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The response of rat colonic mucosa to 5-hydroxytryptamine in health and following restraint stress

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Abstract

The present study characterized the rat colonic secretory response to 5-hydroxytryptamine (5-HT) and determined alterations in this response following stress. 5-HT stimulated rat colonic short-circuit current in a concentration-dependent fashion ($pD_2 = 5.19$). This response was subject to desensitization and was mimicked by the indolealkylamines with a rank order potency of 5-HT ≈ α-methyl-5-HT > 5-carboxytryptamine ≈ 5-methoxytryptamine. 2-Methyl-5-HT was a partial agonist. The colonic response to 5-HT was unaltered by methysergide (10 μM), ritanserin (0.1 μM), ondansetron (1 μM) or clozapine (10 μM), but was antagonized by the 5-HT_4 receptor antagonists SB204070 ($pD'_2 = 9.32$), GR113808 ($pK_b = 8.56$), DAU6285 ($pK_b = 6.07$) and SDZ205557 ($pK_b = 6.80$). The response of colonic epithelial and oesophageal tunica muscularis mucosae to 5-HT is therefore mediated by a similar 5-HT_4 receptor. Following wrap restraint stress, the colonic response to 5-HT became bimodal. Half of the preparations were hyper-responsive, while the rest were hypo-responsive to 5-HT. This 5-HT_4 receptor may therefore be involved in stress related changes in fluid transport.

Keywords: Colon; Epithelium; 5-HT (5-hydroxytryptamine, serotonin); Irritable bowel syndrome; Secretion; Stress

1. Introduction

The 5-hydroxytryptamine (5-HT) receptor family is classified into four well-characterized subpopulations, 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (Humphrey et al., 1993). Molecular cloning studies have identified further subtypes of serotonergic receptors, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6 and 5-HT_7, although their functional correlates remain unclear (Smith and Perker, 1995). More recently, two splice forms of 5-HT_4, 5-HT_{4L} and 5-HT_{4S} have been identified in a rat brain cDNA library (Gerald et al., 1995).

The importance of the 5-HT_4 receptor in the pharmacological treatment of a variety of pathological conditions including altered intestinal motility, functional bowel disorder, emesis and diarrhoea is now accepted (Gaster and Sanger, 1994) and the development of pharmacological tools to study this receptor is expanding rapidly. 5-HT_4 receptors are sensitive to the indolealkylamines 5-HT, α-methyl-5-HT, 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine, and to a much lesser extent 2-methyl-5-HT (Reeves et al., 1991) and to substituted benzamides such as R-Zacopride (Baxter et al., 1991). Up until recently, agonist sensitivity and tropisetron antagonism were the principal tools used to identify 5-HT_4 receptors; however, the interpretation of results was complicated by the fact that these tools display poor selectivity. More recently, the selective 5-HT_4 receptor antagonists, GR113808 (Gale et al., 1994), SB204070 (Wardle et al., 1994), DAU6285 (Waikar et al., 1993) and SDZ205557 (Wardle and Sanger, 1993), have been developed. Two classic assay systems exist for 5-HT_4 receptors in the alimentary tract, namely, 5-HT relaxation of precontracted rat oesophageal tunica muscularis mucosae (Baxter et al., 1991; Reeves et al., 1991) and 5-HT contraction of guinea pig colonic longitudinal muscle (Wardle and Sanger, 1993). The 5-HT-mediated contraction of the guinea pig distal colon and relaxation of the rat oesophagus are antagonized by tropisetron ($pA_2 = 6.4$ and 6.0), GR113808 ($pA_2 = 9.2$ and 9.3) and SB 204070 ($pA_2 = 10.8$ and 10.5) (Wardle and Sanger, 1993; Reeves et al., 1991; Gale et al., 1994; Wardle et al., 1994; Hegde et al., 1995).

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In addition to being an important modulator of intestinal smooth muscle tone, 5-HT also stimulates short-circuit currents across the intestinal epithelium of a number of species. Specifically, in humans, a non-neuronal 5-HT_4 receptor population mediates the secretory response of the jejunum, ileum and proximal colon, while non-neuronal 5-HT_2 receptor activation stimulates the secretory response of proximal and distal colonic mucosa (Budhoo et al., 1996a; Burleigh and Borman, 1993; Borman and Burleigh, 1996). Thus, antagonists of either of these receptor subtypes may be useful in treating diseases associated with hypersecretion. In order to develop such a therapeutic strategy, the guinea pig and rat intestinal epithelium have both been studied as experimental models. In the guinea pig ileum, 5-HT stimulated short-circuit currents through both epithelial and neuronal 5-HT_4 receptors, and neuronal 5-HT_3 receptors (Leung et al., 1995). In contrast, in the guinea pig colon, the response to 5-HT is entirely neural and consists of 5-HT_3 excitatory and 5-HT_4 inhibitory responses (Cooke et al., 1991; Frieling et al., 1991). The rat colon has not been studied in detail, but when it is stripped of its musculature and myenteric plexus, non-neuronal 5-HT_4 receptors appear to play a major role (Bunce et al., 1991; Budhoo et al., 1996a), which makes the rat colon a useful experimental model for the development of anti-secretory 5-HT_4 receptor antagonists. However, confirmation of this point, by using specific pharmacological tools, is required.

