

Altered prejunctional modulation of intestinal cholinergic and noradrenergic pathways by α_2 -adrenoceptors in the presence of experimental colitis

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1 This study investigates the influence of intestinal inflammation on: (1) the control of intestinal neurotransmission and motility by prejunctional α_2 -adrenoceptors and (2) the expression of intestinal α_2 -adrenoceptors. Experimental colitis was induced by intrarectal administration of 2,4-dinitrobenzenesulphonic acid (DNBS) to rats.

2 UK-14,304 inhibited atropine-sensitive electrically evoked contractions of ileal and colonic longitudinal muscle preparations. UK-14,304 acted with similar potency, but higher efficacy, on tissues from DNBS-treated animals; its effects were antagonized with greater potency by phentolamine than rauwolscine.

3 Electrically induced [³H]noradrenaline release from ileal preparations was reduced in the presence of colitis. Tritium outflow was decreased by UK-14,304 and stimulated by rauwolscine or phentolamine: these effects were enhanced in preparations from animals with colitis.

4 Reverse transcription–polymerase chain reaction and Western blot assay demonstrated the protein expression of α_{2A} -adrenoceptors in mucosal and muscular tissues isolated from ileum and colon. The induction of colitis increased α_{2A} -adrenoceptor expression in both ileal and colonic muscular layers, without concomitant changes in mucosal tissues.

5 Induction of colitis reduced gastrointestinal propulsion of a charcoal suspension *in vivo*. In this setting, the gastrointestinal transit was inhibited by intraperitoneal (i.p.) UK-14,304 and stimulated by i.p. rauwolscine. After pretreatment with guanethidine, the stimulant action of rauwolscine no longer occurred, and UK-14,304 exerted a more prominent inhibitory effect that was antagonized by rauwolscine.

6 The present results indicate that, in the presence of intestinal inflammation, prejunctional α_2 -adrenoceptors contribute to an enhanced inhibitory control of cholinergic and noradrenergic transmission both at inflamed and noninflamed distant sites. Evidence was obtained that such modulatory actions depend on an increased expression of α_{2A} -adrenoceptors within the enteric nervous system.

British Journal of Pharmacology (2003) **139**, 309–320. doi:10.1038/sj.bjp.0705249

Keywords: α_2 -Adrenoceptors; acetylcholine; noradrenaline; enteric nervous system; intestinal motility; intestinal inflammation

Abbreviations: ANOVA, analysis of variance; DNBS, 2,4-dinitrobenzenesulphonic acid; EDTA, ethylenediaminetetraacetic acid; L-NAME, N^o-nitro-L-arginine methylester; PCR, polymerase chain reaction; RT, reverse transcription; TBS-T, Tween-20 in Tris-buffered saline; TNBS, 2,4,6-trinitrobenzenesulphonic acid

Introduction

The gastrointestinal tract is extensively innervated by extrinsic noradrenergic neurons, whose projections end in close proximity to several cellular targets (i.e. epithelial cells, neurons, blood vessels). Locally, through their interaction with α_2 -adrenoceptors, endogenous catecholamines modulate a variety of digestive functions, including secretions and motility (Del Tacca *et al.*, 1982; De Ponti *et al.*, 1996; Liu & Coupar, 1997), gastro-colonic sensation (Malcom *et al.*, 2000), and

epithelial cell proliferation (Schaak *et al.*, 2000). Both sympathetic and cholinergic axon terminals of the enteric nervous system are equipped with prejunctional α_2 -adrenoceptors, the activation of which inhibits noradrenaline and acetylcholine release, respectively (Blandizzi *et al.*, 1993; Funk *et al.*, 1995; Colucci *et al.*, 1998).

Inflammatory diseases of the human gut (e.g. inflammatory bowel disease, coeliac disease, infectious gastroenteritis) are commonly associated with alterations of enteric functions, including increased secretion and motor abnormalities, which are thought to contribute to the generation of digestive

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symptoms (Collins, 1996, Giaroni *et al.*, 1999; Sharkey & Kroese, 2001). For this reason, there is currently great interest in understanding the pathophysiological changes occurring in the enteric nervous system as a consequence of intestinal inflammation (Sharkey & Kroese, 2001). Such alterations occur throughout the gut, at both inflamed and noninflamed sites, and they depend, at least in part, on mutual interactions between enteric nerves and the digestive immune system (Collins, 1996).

The role played by adrenoceptors in intestinal inflammation remains poorly understood. Experimental colitis in rats was associated with downregulation of β_3 -adrenoceptors in colonic circular smooth muscle (Zhao *et al.*, 2001). An increased density of α_2 -adrenergic binding sites was detected in cell membrane preparations obtained from jejunal tissues of guinea-pigs with small bowel inflammation (Martinolle *et al.*, 1993). However, data on the possible influence exerted by prejunctional α_2 -adrenoceptors on enteric neurotransmission and digestive motility in the presence of intestinal inflammation are lacking. Accordingly, the present study was designed to assess the influence of experimentally induced intestinal inflammation on: (1) the control of enteric neurotransmission and intestinal motility by prejunctional α_2 -adrenoceptors and (2) the expression pattern of intestinal α_2 -adrenoceptors.

Methods

Animals

Albino male Sprague–Dawley rats, 200–250 g body weight, were used throughout the study. The animals were fed standard laboratory chow and tap water *ad libitum* and were not used for at least 1 week after their delivery to the laboratory. They were housed (five in each cage) in temperature-controlled rooms on a 12-h light–dark cycle at 22–24°C and 50–60% humidity. Their care and handling were in accordance with the provisions of the European Union Council Directive 86-609, recognized and adopted by the Italian Government.

Induction of colitis

Colitis was induced in rats in accordance with the method previously described by Barbara *et al.* (2000). Briefly, during a short anaesthesia with diethyl ether, 30 mg of 2,4-dinitrobenzenesulphonic acid (DNBS) in 0.25 ml of 50% ethanol was administered intrarectally via a polyethylene PE-60 catheter inserted 8 cm proximal to the anus. Control rats received 0.25 ml of 50% ethanol. Animals underwent subsequent experimental procedures 6 days after DNBS administration, in order to allow a full development of histologically evident colonic inflammation.

Assessment of colitis

At 6 days after treatment with DNBS or its vehicle, animals were euthanized and the severity of intestinal inflammation was evaluated macroscopically and histologically in accordance with the criteria previously reported by Wallace & Keenan (1990), as modified by Barbara *et al.* (2000). Briefly, the macroscopic criteria were based on the following: the

presence of adhesions between the colon and other intra-abdominal organs; consistency of colonic faecal material (as an indirect marker of diarrhoea); thickening of the colonic wall; the presence and extension of hyperaemia and macroscopic mucosal damage (assessed with the aid of a ruler). Microscopic criteria for damage and inflammation were assessed by light microscopy on haematoxylin- and eosin-stained histological sections obtained from whole-gut specimens, taken from a region of the inflamed colon immediately adjacent to the gross macroscopic damage and from terminal ileum. Histological criteria included: degree of mucosal architecture changes; cellular infiltration; external muscle thickening; presence of crypt abscess and goblet cell depletion. All the parameters of macroscopic and histological damage were recorded and scored for each rat by two observers blinded to the treatment.

