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SPECIAL REPORT

Functional evidence for a 5-HT_{2B} receptor mediating contraction of longitudinal muscle in human small intestine

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Application of 5-hydroxytryptamine induces contraction of longitudinal muscle strips from human terminal ileum. The response was resistant to antagonism by ketanserin, ondansetron or DAU6285, but was non-surmountably antagonized by methysergide. The selective 5-$HT_{2B/2C}$ receptor antagonist, SB 200646A evoked a concentration-dependent, parallel and dextral displacement of the concentration-response curve to 5-HT, yielding a pA_2 estimate of 7.17. Application of yohimbine, a 5-HT_1 and 5-HT_{2B} receptor antagonist, also induced a rightward shift of the response curve to 5-HT, yielding a pA_2 estimate of 8.10. In conclusion, it appears that a 5-HT_{2B} receptor mediates the contractile response of the longitudinal muscle of human small intestine to 5-HT.

Keywords: Human ileum; longitudinal muscle; contraction; 5-HT_{2B} receptor; SB 200646A; yohimbine

Introduction Physiological and pharmacological studies have indicated that 5-hydroxytryptamine (5-HT) may possess a pivotal role in mediating sensory and reflex responses in the gastrointestinal tract of various species, including man. It has been shown to induce secretory processes in the small and large intestine (Borman & Burleigh, 1993; 1994a), and to influence motility in the human colon (Borman & Burleigh, 1994a). Due to the lack of selective antagonists, previous studies in human small intestine have failed to characterize fully the receptors responsible for the contractile effects of 5-HT (Baxter *et al.*, 1991; Borman & Burleigh, 1994b). In the present study, we have used selective antagonists to classify the receptor responsible for 5-HT-induced contraction in the longitudinal muscle layer of human small intestine.

A preliminary report of these findings was presented to the Autumn 1994 meeting of the British Pharmacological Society in Sunderland.

Methods Human terminal ileum (within 30 cm of the ileocaecal junction) was obtained from specimens of human intestine removed at operation for carcinoma or Crohn's disease. Tissue removed from specimens with Crohn's disease was judged to be macroscopically normal by a Consultant Histopathologist. Tissue was transported in Dulbecco's Modified Eagle's Medium (DMEM; with 3.70 g l^{-1} sodium bicarbonate and 25 mM HEPES buffer, without glutamine) plus Ham's F12 Medium (with 1.24 g l^{-1} phenol red and 1.176 g l^{-1} sodium bicarbonate, without glutamine, 1:1 with DMEM) with 10% foetal bovine serum added, and placed in gassed Krebs solution within 60 min of removal from the patient. Some tissue was stored overnight in Krebs solution at 4°C before use. This treatment did not significantly alter responses to applied agonists.

After removal of mucosa and submucosa by sharp dissection, strips of longitudinal muscle (1.5–2.5 × 15 mm) were prepared from the muscularis externa, and were suspended under isotonic conditions and a preload of 1 g, in gassed (5% CO_2 in oxygen) Krebs solution at 37°C. After 60 min equilibration, 5-HT was applied to the tissue in a cumulative manner, with a 3 min contact time for each concentration. After a further 60 min, the challenge to 5-HT was repeated in the absence (control) or presence of antagonist (incubated for 30 min). The response to 5-HT was expressed as a % of the maximum contraction induced by a supramaximal concentration of acetylcholine (30 μM), applied at the end of each experiment. EC_{50} values for 5-HT were determined graphically from individual concentration-response curves. Concentration-ratios were calculated as the ratio of EC_{50} values between two successive response curves in the same preparation and expressed as geometric mean with 95% confidence limits (95% CL). All other data are given as arithmetic mean ± s.e.mean. Statistical comparisons used the Mann-Whitney U-test, with $P<0.05$ being taken to represent a significant difference.

