Influence of 5-hydroxytryptamine on crypt cell production rate of human duodenal mucosa cultured in vitro

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SUMMARY The effect of 5-hydroxytryptamine (5-HT) on the crypt cell production rate in normal human duodenal mucosa cultured in vitro was studied using a stathmokinetic technique with crypt microdissection. The addition of 5-HT (2 mg/ml) to the culture medium significantly increased the crypt cell production rate ($p < 0.001$), suggesting that this amine may have a trophic effect on crypt cells in vitro. Increased synthesis and release of 5-HT from enterochromaffin cells in patients with coeliac disease may increase the crypt cell production rate and lead to a flattened small intestinal mucosa.

Studies on the kinetics of crypt cells in the flattened upper small intestinal mucosa of patients with untreated coeliac disease have shown a halving of the crypt cell cycle time and an increase in the absolute number of proliferating cells.^{1,2} Similar studies on the jejunal mucosa of laboratory rats in vivo have shown a reduction in the crypt cell cycle time and an increased proliferation of crypt cells after intraperitoneal injection of small doses of 5-hydroxytryptamine (5-HT).³ Increased concentrations of 5-HT have been found in the duodenal mucosa⁴ and blood⁵⁻⁷ both of adults and children with untreated coeliac disease, together with increased urinary excretion of its major metabolite 5-hydroxyindoleacetic acid.⁸⁻¹³ 5-hydroxytryptamine has therefore been implicated in the pathogenesis of this disorder.¹⁴ We have estimated the crypt cell production rate in biopsy specimens of human duodenal mucosa cultured in a basic medium in vitro using a stathmokinetic technique with crypt microdissection.¹⁵ Results were compared with the crypt cell production rate in similar specimens cultured in the same basic medium with the addition of 5-HT to determine whether this amine influenced crypt cell kinetics in vitro.

Material and methods

SMALL INTESTINAL BIOPSY SPECIMENS

Biopsy samples of the mucosa in the third or fourth part of the duodenum were obtained via the mouth from 19 adults who were being routinely investigated

for upper gastrointestinal disorders using a fiberoptic endoscope (Olympus GIF 1T). The tissue specimens were placed in cold Leibowitz L-15 medium and then flattened, serosal surface downwards, using aseptic techniques. Each sample was divided into pieces measuring roughly 3 mm². Samples for routine histopathology were fixed in either 10% buffered formalin or acetified formol sublimate. Sections (4 µm) were cut from each paraffin block and stained with haematoxylin and eosin.

ORGAN CULTURE

Specimens of duodenal mucosa from each patient were placed serosal surface downwards on a stainless steel grid platform, positioned at one end of a well in a Lux rectangular culture dish (Flow Laboratories). Two different culture media were used. The first was a basic medium comprising: 6 ml of Trowell's T-8 medium, 2 ml of NCTC (National Collection of Type Cultures) 135 medium, 1.5 ml of 309 fetal calf serum (GIBCO), 0.2 ml of L-glutamine (200 mM), 0.05 ml of penicillin and streptomycin (5000 U of each), 0.025 ml of amphotericin, and 0.1 ml of HEPES (1 M). The second culture medium consisted of the same basic medium to which 5-hydroxytryptamine creatinine sulphate (Sigma) was added to give a final concentration of 2 mg/ml. After this addition the medium was sterilised by millipore filtration.

Specimens of duodenal tissue from 10 patients (five men and five women) were cultured in basic medium alone, and specimens from nine patients (four men and five women) were cultured in the basic medium with added 5-HT. In each experiment the medium was warmed to 37°C and then 2.5 ml was placed in the culture dish. The dish was covered with a lid (slightly

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raised to allow for gassing) and placed in a controlled atmosphere chamber (Bellco Glass Inc) containing a dish of sterile water to maintain humidity. The chamber was sealed, placed on a rocking apparatus in a 37°C incubator, and rocked at 4 rpm in an atmosphere of 95% oxygen and 5% carbon dioxide to enable only the underside of the tissue to be alternately exposed to the gas phase and to the medium.

CRYPT CELL PRODUCTION RATE

Specimens of duodenal tissue were maintained in organ culture for 18 hours before adding a further 1 ml of the appropriate culture medium to raise the fluid level and ensure good penetration by the metaphase arresting agent. The stathmokinetic experiment was then started by adding vincristine sulphate (Oncovin, Lilly) to each culture well at a concentration of 1 µg/ml. This concentration was derived from earlier studies and was the lowest dose of vincristine sulphate within the range of 0.1–3.0 µg/ml that caused the greatest number of metaphase arrests per crypt over three hours without allowing escape into anaphase to occur. After the addition of vincristine sulphate two explants were removed from the culture at hourly intervals for three hours and were fixed in Carnoy's fluid for four hours. The specimens were then stored in 70% alcohol before staining DNA by the Feulgen technique. Intestinal crypts in each sample were separated by microdissection in 45% acetic acid and squashed under a coverslip. The number of arrested metaphases in each crypt was then counted. As this number varied considerably throughout the tissue, even in adjacent crypts, at least 15 complete crypts from different parts of the tissue were examined. Counts made at the edges of the tissue did not differ appreciably from those in the centre, and preliminary studies after the addition of ³H-thymidine to the culture medium had shown uniform labelling of crypt cell nuclei on autoradiography with no evidence of "edge labelling". The mean number of metaphase arrests per crypt at each sampling time was then plotted for tissue samples cultured in each medium. The slope of the lines joining these points represented the crypt cell production rate (cells/crypt/hour) and was determined by linear regression analysis (least squares). Statistical analysis

of the differences between the slopes was performed using Student's *t* test.