Recording of contractile activity from longitudinal muscle preparations of ileum

Longitudinal muscle strips of proximal ileum containing the Auerbach plexus were prepared as previously reported by Collins *et al.* (1989). In order to record the motor activity of longitudinal muscle, ileal preparations weighing 60–90 mg were set up in organ baths of 10 ml capacity (overflow system), containing oxygenated Krebs solution at 37°C, and connected vertically to isotonic transducers (Basile, Comerio, Italy) under a constant tension of 1 g. The Krebs solution had the following composition (in mM): NaCl 113, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, glucose 11.5 (pH 7.4 ± 0.1). Each preparation was allowed to equilibrate for at least 30 min, with intervening washings at intervals of 10 min. The contractile activity of preparations was recorded by the polygraph Gemini 7080 (Basile, Comerio, Italy). A pair of coaxial platinum electrodes was positioned at distance of 10 mm from the longitudinal axis of each preparation in order to deliver transmural electrical stimulation. Electrical stimuli were applied as 5-s trains of square wave pulses (0.5 ms duration, 5 Hz frequency, 30 mA intensity), applied every 60 s. In preliminary experiments, this pattern of electrical stimulation elicited motor responses consisting of relaxations followed by contractions, which were partly insensitive to atropine. Therefore, all subsequent experiments were performed after incubation of ileal preparations for 30 min with guanethidine (10 μ M), N^o-nitro-L-arginine methylester (L-NAME, 100 μ M) and capsaicin (10 μ M), to suppress the electrically induced motor activity of longitudinal smooth muscle because of the release of noradrenaline, nitric oxide, and sensory peptides (Maggi & Giuliani, 1995). Indeed, once released from intestinal afferent nerves, sensory peptides can induce non-cholinergic contractions through the activation of intrinsic myenteric neurons (Barthò *et al.*, 1999). The α_2 -adrenoceptor agonist UK-14,304 was added cumulatively to the bathing fluid in 0.5-log unit increments. A period of 3–5 min was allowed between subsequent increments of concentration in order to enable a full development of the effect of the agonist. In preliminary experiments, aiming to assay the sensitivity of intestinal preparations to UK-14,304, the agonist was also added to the bathing fluid in a noncumulative manner. In agonist–antagonist interaction experiments, rauwolscine (α_2 -adrenoceptor antagonist) or phentolamine (α_1/α_2 -adrenoceptor antagonist) was added to the bath 20 min before the agonist. Drugs were given in volumes \leq 1% of total bath volume

(10 ml). The effects of test drugs were expressed as percent changes of control cholinergic contractions elicited by electrical stimulation. The potency of UK-14,304 was expressed as EC_{50} (concentration of the agonist that produces 50% of the maximal response for that agonist). The percent maximum inhibition of the control cholinergic motor activity (E_{max}) was also evaluated. Both parameters were calculated from single concentration–response curves and then averaged. The potencies of rauwolscine and phentolamine were expressed as pK_d values from the equation:

$$K_d = [B]/(DR - 1)$$

where B is the molar concentration of the antagonist and DR is the ratio of equally effective concentrations of the agonist (EC_{50}) in the presence and absence of the antagonist (Furchgott, 1972).

Recording of contractile activity from longitudinal muscle preparations of colon

Specimens of distal colon were placed into cold preoxygenated Krebs solution, opened along the mesenteric insertion, and cut along the longitudinal axis into strips of approximately 3 mm width and 30 mm length. The colonic preparations were then used in order to assess the effects of test drugs on electrically induced cholinergic motor activity of longitudinal muscle. The experiments were performed as reported above for ileal preparations. Electrical stimulation was delivered to colonic strips as 5-s trains of square wave pulses (0.5 ms, 5 Hz, 30 mA), applied every 60 s. As also observed for ileal preparations, in preliminary experiments colonic strips developed electrically induced motor responses that could not be completely prevented by atropine. For this reason, all subsequent experiments were carried out in the presence of guanethidine (10 μ M), L-NAME (100 μ M), and capsaicin (10 μ M). The effects of UK-14,304, rauwolscine and phentolamine on cholinergic contractile activity of colonic longitudinal smooth muscle were assayed as described above for the ileum.

Measurement of [3 H]noradrenaline release from longitudinal muscle preparations of ileum

Longitudinal muscle strips of ileum, containing the Auerbach plexus, were prepared as reported above. The ileal preparations were then used to assess the effects of UK-14,304, rauwolscine, and phentolamine on electrically induced [3 H]noradrenaline release, in accordance with the procedure previously described (Blandizzi *et al.*, 2000), with minor modifications. Ileal strips, weighing 60–90 mg, were incubated for 30 min in Krebs solution at 37°C, oxygenated with 95% O_2 + 5% CO_2 (preincubation period), and then loaded with 1-7,8- 3 H]noradrenaline (3.5 μ Ci ml^{-1}) for 60 min in 2 ml of Krebs solution. Ascorbic acid 0.03 mM and disodium ethylenediamine tetraacetic acid (EDTA) 0.1 mM were included in the medium in order to prevent the oxidative breakdown of noradrenaline. At the end of loading period, the ileal strips were repeatedly washed with Krebs solution, transferred into organ baths (5-ml capacity, filled with Krebs solution), and superfused at a flow rate of 1 $ml\ min^{-1}$ with Krebs solution bubbled with 95% O_2 –5% CO_2 at 37°C. The superfusing Krebs solution contained cocaine 5 μ M and hydrocortisone 1 μ M to inhibit the reuptake of noradrenaline. The first 60-min

collection of effluent was discarded (preperfusion), after which 3-min fractions were collected for 90 min. During the superfusion period, the ileal preparations underwent electrical field stimulation (BM-ST6 stimulator, Biomedica Mangoni, Pisa, Italy), delivered as square wave pulses (20 $V\ cm^{-1}$) of 0.5 ms duration at 8 Hz (480 pulses), during the third (S_1) and the 20th (S_2) collection period. At the end of superfusion, the radioactivity contained in each fraction was measured by liquid scintillation counting (Betamatic, Kontron Instruments, Milan, Italy). The radioactive content of ileal strips was also measured. For this purpose, each preparation was weighed and then incubated for 30 min in 1 ml of 10% trichloroacetic acid at room temperature. An aliquot of supernatant (50 μ l) was added to 5 ml of scintillator and the tritium content of the tissue was measured by liquid scintillation spectrometry. The test drugs were added to the superfusion solution in the 12th collection period, namely between S_1 and S_2 , and exposure to each drug continued until the end of experiment. Tritium efflux into the superfusate was calculated as the fraction of tritium present in the ileal strip at the onset of the respective collection period (fractional rate; min^{-1}). The increase in tritium outflow, evoked by electrical stimulation, was calculated as the percentage of tritium content of the tissue at the onset of electrical stimulation. The effects of test drugs on the evoked tritium outflow were expressed as ratio of the percentage release during S_2 over that obtained during S_1 (S_2/S_1), namely in the presence and absence of drug, respectively (Blandizzi *et al.*, 2000).

