Prostaglandin E₂ is a mediator of 5-hydroxytryptamine induced water and electrolyte secretion in the human jejunum

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SUMMARY   Studies in the rat jejunum *in vivo* have shown that 5-hydroxytryptamine (5-HT) causes secretion of fluid and luminal release of prostaglandin (PG) E₂. These effects can be blocked by indomethacin and ketanserin, which suggests that PGE₂ may be an important intermediate in the transduction mechanism leading to 5-HT induced fluid secretion. To test this hypothesis in man 'steady state' perfusions (9 ml/min) were done in eight healthy volunteers using the triple lumen technique. The proximal jejunum was perfused with Ringer's solution which contained ^{51}Cr-EDTA as a non-absorbable marker. Before and after the administration of indomethacin ($1 \cdot 0$ mg/kg *iv*) the effects of exogenous 5-HT (10 µg/kg/min *iv*) on jejunal net transport of fluid and electrolytes and jejunal flow rate (JFR) of PGE₂ were measured in 15-min periods for 2×60 minutes after a 60 minute control period. 5-HT reversed fluid and electrolyte absorption into profuse secretion ($p<0 \cdot 01$, Duncan's multiple range test) and significantly increased JFR of PGE₂ ($p<0 \cdot 01$). Indomethacin partly restored fluid and electrolyte absorption ($p<0 \cdot 01$) and inhibited JFR of PGE₂ ($p<0 \cdot 05$). These results provide further evidence in favour of the theory that PGs are involved in 5-HT induced intestinal fluid secretion.

It is generally accepted that 5-hydroxytryptamine (5-HT) induces calcium dependent intestinal fluid and electrolyte secretion,[1-7] possibly through activation of phosphatidylinositol turnover,[8] but without altering mucosal adenylate cyclase activity[9 10] or cyclic nucleotide contents.[11] Furthermore, prostaglandins (PGs) have been proposed to be important intermediates in 5-HT induced fluid secretion[6] and recent experiments in animals have shown that jejunal fluid secretion and luminal release of PGE₂, mediated by 5-HT, are inhibited by the cyclooxygenase inhibitor, indomethacin, in addition to the 5-HT₂ receptor antagonist, ketanserin.[6]

5-hydroxytryptamine has been implicated as a cause of diarrhoea in patients with carcinoid syndrome[12-14] and triple lumen perfusions in such patients have shown jejunal secretion of fluid, which could be reduced by the 5-HT receptor antagonist, methysergide.[15] Also excess intestinal PG formation has been reported to be responsible for secretion in a variety of diarrhoeal diseases[12 16] and enteral and parenteral administration of PGs elicits intestinal secretion.[17-19]

To clarify further the role of PGs in 5-HT induced intestinal secretion 'steady state' perfusions of the proximal jejunum were carried out in eight healthy volunteers. The relevance of the above observations in animals for the understanding of diarrhoeal disease in man were evaluated by determining the influence of exogenous 5-HT on the transfer of fluid and electrolytes and the flow rate of PGE₂, in addition to the effects of indomethacin on this response.

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Methods

SUBJECTS

Ten healthy volunteers (three women, median age 22 years, range 20–29) free of medication consented to the study protocol, which was carried out in accordance with the Helsinki Declaration II and approved by the regional ethics committee and the Danish National Health Service.

In two cases the study was stopped prematurely: one participant experienced headache, dizziness, and blurred vision before the start of 5-HT infusion; he had a blood pressure of 160/110 mmHg; another had nausea and vomiting, which became intolerable after start of the 5-HT infusion.

