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Determination of prostaglandin synthetase activity in rectal biopsy material and its significance in colonic disease

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SUMMARY A method is described for determining prostaglandin synthetase activity in milligram amounts of tissue. The procedure is based on the conversion of ^{14}C-arachidonic acid to prostaglandin E_2 and $F_2\alpha$-like substances. High levels of prostaglandin synthetase activity occurred in the inflamed mucosa of patients with ulcerative colitis and fell during successful drug therapy, but it is not yet known whether the cause of the inflammation first involves increased PG synthetase activity, or whether inflammation caused increase of PG synthetase.

Prostaglandin (PG) synthetase activity has been studied in several tissues including bovine and ovine seminal vesicles, guinea-pig lung, and tumour cells (Downing, 1972; Levine *et al.*, 1972; Takeguchi and Sih, 1972). The method described below determines PG synthetase activity in biopsy specimens of human gastrointestinal mucosa taken using biopsy forceps during routine examination.

Since PGs can cause diarrhoea (Karim, 1971; Hillier and Embrey, 1972), PG synthetase activity has been studied in the irritable bowel syndrome and in ulcerative colitis, two conditions in which diarrhoea is an important symptom.

Method

Biopsy specimens (10 to 120 mg wet weight) were taken from patients undergoing routine endoscopic examination in the gastroenterology department. The specimens were frozen immediately using liquid nitrogen vapour or solid carbon-dioxide and stored at −70°C until required.

The specimens were weighed and homogenised for no more than 15 seconds in 10 ml of ice-cold 50 mM phosphate buffer containing 10 mM EDTA, 500 μg reduced glutathione, and 0·05% butylated hydroxytoluene at pH 7·4 using a Silverson homogeniser. The homogenate was added to 100 nCi ^{14}C arachidonate (60·2 mCi/mmol, Amersham) and 25 μg arachidonic acid (both in benzene solution, the benzene being removed after addition to the tubes using a stream of nitrogen) in 50 ml tubes in a shaking water bath at 37°C. The tubes were mixed and samples removed for protein estimation using the Folin-Biuret method. Incubations were continued in air for 30 minutes and the reaction was arrested by adding 30 ml cold chloroform:methanol (2:1, −20°C). The solutions were acidified to pH 3 with formic acid and prostaglandins extracted into the chloroform layer (Unger *et al.*, 1971).

The chloroform layer was removed, washed with distilled water until neutral and dried using a rotary vacuum evaporator (Buchii/Rotavapor) at 30°C.

The residue was dissolved in 0·2 ml benzene:ethyl acetate:methanol (60:40:10) and mixed. Then 0·6 ml benzene:ethyl acetate (60:40) was added and mixed. The solution was added to a 150 × 8 mm glass column containing 0·5 g silicic acid and the tube washed with a further 0·2 ml benzene:ethyl acetate (60:40).

PGs were separated according to Jaffe *et al.* (1973). Arachidonic acid was removed in the first elution. Separation was checked using labelled PGs and by thin layer chromatography using the AII system of Green and Samuelsson (1964).

The eluates were evaporated to 1 ml and mixed with 10 ml 0·6% butylphenylbiphenyloxadiazole in toluene in scintillation vials and the radioactivity counted on a Packard Tricarb scintillation counter.

Enzyme activity was expressed as the amount of material running with PGE or PGF, formed per μg protein or per mg, wet weight tissue.

Results

The PG synthetase activities, expressed as the

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Table *Prostaglandin-like material produced in rectal biopsy material**

Diagnosis	*n*	*'PGE' synthetase*		*'PGF' synthetase*	
		ngE/ mg tissue	*ngE/ μg protein*	*ngFformed/ mg tissue*	*ngFformed/ μg protein*
Irritable colon syndrome	37	6·14 ± 2·30	39·4 ± 7·6	4·11 ± 2·00	29·6 ± 8·9
Controlled ulcerative colitis	6	3·74 ± 2·29	33·5 ± 9·1	5·19 ± 3·02	47·2 ± 9·1
Active ulcerative colitis	14	45·4 ± 14·8	440 ± 56	29·0 ± 8·49	249 ± 74

* Mean values ± 1 SEM.

mean ± SEM of material running with PGE or PGF, formed per milligram of tissue or per μg protein are shown in the Table.

The irritable colon group consisted of 37 patients suffering from pain and diarrhoea in whom comprehensive investigations had revealed no specific cause. The rectal mucosa appeared normal on sigmoidoscopy and histological examination. The mean PG synthetase activity per mg tissue in this group was 6·14 ± 2·30 ng PGE and 4·11 ± 2·00 ng PGF.