Reverse transcription–polymerase chain reaction analysis of α_{2A} -adrenoceptors

Experiments were performed on specimens of both ileum and colon, excised as reported above. In both cases, care was taken to carefully dissect the mucosa from muscular layers. Tissue specimens were then used to assess the expression of the gene encoding for α_{2A} -adrenoceptors by reverse transcription (RT) of mRNA followed by polymerase chain reaction (PCR). For this purpose, tissue samples were immediately frozen in liquid nitrogen and stored at $-80^\circ C$ until use. At the time of the extraction, tissues were disrupted in mortars kept cold by filling with liquid nitrogen. Total RNA was isolated by means of Trizol[®] (Life Technologies, Carlsbad, CA, U.S.A.) and chloroform. Total RNA (1 μ g) served as template for single-strand cDNA synthesis in a reaction using 2 μ l random hexamer oligonucleotide primers (0.5 μ g/ μ l) with 200 U of MMLV-reverse transcriptase in manufacturer's buffer containing 500 μ M deoxynucleotide triphosphate mixture (dNTP) and 10 mM dithiothreitol. cDNA samples were subjected to PCR in the presence of specific oligonucleotide primers based on the nucleotide sequence of the previously cloned α_{2A} -adrenoceptor rat gene (sense: 5'-CAGGTCGTGCTGGCG GTGGTGATC-3'; antisense: 5'-GGTCTGTAAGCAGCAGCCCGAG-3'; expected size 264 base pairs) (Wilborn *et al.*, 1998). PCR, consisting of 25 μ l of RT products, *Taq* polymerase 2.5 U, dNTP 100 μ M and oligonucleotide primers 0.5 μ M, was carried out by a PCR-Express thermocycler (Hybaid, Ashford, Middlesex, U.K.) at the following conditions: 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. Aliquots of RNA not subjected to RT were included in PCR reactions to verify the

absence of genomic DNA. The efficiency of RNA extraction, RT and PCR was evaluated by specific sets of oligonucleotide primers for the constitutively expressed rat β -actin gene (sense: 5'-TCATGAAGTGTGACGTTGACATCCGT-3'; antisense: 5'-CTTAGAAGCATTGCGGTGCACGATG-3'; expected size 286 base pairs). The amplified cDNA products were separated by 1.5% agarose gel electrophoresis in a Tris buffer 40 mM containing 2 mM EDTA, 20 mM acetic acid (pH 8), and stained with ethidium bromide. cDNA bands were then visualized by UV light and quantitated by densitometric analysis with NIH Image computer program (Scion Corporation, Frederick, MD, U.S.A.). The relative expression of α_{2A} -adrenoceptor mRNA was normalized to that of β -actin.

Western blot analysis of α_{2A} -adrenoceptors

Experiments were carried out on specimens of both ileum and colon, excised as reported above. In both cases, care was taken to carefully dissect the mucosa from muscular layers. Mucosal and muscular samples were then homogenized in lysis buffer containing: *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid) 10 mM, NaCl 30 mM, EDTA 0.2 mM, phenylmethylsulphonyl fluoride 2 mM, leupeptin 10 μ g ml⁻¹, aprotinin 10 μ g ml⁻¹, sodium fluoride 1 mM, sodium orthovanadate 1 mM, glycerol 2%, MgCl₂ 0.3 mM, Triton X-100 1%, using the homogenizer Powergen 35 (Fisherbrand, Middleton, Manchester, U.K.). Homogenates were centrifuged at 20,000 $\times g$ for 15 min at 4°C. The supernatants were separated from pellets and stored at -80°C. Protein concentration was determined by the Bradford method (Bio-Rad protein assay reagent, Hercules, CA, U.S.A.).

Equivalent amounts of protein lysates (50 μ g) were separated by electrophoresis on a sodium dodecylsulphate-polyacrylamide gel (12%) and transferred onto a nitrocellulose membrane. The blots were blocked overnight with 5% nonfat dried milk in phosphate-buffered saline and incubated for 1 h at room temperature with a polyclonal rabbit antiserum raised against a synthetic peptide corresponding to amino-acid residues 218–235 of human, mouse, rat, and pig α_{2A} -adrenoceptor (dilution 1:500, PA1-048; Affinity Bioreagents, Golden, CO, U.S.A.). After repeated washings with 0.1% Tween-20 in Tris-buffered saline (TBS-T), a peroxide-conjugated goat anti-rabbit antibody (dilution 1:3000, Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) was added for 1 h at room temperature. After repeated washings with TBS-T, the immunoreactive bands were visualized by enhanced chemiluminescence (ECL, Amersham Biosciences Europe, Cologno Monzese, Italy). The relative expression of α_{2A} -adrenoceptors was quantified by densitometric analysis with NIH Image computer program (Scion Corporation, Frederick, MD, U.S.A.).

In vivo evaluation of gastrointestinal transit

The quantitative evaluation of gastrointestinal transit was carried out as previously reported by Singh *et al.* (1996), with minor changes. For this purpose, both DNBS- and vehicle-treated rats were fasted 36 h before the beginning of experiments. Free access to water *ad libitum* was allowed until 2 h before the beginning of experiments. At that time, 2 ml of a charcoal suspension (10% charcoal in 12.5% arabic gum) was injected into the gastric lumen by a polyethylene orogastric

catheter. After 25 min, animals were killed by cervical dislocation and the small intestine was quickly removed avoiding stretching. The gastrointestinal transit was then evaluated by comparing the distance travelled by the charcoal meal from the pyloric sphincter with the total length of the small intestine from the pyloric sphincter to the ileo-caecal junction. In experiments aiming to assay the effects of test drugs on gastrointestinal transit, rauwolscine and UK-14,304 were administered by intraperitoneal (i.p.) route 30 and 20 min before the intragastric injection of charcoal meal, respectively. Control rats received drug vehicles by i.p. route at the same times. In additional experiments, the evaluation of gastrointestinal transit was performed on rats subjected to chemical ablation of sympathetic nervous pathways, according to the procedure previously reported by Ying *et al.* (1998). For this purpose, animals were treated with guanethidine 70 μ mol kg⁻¹ i.p. once daily for two consecutive days. The last dose was administered 3 h before the administration of charcoal meal for the assay of gastrointestinal transit.

