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Adrenergic responses of rat colonic muscularis mucosae

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Abstract

We have investigated adrenoceptor-mediated responses of muscularis mucosae from the proximal, mid and distal regions of the rat colon. Noradrenaline-induced relaxations of the muscularis mucosae in each region were unaffected by atenolol, butoxamine or propranolol, but they were attenuated by the selective β_3-adrenoceptor antagonist cyanopindolol. The β_3-adrenoceptor agonist CL216343 elicited concentration-dependent relaxation of the muscularis mucosae in all regions of the colon. Isoprenaline, a non-selective β-adrenoceptor agonist, evoked concentration-dependent relaxations of the muscularis mucosae in all regions, but only in the proximal colon were these significantly larger than the maximum noradrenaline-induced relaxation. The α_1-adrenoceptor agonist methoxamine caused large contractions of the proximal colonic muscularis mucosae. When proximal tissue was pretreated with phentolamine, an α_1-adrenoceptor antagonist, maximal noradrenaline- and isoprenaline-induced relaxations did not differ significantly. Although the mid colonic muscularis mucosae was also found to possess excitatory α_1-adrenoceptors, these were associated with small contractions and did not modify the muscle's inhibitory responses to noradrenaline. Distal colonic muscularis mucosae lacked excitatory adrenoceptors and only responded to noradrenaline with β_3-adrenoceptor-mediated relaxations. No evidence was obtained for functional α_2-adrenoceptors on the muscularis mucosae in any region of the rat colon. These data demonstrated that noradrenaline-induced relaxation of the rat colonic muscularis mucosae was mediated via β_3-adrenoceptors throughout, but in the proximal region this was modified by concurrent excitatory α_1-adrenoceptor activation. Based upon these observations it appeared unlikely that noradrenaline-induced relaxation of rat colonic muscularis mucosae would be functionally linked to the secretory responses of the corresponding mucosa during periods of increased sympathetic activity.

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Introduction

It is well established that both the longitudinal and circular muscle layers of the rat colon receive functional extrinsic sympathetic innervation that, in a region- and muscle layer-specific manner, can elicit excitation, inhibition or a combination of both, in these tissues (Luckensmeyer & Keast 1998). Sympathetic nerve-induced excitation in the longitudinal and circular muscle layers is believed to be mediated via α_1-adrenoceptors in all regions of the colon. This is because it is phentolamine-sensitive (Luckensmeyer & Keast 1998) and is not mimicked in-vitro by the selective α_2-adrenoceptor agonist clonidine (Umezawa et al 2003). Conversely, muscle relaxation induced by sympathetic nerve stimulation is mediated by both propranolol-sensitive and -insensitive β-adrenoceptors (Luckensmeyer & Keast 1998). The latter were initially labelled 'atypical' (Bianchetti & Manara 1990), but are now believed to be the implied β_3-subtype, as characterized in multiple intestinal smooth muscles by both conventional pharmacological (McLaughlin & MacDonald 1990; MacDonald & Lamont 1993; Kelly & Houston 1996) and molecular techniques (Evans et al 1996; Roberts et al 1999). Thus, while the sympathetic innervation of the rat colonic muscularis propria is well understood and the mechanisms by which it exerts its influence delineated, adrenoceptor-mediated responses of the muscularis mucosae in this organ remain to be characterized.

Histochemical evidence has suggested that within the rat gut the muscularis mucosae is innervated by noradrenaline-containing neurons (Wong 1977) and, in its oesophagus, pharmacological studies have revealed the presence of inhibitory β_2- and β_3-adrenoceptors on this muscle layer (deBoer et al 1993; Kelly & Houston 1996). The functional significance of such observations has yet to be elucidated, but there is evidence that the motor activity of

the muscularis mucosae is linked to epithelial secretion in the rat colon because the epithelium and lamina propria undergo significant physical changes when exposed to secretagogues such as bradykinin (Baron et al 1986). Further, adrenoceptor-mediated mucosal secretion in rat proximal and distal colon occurs in phases that are indometacin- or indometacin- and tetrodotoxin-sensitive (Schultheiss & Deiner 2000). In the rabbit colon, eicosanoids that elicit neurally-mediated epithelial stimulation are generated as a result of muscularis mucosae contraction (Percy et al 2003).

Based upon this background, therefore, the aims of this in-vitro study were to determine the types of adrenoceptors present on the muscularis mucosae of the rat colon, to assess the extent to which these varied along the length of this organ, and to use this knowledge to predict the potential contribution that these adrenoceptors may make to the integrated functioning of this muscle layer and the mucosa to which it is attached.

Materials and Methods

The experiments reported herein were performed in accordance with the principles described in the 'Guide for the Care and Use of Laboratory Animals', Publication No. DHHS (NIH) 86-23.

