Endogenous prostaglandins in peptic ulcer disease

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SUMMARY Plasma concentrations of prostaglandins E and F have been measured by radioimmunoassay in patients undergoing diagnostic upper intestinal endoscopy. The results fail to support a previously reported deficiency of plasma PGE in duodenal ulcer patients. Plasma prostaglandin concentrations failed to correlate with the parameters of gastric secretion studied; and were unaffected by histamine H₂-receptor blockade or the activity of duodenal ulceration. During combined pentagastrin and insulin secretory studies there was a significant correlation between the outputs of PGE and acid into gastric juice.

Prostaglandins of the E series are synthesised by the gastric mucosa and secreted into the gastric juice of man (Bennett *et al.*, 1973; Cheung *et al.*, 1976) and other mammalian species (Bennett *et al.*, 1967; Baker *et al.*, 1977a). Both natural E-type prostaglandins and their synthetic analogues have been shown to inhibit basal and stimulated gastric acid secretion in man, Rhesus monkey, dog, rat, and cat (Carter *et al.*, 1973; Dajani *et al.*, 1975; Wilson *et al.*, 1975; Dajani and Callison, 1976; Robert *et al.*, 1976; Konturek *et al.*, 1976; Baker *et al.*, 1978a); and to inhibit pepsin secretion in man, dog, and cat (Nezamiz *et al.*, 1971; Salmon *et al.*, 1975; Konturek *et al.*, 1976; Baker *et al.*, 1978a). Accordingly it has been postulated that PGE may have a physiological role in the control of gastric secretion, perhaps by means of a negative feedback mechanism (Horton, 1969). We have recently demonstrated in animal studies a positive correlation between the gastric output of immunoreactive prostaglandins E and F and gastric acid secretion stimulated by pentagastrin and insulin (Baker *et al.*, 1978b). A weaker correlation existed between the gastric outputs of these prostaglandins and pepsin secretion. However, prostaglandin F compounds are secreted in smaller quantities than PGE and have been reported as either not inhibiting gastric secretion (Robert *et al.*, 1967) or having a weak or transitory effect (Newman *et al.*, 1975; Baker *et al.*, 1978a).

Administration of PGE has been shown to prevent the formation of gastroduodenal ulcers in several animal models (Robert *et al.*, 1971; 1976; Lee *et al.*, 1973) and has been used to treat peptic ulcers in man (Fung *et al.*, 1974; Fung and Karim, 1976; Gibinski *et al.*, 1977). Hinsdale *et al.* (1974) have reported that duodenal ulcer patients have lower concentrations of PGE in their plasma and basal gastric juice than have normal controls, and it has been suggested that prostaglandin deficiency may be an important aetiological factor in peptic ulcer disease (Robert, 1975). However, other workers studying duodenal ulcer patients have failed to confirm this PGE deficiency in either basal (Tonnesen *et al.*, 1974) or insulin-stimulated (Cheung *et al.*, 1976) gastric juice, but have noted a disruption of rhythms of basal PGE and acid secretion.

This study has been performed further to evaluate the role of endogenous prostaglandins in peptic ulcer disease.

Methods

One-hundred-and-fifty-nine patients, endoscoped for evaluation of upper gastrointestinal symptoms, were studied. All patients were fasted for at least 12 hours before endoscopy, which was performed by experienced endoscopists using both end- and side-viewing instruments as indicated. On the basis of the endoscopic findings the patients were divided into four groups: (1) non-ulcer; (2) inactive duodenal ulcer (definite scarring of the duodenal cap or a previously endoscopically visualised duodenal ulcer); (3) active duodenal ulcer; and (4) active gastric ulcer. Patients with other known

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Table 1 Patient data: age, sex, and endoscopic diagnosis

Endoscopic diagnosis	No.	Mean age		Male	Female
		Years	Range		
Non-ulcer	64	40	16-78	35	29
Inactive duodenal ulcer	19	40	20-69	12	7
Active duodenal ulcer	57	42	16-70	40	17
Active gastric ulcer	19	58	39-77	11	8

diseases, a history of gastrointestinal surgery or of recent ingestion of known prostaglandin synthetase inhibitors or steroids were excluded from the series. Data on patients are summarised in Table 1. Immediately before administration of sedation for endoscopy 5 ml specimens of blood were obtained by antecubital venepuncture (between 1400 and 1600 hours), collected into heparinised tubes, centrifuged at 4°C, and the plasma obtained stored at -20°C until extraction and estimation of radioimmunoassayable prostaglandins.

Plasma prostaglandins were measured in 21 patients with active duodenal ulceration before and after 28 days' treatment with the histamine H₂-receptor antagonist cimetidine (1.6 g/day). All patients were endoscoped immediately before and after treatment and blood samples were collected at the time of this procedure.

Fasting serum gastrin determinations (Blair *et al.*, 1975) were obtained in 13 patients with active duodenal ulceration.