Drugs

The following drugs and reagents were used: diethyl ether, guanethidine, L-NAME, capsaicin, tetrodotoxin, atropine sulphate, phenolamine hydrochloride, ascorbic acid, EDTA, cocaine hydrochloride, disodium hydrocortisone 21-phosphate, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid), phenylmethylsulphonyl fluoride, leupeptin, aprotinin, sodium orthovanadate, glycerol, Triton X-100, sodium dodecylsulphate, polyacrylamide, Tween-20 (Sigma Chemicals Co., St Louis, MO, U.S.A.); hexamethonium 2-chloride, rauwolscine hydrochloride (RBI, Natick, MA, U.S.A.); UK-14,304 [*S*-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine] (Tocris, Balwin, MO, U.S.A.); DNBS dihydrate (ICN Biomedicals, OH, U.S.A.); 1-7,8-[³H]-noradrenaline (specific activity 36 Ci mmol⁻¹; Amersham Pharmacia Biotech, Milan, Italy). Random hexamers, dithiothreitol, MMLV-reverse transcriptase, *Taq* polymerase, dNTP mixture, ethidium bromide (Promega, Madison, WI, U.S.A.). Other reagents were of analytical grade. Unless otherwise stated, all drugs were dissolved in distilled water or in 0.9% sterile saline for *in vivo* treatments. UK-14,304 was dissolved in dimethylsulphoxide and further dilutions were made with distilled water (*in vitro* assays) or 0.9% sterile saline (*in vivo* administrations). Drugs administered i.p. were injected in a volume of 0.5 ml per rat.

Statistical analysis

Results are given as mean \pm s.e.m. The significance of differences was evaluated by: Student's *t*-test for unpaired data (assessment of intestinal inflammation); one-way analysis of variance (ANOVA) for unpaired data (RT-PCR and Western blot assays); two-way ANOVA for unpaired data (noradrenaline release); two-way ANOVA for repeated measures (*in vitro* contractile activity); three-way ANOVA for unpaired data (*in vivo* gastrointestinal transit). *Post hoc* analysis was performed by Tukey or Dunnett test, as appropriate. *P*-values lower than 0.05 were considered significant; '*n*' indicates the number of experiments. In the case of *in vitro* experiments, the ileal and colonic preparations included in each test group were obtained from distinct

animals, and therefore, in the present study, 'n' refers also to the number of animals used per experimental group. EC_{50} values were interpolated from concentration-response curves. All statistical procedures, curve fitting, and calculations of 95% confidence intervals (95% CI) were performed by means of commercial software (GraphPad Prism™, software package version 2.01 for Windows 95, from GraphPad Software Inc., San Diego, CA, U.S.A.; SPSS™, software package version 11, from SPSS Inc., Chicago, IL, U.S.A.).

Results

Assessment of intestinal inflammation

At 6 days after DNBS administration, the distal colon was thickened and ulcerated with evident areas of transmural inflammation. Adhesions were often present and the bowel was occasionally dilated. In contrast, no changes were observed in the terminal ileum. The colitis was characterized by an intense granulocyte infiltrate extending throughout the mucosa and submucosa, and often involving the muscularis propria that appeared thickened. There was a greater than five-fold increase in both macroscopic and microscopic damage scores over ethanol-treated control animals. By contrast, all ileal specimens obtained from animals with colitis did not show any relevant histopathological abnormality. Mean score values of both macroscopic and microscopic damage estimated for ileal and colonic samples are summarized in Table 1.

Contractile activity of longitudinal muscle preparations of ileum

During the resting period, ileal segments developed a pattern of spontaneous contractile activity that did not interfere with electrically induced motor responses. Under the experimental conditions adopted in this study, the application of electrical stimuli to ileal strips, obtained from vehicle-treated or inflamed animals, evoked recurrent phasic contractions that were abolished by tetrodotoxin (1 μ M), or atropine (0.1 μ M), but were unaffected by hexamethonium (10 μ M), indicating an involvement of postganglionic cholinergic neurons. Figure 1 displays typical trace recordings showing the inhibitory effects of atropine either in the absence (Figure 1a) or in the presence (Figure 1c) of colitis.

Table 1 Macroscopic and microscopic damage scores estimated for ileal and colonic specimens 6 days after induction of colitis with DNBS

	Ileum		Colon	
	Vehicle	DNBS	Vehicle	DNBS
Macroscopic damage	0.45±0.09	0.52±0.15	1.85±0.32	10.92±1.71*
Microscopic damage	0.22±0.05	0.27±0.06	1.27±0.23	8.16±1.44*

Each value represents the mean of eight to ten experiments \pm s.e.m. Significant difference from the respective values obtained from vehicle-treated rats: * P <0.05.

UK-14,304 (0.001–10 μ M) caused a concentration-dependent inhibition of electrically evoked cholinergic contractions of ileal strips prepared from vehicle-treated rats (Figures 1b and 2a). In this case, the EC_{50} was 50.9 nM (95% CI = 28.4–91.2), and a maximal inhibition of $-37.8 \pm 5.2\%$ occurred at the concentration of 3 μ M ($n=7$). Rauwolscine (1 μ M) or phentolamine (1 μ M) did not significantly affect the electrically induced motor activity when tested alone, whereas they antagonized the inhibitory actions of UK-14,304 (Table 2). When assayed on ileal preparations obtained from animals with colitis, UK-14,304 was more effective in decreasing the evoked contractile activity of longitudinal muscle (EC_{50} = 46.8 nM, 95% CI = 31.5–69.5; E_{max} = $-82.8 \pm 4.7\%$ at 3 μ M; $n=8$; P <0.05) (Figures 1d and 2a). Under these conditions, the inhibitory effects of UK-14,304 were antagonized by rauwolscine (1 μ M) or phentolamine (1 μ M) (Table 2).

Contractile activity of longitudinal muscle preparations of colon

During the equilibration period, the longitudinal smooth muscle of colonic preparations, obtained from either vehicle- or DNBS-treated rats, developed a spontaneous contractile activity, which remained stable throughout the experiment without interfering with the motor responses elicited by application of electrical stimulation. As already observed for ileal preparations, following incubation with guanethidine, L-NAME and capsaicin, the application of electrical stimuli elicited recurrent phasic contractions, which were sensitive to tetrodotoxin (1 μ M) or atropine (0.1 μ M), but insensitive to hexamethonium (10 μ M). Figure 1 displays typical trace recordings showing the inhibitory effects of atropine either in the absence (Figure 1e) or in the presence (Figure 1g) of colitis.

