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SHORT COMMUNICATIONS

Regional differences of prostaglandin D_2-sensitive adenylate cyclase activity in the human alimentary tract

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In the past, most attention has been focused on prostaglandins of the E- and F-type as the biologically important prostaglandins formed by the gastrointestinal tract [1]. However, several other products of the arachidonic acid cascade are biologically active and, if synthesized by the alimentary tract, could have physiological importance.

Recently Knapp *et al.* [2] presented evidence that quite large amounts of prostaglandin D_2 are produced by whole cell preparations of rat stomach corpus, jejunum and colon. In contrast to PG E_2 and PG $F_{2\alpha}$ there is little information about the gastrointestinal activity of PG D_2.

Prostaglandins of the E-type have been shown to modify the intracellular concentrations of adenosine-3′,5′-monophosphate (*c*AMP) in gastric and colonic mucosa [3–5]. We have investigated the effects of PG D_2 on the adenylate cyclase activities in human gastric and colonic mucosa and have compared the effects with PG E_2.

METHODS AND MATERIALS

(*A*) Stomach tissue was obtained by subtotal gastric resection from six patients suffering from peptic ulcer disease. Scrapings of the gastric mucosa (approximately 2 g) were homogenized in a Teflon–glass homogenizer (Zell-Homogenisator, Colora-Messtechnik GmbH, Lorch, Württemberg, West-Germany) after addition of 20 ml of 5 mM Tris–HCl buffer, pH 7.6 containing 3 mM $MgCl_2$, 1 mM EDTA and 3 mM mercaptoethanol. The homogenate was filtered through a nylon mesh, washed once and assayed for enzyme activity. Only freshly prepared homogenates were used.

(*B*) Biopsy specimens of normal colonic mucosal tissue were obtained from patients suffering from polyposis or colonic neoplasia (n = 6). Biopsies of different parts of the colon were used in our experiments. Single biopsy contained about 0.5 mg tissue. Ten to fifteen biopsy specimens were combined and homogenized in 4–5 ml of 5 mM Tris–HCl buffer, pH 7.6, containing 3 mM $MgCl_2$, 1 mM EDTA and 3 mM mercaptoethanol. Histological studies confirmed this tissue to comprise only colonic mucosa. Tissue was immediately chilled, subsequent preparatory steps were all done at 0–2°C. Each patient gave his informed consent for the removal of biopsies.

The adenylate cyclase activity was determined according to the method of Salomon *et al.* [6] in a final volume of 0.1 ml at 30°. The incubation mixture contained 25 mM Tris–HCl buffer, pH 7.5, 5 mM $MgCl_2$, 20 mM creatine phosphate, 100 U/ml creatine phosphokinase, 1 mM 3′,5′-cyclic AMP, 1 mM α^{32}P-ATP (40–50 d.p.m./pmol). The reaction was initiated by addition of 20 μl of homogenate protein (20–50 μg of protein) and terminated by addition of 0.1 ml of stopping solution composed of 2% (w/v) lauryl sulfate, 1 mM cAMP and 40 mM ATP. Cyclic ^{32}P-*c*AMP was purified by column chromatography using Dowex AG 50 W-X 4 and neutral alumina, as described in detail by Salomon *et al.* [6].

Adenylate cyclase activities were proportional to the amount of protein in both preparations added up to at least 80 μg per sample. Time courses of basal and prostaglandin-stimulated enzyme activities were linear up to 20 min.

When indometacin was added during the homogenization procedure in concentrations up to 1 μmol/l, no change of basal and prostaglandin-stimulated enzyme activities in both tissues was noted.

The protein content of the samples was determined according to Lowry *et al.* [7] using bovine serum albumin as standard. Data are given as picomoles of *c*AMP formed per mg protein per 15 min. Statistical analysis was by the Wilcoxon-test for paired samples.

α-^{32}P-ATP (2–6 Ci/mmol) and ^{3}H-cAMP (27 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, Bucks, England.

Prostaglandin E_2 and prostaglandin D_2 were obtained from the Upjohn Co., Heppenheim, F.R.G. Prostaglandin concentrations were diluted from an ethanolic stock solution (10 mg/ml). The solvent had no effect on enzyme activities in both tissues up to concentrations of 10% (v/v). All other chemicals and reagents were of the highest grade commercially available.

Basal enzyme activity in homogenates of corpus gastric mucosa averaged 500 ± 200 pmol cAMP/mg protein/15 min (Table 1); non-stimulated enzyme activity in biopsy specimens of colonic mucosa was 250 pmol *c*AMP/mg protein/15 min (Table 1).

The cyclase system in *gastric* mucosa could be stimulated dose-dependently by prostaglandin E_2 and prostaglandin I_2 [8], but not by prostaglandin D_2. Maximal effects on enzyme activity were observed at PG E_2-concentrations of 0.28 mM. PG D_2, however, was ineffective in activating the gastric cyclase when tested over the same concentration range (Fig. 1A and Table 1) in the absence and presence of the guanine nucleotide analogue GMP (PNP).

As opposed to the gastric mucosal enzyme, both types of prostaglandins were almost equipotent in stimulating the colonic adenylate cyclase (Fig. 1B and Table 1). An about

Table 1. Effects of prostaglandin D_2 and prostaglandin E_2 on human adenylate cyclase activities in gastric and colonic mucosal homogenates

Additions†	Adenylate cyclase activity* (pmol cAMP/mg protein/15 min) Gastric mucosa	Colonic mucosa
None	500 ± 160	250 ± 80
Prostaglandin D_2	520 ± 170	650 ± 110‡
Prostaglandin E_2	1450 ± 400‡	720 ± 130‡

* Values are mean ± S.D. of six (gastric mucosa) and six (colonic mucosa) separate experiments respectively, carried out in triplicate.