Male Sprague–Dawley rats (250–350 g) were killed with pentobarbitone (100 mg kg^{-1}, i.p.). Following laparotomy the distal 3–4 cm of colon immediately proximal to the pelvic brim was located and removed. Similarly, a 3–4 cm segment of mid colon (defined for the purposes of this study as being a region located mid way between the proximal and distal regions) was excised. A further 3–4 cm of proximal colon approximately 1 cm from the colo-caecal junction was also taken. Each segment was opened along its mesenteric border and rinsed in warm Krebs solution to remove residual faecal material. Preparations were then pinned out, mucosal surface down, in a Sylgard (Dow Corning, Midland, MI)-coated 7-inch Petri dish in oxygenated Krebs solution. Full thickness segments (3 cm × 3 mm) in the longitudinal axis were excised. The muscularis propria of each strip was separated from the mucosa/muscularis mucosae/submucosa by sharp dissection.

Strips of muscularis mucosae with mucosa attached were prepared using the 'sutured edge' technique originally described by Percy & Christensen (1986). Briefly, strips of mucosa, muscularis mucosae and submucosa 3 cm in length were tied in the middle with 5-0 surgical thread and folded, mucosal surfaces inwards, so that they were half their original length. The oral and aboral ends, now side by side, were tied together to form a loop. Since curling of the tissue tends to expose the mucosal surface rather than the submucosal aspect, the vertical edges of the preparation were sutured at four points with 7-0 surgical thread. This held the preparation flat and ensured that the submucosa and muscularis mucosae were fully exposed to the bathing medium, but did not compromise its ability to contract.

Strips prepared in this way were mounted in 2-mL organ baths at 37 ± 0.5°C and one end of the tissue was connected to a stationary mounting point on the bottom of the bath. The other end was connected to a Grass FTO3D force-displacement transducer under a tension equivalent to a 1.0 g load (9.8 mN). The resulting isometric responses were recorded on a Grass Model 7D polygraph. Tissues were allowed to equilibrate for 30 min under these conditions and during this time the bathing medium was replaced at 10-min intervals.

Measurement of resting tone

To assess the possible contribution of regional differences in resting tone (passive tension) to subsequent drug-induced relaxation responses, this parameter was measured at the end of 30-min equilibration. At this time muscular accommodation to the initial stretch elicited by the application of the 1.0 g load was complete.

Responses to pharmacological agents

Following 30-min equilibration the viability of each tissue preparation was assessed by constructing an initial concentration–response curve to acetylcholine (10^{-9}–10^{-3} M). All contractile responses were expressed as a percentage of each tissue's maximum response to acetylcholine; when inhibition was being measured responses were expressed in terms of each tissue's maximum relaxation to sodium nitroprusside (10^{-4} M). The maximum contractile response to acetylcholine was denoted as +100% and the maximum relaxation to sodium nitroprusside as −100%. As responses to higher concentrations of adrenoceptor agonists tended to persist and subsequent responses were then often highly variable, antagonist studies were performed on paired tissues from the same animal where one served as a control and the other was pretreated with an antagonist under investigation. In the case of the β_3-adrenoceptor agonist CL 316243, where tachyphylaxis occurred at even the lowest concentrations, six muscularis mucosae preparations were prepared, two from each of the three colonic regions. Each was exposed to only one of the six concentrations of agent under study. In this way each preparation contributed a single data point on the CL 316243 concentration–response curve. All other concentration–response curves were constructed in a non-cumulative manner, where drugs were washed from the bathing medium and time was allowed for full recovery before further drug additions occurred. At least 30 min was allowed to elapse between the additions of different drugs and these were administered in amounts not exceeding 1% of the total bath volume. Not all pharmacological agents were applied to each individual tissue. The concentrations of agonists and antagonists used in this study were based upon those found to be effective in our previous muscularis mucosae studies (Percy et al 1992, 2002).

Drugs and solutions

All experiments were performed in a Krebs' solution of the following composition (mM): NaCl 118.5, KCl 4.75, $CaCl_2$ 2.54, NaH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25, glucose 11, gassed with a mixture of 95% O_2 and 5% CO_2.

Acetylcholine chloride (Sigma, St Louis, MO) was dissolved in a 5% NaH_2PO_4 solution and serially diluted with Krebs solution taken to pH 4.0 by the addition of 0.1 M HCl.

Atenolol hydrochloride, butoxamine hydrochloride, clonidine hydrochloride, CL 316243 and sodium nitroprusside (all Sigma, St Louis, MO), were dissolved in, and serially diluted with, a modified Krebs' solution of the following composition (mM): NaCl 143, KCl 4.75 and $CaCl_2$ 2.54. Noradrenaline hydrochloride and propranolol hydrochloride (Sigma) were dissolved in, and diluted with, modified Krebs' solution to which ascorbic acid (0.15 mM) was added to act as an antioxidant. Cyanopindolol hemifumarate (Tocris-Cookson, Ballwin, MO), methoxamine hydrochloride (Burroughs-Wellcome, Research Triangle Park, NC) and phentolamine hydrochloride (Ciba-Geigy, Summit, NJ) were dissolved in, and diluted with, distilled water. Dilutions of all drugs were made daily and were kept on ice during experiments.