In the absence of colitis, UK-14,304 (0.001–10 μ M) decreased the cholinergic contractions evoked by electrical stimulation in a moderate, but concentration-dependent manner (Figures 1f and 2b). An EC_{50} value of 54.2 nM (95% CI = 43.4–67.5) was estimated for this agonist, with a maximal inhibition of $-36.7 \pm 6.1\%$ at the concentration of 3 μ M ($n=8$). Rauwolscine (1 μ M) or phentolamine (1 μ M) alone did not modify the pattern of evoked motor responses. However, both these antagonists counteracted the inhibitory effects of UK-14,304 (Table 2).

In the presence of colitis, UK-14,304 caused a marked and concentration-dependent inhibition of electrically evoked cholinergic contractions (Figures 1h and 2b). In this case, the EC_{50} and E_{max} values were 47.9 nM (95% CI = 30.9–74.1) and $-75.3 \pm 4.7\%$ at 3 μ M ($n=8$; P <0.05), respectively. As already observed for vehicle-treated animals, rauwolscine and phentolamine did not interfere with the electrically induced contractile activity, whereas both drugs antagonized the inhibitory actions of UK-14,304 (Table 2).

[³H]noradrenaline release from longitudinal muscle preparations of ileum

In control experiments, performed on ileal preparations from vehicle-treated rats ($n=6$), after a 60-min initial preperfusion, the spontaneous tritium overflow approached a rate of $0.0015 \pm 0.00012 \text{ min}^{-1}$ and did not vary significantly throughout the experiment. When the superfused ileal strips were exposed to electrical stimulation, the tritium efflux

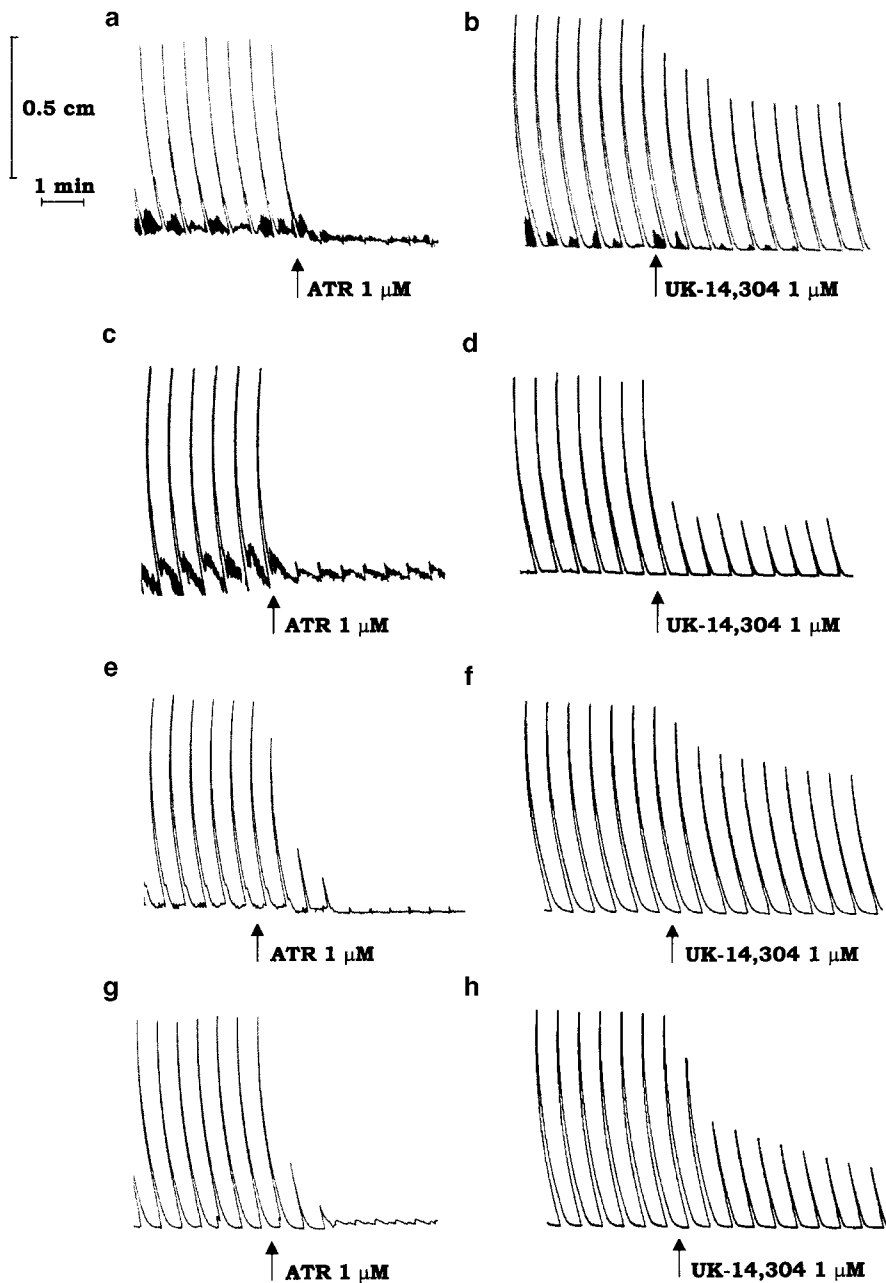


Figure 1 Representative trace recordings showing the effects of atropine (ATR, $1 \mu\text{M}$) or UK-14,304 ($1 \mu\text{M}$) on the contractile activity of longitudinal smooth muscle evoked by transmural electrical stimulation (0.5 ms, 5 Hz, 30 mA, 25 pulses every 60 s) of ileal (a–d) or colonic preparations (e–h), obtained from rats either in the absence (a,b,e,f) or in the presence (c,d,g,h) of DNBS-induced colitis.

increased significantly from 0.0014 ± 0.00010 to $0.0034 \pm 0.00016 \text{ min}^{-1}$ ($P < 0.05$). The increase in tritium outflow was observed usually in four 3-min fractions; the release peaked during this period and then declined exponentially to the prestimulation value. The evoked tritium efflux was $1.72 \pm 0.08\%$ for S_1 and $1.65 \pm 0.06\%$ for S_2 , not significantly different from each other; the S_2/S_1 calculated ratio was $0.96 \pm 0.04\%$ (Figure 3a; Table 3).

In ileal preparations obtained from animals with DNBS-induced colitis ($n=6$), the basal tritium overflow was $0.0017 \pm 0.00014 \text{ min}^{-1}$, and this value did not vary significantly until the end of the experiments. The first electrical

stimulation (S_1) caused a significant increase in tritium efflux, from 0.0017 ± 0.00013 to $0.0029 \pm 0.00024 \text{ min}^{-1}$ ($P < 0.05$). The electrically stimulated increment of tritium outflow was $1.07 \pm 0.09\%$ for S_1 and $1.05 \pm 0.11\%$ for S_2 with a S_2/S_1 ratio of 0.99 ± 0.03 . (Figure 3b; Table 3). The values observed for tritium efflux elicited by S_1 and S_2 were significantly lower than those estimated for ileal preparations obtained from vehicle-treated animals, whereas the S_2/S_1 ratios did not differ (Table 3).