Although Budhoo et al. (1996a) have demonstrated an antagonism of rat colonic secretion by SC53606, a 5-HT_4 receptor antagonist, now classic 5-HT_4 receptor antagonists, have not been studied in this tissue. Thus, one aim of the present study was to further characterize 5-HT_4-mediated colonic epithelial ion transport. This was done by confirming previously reported agonist sensitivity and desensitization (Bunce et al., 1991; Budhoo et al., 1996a) and by studying the sensitivity of the colonic epithelium to the new 5-HT_4-selective receptor antagonists, SB204070, GR113808, DAU6285 and SDZ205557. The activity of these selective antagonists in the colonic epithelium was then compared with their activity in a classic 5-HT_4 model, the rat oesophageal tunica muscularis mucosae, to determine whether the two tissues express similar receptors. Secondly, although 5-HT_4 receptor antagonists are under clinical development for a variety of conditions (see, for example, Gaster and Sanger, 1994), little is known about pathophysiological alterations of 5-HT-related epithelial secretion. Stress may be a contributory factor to pathologies including functional bowel disorder (for review, see Maxwell et al., 1997) and has been shown to induce changes in 5-HT receptor pharmacology in the rat brain (Okuyama et al., 1995). Moreover, stress has been found to alter intestinal fluid transport in humans (Barclay and Turnberg, 1987). Consequently, a second aim of the present study was to determine whether stress altered 5-HT induced secretion. The wrap restraint stress model (Williams et al., 1988) used was modified according to Okuyama et al. (1995), who extended the period of stress from 35 min to 3 days, at which time point changes in central 5-HT receptor function can be seen in vitro.

2. Materials and Methods

2.1. Animals

Male albino rats (300–500 g) were used and were allowed free access to standard rat chow and water throughout this study. In contractility studies, Sprague–Dawley rats (IFFA-CREDO, France) were bled to death following a stunning blow to the head. For epithelial transport studies, Wistar rats (CRF, France) were anaesthetized with sodium pentobarbital (75 mM/kg i.p.) and tissue was removed prior to euthanasia with an overdose of pentobarbital.

2.2. Oesophageal tunica muscularis mucosae preparation

The tunica muscularis mucosae preparation used in the present study has been previously characterized (Baxter et al., 1991). The distal 2 cm of intrathoracic oesophagus was removed and the outer muscle layers were carefully dissected by sharp dissection. The remaining muscularis mucosae/mucosal preparation was mounted in a 20-ml organ bath along its longitudinal axis: one end was attached to an isometric tension transducer (Model K30; Hugo Sachs Elektronic, Germany) connected to a Gould amplifier and the other end was anchored to the base of the bath. The tissue was bathed in Krebs buffer (32 ± 1°C) and continuously gassed with 95%O_2/5%CO_2. An initial resting tension of 0.5 g was applied to the preparation and this was readjusted during the initial 30-min equilibration period, during which the preparation was washed every 10 min. After the 30-min equilibration period, preparations were precontracted with submaximal concentrations of carbachol (0.5 μM), and 15 min later 5-HT was added in a cumulative fashion. To determine the 5-HT receptor subtype that mediates this response, a number of specific antagonists were used. For these experiments, after the initial response to 5-HT had been determined, the tissue was washed and re-equilibrated in the presence of the 5-HT receptor antagonist prior to the re-administration of carbachol and the recording of a second 5-HT response curve. Preliminary studies showed that two consecutive 5-HT concentration–response curves yielded similar maximal responses and EC_{50} values, and that resting tone remained constant throughout the experimental period.

2.3. Colonic epithelial preparation

The colonic epithelial preparation has been described previously (Bunce et al., 1991). Briefly, the distal 5–10 cm

of colon was removed, rinsed in cold Krebs and placed over a glass rod (6 mm diameter). The outer muscle layer was scored along its anti-mesenteric surface, using a dull scalpel blade, and peeled away from the mucosa by using a piece of gauze previously dampened with Krebs buffer. The preparation was opened along its anti-mesenteric surface, and the resultant epithelial preparation was mounted as a flat sheet between two Ussing chambers (exposed area 1.24 cm^2). Two preparations were mounted from each animal, with one preparation always acting as a control. Bunce et al. (1991) have previously shown that adjacent epithelial preparations respond identically to 5-HT. This protocol was chosen, rather than constructing successive response curves in individual preparations, since, as previously reported (Bunce et al., 1991), the colonic epithelium is subject to desensitization to 5-HT. Both the mucosal and serosal surfaces were perfused with 4 ml Krebs buffer using a gas-lift ($95\%O_2/5\%CO_2$; pre-humidified by bubbling through distilled water), and maintained at $37 \pm 1°C$. The short-circuit current generated by the epithelium was continuously monitored using an EVC4000 voltage clamp (WPI, USA). To this end, one voltage detecting electrode and one current passing electrode were inserted into each half chamber, and the electrodes were connected to the EVC4000 via a preamplifier.

