Increased tissue concentrations of 5-hydroxytryptamine in the duodenal mucosa of patients with coeliac disease

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SUMMARY   Tissue concentrations of 5-HT have been measured in the duodenal mucosa of adults and children with coeliac disease and were found to be significantly higher than those from a control group. This finding may be associated with hyperactivity or hyperplasia of enterochromaffin (EC) cells in the duodenum of patients with coeliac disease and could also be directly related to described abnormalities of 5-HT metabolism in this disease.

Increased urinary excretion of 5-hydroxyindole-acetic acid (5-HIAA), a metabolite of 5-hydroxy-tryptamine (5-HT), has been reported in both adults and children with untreated coeliac disease (Haver-back and Davidson, 1958; Haverback *et al.*, 1960; Pimparker *et al.*, 1961; Scriver, 1961; Sleisenger, 1961; Benson *et al.*, 1964; Kowlessar *et al.*, 1964; Challacombe *et al.*, 1972). Clinical recovery after the introduction of a gluten-free diet was accompanied by a fall in urinary 5-HIAA (Sleisenger, 1961; Benson *et al.*, 1964; Kowlessar *et al.*, 1964; Challacombe *et al.*, 1972). Raised blood levels of 5-HT in coeliac disease (Pimparker *et al.*, 1961; Warner and Cohen, 1962) also return to normal after gluten withdrawal from the diet. 5-HT is synthesised in the small intestine by enterochromaffin (EC) cells, and hyperplasia of these cells has been reported in patients with coeliac disease (Challacombe and Robertson, 1976). In the present investigation, tissue levels of 5-HT were estimated in the duodenal mucosa of adults and children with coeliac disease and the results compared with a group of controls.

Methods

SUBJECTS

Coeliac disease
Peroral biopsies were performed on four adults and seven children with untreated coeliac disease. Light microscopic examination of the duodenal mucosa showed subtotal villous atrophy and increased

cellular infiltration of the lamina propria. Introduction of a gluten-free diet to these patients resulted in clinical recovery.

Controls
Four adults and six children underwent peroral duodenal biopsy to investigate suspected mal-absorption. Morphology of the duodenal mucosa on light microscopy in all patients was within normal limits.

Gluten challenge
Three children with a clinical history suggestive of coeliac disease were subjected to biopsy and initial examination of the duodenal mucosa by light micro-scopy showed only minor villous abnormalities. After 10 days on an oral challenge of 10 g gluten powder (Energen Foods, Ltd) three times a day, a second biopsy from the same part of the duodenum showed more severe histopathological changes. These were characterised by increased cellular in-filtration of the lamina propria and partial villous atrophy. Introduction of a gluten-free diet to these patients resulted in clinical recovery.

TECHNIQUES

Small intestinal biopsy
Tissue from a standard site between the third and fourth part of the duodenum was obtained by peroral biopsy under fluoroscopic control using a Watson adult intestinal biopsy capsule. After dividing the specimen, one segment was used for histological examination while the other was weighed on a five-

place micro-balance. This tissue was wrapped in metal foil to prevent drying, placed in a plastic tube, and stored in liquid nitrogen until analysed.

Measurement of tissue 5-hydroxytryptamine

5-HT was extracted from duodenal tissue by the method of Snyder *et al.* (1965) as modified by Small and Holton (1970), and was determined fluorometrically by its reaction with o-phthalaldehyde (Curzon and Green, 1970).

Duodenal biopsy tissue was homogenised in a small glass homogeniser with 2·5 ml ice cold 0·4M perchloric acid, to which had been added 0·05 ml of a solution containing 0·05M disodium EDTA, 0·17M ascorbic acid, and 0·15M sodium chloride. The homogenate was centrifuged at 2000 rpm for 10 minutes and to 2 ml of the supernatant was added 0·5 ml 0·5M sodium borate buffer (pH 10), 0·5 ml 1·5M sodium hydroxide, and 1 g solid sodium chloride. The solution was mixed well after each addition, and especially after adding sodium chloride to ensure complete saturation. Eight millilitres of n-butanol was then added and the mixture shaken for 10 minutes at room temperature. After allowing the two phases to separate, as much as possible of the lower phase was removed. One millilitre of 0·1M sodium borate buffer (pH 10), which had previously been saturated with sodium chloride, was then added and the mixture shaken for three minutes. The lower layer was again removed and the upper layer cleared by a brief period of centrifugation. A 5 ml aliquot of the upper n-butanol layer was then shaken for five minutes with 10 ml n-heptane and 1 ml 8mM cysteine in 0·1M hydrochloric acid. After removing most of the upper layer, two 0·3 ml aliquots of the lower layer were added to two test tubes. To the first tube, the blank, was added 1·8 ml 10M hydrochloric acid and to the second tube, the test, was added 1·8 ml 0·3mM o-phthalaldehyde in 10M hydrochloric acid. After mixing, both tubes were heated in a boiling water bath for 15 minutes. The tubes were cooled in cold water and fluorescence measured in a Perkin-Elmer MPF-3 fluorescence spectrophotometer at activation and emission wavelengths of 360 nm and 470 nm respectively. A stock standard solution of serotonin creatinine sulphate (23 mg in 100 ml water) was diluted 1 in 100 with 8 mM cysteine in 0·1M hydrochloric acid. This solution was further diluted 1 in 5 with water just before use. 0·5 ml aliquots of this working standard (200 ng/ml) and 0·5 ml water were mixed with 2·5 ml ice-cold 0·4M perchloric acid, and 2 ml of these solutions were taken through the entire extraction and assay, as standard and blank. Two reference standards (0·3 ml of the 200 ng/ml working standard) and a reference blank (0·3 ml of 8 mM cysteine in