EXPERIMENTAL DESIGN

'Steady state' perfusions were carried out as described by Cooper *et al.*[20] After an overnight fast the jejunum was intubated with a triple lumen polyvinyl tube (Meadox Surgimed Ltd, Denmark), the position of which was checked fluoroscopically before and after the perfusion. The infusion port was placed 5–10 cm aborally to the ligament of Treitz. The mixing segment was 10 cm and the test segment was 30 cm. Another tube was positioned in the antrum of the stomach, which was continously emptied. The jejunum was perfused at a rate of 9 ml/min (LKB 2115 Multiperspex pump, Bromma, Sweden) with an isotonic solution containing Na^+ 145 mmol/l, K^+ 5 mmol/l, Cl 140 mmol/l, HCO_3 10 mmol/l, and as a non-absorbable marker ^{51}Cr-EDTA 30 µCi/l. Proximal aspiration was by siphonage (range 0·5–2·0 ml/min), while distal aspiration was by intermittent suction (range 0·8–4·0 ml/min; Pump AB, Einar Engell, Sweden).

After a 60 minute equilibration period samples were collected every 15 minutes and immediately stored at −20°C for analysis within three weeks. After a 60 minute control period 5-HT (5-hydroxytryptamine creatininesulphate (Serva Chemicals, FRG) dissolved in saline) was infused into a cubital vein at a rate of approximately 100 ml/h equal to 5-HT 10 µg/kg/min. Sixty minutes after start of 5-HT infusion an intravenous bolus injection of indomethacin (Dumex, Copenhagen, Denmark) 1·0 mg/kg was administered over 10 minutes and the 5-HT infusion was continued for another 60 minutes. Because of the lag phase of drug response, and because no equilibration period was interposed between the test periods, the results of the first 15 minute collections in each test period were added to those obtained in the previous period for calculation of the average transport/flow rate in that period. The participants were monitored in a cardioscope and their blood pressure and heart rate were determined regularly and at the start of 5-HT infusion each minute for 10 minutes.

ANALYTICAL PROCEDURES

Net fluid and electrolyte transfer was determined as previously described.[20] Sodium and potassium were determined by flame photometry (Lange, Berlin, FRG) and chloride was measured in a chloride meter (Chloro-counter, Mark II, Labo International, Utrecht, Belgium). ^{51}Cr-activity was determined by gammaspectrometry (Model 1185, Searle Nuclear Chicago Division, Chicago, USA). The values were expressed as ml/h or meq/h/cm length of jejunum. Positive values represent absorption and negative values secretion.

PGE_2 concentrations in aspirates were measured by a radioimmunological method validated by gas chromatography-mass spectrometry as previously described,[21 22] because the mucosal 'overflow' of PGE_2 presently appears to provide the most reliable index of the balance between intestinal PGE_2 synthesis and degradation *in vivo*.[23] The jejunal flow rate (JFR) of PGE_2 – that is, the amount of PGE_2 passing a cross section of the jejunum/minute – was calculated as the concentration of PGE_2 in the aspirate from the proximal aspiration port times the flow rate of fluid passing this port. Transepithelial secretion rates of PGE_2 are not given because the differences between the amounts passing the
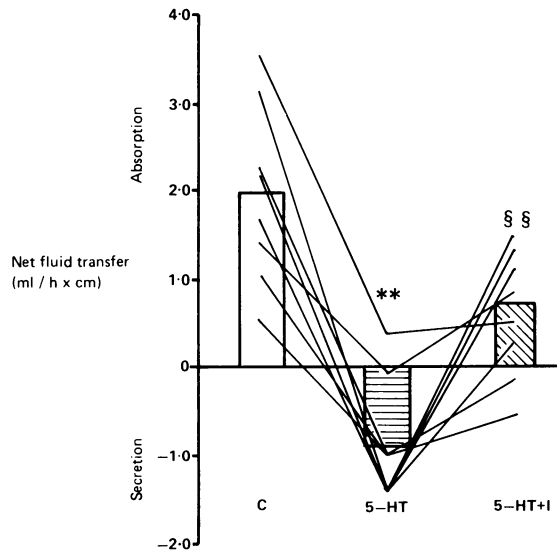

Fig. 1 *Net fluid transfer in proximal human jejunum during control conditions (C), in response to 5-HT infusion (10 µg/ kg×min iv) and following the administration of indomethacin (I, 1 mg/kg iv). **=p<0·01 v control and §§=p<0·01 v 5-HT.*

proximal and the distal aspiration ports were negligible and not significantly different from zero (p>0·05). This observation suggests that degradation of luminal PGE_2 occurs rapidly and/or that the ratio between PGE_2 synthesis and degradation varies along the jejunum. The values of PGE_2 determinations are given as ng/min.

