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Investigation of the 5-HT receptor mediating relaxation in guinea-pig proximal colon

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Abstract—5-Hydroxytryptamine (5-HT) induced concentration-related relaxations (EC50 = 9·1 μM) of guinea-pig proximal colon pretreated with ketanserin (1 μM) and ondansetron (10 μM). This 5-HT-induced effect was neuronally mediated since it was blocked by tetrodotoxin (0·3 μM). 5-Carboxamidotryptamine was a full agonist and ten times more potent than 5-HT. α-Methyl-5-HT was a partial agonist. 2-Methyl-5-HT was without effect. Methysergide and metergoline were antagonists of 5-HT producing parallel shifts at 0·1 μM but unsurmountable antagonism at higher concentrations. pK_B values of 8·0 and 7·3 were calculated for methysergide and metergoline, respectively. This study has identified a 5-HT-induced relaxation of guinea-pig proximal colon which is mediated via a neuronal 5-HT_1-like receptor. However, the subtype has yet to be established.

It has been reported previously that 5-hydroxytryptamine (5-HT) induces relaxation of guinea-pig proximal colon through a mechanism which is neuronal since it is blocked by tetrodotoxin (TTX) (Costa & Furness 1979; Kojima & Shimo 1986). However, the pharmacological characteristics of the 5-HT receptor mechanism involved have not been investigated, although Kojima & Shimo (1986) suggested that it might be 5-HT_3-like. The purpose of the present study was to characterize the 5-HT receptor mediating the relaxation response in this tissue.

Methods and materials

Tissue preparation. Female Dunkin Hartley guinea-pigs, 300–400 g, were killed by cervical dislocation and the most proximal portion of the colon (a 10 cm segment starting 1 cm from the caecum) removed. The colon was divided into 3 cm segments and opened longitudinally. Faecal matter was removed and the mucosa dissected away. The muscle strip was then set up in the longitudinal plane in a 20 mL gut bath filled with Krebs solution containing ondansetron (10 μM) and ketanserin (1 μM) to block contractions mediated by the 5-HT_3- and 5-HT_2-receptors, respectively. The Krebs solution was maintained at 32°C and gassed with 95% O_2 − 5% CO_2 and had the following ionic composition (mM): NaCl 118·5, $NaHCO_3$ 25·0, KCl 4·7, $MgSO_4$ 0·6, KH_2PO_4 1·2, $CaCl_2$ 1·3, glucose 11.1. Tissues were allowed to settle for 40 min under a basal tension of 1 g. Responses were measured via an isometric transducer.

Establishment of agonist concentration-response curves and antagonist potencies. Tissues were contracted with a just maximal concentration of carbachol (0·3 μM) and a plateau response allowed to develop; a single agonist concentration was then applied. When the maximum relaxation response had been obtained the tissue was washed and left for 10 min before recontracting with carbachol and administration of a different agonist concentration. Tissues were initially given two priming doses of 5-HT (100 μM) before construction of sequential agonist concentration-response curves. Thirty minutes were allowed between agonist concentration-response curves. Tissues were paired (from adjacent portions of colon) so that for the second agonist concentration-response curves, one tissue was used for the test compound (either agonist or antagonist) and the second tissue was used to repeat the control 5-HT curve. Antagonists were incubated for 30 min before repeating the agonist concentration-response curve.

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Expression of results. EC50 values (the concentration which produces 50% of the maximum response), equipotent molar ratios for agonists (EPMRs) and concentration ratios produced by antagonists were calculated graphically for each tissue and the results are presented as a geometric mean with 95% confidence limits. pK_B values for antagonists were calculated using the Schild equation and are expressed as an arithmetic mean with standard error of the mean.

Compounds. 5-HT hydrochloride, tetrodotoxin and 5-methoxytryptamine hydrochloride were obtained from Sigma, UK. Ketanserin tartrate and spiperone were from Janssen, Belgium, methysergide hydrogen maleate from Sandoz, Switzerland, carbachol chloride from BDH Chemicals, UK and metergoline was obtained from Farmitalia, Italy; citalopram hydrogen bromide was obtained from Lundbeck, UK and desipramine hydrogen chloride was obtained from Ciba-Geigy, USA.

