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Comparison of 5-HT_4 and 5-HT_7 receptor expression and function in the circular muscle of the human colon

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Abstract

Serotonin receptors are potential targets for treating functional bowel disorders. This study investigated the functional roles and expression of the 5-HT_4 and the 5-HT_7 receptor, which coexist in human colon circular smooth muscle. 5-HT_3 receptor expression was also investigated. Part of the relaxant response to 5-HT was due to activation of 5-HT_4 receptors as the apparent pK_B value of the selective 5-HT_4 antagonist, GR 113808, was 9.36. 5-HT_4 mRNA levels were low in five tissues and undetectable in four others, but all responded to 5-HT with an EC_{50} value of 102.54± 19.32 nM. The contribution of 5-HT_7 receptors to the response was not readily demonstrated using the selective 5-HT_7 antagonist, SB-269970, as its apparent pK_B value of 7.19 (5-HT_4 block with 1 μM GR 113808) was lower than the value obtained using the 5-HT_7 guinea pig ileum assay (8.62). Nevertheless, the 5-HT_7 receptor was expressed more consistently than the 5-HT_4, but at similar levels. The 5-$HT_{3Ashort}$ and 5-HT_{3B} subunits were co-expressed at similar levels, but the 5-HT_{3Along} subunit was detected in only five of the nine samples tested. The findings show that 5-HT_4-induced relaxation occurs at low to undetectable levels of tissue mRNA, as measured by qPCR. Although 5-HT_7 receptor mRNA is detected at low, but consistent levels, the functional activity of this receptor is not readily identified given the currently available drugs.

Keywords: Serotonin receptors; 5-HT_3 receptors; 5-HT_4 receptors; 5-HT_7 receptors; Gastrointestinal tract; Human tissue; Colonic smooth muscle

Introduction

Serotonin (5-HT) has diverse physiological effects that are mediated by at least 14 different receptor subtypes (Hoyer et al., 1994). The largest amount of 5-HT in the body is present along the length of the gastrointestinal tract (60–90%), where most occurs in enterochromaffin cells of the mucosal layer with the remainder in the enteric nervous system (Gaginella and Galligan, 1995). When 5-HT is released it alters the rate at which contents travel down the digestive tract and also the rate at which fluid is absorbed (Gaginella and Galligan, 1995). Consequently, 5-HT_3 antagonists, 5-HT_4 agonists and SSRI antidepressants have been the subject of intense investigation for the treatment of functional bowel disorders such as irritable bowel syndrome (IBS; Crowell, 2004). For example, the 5-HT_4 agonist, tegaserod, has been shown to reduce abdominal pain and give a degree of relief from other symptoms in patients with constipation-predominant IBS (Müller-Lissner et al., 2001). 5-HT_4 receptors are present in several discrete tissue locations in the human colon. These include the mucosa where the response to 5-HT released by enterotoxins induces Cl^- secretion resulting in diarrhoea (Borman and Burleigh, 1996). 5-HT_4 receptors are also present on cholinergic nerve endings, where their function is to enhance transmitter release to the longitudinal muscle bands (Prins et al., 2000). Another less well established location of the 5-HT_4 receptor in the human colon is on sensory nerve endings, where its function may be to increase sensory perceptions arising from the abdomen leading to altered motility patterns. A recent clinical study lends some support to this hypothesis, which

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showed that IBS patients have significantly lower perception and defecation thresholds to rectal thermal and pressure stimuli compared to age and gender matched control subjects (Li et al., 2004). The 5-HT_4 receptor is also present in the circular smooth muscle cells of the human colon. 5-HT_4 agonists induce relaxation and inhibition of spontaneous contractions by activating adenylyl cyclase to increase intracellular levels of cyclic-3,5-AMP (McLean et al., 1995; McLean and Coupar, 1996). Paradoxically, 5-HT_4 receptors expressed by cholinergic neurones in the human colon oppose the effect of this inhibitory postsynaptic 5-HT_4 population by enhancing acetylcholine release (Leclere et al., 2005). Prins et al. (2000) demonstrated that the 5-HT_4 receptors of cholinergic neurones also function to enhance transmitter release to the longitudinal muscle bands (taenia coli). They suggested that the well established effects of 5-HT itself and 5-HT_4 agonists to facilitate colonic propulsion are partly achieved by a coordinated combination of circular muscle relaxation and longitudinal muscle contraction. The human small intestine and colon express various h5-HT_4 splice variants (Blondel et al., 1998; Bender et al., 2000). In one study the amount of all (or pan) 5-HT_4 receptor mRNA was highest in the intestine compared to other body organs (Medhurst et al., 2001). However, there is a lack of quantitative data for expression relative to the colon.