Statistical analysis

Regional variations in resting tone were compared by one-way analysis of variance with Bonferroni correction. However, due to the inherent variability of relaxation responses, some of these data sets exhibited large differences in their respective standard deviations. For this reason statistical comparisons between multiple maximal responses were made using the non-parametric Mann–Whitney U test. Comparisons between individual pairs of data sets were made using a non-paired *t*-test or, where these had significantly different standard deviations, a non-paired *t*-test with Welch correction. Statistical analyses were performed using GraphPad Instat Version 3 (GraphPad Software Inc., San Diego, CA) and a P value of less than 0.05 was considered to represent a significant difference. In all cases n equals one replicate of one experiment using one tissue from one animal.

Results

Resting tone

Following 30-min equilibration and before commencing any drug-addition protocols, the resting tone in each region was; proximal 3.93 ± 0.13 mN (n = 23), mid 3.30 ± 0.11 mN (n = 27) and distal 2.89 ± 0.11 mN (n = 25). Distal colonic muscularis mucosae maintained a significantly lower resting tone compared with that found in either the mid colon ($P < 0.05$) or the proximal colon ($P < 0.001$); similarly, mid colonic muscularis mucosae maintained a lower resting tone than that found in the proximal region ($P < 0.01$).

Agents acting at β-adrenoceptors

Muscularis mucosae from the proximal, mid and distal colon exposed to noradrenaline (10^{-9}–10^{-3} M) exhibited relaxations that reached respectively −90.1 ± 6.4% (n = 16), −145.8 ± 9.7% (n = 15) and −213.4 ± 15.9% (n = 16) of each tissue's maximal relaxation to sodium nitroprusside (10^{-4} M) (Figure 1). It was also noted that, over all experimental paradigms, in 14 of 27 proximal preparations exposed to 10^{-3} M noradrenaline, the relaxation response at this concentration was preceded by a contraction above baseline of 62.5 ± 10.0% of the acetylcholine maximum. In addition, all proximal preparations exhibited some degree of excitation during one or more noradrenaline-induced relaxations, but this was never of sufficient magnitude to overcome the predominant inhibitory response (see, for example, Figure 1, top panel). The latter phenomenon was not observed in muscularis mucosae from either the mid or the distal region.

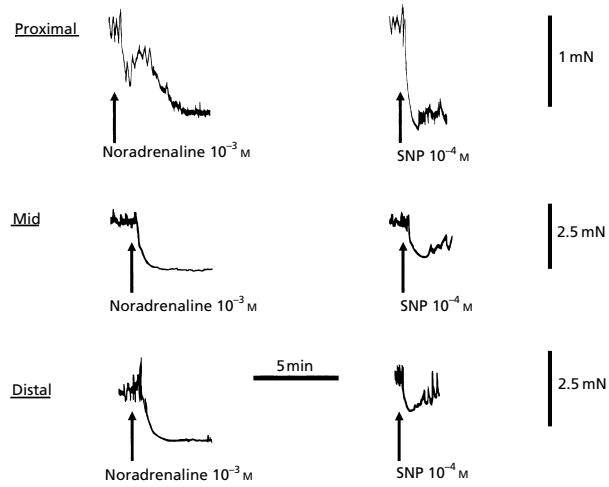

Figure 1 Representative tracings showing proximal, mid and distal colonic muscularis mucosae maximal relaxations to noradrenaline (10^{-3} M), relative to that elicited by sodium nitroprusside (10^{-4} M) (SNP) on the same tissue. Note the transient superimposed excitation during the initial phase of the relaxation response in the proximal tissue and the ephemeral nature of the SNP-induced inhibition in the mid and distal regions. These regional differences were not simply a function of resting tone, because this was greatest in the proximal and least in the distal colon (see text for details).

Although not always numerically identical, the responses of the proximal, mid and distal colonic muscularis mucosae to noradrenaline were found not to differ significantly from controls following a 30-min pretreatment with, and in the continuous presence of, either atenolol (10^{-5}M, β_1-adrenoceptor antagonist), butoxamine (10^{-5}M, β_2-adrenoceptor antagonist) or propranolol (10^{-6}M, non-selective β_1- and β_2-adrenoceptor antagonist). However, cyanopindolol at 10^{-6}M (a selective β_3-adrenoceptor antagonist (MacDonald et al 1994; Hoey et al 1996)) shifted the concentration–response curves for noradrenaline in each region to the right, with no significant change in the magnitude of their respective maximal relaxations (Figure 2).

Analysis of the data from the individual preparations that had each been exposed to a single concentration of the selective β_3-adrenoceptor agonist CL216343 (5.36×10^{-10}–5.36×10^{-4}M) to avoid desensitization showed that the colonic muscularis mucosae was relaxed in a concentration-dependent manner by this agent. The respective maxima were −67.0 ± 7.7% (n = 6), −94.1 ± 8.5% (n = 8) and −105.0 ± 3.3% (n = 6), in the proximal, mid and distal regions. In preparations from the mid and distal colon these relaxations were significantly smaller than the corresponding maximal responses to noradrenaline, $P < 0.01$ and $P < 0.001$, respectively.