STATISTICAL ANALYSES

All results are given as mean (SE). The data were analysed by Duncan's multiple range test and probability values less than 0·05 were considered significant.

Results

FLUID TRANSFER

Fluid was absorbed in all control periods. 5-HT reversed (p<0·01) net fluid absorption (+1·99 ml/h× cm (0·31)) into profuse secretion (−0·91 ml/h×cm (0·22)). Indomethacin significantly (p<0·01) inhibited this effect of 5-HT, but fluid absorption (+0·73 ml/h×cm (0·27)) was not completely restored (Fig. 1).

ELECTROLYTE TRANSFER

Electrolytes were absorbed during the control period (Na^+: +29·1 mmol/h×cm (6·3); Cl^-: +24·7 mmol/ h×cm (5·5); K^+: +1·7 mmol/h×cm (0·4)). 5-HT elicited net secretion of electrolytes (Na^+: −17·8 mmol/h×cm (9·4); Cl^-: −18·3 mmol/h×cm (6·9); K^+: −0·8 mmol/h×cm (0·3); p<0·01) and this effect was partially reversed (Na^+: +8·5 mmol/h×cm (7·9); Cl^-: +7·0 mmol/h×cm (6·5); K^+: +0·5 mmol/ h×cm (0·3); p<0·01) by indomethacin (Fig. 2).

FLOW RATE OF PROSTAGLANDIN E_2

The fasting jejunal flow rate of PGE_2 at the proximal aspiration port was low during control periods (0·130 ng/min (0·031)) and 5-HT markedly increased JFR of

Fig. 3 *Jejunal flow rate of PGE_2 in proximal human jejunum during control conditions (C), in response to infusion of 5-HT (10 μg/kg×min iv) and following the administration of indomethacin (I, 1 mg/kg iv). ***=p<0·01 v control and §§=p<0·01 v 5-HT.*

PGE_2 (0·494 ng/min (0·131); p<0·01). Indomethacin significantly inhibited the stimulatory effect of 5-HT on JFR of PGE_2 (0·183 ng/min (0·041); p<0·05) (Fig. 3).

ADVERSE EFFECTS

The infusion of 5-HT caused tenderness and redness at the infusion site and in a single case venous spasm, which necessitated a change of the infusion site. The alterations in systolic blood pressure observed during 5-HT infusion were transient and did not exceed 10 mmHg. No significant effect on heart rate was observed. All participants, but three, experienced nausea and slight abdominal discomfort. The administration of indomethacin transiently intensified nausea in all subjects and provoked vomiting in a few cases, but within a few minutes the abdominal symptoms were relieved.