Compounds used were 5-hydroxytryptamine creatinine sulphate (Sigma), acetylcholine chloride (Sigma), ondansetron hydrochloride (GR38032F, Glaxo), methysergide hydrogen maleate (Sandoz), yohimbine hydrochloride (Sigma), ketanserin tartrate (Janssen) and DAU6285 (1H-benzimidazole-1-carboxylic acid,2,3-dihydro-6-methoxy-2-oxo-8-methyl-8-azabicyclo (3,2,1) oct-3-yl ester chloridate, Boehringer Ingelheim Italia). DMEM, Ham's F12 Medium and foetal bovine serum were obtained from ICN Flow Laboratories, Cat. No. 12-331-54, 12-422-54 and 29-101-54 respectively. SB 200646A (N-(1-methyl-1*H*-indol-5-yl)-*N'*-(3-pyridyl) urea hydrochloride) was generously donated by SmithKline Beecham Pharmaceuticals. All these compounds were dissolved in distilled water and diluted in Krebs solution of the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.5.

Results Cumulative application of 5-HT produced a contraction of longitudinal muscle strips of human terminal ileum. The maximum response to 5-HT was 41.4 ± 2.8% of the response to acetylcholine, with an EC_{50} for 5-HT of 34.9 nM (95% CL 26.3–46.2). The response to 5-HT showed desensitization on repetition of the challenge, with a significant dextral shift of the second control concentration-response curve, but with no alteration of the maximum response. This led to a concentration-ratio between successive control response curves of 2.3 (95% CL 1.5–3.7).

The contractile response to 5-HT was shown to be insensitive to antagonism by ketanserin (1 μM; 5-HT_{2A} receptor antagonist), ondansetron (1 μM; 5-HT_3 receptor antagonist) or DAU6285 (1 μM; 5-HT_4 receptor antagonist; Schiavone *et al.*, 1992) ($n \geq 4$ all treatments). The response was, however, antagonized in a non-surmountable manner by methysergide (10 pM to 1 nM; Figure 1a).

Application of SB 200646A (0.3 to 3 μM; a 5-$HT_{2B/2C}$ receptor antagonist; Baxter *et al.*, 1994) or yohimbine (30 to 300 nM; a 5-HT_1 and 5-HT_{2B} receptor antagonist) evoked

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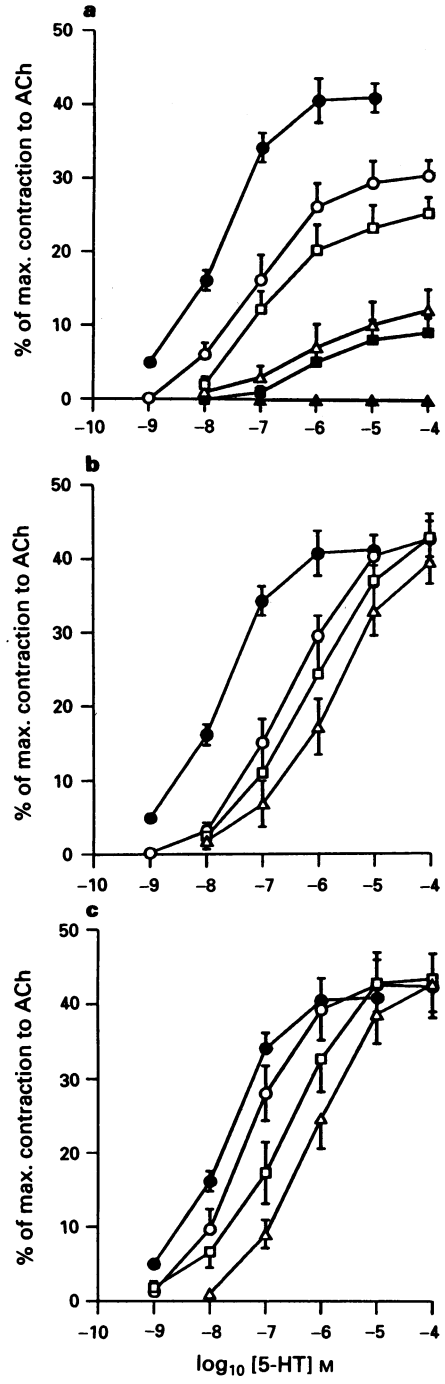