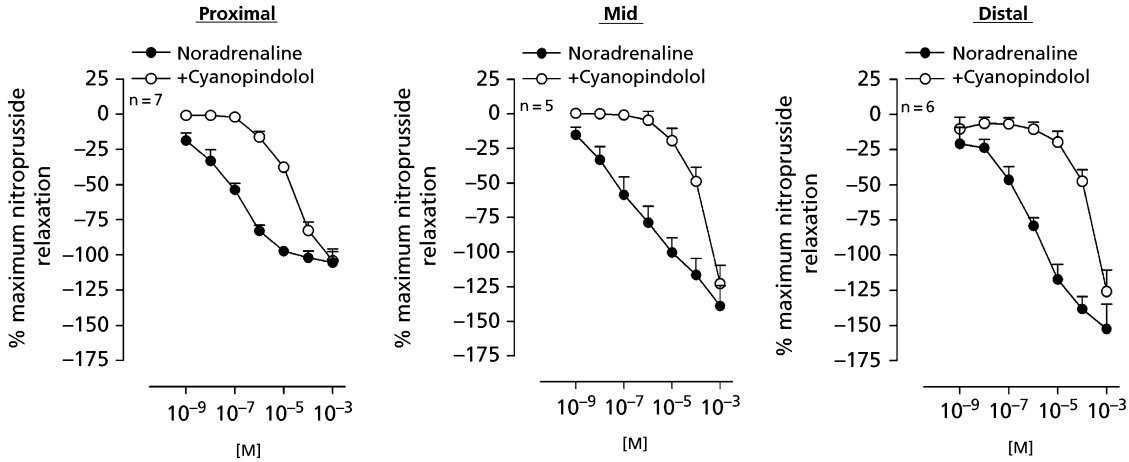

Figure 2 Effects of the β_3-adrenoceptor antagonist cyanopindolol (10^{-6} M) on rat proximal, mid and distal colonic muscularis mucosae responses to noradrenaline. Note that in all regions of the colon noradrenaline-induced relaxation responses were attenuated in the presence of this antagonist. Data are expressed as a percentage of each tissue's maximum relaxation (−100%) to sodium nitroprusside (10^{-4} M) and are the mean and s.e. of the number of observations indicated (n).

Table 1 Comparison of maximal relaxations elicited by noradrenaline, by noradrenaline in the presence of phentolamine and by isoprenaline in rat proximal, mid and distal colonic muscularis mucosae

Region of colonic muscularis mucosae	Noradrenaline	Noradrenaline + phentolamine	Isoprenaline
Proximal	–90.10 ± 3.78 (16)	–229.31 ± 47.65 (6)**	–168.50 ± 21.71 (9)**
Mid	–145.80 ± 9.74 (15)	–170.45 ± 35.81 (6)	–169.60 ± 22.61 (5)
Distal	–221.00 ± 16.05 (12)	–229.31 ± 47.65 (6)	–161.45 ± 38.17 (6)

**$P < 0.01$, significantly larger than noradrenaline alone in this region. Note that although numerically smaller, the maximal isoprenaline-induced relaxation in the distal region was not significantly different to that elicited by noradrenaline alone ($P = 0.2$). Data shown are the mean ± s.e. of the number of observations indicated in parentheses.

Isoprenaline (10^{-9}–10^{-3} M), a non-selective β-adrenoceptor agonist, evoked concentration-dependent relaxations of the proximal, mid and distal colonic muscularis mucosae (Table 1). In the mid and distal regions isoprenaline-induced maximal relaxations did not differ significantly from those elicited by noradrenaline (Table 1). In the proximal region the maximal isoprenaline-induced relaxation was significantly larger compared with that induced by noradrenaline ($P = 0.003$) (Table 1). However, when the maximal isoprenaline-induced responses of muscularis mucosae from this region were compared with those achieved by noradrenaline in the continuous presence of phentolamine (10^{-6} M, 30 min prior exposure) their respective maxima were then found not to be significantly different from each other ($P = 0.455$) (Table 1). In the mid and distal regions the muscularis mucosae responses to noradrenaline were unchanged by phentolamine (10^{-6}M, 30 min prior exposure) (Table 1).

Agents acting at α-adrenoceptors

The α_1-adrenoceptor agonist methoxamine (10^{-9}–10^{-3} M) evoked concentration-dependent contractions of the proximal colonic muscularis mucosae that reached 113.6 ± 25.3% (n = 9) of these tissues' largest responses to acetylcholine. Clonidine (10^{-9}–10^{-3}M), a selective α_2-adrenoceptor agonist, was without effect in this region, eliciting a maximal response of only 4.5 ± 2.0% of the acetylcholine maximum (n = 6). Mid colonic muscularis mucosae contracted weakly in response to methoxamine (37.3 ± 23.1%; n = 5), but was unresponsive to clonidine (3.3 ± 2.0%; n = 7). Neither methoxamine (6.8 ± 2.0%; n = 6) nor clonidine (0.9 ± 0.7%; n = 6) elicited a significant motor response from the distal colonic muscularis mucosae.

Discussion

It is tempting to speculate that the properties of the muscularis mucosae in any region of the intestine could simply be predicted from the known pharmacological responses of the corresponding longitudinal and circular muscle layers. However, regardless of species, the characteristics of the muscularis propria at any location within the gut have universally been found to differ significantly from those of the muscularis mucosae and, as such, it has a poor predictive value in this regard.