The voltage generated by the epithelium was continuously short-circuited by passing current across the tissue, using the current passing electrodes. The short-circuit current is expressed as $\mu A/1.24\ cm^2$. In a control tissue, following a 30-min equilibration period, 5-HT was added in a cumulative fashion to the serosal solution. In a paired tissue, a cumulative concentration–response curve was recorded for either 5-HT following a 30-min equilibration in the presence of an antagonist or for one of the indolealkylamines. In certain experiments, concentration–response curves were also made for acetylcholine.

2.4. Stress induction

Animals were restrained by immobilizing their forepaws using adhesive tape (Williams et al., 1988). This procedure was performed over a 2-h period, starting at 0930, on three consecutive days. After the final period of stress, animals were anaesthetized and the most distal 10 cm of colon was removed and epithelial sheets were then prepared as described above.

2.5. Data handling

Data were continuously collected by using JAD (Notocord, France) or Scope (AD Instruments, Australia) acquisition packages for contractile and short-circuit current studies, respectively. These automatically determined agonist responses in grams of tension or short-circuit current in μA. Data are expressed as a percentage of the maximum control response to 5-HT or as absolute values as appropriate, plotted and pD_2 and E_{max} values were calculated. E_{max} values are expressed as a percentage of control E_{max} values. Variations in the E_{max} were assessed statistically, using a one-sample *t*-test with $P < 0.05$ being considered significant. To compare agonist potencies, the equipotent molar ratio was calculated by dividing the EC_{50} for each of the agonist response curves by the EC_{50} for 5-HT in its paired tissue. To determine antagonist effect, it was first determined whether the E_{max} was significantly reduced as described above, and if this was the case, the pD'_2 value ($-\log$ of the antagonist concentration required to reduce the E_{max} by 50%) was calculated using the method of Van Rossum (1963). In cases where an antagonist failed to alter the E_{max}, individual curves were fitted to a sigmoid curve and the dose ratio (DR) for the response to 5-HT was calculated. Where the DR was significantly ($P < 0.05$) greater than 2, as determined using a one-sample *t*-test, pK_b values were then calculated as:

$$pK_b = -\log([\text{antagonist}]/DR - 1).$$

Data are described as mean values ± standard error of the mean for the number of tissue preparations indicated. Only one tissue preparation per animal was used for any one set of experimental conditions.

2.6. Drugs and solutions

The following antagonists, which were synthesized by the chemistry department of Synthélabo Recherche, were used: methysergide (10 μM), which antagonizes 5-HT_{1A-F}, 5-HT_{2A-C}, 5-HT_{5A-B} and 5-HT_7 receptor binding or activity (Briejer et al., 1995; Matthes et al., 1993; Carter et al., 1995); the 5-HT_2 receptor antagonist ritanserin (0.1 μM; Briejer et al., 1995); the 5-HT_3 receptor antagonist ondansetron (1 μM; Butler et al., 1988); the 5-HT_4 receptor antagonists GR113808 (10 nM; Gale et al., 1994), SB204070 (0.1–10 nM; Wardle et al., 1994), DAU6285 (Waikar et al., 1993) and SDZ205557 (Wardle and Sanger, 1993); the mixed 5-HT_3/5-HT_4 receptor antagonist tropisetron (0.1–1 μM; Craig et al., 1990) and clozapine, which at the concentration used (1 μM) antagonizes 5-HT_6 receptor binding (Schoeffter and Waeber, 1994). In addition, 5-HT (creatine sulphate complex; Sigma), 5-CT maleate (RBI, USA), 5-methoxytryptamine hydrochloride (Sigma), α-methyl-5-HT maleate (RBI) and 2-methyl-5-HT maleate (RBI) were used as 5-HT agonists. Acetylcholine chloride was purchased from Sigma.