In experiments performed on ileal strips from vehicle-treated animals, UK-14,304 significantly inhibited the electrically induced outflow of tritium in a concentration-dependent

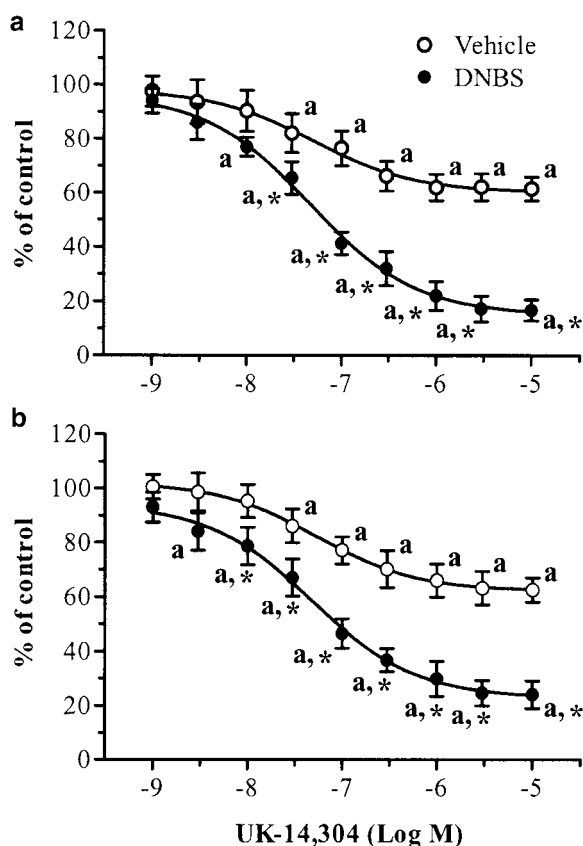


Figure 2 Effects of increasing concentrations of UK-14,304 on cholinergic contractile activity of longitudinal smooth muscle evoked by transmural electrical stimulation of ileal (a) or colonic (b) preparations obtained from rats either in the absence or in the presence of DNBS-induced colitis. Each point represents the mean of seven to eight experiments \pm s.e.m. (vertical bars). Significant difference from the respective values obtained in vehicle-treated animals: * $P < 0.05$; significant difference from the respective control values: ^a $P < 0.05$.

Table 2 Antagonism of rauwolscine or phentolamine against the inhibitory effects of UK-14,304 on cholinergic contractions

	pK_d (ileum)	pK_d (colon)
Vehicle		
Rauwolscine 1 μ M	7.61 (7.20–8.02)	7.73 (7.44–8.02)
Phentolamine 1 μ M	8.94 (8.61–9.27)	9.06 (8.64–9.48)
DNBS		
Rauwolscine 1 μ M	7.55 (7.23–7.87)	7.68 (6.87–8.05)
Phentolamine 1 μ M	9.13 (8.75–9.51)	9.24 (8.89–9.59)

Ileal and colonic preparations were obtained from rats either in the absence or in the presence of DNBS-induced colitis. The cholinergic contractile activity of longitudinal muscle was evoked by electrical transmural stimulation of intestinal tissues (0.5 ms, 5 Hz, 30 mA, 25 pulses every 60 s). Values reported for pK_d , with 95% CI in brackets, were determined from the rauwolscine- or phentolamine-induced shift of the concentration-response curves ($n=6$ for each point) of UK-14,304 at the level of EC_{50} .

manner, with apparent EC_{50} and E_{max} values of 115.4 nM and $-52.5 \pm 3.6\%$ (at 3 μ M), respectively (Figure 4; Table 3). When tested on ileal preparations from rats with colitis, UK-14,304 caused a more prominent inhibition of electrically stimulated

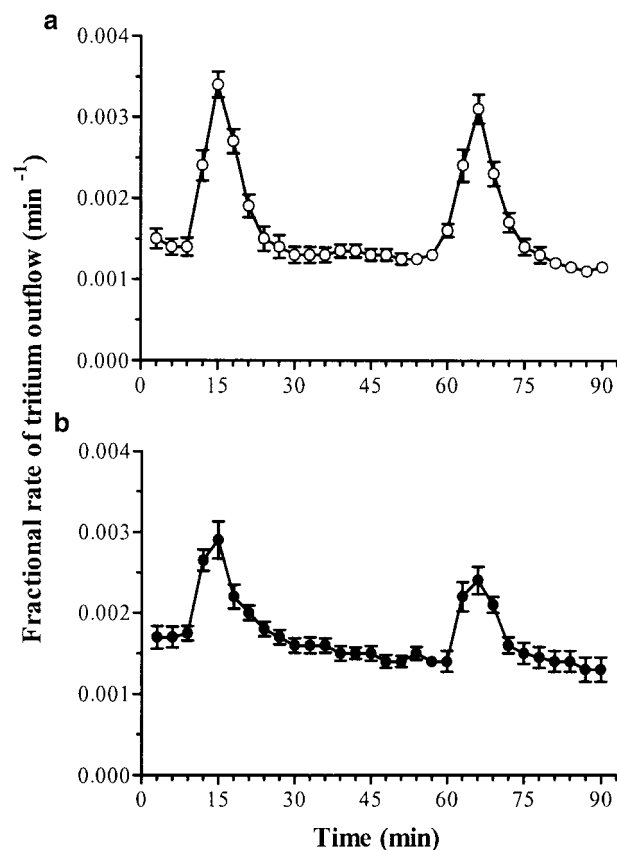


Figure 3 Tritium efflux from longitudinal muscle preparations of rat ileum preincubated with [³H]noradrenaline. (Abscissa): Time of superfusate collection. (Ordinate): Efflux of tritium per min, expressed as fraction of tissue tritium at the onset of the respective collection period. Electrical field stimulation during S_1 and S_2 consisted of 480 pulses (20 V cm^{-1} , 0.5 ms) at 8 Hz. Ileal preparations were obtained from rats either in the absence (a) or in the presence (b) of colitis. Each point represents the mean of six experiments \pm s.e.m. (vertical bars).

tritium efflux (Figure 4; Table 3). In this case, EC_{50} and E_{max} were 97.7 nM and $-70.1 \pm 4.8\%$ (at 3 μ M), respectively. The latter value was significantly higher than that estimated for intestinal tissue isolated from rats in the absence of colitis ($P < 0.05$). Additional experiments were carried out to assay the effects of α_2 -adrenoceptor antagonists on [³H]noradrenaline release. Rauwolscine (1–10 μ M) or phentolamine (1–10 μ M) significantly increased the tritium outflow evoked by electrical stimulation of ileal strips prepared from vehicle-treated rats. The stimulant actions exerted by both antagonists were enhanced when these drugs were assayed on ileal preparations isolated from animals with experimental colitis (Table 3).