† The concentrations of prostaglandin D_2 and prostaglandin E_2 were 0.28 mM.

‡ Significantly higher than the corresponding basal adenylate cyclase activities ($P \leq 0.05$).

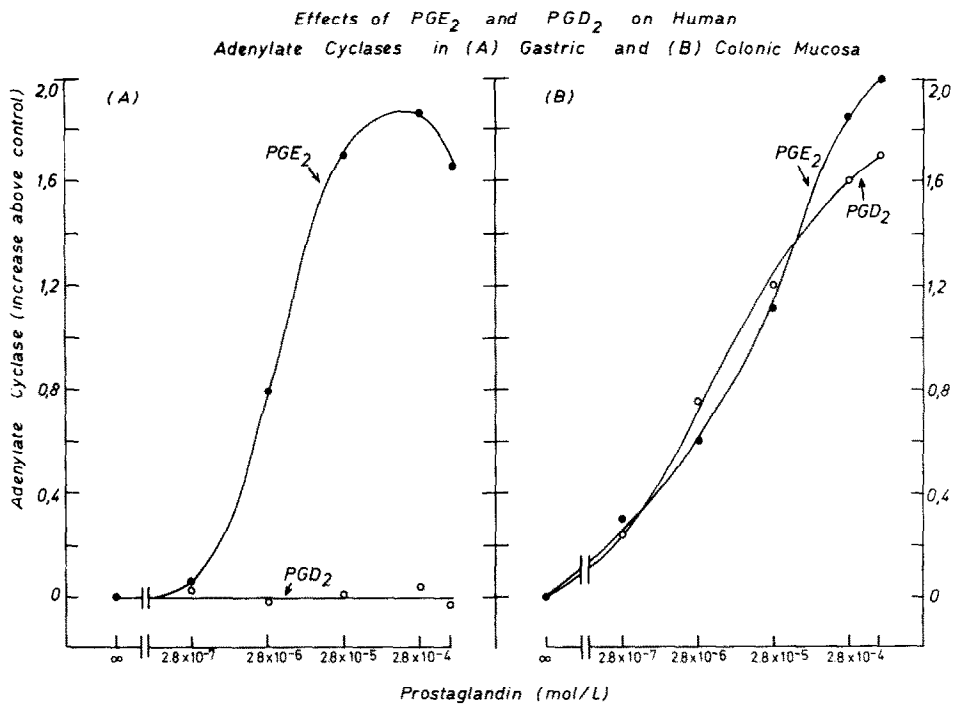

Fig. 1. Effect of prostaglandin D_2 and prostaglandin E_2 on the adenylate cyclase activities in human gastric (Fig. 1A) and colonic (Fig. 1B) mucosal homogenates.

Adenylate cyclase activity is expressed as the relative increment above basal levels.

Basal enzyme activity in homogenates of Billroth II resection material averaged 500 pmol cAMP/mg prot./15 min; non-stimulated enzyme activity in biopsy specimens of colonic mucosa was 250 pmol cAMP/mg prot./15 min.

A representative experiment out of six individual preparations is shown.

1.8–2.0-fold increase of enzyme activity was observed at prostaglandin concentrations of 0.28 mM. Dose–response curves revealed that the concentrations needed for one-half maximal activation were approximately in the same range for both hormones (10 μM) (Fig. 1B). When PG E_2 and PG D_2 in submaxillary concentrations were added into the assay medium, the activating effects of both prostaglandins were additive (not shown).

The present study suggests that in human large bowel cyclic AMP is implicated as the mechanism in the response to prostaglandin D_2. The failure to demonstrate PG D_2-receptor sites coupled to cyclase in human corpus mucosa is surprising and cannot be explained by the pathological resection material used in the present study: an unresponsiveness of the adenylate cyclase towards PG D_2 (not to PG E_2) could also be demonstrated in histologically intact biopsies of the corpus and antral region of the stomach. Since large quantities of prostaglandin D_2 are synthesized in the rat stomach wall [2] our findings are compatible with the concept that prostaglandin D_2 is acting on non-mucosal components of this organ.

Our results, therefore, emphasize that prostaglandin receptor populations coupled to the adenylate cyclases in human *gastric* and *colonic* mucosa are different. The possibility exists that similar to the platelets [9] the gastrointestinal mucosa of human beings contains separate and specific sites for prostaglandin E_2 and prostaglandin D_2.

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REFERENCES

1. A. Bennett, in *Prostaglandins* (Ed. S. M. Karim), pp. 247–276. MTP Press, Lancaster (1976).
2. H. R. Knapp, O. Oelz, B. J. Sweetman and J. A. Oates, *Prostaglandins* **15**, 751 (1978).
3. C. V. Perrier and L. Lester, *Eur. J. clin. Invest.* **6**, 1548 (1976).
4. A. Wollin, Ch. F. Code and Th. P. Dousa, *J. clin. Invest.* **57**, 1548 (1976).
5. B. Simon, P. Czygan, G. Spaan, J. Dittrich and H. Kather, *Digestion* **17**, 541 (1978).
6. Y. C. Salomon, C. Londos and M. Rodbell, *Analyt. Biochem.* **58**, 541 (1974).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. B. Simon and H. Kather, *Digestion* **19**, 137 (1979).
9. B. J. R. Whittle, S. Moncada and R. Vane, *Prostaglandins* **16**, 373 (1978).