0·1M hydrochloric acid), were set up for the final assay procedure to calculate recovery of the extracted standard. During the initial development of the method is was found that after reacting the final tissue extracts with o-phthalaldehyde, the absorption and emission spectra produced were similar to those produced by reacting o-phthalaldehyde with authentic 5-hydroxytryptamine. The insoluble material remaining in the homogeniser was transferred with three separate 3 ml portions of 0·5M perchloric acid, to the centrifuge tube containing the bulk of the acid-insoluble tissue homogenate. The contents of the tube were mixed well and centrifuged. The supernatant was removed and the tube drained. The residue was mixed with 1 ml 0·3M sodium hydroxide and incubated at 37°C for one hour. After re-mixing on a vortex mixer, the contents of the tube were centrifuged and 0·2 ml of the clear supernatant was transferred to a clean tube to await protein estimation (Lowry *et al.*, 1951). Tissue concentrations of 5-HT were expressed per gram of tissue weight and per gram of tissue protein.

Results

Tissue concentrations of 5-HT in patients with coeliac disease and in controls are shown in Table 1. Results from the two groups were compared statistically using Student's *t* test. Wet weight of duodenal tissue (mg) and protein content (mg/g tissue) were not significantly different in the two groups of patients. ($p > 0.3$). Tissue concentrations of 5-HT related either to tissue protein (μg/g tissue protein) or to wet weight of tissue (μg/g of tissue) were significantly higher in patients with coeliac disease than in the controls ($p < 0.001$). There was no significant difference between tissue 5-HT concentrations in the adults and children in either the controls ($p > 0.7$) or in patients with coeliac disease ($p > 0.7$). Results from three children who were challenged with gluten are shown in Table 2. Tissue concentrations of 5-HT in the pre-challenge biopsies were not significantly different from the controls ($p > 0.7$). Post-challenge 5-HT concentrations in each patient were greater than pre-challenge values, both in relation to tissue weight and to tissue protein. Although mean 5-HT concentrations were greater in the post-challenge biopsies than in the controls, levels did not attain the values found in patients with coeliac disease who had unequivocal histopathological changes in their duodenal biopsies.

Discussion

5-HT is synthesised from dietary tryptophan by enterochromaffin (EC) cells, situated mainly between

Table 1 *Tissue concentration of 5-HT*

Patients	Age	Tissue weight (mg)	Tissue protein (mg/g tissue)	5-HT/tissue weight (μg/g)	5-HT/tissue protein (μg/g)
Controls					
1	3 m	4·5	122	6·9	57
2	3 m	2·6	102	6·5	63
3	1 yr 6 m	8·0	72	4·9	68
4	1 yr 8 m	9·0	71	2·6	37
5	1 yr 9 m	5·2	75	5·4	72
6	5 yr 2 m	10·6	77	3·4	45
7	Adult	12·3	60	4·6	77
8	Adult	14·0	39	1·6	41
9	Adult	16·0	49	1·1	23
10	Adult	19·8	68	4·8	70
Mean		10·2	73·5	4·2	55·3
SD		5·4	24·0	2·0	17·9
Coeliacs					
1	5 m	7·0	52	8·2	158
2	6 m	13·0	83	8·9	107
3	9 m	15·8	79	19·6	248
4	9 m	8·6	83	9·7	117
5	1 yr 2 m	27·8	84	8·8	105
6	3 yr 9 m	8·3	77	10·0	130
7	5 yr 9 m	1·6	138	17·5	127
8	Adult	13·2	53	7·2	137
9	Adult	19·0	74	10·4	141
10	Adult	12·3	55	8·3	152
11	Adult	12·7	63	10·6	168
Mean		12·7	76·5	10·8	144·5
SD		6·9	23·9	4·0	39·8
P		> 0·3	> 0·7	< 0·001	< 0·001