Discussion

5-hydroxytryptamine has been shown to induce intestinal secretion in animals[1-7] by eliciting chloride secretion, in addition to decreasing the electroneutral absorption of sodium chloride.[2,4,24] Furthermore, enterochromaffine cells[14] and nerves in the myenteric plexus[25] contain 5-HT. Although specific 5-HT receptors are present in the submucosal ganglia,[26] enterocytes do not seem to possess specific receptors for 5-HT.[27,28] These observations suggest that the action of the secretagogue is an indirect one
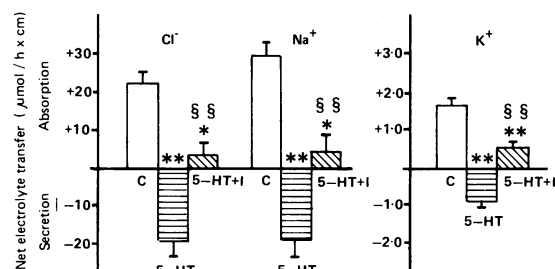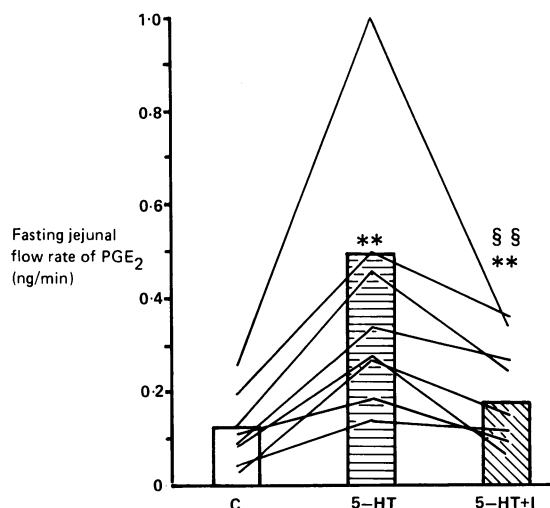

Fig. 2 *Net transfer of electrolytes (mean ± SE) in proximal human jejunum during control conditions (C), in response to infusion of 5-HT (10 μg/kg×min iv) and following the administration of indomethacin (I, 1 mg/kg iv). ***=p<0·01 v control, *=p<0·05 v control, and §§=p<0·01 v 5-HT.*

and that 5-HT requires a paracellular mediator to elicit the final secretory response.

In the morphine withdrawal syndrome observed in the rat the intestinal secretion is mediated by 5-HT[9][29] through stimulation of local PG formation in the absence of increased mucosal cyclic AMP contents.[9] The secretion of fluid and the release of PGE_2 are inhibited by the specific 5-HT_2-receptor antagonist, ketanserin, and the cyclooxygenase-inhibitor, indomethacin, without affecting the release of 5-HT.[9] Intestinal fluid secretion mediated by 5-HT appears to be calcium dependent[2][3][5-7] and the secretory effects of physiologically low doses of exogenous PGE_2 are prevented by the calcium antagonist, verapamil.[6] Therefore, PGE_2 may be an important intermediate in the transduction mechanism leading to fluid secretion.[16]

Although the application of intestinal steady state perfusion in man may only add little to the elucidation of the mechanisms responsible for 5-HT induced secretion, the relevance of the above observations for the understanding of diarrhoeal disease should be judged from data obtained in man. We chose the triple lumen technique to avoid the influence of mechanical stimulation, if any, and/or the possible activation of local PG formation through nervous reflex mechanisms provoked by occluding balloons.

The results of the present study unequivocally show that exogenous 5-HT induces profuse jejunal secretion of fluid and electrolytes in man, in addition to increasing JFR of PGE_2. These effects were only partially inhibited by indomethacin. The infusion rate of 5-HT used in this study was chosen to obtain a local mucosal concentration similar to that reported in previous *in vivo* experiments on the rat jejunum.[6] The local jejunal plasma concentration of 5-HT was calculated roughly to be approximately 85 ng/ml, assuming pulmonary and hepatic clearances of 40%[30] and 100%, respectively, and a cardiac output of 5 l/min.

The apparent discrepancy between the effects of conventional doses of indomethacin and those previously obtained in the rat[6] is explained by the use of a 10-fold higher dose in the latter experiments. We would tentatively conclude, therefore, that the secretory action of 5-HT observed in the present study provides further evidence for the hypothesis that PGE_2 is involved in 5-HT induced intestinal secretion and may prove relevant for the understanding of the pathogenesis of certain diarrhoeal disorders, such as cholera[31] and the carcinoid syndrome.[13]

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