The Krebs solution used for both contractile and epithelial transport studies was of the following composition (mM): NaCl, 118; KCl, 4.70; $MgSO_4 \cdot 7H_2O$, 1.64; KH_2PO_4, 1.18; glucose, 11.5; $NaHCO_3$, 24.9; $CaCl_2 \cdot 2H_2O$, 2.52. All drugs were dissolved and diluted in distilled water on a daily basis, with the exception of ritanserin, which was dissolved in 0.1% tartaric acid, and

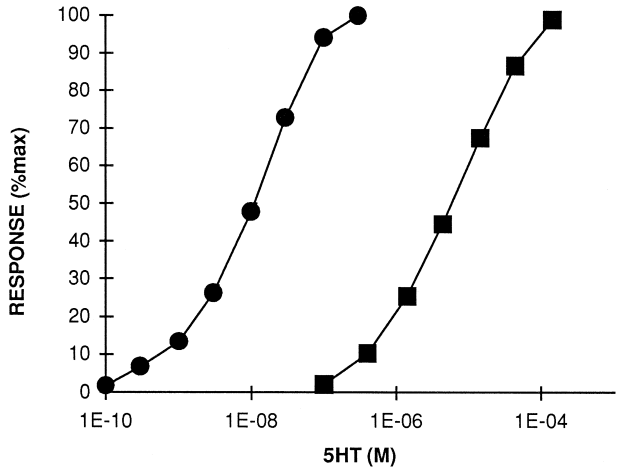

Fig. 1. A comparison of the response of oesophageal muscularis mucosae (●; $n = 17$) and colonic epithelium (■; $n = 73$) to 5-HT. Data represent the pooled results of all control responses reported in the characterization of the 5-HT response in non-stressed tissue.

GR113808, which was reacted with 40 μl HCl (1 M) prior to being diluted in distilled water to facilitate solution.

3. Results

3.1. Agonist responsiveness

5-HT relaxed the carbachol-precontracted oesophageal tunica muscularis mucosae and stimulated the colonic short-circuit current in a concentration-dependent fashion (Fig. 1). There were, however, differences in the pharmacology of these responses. First, after 30 min, the oesophageal tunica muscularis mucosae displayed no desensitization to a subsequent addition of 5-HT ($104 \pm 5\%$ of the initial curve). This contrasted with the colonic epithelium, because if two curves were constructed 30 min apart, the E_{max} of the second was significantly reduced to $52 \pm 7\%$ of the initial curve. Second, the colonic epithelium was much less sensitive than oesophageal tunica muscularis mucosae to 5-HT (Fig. 1): pooled pD_2 values were calculated to be 5.19 ± 0.05 ($n = 62$) and 7.89 ± 0.04 ($n = 17$), respectively. In addition to 5-HT, the colonic epithelium was also sensitive to the indolealkylamines with DRs of 5-HT ≈ α-methyl-5-HT (2.38 ± 0.78; $n = 3$) < 5-CT (14.08 ± 3.14; $n = 7$) ≈ 5-methoxytryptamine (15.21 ± 10.21; $n = 4$). 2-Methyl-5-HT stimulated the short-circuit current; however the maximal response was significantly less than that of 5-HT ($38.08 \pm 5.4\%$ of E_{max}; $n = 3$).

3.2. 5-HT$_4$ receptor antagonist sensitivity

As previously demonstrated, GR113808 antagonized the contractile response of oesophageal smooth muscle to 5-HT. Although the E_{max} was significantly reduced (Fig. 2a), this effect was minor and not concentration-dependent (data not shown) and a pK_b of 9.64 ± 0.06 was thus calculated. SB 204070 dose dependently (0.1–10 nM; Fig. 3a) reduced the E_{max} of the contractile response, yielding a pD'_2 of 9.01. These data confirm that the oesophageal tunica muscularis mucosae is a good model to study 5-HT$_4$ receptors. The sensitivity of the colonic epithelium to 5-HT receptor antagonists was then examined and compared to that of oesophageal tunica muscularis mucosae. Like the oesophageal tunica muscularis mucosae, the colonic epithelial response to 5-HT was antagonized by GR113808 (Fig. 2b) and in a non-surmountable fashion by SB204070 (0.1–10 nM; Fig. 3b). The pK_b and pD'_2 values were 8.56 ± 0.16 and 9.32, respectively. Furthermore, the colonic epithelium was found to be sensitive to

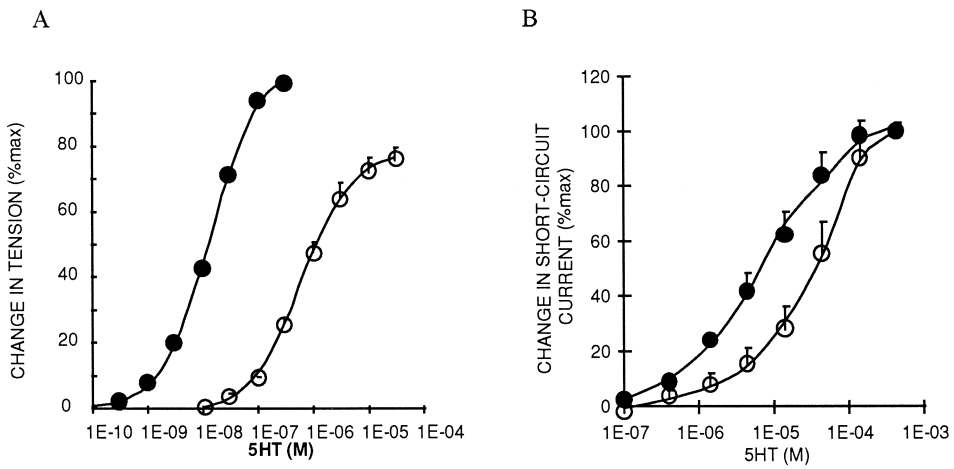

Fig. 2. Effect of 10 nM GR113808 (○; $n = 4$) on the response of (A) the oesophageal muscularis mucosae and (B) the colonic epithelium to 5-HT. Each response is compared to a paired control response (●; $n = 4$).