Table 2 *Gluten challenge*

Patients	Age (m)	Tissue weight (mg)		Tissue protein (mg/g tissue)		5-HT/tissue weight (μg/g)		5-HT/tissue protein (μg/g)	
		Pre-chall.	Post chall.	Pre-chall.	Post chall.	Pre-chall.	Post chall.	Pre-chall.	Post chall.
1	15	8·8	12·7	99	98	7·4	9·2	75	93
2	20	9·7	14·4	89	88	4·4	5·7	49	65
3	20	11·0	10·0	89	104	2·4	7·0	27	67
Mean		9·8	12·4	92·3	96·7	4·7	7·3	50·3	75·0
SD		1·1	2·2	5·8	8·1	2·5	1·8	24·0	15·6

epithelial cells in the crypts of the small intestine. Increased numbers of EC cells have been reported in the duodenal mucosa of children with coeliac disease (Challacombe and Robertson, 1976). In the present study increased tissue concentrations of 5-HT were found in the duodenal mucosa of adults and children with coeliac disease. In three children with coeliac disease who initially had only minor histological abnormalities in the duodenal mucosa, increased tissue levels of 5-HT were found after a 10-day gluten challenge. Further studies will be necessary to determine whether raised tissue levels of 5-HT are due to hyperplasia or hyperactivity of EC cells, or to both. 5-HT is metabolised by the enzyme monoamine oxidase in the tissues to 5-hydroxy-indolylacetaldehyde, which is further oxidised and excreted in the urine as 5-HIAA. Raised levels of 5-HT in the blood (Pimparker *et al.*, 1961; Warner and Cohen, 1962) and 5-HIAA in the urine (Haverback and Davidson, 1958; Haverback *et al.*, 1960; Pimparker *et al.*, 1961; Scriver, 1961; Sleisenger, 1961; Benson *et al.*, 1964; Kowlessar *et al.*, 1964; Challacombe *et al.*, 1972; Challacombe *et al.*, 1975) have been reported in patients with untreated coeliac disease. Clinical recovery after the introduction of a gluten-free diet was accompanied by a fall in blood 5-HT (Pimparker *et al.*, 1961) and urinary 5-HIAA (Sleisenger, 1961; Benson *et al.*, 1964; Kowlessar *et al.*, 1964; Challacombe *et al.*, 1972). The metabolic pathway leading to the

production of 5-HIAA has been studied in adults with coeliac disease using L-tryptophan loading tests (Kowlessar *et al.*, 1964). Significantly increased excretion of 5-HIAA was found in patients with coeliac disease compared with controls, and an augmented shunt of the 5-HT to 5-HIAA pathway in untreated coeliac disease was proposed. Raised urinary excretion of 5-HIAA in coeliac disease could therefore result from increased synthesis of 5-HT in the small intestinal mucosa, with increased release of this amine into the circulation and subsequent breakdown to 5-HIAA. Increased tissue monoamine oxidase activity in the body may also contribute to raised urinary levels of 5-HIAA in coeliac disease. Low levels of this enzyme were found in the duodenal mucosa of children with coeliac disease but platelet concentrations were within the normal range (Challacombe *et al.*, 1971). The intestinal enzyme defect is probably a consequence of local tissue damage. Although monoamine oxidase activity in other tissues has not been estimated, these findings do not suggest that increased enzyme activity is primarily responsible for the increased urinary excretion of 5-HIAA.

Increased duodenal tissue levels of 5-HT may be a non-specific response of the small intestine in susceptible individuals, to many toxic dietary factors, including dietary gluten. An association with villous flattening in other gastrointestinal disorders has not yet been excluded. However, previous studies of urinary 5-HIAA excretion in children suggest that raised excretion may be a specific manifestation of coeliac disease (Challacombe *et al.*, 1972).

Small doses of 5-HT (10 μg/kg) injected into rat peritoneum have been reported to cause an acceleration of crypt cell proliferation and shortening of cell cycle time in the jejunal mucosa when compared with control animals (Tutton, 1974). Conversely, partial 5-HT depletion after injection of 6-fluorotryptophan (a tryptophan hydroxylase inhibitor, which depletes 5-HT stores in the small intestine) retards crypt-cell cycle time (Tutton, 1974). Shortening of cell-cycle time and an expanded proliferative compartment have also been reported in cell kinetic studies on the duodenal mucosa of adults with coeliac disease (Trier and Browning, 1970; Wright *et al.*, 1973, a, b). Altered cell kinetics in rats in response to 5-HT may be a species related phenomenon and inappropriate to man. If, however, a similar response to 5-HT was also present in man, increased local release of 5-HT in the small intestinal mucosa might be related to the histopathological changes found in coeliac disease. Treatment of coeliac disease by the use of pharmacological agents which antagonise the action of 5-HT or inhibit its release from EC cells could then be considered.

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