Our previous functional experiments with human colonic circular muscle showed that the relaxation induced by 5-HT is not entirely attributed to a homogeneous population of 5-HT_4 receptors. Our evidence was that the 5-HT_4 antagonist, SB-207710, displayed a limited ability to competitively antagonise 5-HT, indicating the presence of another 'low affinity' receptor (McLean et al., 1995). The 5-HT_4 receptor-resistant relaxation of the colon has since been attributed to a 5-HT_7 receptor population located in the circular smooth muscle (Prins et al., 1999). The main evidence was that the rank order of potency on the 5-HT_4-resistant part of the response was 5-CT = 5-MeOT = 5-HT > 2-Me-5-HT and that the affinity values of mesulergine and methysergide were characteristic of antagonism at 5-HT_7 receptors. The result that the relaxations were tetrodotoxin-resistant indicated that the receptors were localised to the smooth muscle. It has been shown subsequently that all 5-HT_7 receptor slice variants are expressed in the human small intestine and colon including a certain amount of the h5-$HT_{7(d+5)}$ fragment (Krobert et al., 2001). The consequences of these findings are that 5-HT_7 receptors may need to be considered in the future when developing serotonin-modifying pharmacotherapies for conditions of the colon. Like 5-HT_4 receptors, 5-HT_7 receptors preferentially couple to adenylyl cyclase via $G_s\alpha$ (Adham et al., 1998).

There is considerable evidence to show that 5-HT_3 receptors are present on extrinsic sensory nerve endings of the intestine and that their activation leads to enhanced visceral sensitivity (Gershon, 2004). The genes encoding the 5-HT_3 receptor have been detected in the human colon (Miyake et al., 1995; Niesler et al., 2003) and a 5-HT_3 antagonist, alosetron, was shown to improve the symptoms of female IBS patients (Camilleri et al., 1999). Recent functional and immunohistochemical experiments have identified 5-HT_3 receptors in submucous plexus of the human colon (Michel et al., 2005).

This study focuses on the relative participation of the 5-HT_4 and the 5-HT_7 receptor in 5-HT-induced relaxation of the circular muscle of the human colon. The functional results are complemented by molecular data of the relative expression levels of mRNA for these receptors. Since current therapies for functional bowel disorders, such as irritable bowel syndrome, are aimed at targeting 5-HT_4 or 5-HT_3 receptors, we also investigated the relative expression of the h5-HT_3 receptor subtypes in our human colon preparations.

Materials and methods

Chemicals

GR 113808 A (1-{2[(methylsulfonyl)amino]ethyl}piperidin-4-yl) methyl 5-fluoro-2-methoxy-1*H*-indole-3-carboxylate) and SB-269970-A ((*R*)-3-(2-(2-(4-methyl-piperidin-l-yl) ethyl)-pyrrolidine-l-sulphonyl)-phenol)) were from Glaxo SmithKline (Hertfordshire, UK), cocaine hydrochloride from Glaxo (Melbourne, Australia) and indomethacin from Merck Sharp and Dohme (Melbourne, Australia). Substance P was purchased from Auspep (Parkville, Australia) and atropine methyl bromide, hydrocortisone 21-hemisuccinate sodium salt, 5-hydroxytryptamine creatinine sulphate (5-HT), and pargyline hydrochloride and analytical grade reagents were purchased from Sigma Chemical Company (Sydney, Australia).

Human tissue

This project was approved by the Monash University Standing Committee on Ethics in Research Involving Humans (Protocol number: 98/115) and by the Human Research Ethics Committee of St. Vincent's Hospital, Melbourne (Protocol number: 001/01). Specimens of human sigmoid colon were obtained from patients who gave consent prior to surgical resection for colonic cancer at St. Vincent's Hospital Melbourne. The specimens were taken as far from the tumour as possible and appeared to be normal by gross visual inspection. Tissues were obtained from 11 patients (8 from the sigmoid colon and 1 each from the ascending, transverse and descending regions). The age of these patients ranged from 50 to 88 (median 74) of which there were 7 male and 4 female patients. The patients received pethidine premedication and standard anaesthetics and muscle relaxants.

The specimens were acquired from the surgical theatre immediately after resection and transported to the laboratory in 4 °C Krebs–Henseleit solution (composition in mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, (D)-glucose 11.1 and $CaCl_2$ 2.5) pre-oxygenated with carbogen (95% O_2/5% CO_2). The specimens were dissected to remove the mucosa and associated mesentery plus fat. The remaining intertaenial tissue was then cut into strips in the orientation of the circular smooth muscle (1.5–2 cm in length and 2–3 mm in width) and stored at 4 °C in fresh Krebs–Henseleit solution overnight. Separate adjacent samples were taken and stored in RNALater® (Ambion) at −80 °C for qPCR experiments. Four circular smooth muscle strips were each mounted in a 20 ml organ bath containing Krebs–Henseleit solution (37 °C and oxygenated with carbogen) under