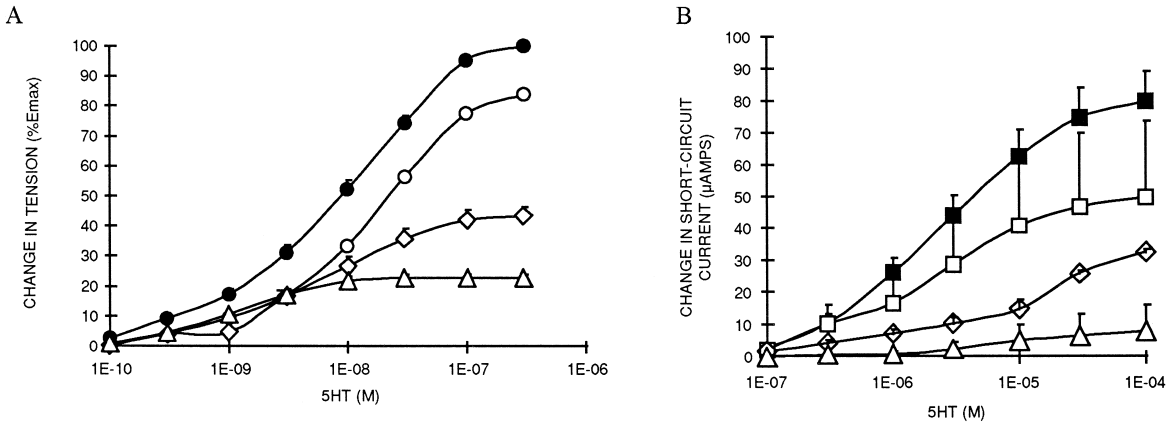

Fig. 3. (A) Effect of 0.1 nM (○; $n = 2$), 1.0 nM (◇; $n = 4$) and 10 nM (△; $n = 4$) SB204070 on the response of the oesophageal muscularis mucosae to 5-HT. Each response is compared to a control response elicited in the same tissue 30 min earlier. Data for control responses were pooled (●; $n = 10$). (B) Effect of 0.1 nM (□; $n = 4$), 1.0 nM (◇; $n = 4$) and 10 nM (△; $n = 2$) SB204070 on the response of the colonic epithelium to 5-HT. Each response is compared to a control response elicited in a paired segment of tissue. Control data were pooled (■; $n = 10$).

the 5-HT_4 receptor antagonists, DAU 6285 and SDZ205557, with pK_b values calculated as 6.07 ± 0.62 and 6.80 ± 0.42, respectively (Fig. 4).

The response of the colonic epithelium to 5-HT was insensitive to a range of other 5-HT receptor antagonists. These included methysergide (10 μM; DR = 0.59 ± 0.29; $n = 4$), ritanserin (0.1 μM; DR = 1.74 ± 0.72; $n = 4$), ondansetron (1 μM; DR = 1.59 ± 0.6; $n = 4$) and clozapine (10 μM; DR = 0.50 ± 0.07; $n = 3$). Likewise, none of these antagonists significantly altered the maximal response to 5-HT (136 ± 51, 104 ± 17, 126 ± 33 and $120 \pm$ 38% of control E_{max} values, respectively).

3.3. Effect of chronic stress on colonic response to 5-HT

Chronic stress had a dramatic effect on the E_{max} of the response of the colonic epithelium to 5-HT, with some tissues displaying a reduced response and others an increased response. To quantify this bimodality, a cut-off point of 60 μA was chosen based on the mean E_{max} value determined in a preliminary study of tissue taken from control animals. On this basis, compared to control tissue ($E_{max} = 44 \pm 6$ μA; $n = 7$), 53% of tissues responded with a significantly lower E_{max} (17 ± 1 μA; $n = 8$) and 47% responded with a significantly higher E_{max} (121 ± 18