Important examples that illustrate this point include: the observations that rabbit distal colonic muscularis mucosae was refractory to cholecystokinin (Percy et al 1992), which contracted the longitudinal muscle in the same region (Yagi et al 1991); γ-amino butyric acid relaxed rabbit colonic longitudinal and circular muscle (Tonini et al 1989), but was inactive on the corresponding muscularis mucosae (Percy et al 1992); leukotriene D4 was a potent contractile agent on the rabbit distal colonic muscularis mucosae while being without effect on the muscularis propria (Percy et al 1990); and rabbit gastric muscularis mucosae possessed excitatory P2 purinoceptors (Percy et al 1999) whereas the corresponding muscularis propria had only inhibitory P1 receptors (Hyman et al 1993). In the rat colon itself, bradykinin relaxed the longitudinal muscle layer (Gaddum & Horton 1959), but it contracted the muscularis mucosae (Percy 2005). Similarly, rat colonic longitudinal muscle was relaxed by adenosine acting via A2 receptors (Bailey & Hourani 1990), whereas the muscularis mucosae was contracted by the same purine acting at A1 receptors (Bailey et al 1992).

In addition to its many pharmacological differences from the muscularis propria, a second notable finding concerning the muscularis mucosae both throughout the gut and across species is that it undergoes significant modifications in its responses to pharmacological agents along the length of several organs including the oesophagus (Christensen & Percy 1984), stomach (Percy et al 1999, 2002) and colon (Percy et al 1992; Appleyard et al 2006). Interestingly, therefore, in this study the most striking feature of the colonic muscularis mucosae was that the predominant response to noradrenaline was relaxation, regardless of the region under study. While the absolute magnitude of the maximum noradrenaline-induced relaxation increased in a proximal to distal fashion, this could not be attributed to differences in resting tone because, paradoxically, the region exhibiting the largest relative relaxation responses to this agent, the distal colon, had the lowest resting tone of the three regions under study.

Neither the non-selective β-adrenoceptor antagonist propranolol nor the selective β_1- and β_2-adrenoceptor antagonists atenolol and butoxamine significantly modified muscularis mucosae responses to noradrenaline in any region of the colon. When coupled to the rightward shift in each concentration–response curve produced by the selective β_3-adrenoceptor antagonist cyanopindolol, and the ability of the selective β_3 agonist CL216343 to elicit muscularis mucosae inhibition, these data provide good evidence that the adrenoceptor responsible for noradrenaline-induced relaxation of this muscle layer was the β_3 subtype. This demonstrates pharmacologically that the β_3-adrenoceptor mRNA previously detected in the rat colonic mucosa/submucosal layers (Evans et al 1996) represented, in part, a population of functional receptors linked to muscularis mucosae motor activity, with the remainder presumably attributable to those located on the epithelium itself (Schultheiss & Diener 2000).

However, it was interesting to observe that direct stimulation of the rat colonic muscularis mucosae with CL216343 led to significant tachyphylaxis, a phenomenon previously noted in rat colonic longitudinal muscle exposed to BRL37344, another selective β_3-adrenoceptor agonist (Kelly & Houston 1996). In preparations of muscularis mucosae from each colonic region the loss of responsiveness to CL216343 occurred to such an extent that it was not possible to see concentration-related responses if any preparation was exposed to this agent more than once. Even using the single exposure protocol adopted here, it is still likely that the true magnitude of the CL216343-induced relaxations had been underestimated. This likely explains why in the mid and distal regions noradrenaline-induced inhibitory responses were large, but the maximal CL216343-induced responses were significantly smaller, remaining comparable in magnitude with those seen in the proximal region.

These data demonstrate that rat colonic muscularis mucosae differs from the longitudinal muscle where, in addition to β_3-adrenocptors (Kelly & Houston 1996), β_2-adrenoceptors could contribute to relaxation responses (McKean & MacDonald 1995); although there has been evidence for an additional contribution to this by β_1-adrenoceptors (McKean & MacDonald 1995), this finding has not been unequivocal (MacDonald & Lamont 1993). These findings illustrate an important contrast between the ability to detect β-adrenoceptor mRNA in a heterogeneous tissue sample (Roberts et al 1999) and the functional role of the receptors whose expression this represents. The role of β_3-adrenoceptors in mediating rat colonic muscularis mucosae relaxation was, however, similar to that described in the rabbit colon (Percy et al 1992), suggesting, therefore, that this may be a consistent feature of this muscle layer in the colon of multiple species.