Figure 1 Effect of antagonists on the contractile response to 5-hydroxytryptamine (5-HT) of the longitudinal muscle of human small intestine. Results shown as % of maximum contraction to acetylcholine (ACh). Figure shows a second concentration-response curve to 5-HT in the absence (●) and presence of (a) methysergide at concentrations of 1pM (○), 10 pM (□), 100 pM (△), 1nM (■) and 10 nM (▲); (b) SB 200646A at concentrations of 0.3 μM (○), 1 μM (□) and 3 μM (△), and (c) yohimbine at concentrations of 30 nM (○), 100 nM (□) and 300 nM (△). Data are mean ± s.e.mean for $n \geqslant 6$ determinations

concentration-dependent dextral shifts of the second concentration-response curve to 5-HT, with no alteration of the maximum response (Figure 1b and 1c respectively). Schild analysis of the antagonism produced by SB 200646A yielded a Schild plot with a slope of 0.96 (95% CL 0.66–1.25) and an apparent pA_2 estimate of 7.17 ± 0.16. For yohimbine, Schild analysis produced a plot with a slope of 1.10 (95% CL 0.78–1.42) and a pA_2 estimate of 8.10 ± 0.06 (slope constrained to unity for both pA_2 estimates).

Discussion The 5-HT_{2B} receptor has been shown to mediate the contractile effect of 5-HT in the rat stomach fundus (Baxter *et al.*, 1994). The receptor mRNA transcript has subsequently been identified in the rat (Kursar *et al.*, 1992), mouse (Loric *et al.*, 1992) and more recently, in the human small intestine (Kursar *et al.*, 1994). The receptor has proved difficult to classify definitively due to the lack of selective antagonists, with few compounds able to differentiate clearly between receptor sub-types. The close similarity of the 5-HT_{2B} receptor with both the 5-HT_{2C} receptor, and the 5-HT_{1D} receptor (the two receptors share a comparable tryptamine fingerprint) has further complicated investigations. Of those compounds which do show some selectivity, yohimbine and rauwolscine have proved most useful in differentiating 5-HT_{2B} receptors from the closely related 5-HT_{2C} receptor. Both of these compounds, however, have been shown to have effects at other 5-HT receptor sub-types, as well as at other non-5-HT receptors. More recently, the discovery of a novel 5-$HT_{2B/2C}$ receptor antagonist, SB 200646A (Baxter *et al.*, 1994), has greatly facilitated the characterization of the 5-HT_{2B} receptor.

In the longitudinal muscle of human small intestine, both yohimbine and SB 200646A were shown to antagonize competitively the contractile response to 5-HT, with pA_2 estimates similar to those reported at the rat 5-HT_{2B} receptor (Baxter *et al.*, 1994). Although yohimbine is not uniquely selective for the 5-HT_{2B} receptor and has been shown to antagonize responses at 5-HT_1 receptors as well as α_2-adrenoceptors, the relative selectivity of SB 200646A for the 5-HT_{2B} receptor over these other subtypes ($pK_i < 5.0$; Kennett *et al.*, 1994), and the lack of effect of yohimbine at the 5-HT_{2C} receptor ($pK_i < 6.0$; Baxter *et al.*, 1994), provides firm evidence for the involvement of a 5-HT_{2B} receptor in the contractile response to 5-HT of human small intestinal longitudinal muscle. This confirms the results of Kursar *et al.* (1994), whose studies revealed 5-HT_{2B} receptor mRNA to be expressed in human small intestine, and provides the first functional evidence for a human 5-HT_{2B} receptor.

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