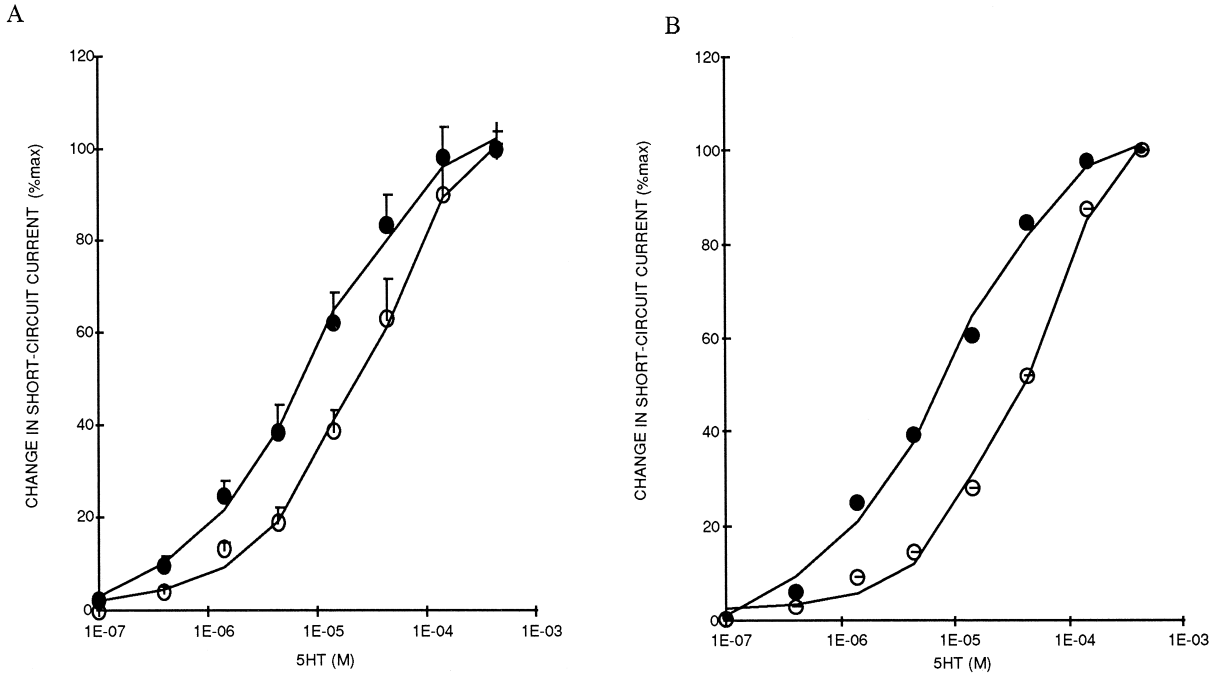

Fig. 4. Response of the colonic epithelium to 5-HT in paired segments of tissue ($n = 4$) in the presence (○) or absence of (A) DAU6285 (●; 1 μM) or (B) SDZ20555 (●; 1 μM).

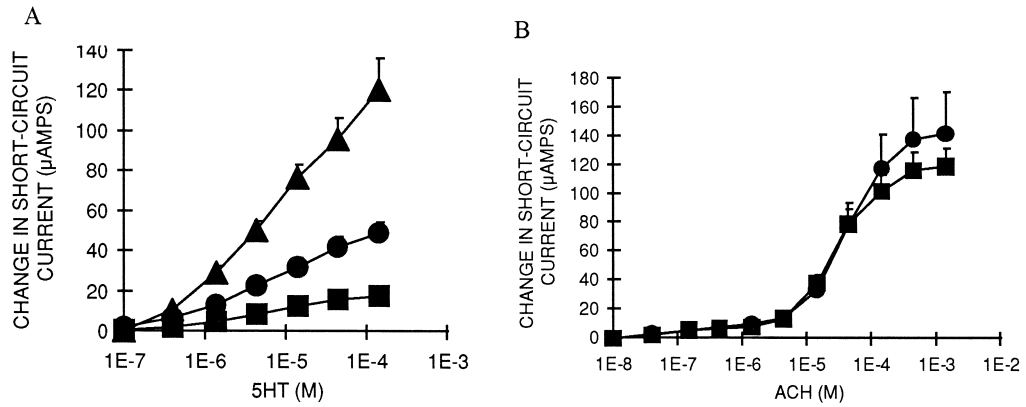

Fig. 5. Effect of chronic stress on (A) the response to 5-HT. The response of hyper- (▲; $n = 7$) and hypo-responsive (■; $n = 8$) tissue taken from animals stressed by forelimb immobilization over a period of 2 h on 3 successive days was compared to that of unstressed controls (●; $n = 6$); and (B) the response to acetylcholine. Tissue taken from animals stressed by forelimb immobilization over a period of 2 h on 3 successive days (■; $n = 11$) and was compared to tissue from unstressed controls (●; $n = 8$).

μA; $n = 7$) (Fig. 5a). If the control population was treated in a similar fashion using the same cut-off point (60 μA), two significantly different groups could not be identified. In contrast, the response to acetylcholine was unaltered (142 ± 29 vs. 119 ± 12 μA in control and stressed tissue, respectively; $n \geq 8$; Fig. 5b) and appeared homogeneous.