The following compounds were made in the Chemical Research Division of Glaxo Group Research: ondansetron hydrochloride, 5-carboxamidotryptamine maleate, α-methyl-5-HT maleate, 2-methyl-5-HT hydrochloride monohydrate, sumatriptan succinate.

Results

5-HT produced concentration related relaxations with an EC50 value of 9·1 (6·5, 12·7) μM (n = 15). The maximum inhibition of the carbachol tone was 46·8 ± 4·1%. Two control 5-HT curves could be repeated in each preparation (concentration ratio (CR) = 0·75 (0·39–1·44; n = 12). Responses to 5-HT were unaffected by the 5-HT uptake blocker citalopram (1 μM) (CR = 0·95 (0·3–2·8)) or the catecholamine uptake blocker desipramine (0·1 μM) (CR = 0·49 (0·18–1·30)). The effects of the 5-HT receptor agonists are summarised in Fig. 1 with details of relative potencies given in Table 1. 5-Carboxamidotryptamine (5-CT) was a full agonist and 10 times more potent than 5-HT. α-Methyl-5-HT behaved as a weak partial agonist and 2-methyl-5-HT (up to 300 μM) was not an agonist in this preparation. 5-Methoxytryptamine and sumatriptan also produced large relaxations of the carbachol tone with a greater maximum response than 5-HT (Table 1).

The relaxation responses to 5-HT and 5-CT were abolished by TTX (0·3 μM). The relaxations were also antagonised by the 5-HT_1-like receptor antagonists methysergide (0·1–1 μM) and metergoline (0·1–1 μM). Both compounds produced concentration-related rightward shifts of the agonist concentration-response curve (Fig. 2). From the rightward shifts produced by methysergide (0·1 μM) and metergoline (0·1 μM), pK_B values of 8·0 ± 0·3 and 7·3 ± 0·1, respectively, were calculated. A higher concentration of methysergide or metergoline (1 μM) produced further rightward shifts, but these responses were apparently

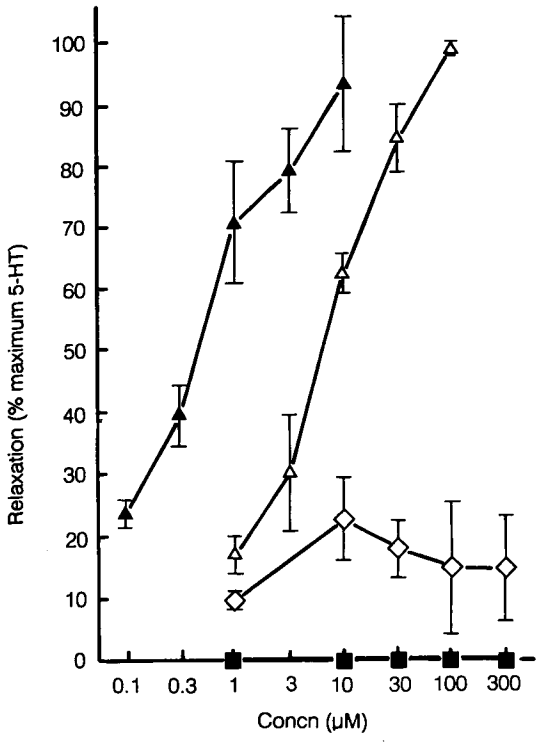

FIG. 1. The agonist effects of selected indoles: 5-HT, △; 5-CT, ▲; α-methyl-5-HT, ◇; 2-methyl-5-HT, ■. Results are expressed as mean ± s.e., n = 4–5.

unsurmountable. However, even at 1 μM, both methysergide and metergoline were selective since they did not inhibit relaxations induced by isoprenaline (3–300 nM). Methysergide (0·1 μM) also antagonised responses to 5-CT but caused a large depression in maximum response (64.2 ± 9·4%, n = 5). However, the relaxations produced by 5-methoxytryptamine and sumatriptan were unaffected by methysergide even at a high concentration (10 μM). Spiperone (0.3 μM), another 5-HT_1-like receptor antagonist, had little effect on the 5-HT response, reducing the maximum response by 20·0 ± 9·3% but causing a rightward shift of only 2·2 (0·6, 7·6)-fold. In addition, increasing the concentration of ketanserin, from 1 to 10 μM, had no significant effect on the 5-HT-induced responses (CR = 0·64 (0·17–2·4) n = 3).