In the rabbit there are significant regional differences in the responses of the colonic muscularis mucosae to noradrenaline, such that the proximal region relaxes at low concentrations while contracting at higher concentrations. This is because, in addition to the inhibitory β-adrenoceptors found on the muscularis mucosae throughout the colon, excitatory α-adrenoceptors in the proximal region are able to modify the inhibitory responses elicited by concurrent β-adrenoceptor stimulation (Percy et al 1992). In contrast, although excitatory α_1-adrenoceptors can be shown to be present elsewhere on the rabbit colonic muscularis mucosae through the use of selective agonists, only β-adrenoceptor-mediated inhibition is seen when these tissues are exposed to noradrenaline (Percy et al 1992). The results of this study indicate a similar pattern of adrenoceptor distribution in the muscularis mucosae in the rat colon, even though the overall mechanical responses to noradrenaline in the two species were not identical. In the rat proximal colon, contractions elicited by the selective α_1-adrenoceptor agonist methoxamine were equal in magnitude to the largest contractions produced by acetylcholine. Despite high concentrations of noradrenaline being able to elicit an initial excitation in approximately 50% of proximal muscularis mucosae preparations, this was never of sufficient magnitude or duration to overcome the concurrent β_3-adrenoceptor-mediated inhibitory response, as was seen in the corresponding region of the rabbit colon (Percy et al 1992). However, in the presence of phentolamine, a selective α_1-adrenoceptor antagonist, noradrenaline-induced relaxations were increased in magnitude to equal those elicited by the non-selective β-adrenoceptor agonist isoprenaline, showing that α_1-adrenoceptors did act to modify noradrenaline-induced responses in the proximal region. These data together provide strong evidence that the rat proximal colonic muscularis mucosae

possesses functional excitatory α_1-adrenoceptors in addition to the inhibitory β_3-adrenoceptors found on this muscle layer throughout the colon. In contrast, although it was possible to show the presence of excitatory α_1-adrenoceptors in the mid colon, the inability of phentolamine to augment noradrenaline-induced relaxations in this region suggests that these did not act to modify the concurrent β_3-adrenoceptor-mediated inhibitory responses. No evidence was found for excitatory α_1-adrenoceptors in the distal colon and, as in the rabbit (Percy et al 1992), α_2-adrenoceptor-mediated changes in muscularis mucosae contractile activity appeared to be absent in all regions of the rat colon.

In the rabbit distal colon, mucosal secretion is linked to muscularis mucosae contraction via prostaglandin production and the ensuing stimulation of non-cholinergic secretomotor neurons (Percy et al 2003). In this regard, therefore, it was interesting to note that in the rat colon bradykinin elicited mucosal secretion in a manner that was associated with a physical deformation of the mucosa itself (Baron et al 1986). Although it was originally thought that this could not be the result of muscularis mucosae contraction, that conclusion was based upon the erroneous assumption that, as had been found in the rat colonic longitudinal muscle (Gaddum & Horton 1959), bradykinin would relax this muscle layer (Baron et al 1986). However, because bradykinin is now known to be a potent stimulant of muscularis mucosae contraction in all regions of the rat colon (Percy 2005), this suggests that rat colonic mucosal secretion, like that in the rabbit (Percy et al 2003), may also be linked to increased motor activity in this muscle layer.

Based upon these observations and the data obtained in this study, it seems unlikely that muscularis mucosae-mediated modulation of rat colonic mucosal secretion could be achieved via concurrent activation of their respective noradrenergic innervation. Under these conditions noradrenaline-induced relaxation of rat colonic muscularis mucosae would not act to promote or augment the initial β_3-adrenoceptor-mediated increase in short circuit current (I_{sc}) that is known to occur throughout the colon via epithelial Cl^- secretion (Schultheiss & Diener 2000). This conclusion is based upon current evidence which suggested that contraction (Percy et al 2003), rather than relaxation, of this muscle would be linked to generation of the prostaglandin(s) known to be associated with this process (Schultheiss & Diener 2000). Furthermore, it is also improbable that noradrenaline-induced muscularis mucosae relaxation could be linked to the K^+ secretion thought to be responsible for the second component of the β_3-adrenoceptor-mediated rat colonic mucosal response to catecholamines, namely a decreased I_{sc} (Schultheiss & Diener 2000).

It has been known for 30 years that there are alterations in the adrenergic submucosal innervation of the human large intestine in inflammatory bowel disease (Kyösola et al 1977). More recently it has been established that colonic mucosal levels of noradrenaline, but not adrenaline, were significantly reduced during active inflammation in both Crohn's disease and ulcerative colitis (Magro et al 2002) but, interestingly, not in the non-inflamed state in ulcerative colitis patients. In the rat trinitrobenzenesulfonic acid colitis model, altered colonic circular muscle motor activity has been attributed to decreased inhibitory adrenergic modulation via β_3-adrenoceptors on the muscle itself (Zhao et al 2001) although, in the corresponding rat acetic acid model, colonic β_3-adrenoeptor expression was found to be unchanged, as was the affinity of the β_3 receptor agonist BRL37344 (Khan et al 2002). The presence of β_3-adrenoceptors that can influence the motor activity of the rat colonic muscularis mucosae and their previous localization in the muscularis mucosae of both the human small and large intestine (Anthony et al 1998) suggests that that these too may play a role in the functional changes associated with colonic inflammation. This strongly suggests that, with respect to integrated muscularis mucosae and mucosal function in colitis, further studies of this muscle, these receptors and the changes that they undergo in such pathophysiological conditions are warranted.

