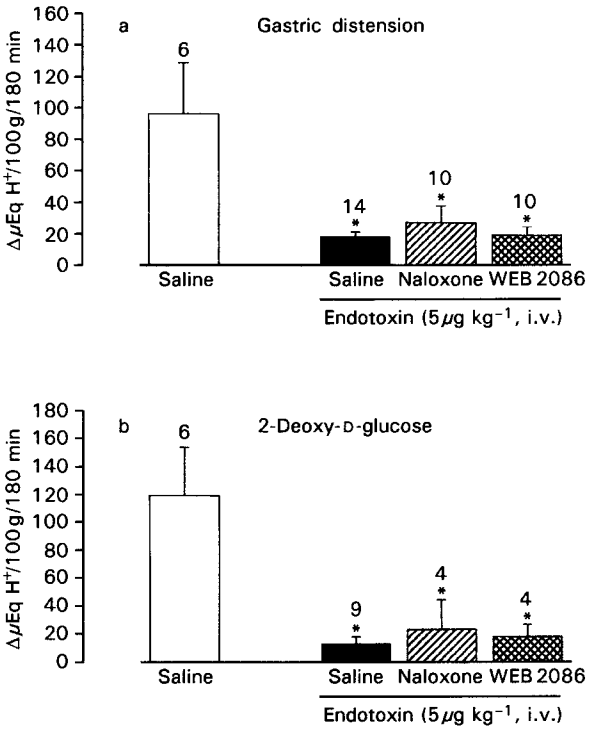

FIG. 1. Pretreatment with the opioid antagonist naloxone (1 mg kg^{-1}, i.v.) or PAF antagonist WEB 2086 (2 mg kg^{-1}, i.v.) did not significantly modify the inhibitory effect of endotoxin (5 $\mu g\ kg^{-1}$, i.v.) on acid secretion induced by distension of the stomach with an intragastric pressure of 20 cm H_2O (a) or by an intravenous bolus injection of 2-deoxy-D-glucose (150 mg kg^{-1}; b) in the anaesthetized rat. Each column shows mean ± s.e.m.; the level of statistical difference from the saline-treated group is shown by $*P < 0.001$. Numbers above the columns indicate the number of animals used.

Effects of PAF receptor antagonist on endotoxin-induced inhibition of gastric acid secretion

In endotoxin-treated rats the inhibition of gastric acid secretion stimulated by distension (96%) or by intravenous injection of 2-deoxy-D-glucose (89·4%) was not significantly modified by pre-treatment with the PAF receptor antagonist WEB 2086 (81·5% inhibition, n = 4, or 84·7% inhibition, n = 4, respectively) (Figs 1a and 1b).

Effects of endotoxin on systemic blood pressure

The administration of endotoxin (5 $\mu g\ kg^{-1}$, i.v.) did not significantly modify systemic arterial blood pressure, which remained between 95 and 105 mmHg (n = 5) during the 180-min evaluation period. In rats treated with naloxone or WEB 2086 before endotoxin, systemic blood pressure was similar to that observed for saline-treated animals throughout the experiment.

Discussion

This study has demonstrated that peripheral endotoxin dose-dependently reduces distension-stimulated gastric acid secretion and extends our previous observations (Martínez-Cuesta et

al 1992) showing the inhibitory effects of much higher doses of endotoxin on pentagastrin-stimulated gastric acid secretion. The study also shows that another stimulant of neurally mediated acid secretion, 2-deoxy-D-glucose, is sensitive to the effects of doses of endotoxin as low as 5 $\mu g\ kg^{-1}$; this dose is similar to that previously reported to inhibit gastric acid secretion in pylorus-ligated rats (Tsuji et al 1992). It seems that neurally-mediated gastric acid secretion is more sensitive than direct stimulants of the oxyntic cell to the inhibitory effects of endotoxin. These results are in agreement with recent studies showing that exogenously administered nitric oxide, one of the main mediators involved in the inhibitory effects of endotoxin, preferentially inhibits neurally mediated gastric acid secretion (Barrachina et al 1994). The low dose of endotoxin used in this study did not induce any significant fall in blood pressure during the experimental period (180 min), suggesting that the action of endotoxin on gastric acid production is independent of any vascular changes.