4. Discussion

The oesophageal tunica muscularis mucosae is considered a classic model for 5-HT_4 receptors and, like the guinea pig colonic longitudinal muscle, has been shown to be sensitive to antagonism by the 5-HT_4 receptor antagonists, GR113808 and SB 204070 (Gale et al., 1994; Wardle et al., 1994). The potencies of these antagonists in the oesophageal tunica muscularis mucosae preparation in the present study were similar to those previously reported. The present study is the first to show that these and other well-characterized and specific 5-HT_4 receptor antagonists are active in both the colonic epithelium and the oesophageal tunica muscularis mucosae. Furthermore we have shown that the colonic epithelial response to 5-HT is altered following stress.

As previously reported, the oesophageal tunica muscularis mucosae is sensitive to a range of 5-HT_4 receptor antagonists including SB204070, GR113808, DAU6285 and SDZ205557 (Gale et al., 1994; Hegde et al., 1995; Waikar et al., 1993; Wardle and Sanger, 1993), but insensitive to both methysergide and ondansetron (Reeves et al., 1991). Methysergide is a broad-spectrum antagonist, which at the concentration used antagonizes, 5-HT_{1A-F}, 5-HT_{2A-C}, 5-HT_{5A-B} and 5-HT_7 receptor binding or activity (Briejer et al., 1995; Matthes et al., 1993; Carter et al., 1995), while ondansetron is a specific 5-HT_3 receptor antagonist (Butler et al., 1988). Similarly, the neurotoxin, tetrodotoxin, had no effect on the response to 5-HT (Reeves et al., 1991). The sensitivity to tetrodotoxin and selectivity towards 5-HT_4 receptor antagonists suggests that there is a single population of non-neuronal 5-HT_4 oesophageal tunica muscularis mucosae receptors. Likewise, the colonic epithelial response is insensitive to tetrodotoxin (Bunce et al., 1991), and we, like others (Bunce et al., 1991), showed that the colonic epithelial response to 5-HT was unaffected by methysergide and ondansetron. Furthermore, we showed that this response was insensitive to ritanserin, confirming the lack of involvement of 5-HT_2 receptors, and to clozapine, which is one of the more active antagonists at the 5-HT_6 receptor (Schoeffter and Waeber, 1994). In contrast, the colonic epithelial response was antagonized by the 5-HT_4 receptor antagonists SB204070 and GR113808, and thus, like the oesophageal tunica muscularis mucosae, the rat colonic epithelium possesses a relatively pure population of non-neuronal 5-HT_4 receptors. Likewise, as previously shown for the tunica muscularis mucosae (Waikar et al., 1993; Wardle and Sanger, 1993), the colonic epithelium was also sensitive to DAU6285 and SDZ205557. Thus, on the basis of antagonist sensitivity, the colonic epithelium contains a 5-HT_4 receptor similar to that reported in the oesophageal tunica muscularis mucosae preparation. This conclusion is in agreement with those made by Bunce et al. (1991) and Budhoo et al. (1996a), who used the non-selective 5-HT_4 receptor antagonist tropisetron and the relatively uncharacterized 5-HT_4 receptor antagonist SC53606, respectively. It should be noted that Sprague–Dawley and Wistar rats were used for contractile and secretory studies, respectively. Species differences are unlikely to alter our conclusions, however, because Sprague–Dawley and Wistar rats show similar sensitivity to 5-HT_4 agonists and receptor antagonists (Gale et al., 1994). This suggests that this preparation may be of use in the study of 5-HT_4 receptors and in the development of specific antagonists.

The 5-HT_4 receptor in the rat oesophageal tunica muscularis mucosae and in the guinea pig colonic circular muscle is extremely sensitive to 5-HT, displaying pD_2

values of 8 and 9.2 (Wardle and Sanger, 1993), respectively. In contrast, we show, in agreement with Bunce et al. (1991), that the colonic epithelium is less responsive to 5-HT, with a pD_2 value of around 5. A number of explanations could account for this difference, including multiple 5-HT_4 receptor subtypes, differences in efficiency of coupling to the second messenger system, or coupling to different second messenger systems. The third possibility is unlikely, however, because cAMP has been shown to be involved in both the oesophageal tunica muscularis mucosae and the colonic epithelium (Reeves et al., 1991; Budhoo et al., 1996a). Furthermore, on the basis of the antagonist sensitivities described above, it is unlikely that different receptor subtypes exist in the oesophageal tunica muscularis mucosae and colonic epithelial preparation. Thus, it is most likely that differences occur at the level of receptor coupling. Like 5-HT, a variety of indolealkylamines were found to stimulate the short-circuit current. The rank order of potency (5-HT ≈ α-methyl-5-HT < 5-CT ≈ 5-methoxytryptamine) was similar to that described for the tunica muscularis mucosae 5-HT_4 receptor (Reeves et al., 1991). Most importantly, 5-methoxytryptamine, which is inactive at the 5-HT_3 receptor, stimulated the short-circuit current while the 5-HT_3 agonist 2-methyl-5-HT was much less effective. Thus, in support of antagonist data, the rank order of potency of agonists confirms that 5-HT stimulation of the colonic short-circuit current is unlikely to be mediated by 5-HT_3 receptors, and instead implies the involvement of a 5-HT_4 receptor population. Like 5-HT, these agonists were found to be much less potent in the epithelium than in the oesophageal tunica muscularis mucosae, once again suggesting that there are differences in receptor coupling.