Results

Histological sections of duodenal mucosa from all the patients studied initially appeared normal when examined by light microscopy (fig 1). After culture for 21 hours the appearances were characterised by shortening of some villi, good preservation of the superficial epithelial cells, and a mantle of cellular debris and mucin on the mucosal surface (fig 2). Any evidence of cell necrosis in the tissue was mainly limited to one or two crypts towards the periphery of the specimen and these were not counted. The overall success of the culture system may have been due to the use of a rocking platform, and to ensuring that the underside of the tissue was alternately exposed to the gas phase and to the medium.

The table and fig 3 show the results of the stathmokinetic studies on specimens cultured in the two media. The crypt cell production rate in the specimens from 10 patients cultured in the basic medium alone was estimated to be 1.2 cells/crypt/hour; in contrast, the rate in specimens from nine patients cultured in the basic medium with added 5-HT was significantly increased to 2.7 cells/crypt/hour (*p* < 0.001). A close correlation was found between the number of metaphase arrests and time in tissues cultured in the basic medium (*r* = 0.99) and in those cultured in the basic medium with added 5-HT (*r* = 0.98).

Discussion

The results of this study suggest that 5-HT has a trophic effect on crypt cells in normal human duodenal mucosa when cultured in vitro. Further dose related studies are necessary, however, as similar experiments on laboratory rats in vivo showed that small doses of 5-hydroxytryptamine creatinine phosphate (10–100 µg/kg body weight) increased crypt cell proliferation and shortened the cell cycle time in the jejunal mucosa, while larger doses of this amine (300 mg/kg body weight) inhibited crypt cell proliferation.³ Previous estimates of the crypt cell production rate in the

Table Arrested metaphases per crypt

Time after addition of vincristine (minutes)	Tissue cultured in basic medium (n = 10)		Tissue cultured in basic medium + 5-HT (n = 9)	
	Mean	SEM	Mean	SEM
60	1.3	0.26	1.9	0.52
120	2.6	0.29	3.7	0.50
180	3.6	0.34	7.3	1.10

Fig 1 Human duodenal mucosa before culture. (Haematoxylin and eosin.)

normal duodenal mucosa have been derived from morphometric data obtained from histological sections or from histological sections prepared after stathmokinetic studies performed in vivo on two patients.¹⁶ The crypt cell production rate derived from the histological sections was 17 cells/crypt/hour and was 11 and 14 cells/crypt/hour, respectively, from the sections after the stathmokinetic studies. These values are considerably higher than those obtained in the present study using stathmokinesis and crypt microdissection. It may be possible to increase the crypt cell production rate in vitro by improving the techniques used for culturing the small intestinal mucosa.

In the gastrointestinal tract 5-HT is normally synthesised from dietary tryptophan by enterochromaffin cells, which are present mainly in the crypts of the upper small intestine. Raised concentrations of 5-HT

have been found in the duodenal mucosa⁴ and blood⁵⁻⁷ of patients with untreated coeliac disease, together with increased urinary excretion of its major metabolite 5-hydroxyindoleacetic acid.⁸⁻¹³ These values return to normal after treatment with a gluten free diet. These metabolic abnormalities may be caused by increased synthesis and release of 5-HT by greater numbers of enterochromaffin cells in the upper small intestinal mucosa, which may be secondary to the hyperplasia of all crypt cells in coeliac disease.^{17,18} Recent cytofluorometric¹⁹ and immunohistochemical²⁰ studies of enterochromaffin cells also suggest, however, that each cell may synthesise and release greater amounts of 5-HT in coeliac disease. As the crypt cell production rate is increased in the mucosa of the small intestine of patients with coeliac disease² and 5-HT increased the crypt cell production rate in the present study, this amine could be impli-

Fig 2 Human duodenal mucosa cultured in basic medium for 24 hours, with cellular debris removed by gentle brushing. (Haematoxylin and eosin.)

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Fig 3 Accumulation of metaphase arrests during stathmokinetic experiments on tissues cultured in both media. Each point represents mean number of arrests in 15 crypts; two tissue samples were analysed at each time point.

cated in the pathogenesis of this disease. It is possible that dietary gluten in patients with coeliac disease stimulates enterochromaffin cells to synthesise and release increased amounts of 5-HT, which increases the crypt cell production rate and leads to a flattened small intestinal mucosa.

Support for this hypothesis might be expected to be found in patients with carcinoid tumours that synthesise increased quantities of 5-HT, but in an earlier study the appearance of the jejunal mucosa on light microscopy in two such patients was normal.²¹ Two recent reports, however, have described patients with carcinoid tumours associated with a flat small intestinal mucosa and coeliac disease,^{22,23} suggesting that further study of the small intestinal mucosa in such patients is indicated.

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