Gastric secretory studies were performed in 14 patients with active duodenal ulceration. After an overnight fast and the withholding of all medications for 24 hours, a double-lumen nasogastric tube was passed into the stomach and its satisfactory position confirmed by the recovery of a known amount of injected fluid. A 'wash-out' technique employing phenol red as marker was used to estimate the amount of secretion escaping collection (Venables, 1972). Resting gastric secretion was aspirated and discarded. After a preliminary wash-out period of at least 15 minutes basal secretion was collected over an additional 15 minute period. In seven patients, after the basal collection, pentagastrin (Peptavlon, ICI, England) 6 µg kg⁻¹ was given by intramuscular injection and the stimulated secretion collected over the next 60 minutes. At the end of this period soluble insulin (Wellcome, England) 0.2 µg kg⁻¹ was given intravenously and secretion collected for another 75 minutes. Blood glucose estimations performed on samples obtained immediately before and 45 minutes after the insulin injection confirmed an adequate hypoglycaemic response, mean pre- and post-insulin blood glucose levels being 4.3 and 1.2 mmol l⁻¹ respectively. Plasma samples were

obtained for prostaglandin estimation in the fasting state (before nasogastric intubation), one hour after the pentagastrin injection, and 45 minutes after the insulin injection. Acid output was determined by electrometric titration of 1 ml samples of gastric juice to pH 7.0 with 0.1 M NaOH (Radiometer, Denmark) and expressed as mmol H⁺ h⁻¹. Pepsin activity was determined by a haemoglobin digestion method (Chiang *et al.*, 1966) and expressed as milligram equivalents to bovine pepsin h⁻¹. In five patients samples of the collected gastric juice were stored at -20°C until extraction and estimation of prostaglandin by radioimmunoassay. Prostaglandin output into gastric juice was expressed as ng 15 min⁻¹.

PROSTAGLANDIN EXTRACTION AND RADIOIMMUNOASSAY

One millilitre samples of plasma and gastric juice were extracted by the addition of 3.0 ml of an organic solvent solution (ethyl acetate: isopropanol: 0.1 M-HCl, 3:3:1) and two phases created by the addition of 2.0 ml ethylacetate and 3.0 ml water. The organic phases were aspirated, evaporated at 37°C, and dissolved in benzene: ethyl acetate: methanol, 60:40:2. Fractions containing prostaglandins of the E and F groups were eluted separately from microsilicic acid columns by increasing concentrations of methanol (2% for PGEs and 17% for PGFs respectively) (Jaffe and Parker, 1972). Concentrations of immunoreactive PGE and PGF were measured using specific homologous radioimmunoassay systems (Jaffe *et al.*, 1973). Antibodies to PGE were raised in rabbits immunised with PGE₁ conjugated to Keyhole Limpet haemocyanin using ethyl chloroformate (Jaffe *et al.*, 1971); antibodies to PGF_{2α} were elicited in rabbits immunised with PGF_{2α} conjugated to bovine serum albumin using a water-soluble carbodiimide (Jaffe *et al.*, 1971). Tritiated markers ([³H] PGE₁ and [³H] PGF_{2α}) were purchased from the New England Nuclear Company and stored at -20°C under nitrogen until they were used. Separation of antibody-bound and free labelled ligand was performed using dextran-coated charcoal. By this method, separation of PGEs and PGFs from each other, and from PGAs, PGBs, and thromboxane B₂, is >99%. The sensitivity of each radioimmunoassay is 5 pg ml⁻¹. Intra- and interassay coefficients of variation are consistently less than 10%.

Mean results for each group were compared and the significance of differences tested by Student's *t* test for paired or unpaired data as appropriate, where significant differences between the variances of the groups occurred Cochran's correction factor was applied. Possible correlations were tested by the

Table 2 Plasma prostaglandin concentrations (pg ml^{-1}) in dyspeptic patients ($\bar{x} \pm 1 \text{ SEM}$)

	Non-ulcer (n = 64)	Inactive DU (n = 19)	Active DU (n = 57)	Active GU (n = 19)
PGE	233 $\pm$ 17	272 $\pm$ 48	283 $\pm$ 21	276 $\pm$ 47
PGF	59 $\pm$ 5	75 $\pm$ 12	57 $\pm$ 6	66 $\pm$ 19

Table 3 Plasma prostaglandin concentrations (pg ml^{-1}) in cimetidine treated patients ($\bar{x} \pm 1 \text{ SEM}$)

	Ulcer healed (n = 18)		Ulcer unhealed (n = 3)	
	PGE	PGF	PGE	PGF
Pre-treatment	215 $\pm$ 21	42 $\pm$ 9	240 $\pm$ 55	24 $\pm$ 5
Post-treatment	257 $\pm$ 25	35 $\pm$ 3	275 $\pm$ 59	28 $\pm$ 9

method of least squares (r = correlation coefficient) and the Spearman rank test. Significance was set at $P < 0.05$.

Results

The age distributions of the patient groups were similar with the exception of the gastric ulcer group, which was significantly older (Table 1). There were wide variations in individual plasma PGE and PGF concentrations within all the groups, but no significant difference between any of the groups (Table 2). There was no correlation between age and plasma PG, or between sex and plasma PG, irrespective of the endoscopic diagnosis, or within any of

the diagnostic groups.