In addition to variations in agonist sensitivity, we have shown differences in the susceptibility of the oesophageal tunica muscularis mucosae and colonic epithelium to 5-HT desensitization. The oesophageal tunica muscularis mucosae rapidly desensitizes to 5-HT (Ronde et al., 1995); however, following a 30-min washout, the response of this preparation is fully restored (Baxter et al., 1991). Likewise, in the present study, we demonstrated that if there was a 30-min interval between successive 5-HT response curves, the oesophageal tunica muscularis mucosae showed no evidence of desensitization. This contrasts with the colonic epithelium, which exhibited a prolonged and profound desensitization, since even 30 min after a prior challenge with 5-HT, responsiveness was diminished. Further studies are required to ascertain whether this represents homologous desensitization. If this is the case, it may result from differences in coupling of the 5-HT_4 receptor to adenylate cyclase. Alternatively, the biochemical pathways responsible for desensitization may differ between the preparations studied.

Having characterized the colonic epithelial 5-HT_4 receptor, we attempted to determine its pathophysiological significance. Stress has been shown to induce hypersecretion in human ileum (Barclay and Turnberg, 1987). In the present study, we showed that the in vitro response of the colonic epithelium to 5-HT was altered by prior whole-animal stress, with a bimodal effect being detected. Tissue from approximately 50% of animals displayed a reduced responsiveness to 5-HT, which is a phenomenon indicative of a reduction in receptor number, desensitization of the receptor or the induction of inhibitory pathways. Further studies are required to distinguish between these possibilities. Tissue from a similar number of animals was hyperresponsive to 5-HT, and this may be due to upregulation of the second messenger system or to an increase in receptor expression. Once again, further studies are required to explore these possibilities. It is unclear whether these effects were immediate after-effects of the final period of stress, or whether they were due to the chronic nature of the stress. Furthermore, studies are required to determine whether the observations reported in the present study are representative of changes throughout the alimentary tract. In contrast to the changes in the response to 5-HT, acetylcholine-induced ion transport was unaltered by stress. This suggests that the secretory responsiveness of the epithelium does not undergo a general change; however studies with other secretagogues are awaited to ascertain the specificity of the phenomenon reported in the present study.

The secretory activity of 5-HT is well-accepted (Franks et al., 1995), and it has been shown that the human colonic epithelium responds to 5-HT (Borman and Burleigh, 1996). Reduced responsiveness to 5-HT could contribute to constipation, while increased responsiveness could contribute to diarrhoea. In extrapolating our data to the human, it should be remembered that the receptor pharmacology of the human and rat colonic epithelium differs. Although 5-HT_4 receptors mediate the response to 5-HT in the human jejunum and proximal colon (Budhoo et al., 1996b; Borman and Burleigh, 1996), in the distal colon, the response is mediated by 5-HT_2 receptors (Borman and Burleigh, 1996). Thus, our data could explain changes in stress-related secretion if the changes reported in the present study are also seen in the human jejunum or proximal colon, or if changes similar to those seen in 5-HT_4 pharmacology in the present study are seen for the 5-HT_2 subtype in the human colon. With respect to the second point, it is of interest that stress is proposed to enhance the response to 5-HT_2 stimulation in rat brain (Okuyama et al., 1995). In order to investigate either of these possibilities, it would be necessary to conduct ion transport studies on mucosal biopsies taken from chronically stressed patients.

Irrespective of the role of 5-HT_4 receptors in disease states, we have confirmed that they are involved in the control of colonic ion transport. We arrived at this conclusion through the use of specific and well-characterized antagonists, and by determining the rank order of potency of a number of agonists. This therefore extends and confirms earlier studies that did not have the benefit of specific pharmacological tools. Furthermore, our data de-

scribe for the first time the anti-secretory potency of a number of 5-HT_4 receptor antagonists in clinical development. Finally, we have shown that the colonic response to 5-HT is altered in an animal model of stress, which may contribute to our understanding of stress-related hypersecretion in humans. 5-HT_4 receptor antagonists are in clinical development for functional bowel syndrome, and if the diarrhoea experienced by these patients is in part stress-related (Maxwell et al., 1997), the anti-secretory properties of these compounds may be an important factor in determining their use.

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