Table 1. Effects of 5-HT receptor agonists at the relaxant 5-HT receptor in guinea-pig proximal colon.

Agonist (μM)	Concn range (μM)	n	EPMR (95% confidence limits) (5-HT = 1)	Max response % of 5-HT (mean ± s.e.m.)
5-Hydroxy-tryptamine	1–100		1 (EC50 = 9·1 (6·5, 12·6) μM)	100
5-Carboxamido-tryptamine	0·1–100	4	0·1 (0·03, 0·4)	95·0 ± 9·5
5-Methoxy-tryptamine	1–300	5	4·7 (2·0, 11·1)	131·6 ± 23·4*
α-Methyl-5-HT	1–300	4	—	36·0 ± 3·9
2-Methyl-5-HT	1–100	4	—	5·8 ± 5·8
Sumatriptan	0·1–300	7	3·2 (1·2, 8·9)	166·7 ± 17·8*

* Not antagonized by methysergide (10 μM).

Discussion

The present study confirms previous observations (Costa & Furness 1979; Kojima & Shimo 1986) that 5-HT induces relaxation of guinea-pig proximal colon through a neuronal mechanism that is sensitive to TTX. This response cannot be attributed to 5-HT_2- or 5-HT_3-receptors since ketanserin (1 μM)

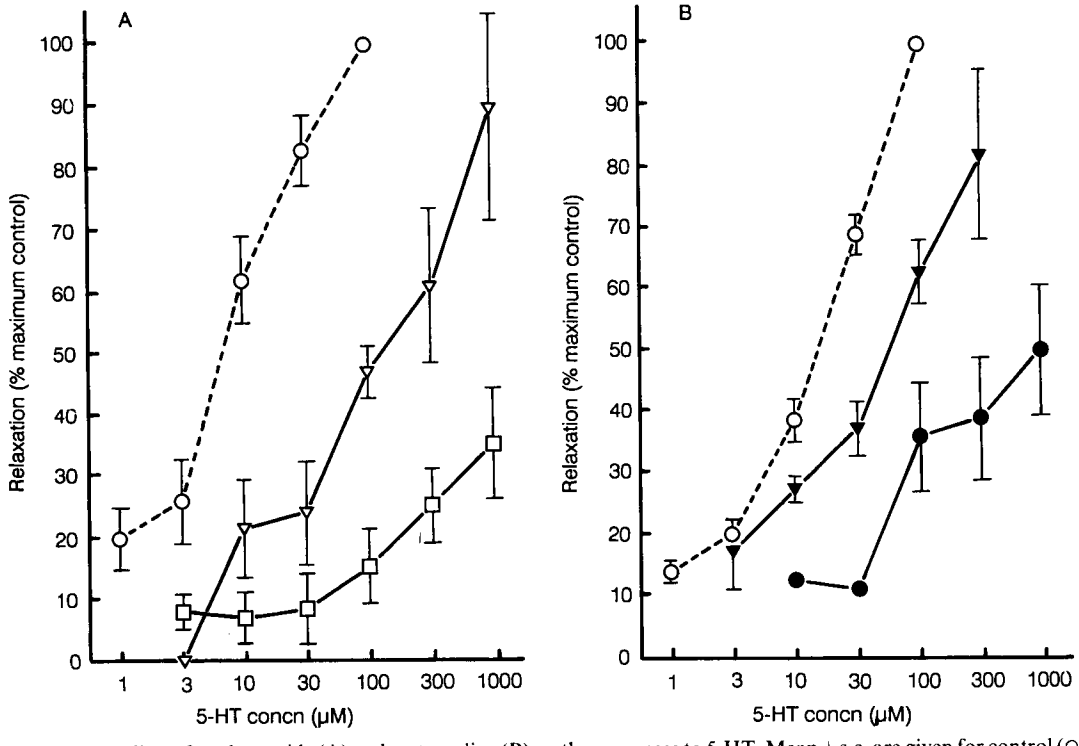

FIG. 2. The effect of methysergide (A) and metergoline (B) on the responses to 5-HT. Mean ± s.e. are given for control (○) and responses in the presence of the antagonist at 0·1 (▽) and 1 μM (□), and metergoline at 0·1 (▼) and 1 μM (●), n = 4.

and ondansetron (10 μM) were routinely added to the Krebs solution. In addition, the 5-HT_2- and 5-HT_3-receptor selective agonists, α-methyl-5-HT and 2-methyl-5-HT exhibited little or no agonist activity under the conditions of the present experiments.