The results of this study add to the growing body of evidence showing that, regardless of the model utilized or the anatomical location of the tissue within the gut, the muscularis mucosae is pharmacologically distinct from the muscularis propria. Furthermore, as noted in other organs and in other species, this muscle layer is not a homogenous entity along the length of the rat colon. These observations add to our understanding of the physiological role of the muscularis mucosae by demonstrating that endogenous catecholamine-induced changes in its motor activity in the rat colon are unlikely to be physiologically correlated with the mucosal events that take place under the same circumstances.

Conclusion

The rat colonic muscularis mucosae differed from the corresponding longitudinal and circular muscle layers in possessing only a single subclass of adrenoceptors that mediated its relaxation responses to noradrenaline. Although this was a uniform finding along the length of the rat colon, in the proximal region β_3-adrenoceptor-mediated relaxation elicited by noradrenaline was opposed by the concurrent activation of excitatory α_1-adrenoceptors, a phenomenon absent in the mid and distal regions. α_2-adrenoceptors, in contrast, did not appear to have a functional role in muscularis mucosae contractile activity in any region of the rat colon. The types of adrenoceptors present on the rat colonic muscularis mucosae had a regional distribution analogous to that found on this muscle layer in the rabbit colon, suggesting the possibility of inter-species homology in this regard. Although adrenoceptors can also mediate stimulation of the rat colonic mucosa (Schultheiss & Diener 2000), concurrent β_3-adrenoceptor mediated muscularis mucosae relaxation was unlikely to be a pro-secretory change in its motor activity in-vivo during periods of increased sympathetic activity. This is because, in the rabbit colon at least, contraction and not relaxation of this muscle layer was associated with the generation of eicosanoids that acted as the secretagogues linking muscular activity to epithelial function (Percy et al 2003). The results of this study, when added to the current knowledge about the muscularis mucosae, suggest that regional variations in its pharmacological properties are indicative of highly significant modifications that are necessary for it to perform its physiological functions, not only in successive regions of the colon, but also throughout the gut as a whole.

References

Anthony, A., Schepelmann, S., Guillaume, J.-L., Strosberg, A. D., Dhillon, A. P., Pounder, R. E., Wakefield, A. J. (1998) Localization of the β(beta)$_3$-adrenoceptor in the human gastrointestinal tract: an immunohistochemical study. *Aliment. Pharmacol. Ther*. **12**: 519–525

Appleyard, C. B., Morales, M., Percy, W. H. (2006) Regional variations in neurokinin receptor subtype contributions to muscularis mucosae and epithelial function in rat colon. *Dig. Dis. Sci*. **51**: 506–516

Bailey, S. J., Hourani, S. M. O. (1990) Effects of purines on the longitudinal muscle of the rat colon. *Br. J. Pharmacol*. **105**: 885–892

Bailey, S. J., Hickman, D., Hourani, S. M. O. (1992) Characterization of the P1-purinoceptor mediating contraction of the rat colon muscularis mucosae. *Br. J. Pharmacol*. **105**: 400–404

Baron, D. A., Miller, D. H., Margolius, H. S. (1986) Kinins induce rapid structural changes in colon concomitant with chloride secretion. *Cell Tissue Res*. **246**: 589–594

Bianchetti, A., Manara, L. (1990) In vitro inhibition of intestinal motility by phenylethanolaminotetralines: evidence of atypical β-adrenoceptors in rat colon. *Br. J. Pharmacol*. **100**: 831–839

Christensen, J., Percy, W. H. (1984) A pharmacological study of oesophageal muscularis mucosae from the cat, dog and American opossum (*Didelphis virginiana*). *Br. J. Pharmacol*. **83**: 329–336

deBoer, R. E., Brouwer, F., Zaagsma, J. (1993) The beta adrenoceptors mediating relaxation of rat oesophageal muscularis mucosae are predominantly of the beta3-, but also the beta2-subtype. *Br. J. Pharmacol*. **110**: 442–446

Evans, B. A., Papaioannou, M. Bonazzi, V. R., Summers, R. J. (1996) Expression of β3-adrenocpetor mRNA in rat tissues. *Br. J. Pharmacol*. **117**: 210–216

Gaddum, J. H., Horton, E. W. (1959) The extraction of human urinary kinin (substance Z) and its relation to the plasma kinins. *Br. J. Pharmacol*. **14**: 117–124

Hoey, A. J., Jackson, C. M., Pegg, G. G., Sillence, M. N. (1996) Characteristics of cyanopindolol analogues active at the beta 3-adrenoceptor in rat ileum. *Br. J. Pharmacol*. **119**: 564–568

Hyman, P. E., Hou, H. X., Willenbucher, R., Snape, W. J., Tomomasa, T. (1993) Postnatal changes in receptor-mediated rabbit gastric smooth muscle relaxation. *Biol. Neonate* **64**: 310–317

Kelly, J., Houston, G. (1996) β3-Adrenoceptors mediating relaxation of the oesophageal tunica muscularis mucosae and distal colon in the rat: comparative pharmacology and their desensitization by BRL 37344. *J. Auton. Pharmacol*. **16**: 205–211