The controlled ulcerative colitis group consisted of six patients with ulcerative colitis diagnosed by barium enema and sigmoidoscopy, treated with sulphasalazine. The rectal mucosa appeared normal sigmoidoscopically and histology showed no active inflammation. The mean PG synthetase activity per mg tissue in this group was 3·74 ± 2·29 ng PGE and 5·19 ± 3·02 ng PGF. These values were not statistically different from corresponding values in the irritable colon syndrome group ($P = 0{\cdot}1$, Student's *t* test).

The active ulcerative colitis group consisted of 14 patients before treatment. Each had diarrhoea, inflamed rectal mucosa, and histological findings of typical crypt abscesses and round cell infiltration. The mean PG synthetase activity per mg tissue was 45·5 ± 14·8 ng PGE and 29·0 ± 8·49 ng PGF. These values were significantly greater than corresponding values in the previous two groups ($P < 0{\cdot}01$ in each case).

Discussion

The method described permits PG synthetase activity to be determined in small amounts of intestinal mucosa and allows the involvement of PGs in colonic disease to be studied. Measurements of circulating levels of PGs in peripheral blood are of questionable value, as the site of production is not indicated and because some PGs are deactivated in various vascular beds (Piper *et al.*, 1970; Green, 1971). Increased PGs extracted from blood may be due to formation during handling or processing, or due to a reduced breakdown. Measurement of PG synthetase, however, may reflect local formation of PGs. Patients with active ulcerative colitis had high PG synthetase activity in rectal mucosa, while, in remission, the mucosal PG synthetase activities were similar to those in patients with inflammatory bowel disease. Similarly, Gould (1975, 1976) found higher levels of PG-like material in the stools of patients with active ulcerative colitis, compared with the amounts in control subjects. It has also been shown that high levels of PG activity can be extracted from peripheral venous blood of ulcerative colitis patients and this is reduced with successful drug therapy (Harris *et al.*, 1978). Amounts of PG-like material separated from venous plasma of patients with ulcerative colitis are similar to those in patients with acute gastroenteritis and might be responsible for the diarrhoea associated with these two conditions. Overproduction of PGs in the inflamed colonic mucosa associated with active ulcerative colitis may contribute to the pathophysiology of the condition. As the inflammation was reduced so the PG synthetase activity diminishes and symptoms subside.

We believe that an overproduction of PGs contributes to the inflammation and diarrhoea associated with active ulcerative colitis. However, it is not possible to say whether an increase in PG synthetase activity causes the inflammation or whether the inflammation leads to increased PG synthetase.

References

Downing, D. T. (1972). Differential inhibition of prostaglandin synthetase and soybean lipoxygenase. *Prostaglandins*, **1**, 437-441.

Gould, S. R. (1975). Prostaglandins, ulcerative colitis, and sulphasalazine (Letter). *Lancet*, **2**, 988.

Gould, S. R. (1976). Assay of prostaglandin-like substances in faeces and their measurement in ulcerative colitis. *Prostaglandins*, **11**, 489-497.

Green, K. (1971). Metabolism of prostaglandin E_2 in the rat. *Biochemistry*, **10**, 1072-1086.

Green, K., and Samuelsson, B. (1964). Prostaglandins and related factors. XIX. Thin layer chromatography of prostaglandins. *Journal of Lipid Research*, **5**, 117-120.

Harris, D. W., Smith, P. R., and Swan, C. H. J. (1978). Venous prostaglandin-like activity in diarrhoeal states. *Gut*. (In press).

Hillier, K., and Embrey, M. P. (1972). High dose intravenous administration of prostaglandin E_2 and $F_{2\alpha}$ for the termination of mid-trimester pregnancies. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **79**, 14-22.

Jaffe, B. M., Behrman, H. R., and Parker, C. W. (1973). Radioimmunoassay measurement of prostaglandins E, A, and F in human plasma. *Journal of Clinical Investigation*, **52**, 398-405.

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Karim, S. M. M. (1971). Effects of oral administration of prostaglandins E_2 and $F_{2\alpha}$ on the human uterus. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **78**, 289-293.

Levine, L., Hinkle, P. M., Voelkel, E. F., and Tashjian, A. H., Jr. (1972). Prostaglandin production by mouse fibrosarcoma cells in culture: inhibition by indomethacin and aspirin. *Biochemical and Biophysical Research Communications*, **47**, 888-896.

Piper, P. J., Vane, J. R., and Wyllie, J. H. (1970). Inactivation of prostaglandins by the lungs. *Nature*, **225**, 600-604.

Takeguchi, C., and Sih, C. J. (1972). A rapid spectrophotometric assay for prostaglandin synthetase: application to the study of non-steroidal antiinflammatory agents. *Prostaglandins*, **2**, 169-184.

Unger, W. G., Stamford, I. F., and Bennett, A. (1971). Extraction of prostaglandins from human blood. *Nature*, **233**, 336-337.

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