The observation that 5-CT was a full agonist, but more potent than 5-HT, is consistent with the contention that the response was mediated by 5-HT_1-like receptors (Bradley et al 1986), and this conclusion is corroborated by the observation that the responses to both 5-HT and 5-CT were blocked by methysergide and metergoline. The pK_B values calculated for these compounds against 5-HT as the agonist (8·0 and 7·3, respectively) do not match precisely with the published data for any 5-HT_1 binding site (Hoyer 1989) or with functional 5-HT_1-like receptors in dog saphenous vein (Apperley et al 1980; Humphrey et al 1988) and piglet vena cava (Sumner et al 1989). However, there are some similarities between the results in the present study and the findings of Kalkman et al (1986) in guinea-pig ileum. In the latter study Kalkman et al (1986) identified a 5-HT receptor-type mediating smooth muscle relaxation through a direct non-neuronal mechanism. 5-CT was a full agonist with an EPMR of 0·18 and metergoline and methysergide were antagonists with estimated affinity constants of 7·9 and approximately 8 respectively, values close to those obtained in the present study.

The relaxant responses to 5-methoxytryptamine and sumatriptan, with maximum responses significantly greater than those produced by 5-HT and 5-CT are difficult to interpret. Indeed, these responses were insensitive to high concentrations of methysergide (10 μM), suggesting a separate mechanism of action.

The antagonist data obtained in the present study does not agree well with published data (Costa & Furness 1979; Kojima & Shimo 1986). In the later studies methysergide (up to 2 μM) was without effect, whereas, in the present work, methysergide (0·1–1 μM) clearly antagonised the responses to 5-HT and 5-CT. The reason for this discrepancy is difficult to explain, although the different conditions used may provide some explanations; whereas in this study, relaxations were investigated using preparations with elevated tone, both of the other studies (see above) looked at relaxations of basal tone.

In summary, the present study has identified a neuronal receptor in guinea-pig proximal colon which mediates relaxation mediated by 5-HT, and which has been provisionally characterized as a 5-HT_1-like receptor. This receptor exhibits properties similar to that identified by Kalkman et al (1986) in guinea-pig ileum.

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Increase in susceptibility to *Eco*RII restriction of bacteriophage λ produced by propagation on host cells growing in 5-azacytidine: a new in-vivo method for demonstration of DNA-methylation inhibition

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Abstract—The efficiency of plating on *Eco*RII-restricting cells of bacteriophage λ_{vir} propagated on an *Escherichia coli* K-12 *dcm*$^+$ host decreased with increase in concentration of 5-azacytidine (5-azaC) in the propagating medium. This illustrates, in-vivo, the inhibition of DNA-cytosine methylation induced by 5-azaC and provides a simple system for the detection of DNA-methylation inhibitors.

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5-Azacytidine (5-azaC) is incorporated into nucleic acids of both eukaryotic and prokaryotic cells, producing defective rRNAs and tRNAs, and inhibiting protein synthesis (Cedar & Razin 1990). Uptake into DNA inhibits DNA synthesis and blocks DNA-cytosine methylation (Friedman 1981) by noncompetitive inhibition of DNA methyl transferases (Santi et al 1983, 1984). The chromosomal *dcm* gene of *Escherichia coli* codes for a DNA cytosine methylase that methylates, postreplicationally, the internal cytosine in the sequence 5′-CC(A/T)GG-3′ (May & Hattman 1975a, b). To date no phenotypic abnormality has been attributed to mutation within the *dcm* gene. However, 5-methylcytosine deaminates spontaneously to thymine, which upon replication introduces T. G mispairs into DNA. These are