Khan, I., Hussein, S. M. K., Oriowo, M. A. (2002) Studies on the expression and function of beta-3-adrenoceptors in the colon of rats with acetic acid-induced colitis. *Pharmacology* **64**: 98–105

Kyösola, K., Penttilä, O., Salaspuro, M. (1977) Rectal mucosal adrenergic innervation and enterochromaffin cells in ulcerative colitis and irritable colon. *Scand. J. Gastroenterol*. **12**: 363–367

Luckensmeyer, G. B., Keast, J. R. (1998) Activation of α- and β-adrenoceptors by sympathetic nerve stimulation in the large intestine of the rat. *J. Physiol. (Lond.)* **510**: 549–561

MacDonald, A., Lamont, M. (1993) Effects of selective antagonism of β-adrenoceptor sub-types on responses to isoprenaline in rat distal colon in vitro. *Br. J. Pharmacol*. **110**: 1551–1555

Macdonald, A., Forbes, I. J., Gallacher, D., Heeps, G., McLaughlin, D. P. (1994) Adrenoceptors mediating relaxation to catecholamines in rat isolated jejunum. *Br. J. Pharmacol*. **112**: 576–578

McKean, J., MacDonald, A. (1995) Contribution of β-adrenoceptor subtypes to responses to isoprenaline in rat isolated distal colon. *J. Pharm. Pharmacol*. **47**: 388–391

Magro, F., Vieira-Coelho, M. A., Fraga, S. Serrão, M. P., Tavarela Veloso, F., Ribeiro, T., Soares-da-Silva, P. (2002) Impaired synthesis or cellular storage of norepinephrine, dopamine and 5-hydroxytryptamine in human inflammatory bowel disease. *Dig. Dis. Sci*. **47**: 216–224

McLaughlin, D. P., MacDonald, A. (1990) Evidence for the existence of "atypical" β-adrenoceptors (β3-adrenoceptors) mediating relaxation in the rat distal colon in vitro. *Br. J. Pharmacol*. **101**: 569–574

Percy, W. H. (2005) Region-specific mechanisms underlying the excitatory effects of bradykinin and ATP on rat colonic muscularis mucosae. *Neurogastro. Motil*. **17**: 33

Percy, W. H., Christensen, J. (1986) Pharmacological characterization of opossum distal colonic muscularis mucosae in vitro. *Am. J. Physiol*. **250**: G98–G102

Percy, W. H., Lee, S. Y., Burton, M. B., Tolentino, T., Burakoff, R. (1990) Leukotriene D4 excitation of rabbit distal colon arises in the region of the muscularis mucosae. *Am. J. Physiol*. **259**: G753–G759

Percy, W. H., Rose, K., Burton, M. B. (1992) Pharmacologic characterization of the muscularis mucosae in three regions of the rabbit colon. *J. Pharmacol. Exp. Ther*. **261**: 1136–1142

Percy, W. H., Warren, J. M., Brunz, J. T. (1999) Characteristics of the muscularis mucosae in the acid-secreting region of the rabbit stomach. *Am. J. Physiol*. **276**: G1213–G1220

Percy, W. H., Kittelsrud, J. M., Brunz, J. T. (2002) Types of adrenoceptors mediating responses of rabbit gastric muscularis mucosae. *Dig. Dis. Sci*. **47**: 356–364

Percy, W. H., Fromm, T. H., Wangsness, C. E. (2003) Muscularis mucosae contraction evokes colonic secretion via prostaglandin synthesis and nerve stimulation. *Am. J. Physiol*. **284**: G213–G220

Roberts, S. J., Papaioannou, M., Evans, B. A., Summers, R. J. (1999) Characterization of β-adrenoceptor mediated smooth muscle relaxation and the detection of mRNA for β1-, β2- and β3-adrenoceptors in rat ileum. *Br. J. Pharmacol*. **127**: 949–961

Schultheiss, G., Diener, M. (2000) Adrenoceptor-mediated secretion across the rat colonic epithelium. *Eur. J. Pharmacol*. **403**: 251–258

Tonini, M., Crema, A., Frigo, G. M., Rizzi, C. A., Manzo, L., Candura, S. M., Onori, L. (1989) An in vitro study of the relationship between GABA receptor function and propulsive motility in the distal colon of the rabbit. *Br. J. Pharmacol*. **98**: 1109–1118

Umezawa, T., Guo, S., Jiao, Y., Hisamitsu, T. (2003) Effect of clonidine on colonic motility in rats. *Auton. Neurosci*. **107**: 32–36

Wong, W. C. (1977) Ultrastructural localization of adrenergic nerve terminals in the circular muscle layer and muscularis mucosae of rat duodenum after acute treatment with 6-hydroxydopamine. *J. Anat*. **124**: 637–642

Yagi, H., Snape, W. J., Hyman, P. E. (1991) Developmental changes in agonist-mediated colonic smooth muscle contraction in the rabbit. *Pediatr. Res*. **29**: 20–23

Zhao, A., Bossone, C., Piñeiro-Carrero, V., Shea-Donohue, T. (2001) Colitis-induced alterations in adrenergic control of circular smooth muscle in vitro in rats. *J. Pharmacol. Exp. Ther*. **299**: 768–774