RT-PCR analysis of α_{2A} -adrenoceptors

RT-PCR assay of intestinal tissues revealed the expression of α_{2A} -adrenoceptor mRNA at the level of mucosal and muscular layers of both ileum and colon retrieved from vehicle- or DNBS-treated animals (Figure 5). The densitometric analysis of the amplified cDNA bands showed a significant enhancement of α_{2A} -adrenoceptor expression in ileal and colonic muscular layers obtained from animals with colitis (+54.7 and +59.2%, respectively). However, the

Table 3 Effects of UK-14,304, rauwolscine or phentolamine on tritium overflow

	Evoked tritium overflow	
	S_1	S_2/S_1
Vehicle control	1.72 ± 0.08	0.96 ± 0.04
UK-14,304 1 μ M	1.68 ± 0.06	0.52 ± 0.03 ^a
Rauwolscine 1 μ M	1.65 ± 0.09	1.68 ± 0.18 ^a
Rauwolscine 10 μ M	1.76 ± 0.09	2.21 ± 0.23 ^a
Phentolamine 1 μ M	1.73 ± 0.07	1.97 ± 0.15 ^a
Phentolamine 10 μ M	1.70 ± 0.05	2.84 ± 0.17 ^a
DNBS control	1.07 ± 0.09*	0.99 ± 0.03
UK-14,304 1 μ M	1.03 ± 0.07*	0.35 ± 0.06 ^{a,*}
Rauwolscine 1 μ M	1.02 ± 0.05*	2.06 ± 0.11 ^{a,*}
Rauwolscine 10 μ M	1.05 ± 0.05*	2.73 ± 0.13 ^{a,*}
Phentolamine 1 μ M	1.03 ± 0.08*	2.92 ± 0.15 ^{a,*}
Phentolamine 10 μ M	1.11 ± 0.06*	3.66 ± 0.11 ^{a,*}

Longitudinal muscle strips of ileum were obtained from rats either in the absence or in the presence of DNBS-induced colitis. Each preparation was preincubated with [³H]noradrenaline, superfused with Krebs solution, and subjected twice to electrical stimulation (0.5 ms, 20 V cm⁻¹, 8 Hz, 480 pulses). The effects of test drugs on tritium outflow are expressed as S_1 (percentage of the tritium content of the tissue at the onset of the first electrical stimulation), or S_2/S_1 (ratio of the percentage release during the second stimulation over that obtained during the first stimulation). Each value represents the mean of five to six experiments ± s.e.m. Significant difference from the respective values obtained from vehicle-treated rats: * P < 0.05; significant difference from the respective control values: ^a P < 0.05.

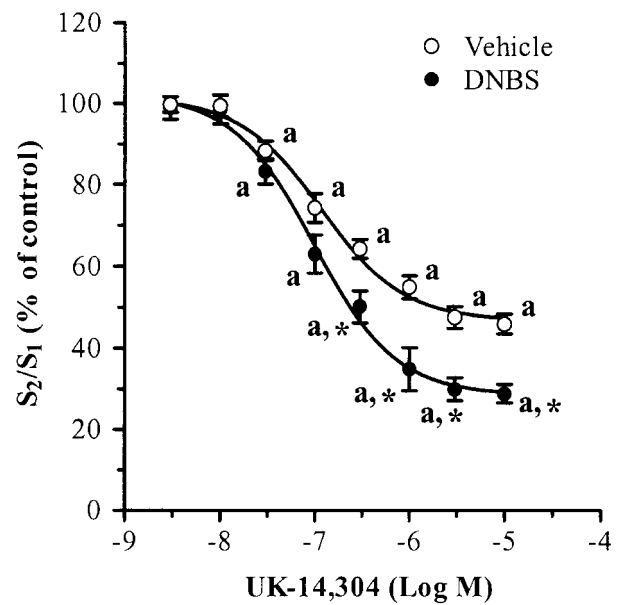


Figure 4 Effects of increasing concentrations of UK-14,304 on tritium outflow evoked by electrical field stimulation of longitudinal muscle preparations of rat ileum preincubated with [³H]noradrenaline. Electrical stimulation during S_1 and S_2 consisted of 480 pulses (20 V cm⁻¹, 0.5 ms) at 8 Hz. Ileal preparations were obtained from rats either in the absence or in the presence of DNBS-induced colitis. Each point represents the mean of five to six experiments ± s.e.m. (vertical bars). Significant difference from the respective values obtained in vehicle-treated animals: * P < 0.05; significant difference from the respective control values: ^a P < 0.05.

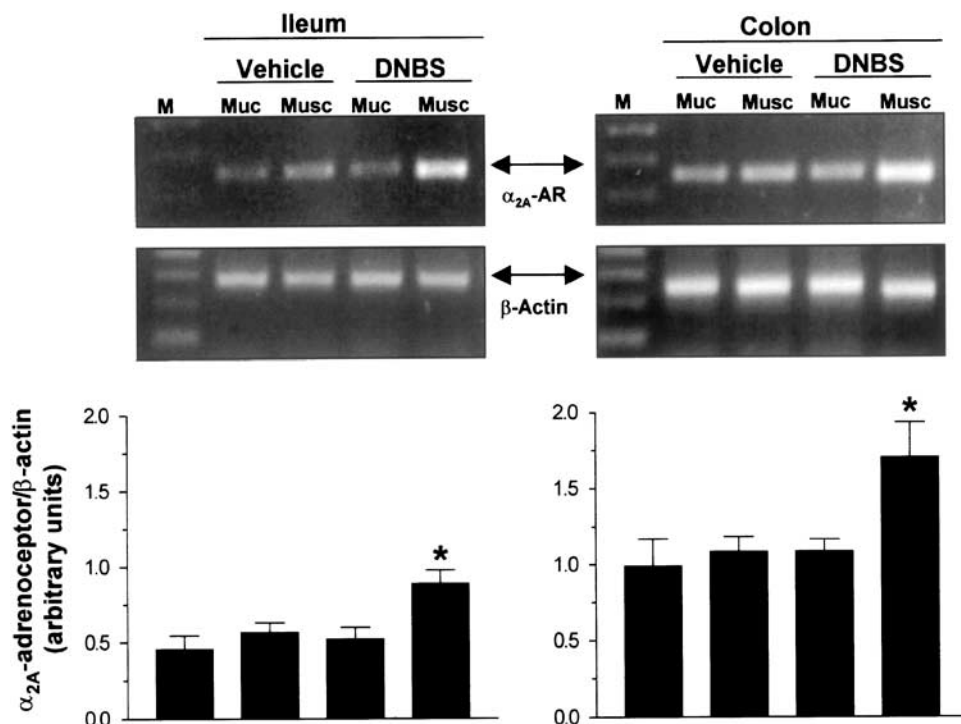


Figure 5 RT-PCR analysis of α_2A -adrenoceptor (α_2A -AR) and β -actin mRNA expression in mucosal (Muc) and muscular (Musc) layers of ileum or colon isolated from animals in the absence (vehicle) or in the presence of DNBS-induced colitis (DNBS). Each panel displays two representative agarose gels, referring to the amplification of α_2A -adrenoceptor and β -actin cDNAs, and a column graph referring to the densitometric analysis of α_2A -adrenoceptor cDNA bands normalized to the expression of β -actin. M = size markers. Each column represents the mean of five separate experiments ± s.e.m. (vertical bars). Significant difference from the respective values obtained in vehicle-treated animals: * P < 0.05.

induction of colitis did not significantly alter the expression of α_{2A} -adrenoceptor mRNA in the mucosa of ileum or colon (+12.9 and +9.8%, respectively) (Figure 5).