As determined endoscopically 18 of the 21 patients treated with cimetidine obtained duodenal ulcer healing. Mean fasting plasma levels of PGE and PGF did not alter significantly during the course of therapy (Table 3).

Fasting serum immunoreactive gastrin concentrations measured in 13 active duodenal ulcer patients were all within the normal range (mean $\pm$ SEM = $52 \pm 6 \text{ pg ml}^{-1}$) and showed no correlation with plasma concentrations of PGE or PGF (Fig. 1).

In 14 duodenal ulcer patients the mean basal gastric outputs of acid and pepsin were $13 \pm 2 \text{ mmol h}^{-1}$ and $221 \pm 33 \text{ mg h}^{-1}$ ($\bar{X} \pm \text{SEM}$) respectively. Mean fasting plasma concentrations of PGE and PGF were 200 ± 32 and $62 \pm 13 \text{ pg ml}^{-1}$ respectively. There was no correlation between fasting plasma prostaglandin concentration and basal acid or pepsin concentrations or outputs.

In seven patients plasma concentrations of PGE and PGF obtained during stimulation with pentagastrin and insulin did not correlate with either acid (Figs. 2 and 3) or pepsin output at that time.

Concentrations of PGE and PGF were measured in the gastric juice of five patients undergoing combined pentagastrin and insulin secretory studies (Fig. 4). There was a significant positive correlation between the gastric output of PGE ($\text{pg } 15 \text{ min}^{-1}$) and that of acid ($\text{mmol } 15 \text{ min}^{-1}$) ($r = 0.563$) (Fig. 5), but no correlation between the outputs of PGE and pepsin. The output of PGF correlated with neither acid nor pepsin output.

Fig. 1 The relationship between serum gastrin concentrations and plasma concentrations of PGE (○) and PGF (●) in 13 duodenal ulcer patients.

Fig. 2 The relationship between plasma concentrations of PGE and gastric acid output in the basal state (○: 14 subjects) and after pentagastrin (●: seven subjects) and insulin (□: seven subjects) in duodenal ulcer patients.

Fig. 3 The relationship between plasma concentrations of PGF and gastric acid output in the basal state (○: 14 subjects) and after pentagastrin (●: seven subjects) and insulin (□: seven subjects) in duodenal ulcer patients.

Discussion

These results show that plasma PGE concentrations in duodenal ulcer patients are not lower than normal as previously suggested by Hinsdale and his colleagues (1974), or higher than normal as suggested by our preliminary results (Baker *et al.*, 1977b). Plasma prostaglandins do not appear to be related to gastric secretory status, serum gastrin concentration, or a specific diagnosis in dyspeptic patients. Furthermore, plasma prostaglandin concentrations are unaltered by histamine H₂-receptor blockade or the activity of duodenal ulceration.

Although prostaglandins are relatively stable in blood (Ferreira and Vane, 1967), their rapid clearance from the circulation, up to 95% in a single passage through the lungs and liver (Ferreira and Vane, 1967; Piper *et al.*, 1970; Hammond *et al.*, 1977), makes it unlikely that changes in gastric prostaglandin metabolism will be reflected in the plasma or that plasma prostaglandin levels will influence gastric secretory status (with the exception of relatively large prostaglandin infusions).

As prostaglandins are synthesised and can be inactivated within the same tissues (Pace-Asciak and Wolfe 1970; Pace-Asciak, 1972; Peskar and Peskar 1976), and are rapidly cleared from the circulation, it is likely that their action is a local one. Variations in the gastric output of prostaglandins occur under differing stimulatory conditions (Baker *et al.*, 1978b, c) and it seems reasonable to assume that changes in gastric mucosal prostaglandin metabolism

Fig. 4 The relationships between the gastric outputs of acid, pepsin, PGE, and PGF in the basal state and after pentagastrin and insulin stimulation in five duodenal ulcer patients. Responses are mean $\pm$ 1 SEM.

will be paralleled by changes in the gastric juice. In the duodenal ulcer patients who were studied the output of PGE into gastric juice correlated significantly with gastric acid output. These results are compatible with the proposed role of locally produced PGE in the physiological control of gastric acid secretion. However, PGE output into gastric juice may be volume dependent and so provide a spurious correlation with H^+ output—indeed, animal studies suggest that this may well be the case (Baker *et al.*, 1978c and unpublished data). Furthermore, although allowing of the hypothesis that gastric PGE output is related to gastric acid output, these results do not preclude some other role for PGE in gastric juice—for example, their cytoprotective effect (Robert *et al.*, 1971).

More recently it has been demonstrated that

Fig. 5 The relationship between the gastric outputs of PGE and acid during secretory function tests in five duodenal ulcer patients. The secretory data are illustrated in Fig. 4. r = the correlation coefficient and p = the significance of the regression line.

prostacyclin is generated by the gastric mucosa and can inhibit gastric acid output (Whittle *et al.*, 1978). Under certain experimental conditions prostacyclin is a more potent inhibitor of gastric secretion than is PGE_2 , but differences in the stability and pulmonary inactivation of these compounds make direct comparison difficult. Further evaluation of prostacyclin and its relationship to PGE is required before their relative roles can be assessed.

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