Many endogenous mediators have been involved in the host reaction to infection. Among them prostaglandins, nitric oxide and PAF have been shown to mediate the anti-secretory effects of endotoxin in different experimental models (Martínez-Cuesta et al 1992; Tsuji et al 1992; Barrachina et al 1995). In-vivo and in-vitro studies have shown that release of endogenous opioids is induced by endotoxin (Bone et al 1981; Harbour et al 1985; Johansen et al 1995). Exogenous administration of opioid peptides inhibits either distension- or 2-deoxy-D-glucose-stimulated gastric acid secretion in rats, although the location of the receptors involved seems to be different. Whereas the inhibition of distension-stimulated gastric acid secretion has been shown to be exerted at peripherally-located opioid receptors, the inhibition of 2-deoxy-D-glucose by opioids seems to be mediated at CNS level (Esplugues et al 1992). However, the current study shows that either centrally or peripherally located opioid receptors are not involved in the inhibitory effects of endotoxin on distension- or 2-deoxy-D-glucose-stimulated gastric acid secretion, because naloxone, an opioid antagonist that crosses the blood-brain barrier, did not reverse the anti-secretory effects of endotoxin.

Previous studies have shown that nitric oxide and PAF participate in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion (Martínez-Cuesta et al 1992). However, the current results, obtained using doses of the PAF receptor antagonist similar to that previously used, do not suggest participation of PAF in endotoxin-induced inhibition of distension- or 2-deoxy-D-glucose-stimulated gastric acid secretion. The doses of endotoxin necessary to inhibit pentagastrin-stimulated gastric secretion are larger than those required to inhibit neurally-mediated gastric acid secretion; this could account for the differences observed.

This study shows similar sensitivity of two different neurally-mediated stimulants of gastric acid secretion to the acute inhibitory effects of endotoxin in rats. Endogenous opioids or the release of PAF are not involved in the inhibitory mechanisms of peripheral endotoxin on either distension- or 2-deoxy-D-glucose-stimulated gastric acid secretion.

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References

Barrachina, M. D., Calatayud, S., Esplugues, J., Whittle, B. J. R., Moncada, S., Esplugues, J. V. (1994) Nitric oxide donors preferentially inhibit neuronally mediated rat gastric acid secretion. Eur. J. Pharmacol. 262: 181–183

Barrachina, M. D., Whittle, B. J. R., Moncada, S., Esplugues, J. V. (1995) Endotoxin inhibition of distension-stimulated gastric acid secretion in rat: mediation by NO in the central nervous system. Br. J. Pharmacol. 114: 8–12

Bone, R. C., Jacobs, E. R., Potter, D. M., Hiller, F. C., Wilson, F. J. (1981) Endorphins in endotoxin shock. Microcirculation 1: 285–295

Bueno, L., Fioramonti, J. (1988) Action of opiates on gastrointestinal function. Baillieres Clin. Gastroenterol. 2: 123–139

Esplugues, J. V., Barrachina, M. D., Esplugues, J. (1992) Modulation by peripheral opioids of basal and distension-stimulated gastric acid secretion in the rat. Br. J. Pharmacol. 106: 33–38

Harbour, M. D., Smith, E. M., Blalock, J. E. (1985) Bacterial lipopolysaccharide induction of leukocyte-derived corticotropin and endorphins. Infect. Immun. 48: 813–817

Johansen, O., Jorde, R., Tonnesen, T., Jenssen, T., Burhol, P. G., Reikeras, O. (1995) Effects of naloxone on glucagon and insulin concentrations after injection of endotoxin in rats. Eur. J. Surg. 161: 635–638

Kromer, W. (1988) Endogenous and exogenous opioids in the control of gastrointestinal motility and secretion. Pharmacol. Rev. 40: 121–162

Martínez-Cuesta, M. A., Barrachina, M. D., Piqué, J. M., Whittle, B. J. R., Esplugues, J. V. (1992) The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. Eur. J. Pharmacol. 218: 351–354

Tsuji, K., Uehara, A., Okumura, T., Taniguchi, Y., Kitamori, S., Takasugi, Y., Namiki, M. (1992) The gastric anti-secretory action of lipopolysaccharide is blocked by indomethacin. Eur. J. Pharmacol. 210: 213–215

Uehara, A., Okumura, T., Okamura, K., Takasugi, Y., Namiki, M. (1990) Lipopolysaccharide-induced inhibition of gastric acid and pepsin secretion in rats. Eur. J. Pharmacol. 181: 141–145

Wallace, J. L., Steel, G., Whittle, B. J. R., Lagente, V., Vargaftig, B. (1987) Evidence for platelet-activating factor as mediator of endotoxin-induced gastrointestinal damage in the rat. Effects of three platelet-activating factor antagonists. Gastroenterology 93: 765–773

Watanabe, K., Yano, S., Minikawa, Y. (1987) Morphine inhibits the gastric acid secretion stimulated by 2-deoxy-D-glucose via a central mechanism in the anesthetized rats. Eur. J. Pharmacol. 143: 293–298