Western blot analysis of α_{2A} -adrenoceptors

Western blot analysis of intestinal tissues, excised from vehicle- or DNBS-treated animals, showed that the expression of α_{2A} -adrenoceptors (45 kDa) could be detected at the level of mucosal and muscular layers of both ileum and colon (Figure 6). The densitometric analysis of immunoreactive bands revealed a significant increase of α_{2A} -adrenoceptor

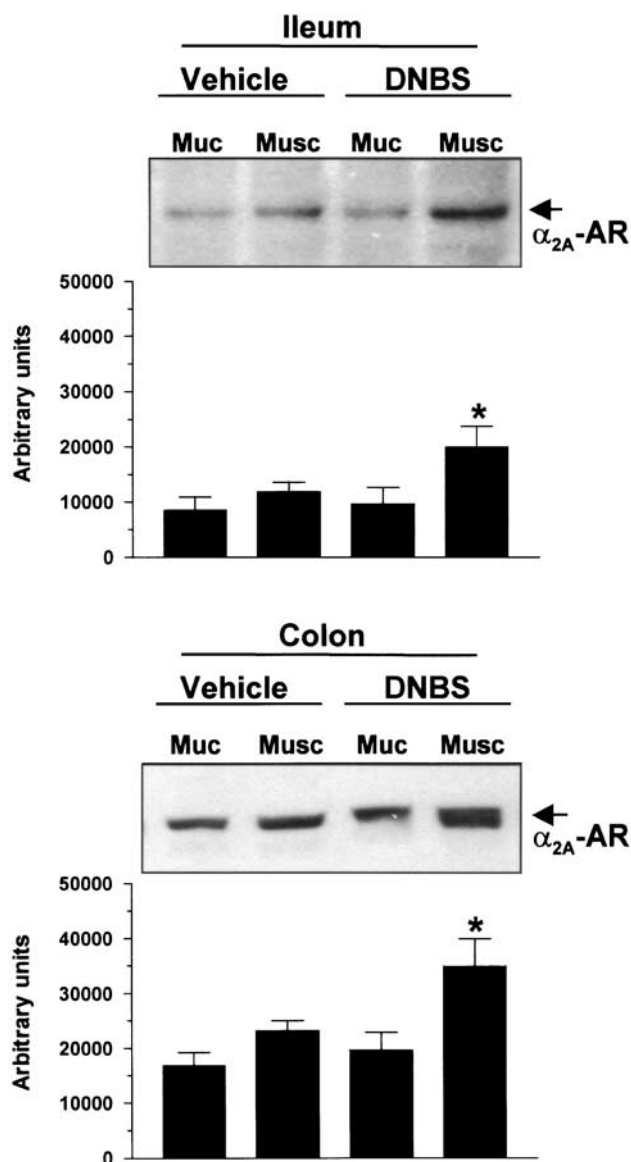


Figure 6 Western blot analysis of α_{2A} -adrenoceptor (α_{2A} -AR) expression in mucosal (Muc) and muscular (Musc) layers of ileum or colon isolated from animals in the absence (vehicle) or in the presence of DNBS-induced colitis (DNBS). Each panel displays a representative blot and a column graph referring to the densitometric analysis of immunoreactive bands. Each column represents the mean of four separate experiments \pm s.e.m. (vertical bars). Significant difference from the respective values obtained in vehicle-treated animals: * $P < 0.05$.

expression in both ileal and colonic muscular layers retrieved from animals subjected to DNBS-induced colitis (+67.8 and +50.8%, respectively). By contrast, treatment with DNBS did not significantly affect the expression of α_{2A} -adrenoceptors in the mucosal layer of both ileum and colon (+12.8 and +16.5%, respectively) (Figure 6).

Gastrointestinal transit

The first series of experiments was aimed to assess the gastrointestinal transit in the absence of colitis. In vehicle-treated animals, the distance travelled by the charcoal suspension accounted for $72.9 \pm 6.4\%$ ($n = 10$) of the total length of small intestine. UK-14,304 ($0.2 \mu\text{mol kg}^{-1}$ i.p.) caused a significant inhibition of gastrointestinal transit, whereas rauwolscine ($1 \mu\text{mol kg}^{-1}$ i.p.) was without effect (Figure 7a). Similar results were obtained with UK-14,304 and rauwolscine when they were assayed on animals subjected to pretreatment with guanethidine in order to ablate the sympathetic nervous pathways (Figure 7c). In both cases, the inhibitory effects of UK-14,304 on gastrointestinal transit were antagonized by rauwolscine (Figure 7a and c).

In the second series of experiments, the gastrointestinal transit was evaluated in rats with DNBS-induced colitis. In this case, the distance travelled by the charcoal suspension was $54.3 \pm 4.4\%$ of the total small intestine ($n = 8$) (Figure 7b). Such a value was significantly lower than that measured in the absence of experimental colitis ($P < 0.05$). Under these conditions, UK-14,304 ($0.2 \mu\text{mol kg}^{-1}$ i.p.) was less effective in reducing the gastrointestinal transit, whereas rauwolscine ($1 \mu\text{mol kg}^{-1}$ i.p.) caused a significant enhancement (Figure 7b). When animals with colitis were subjected to pretreatment with guanethidine, the gastrointestinal transit was $67.2 \pm 2.8\%$ ($n = 7$), this value being not significantly different from that estimated in the absence of colitis. In this setting, the inhibitory effect of UK-14,304 on the gastrointestinal transit was significantly higher than that estimated in vehicle-treated rats, whereas the stimulant action of rauwolscine no longer occurred (Figure 7d). As observed in the absence of colitis, the inhibitory actions of UK-14,304 on both guanethidine-untreated and guanethidine-treated animals were counteracted by pretreatment with rauwolscine (Figure 7b, d).

Discussion

Intestinal inflammation is associated with alterations of gut functions (motility, absorption/secretion), which may contribute to generation of various symptoms. Diarrhoea is not the only symptom experienced by patients with inflammatory bowel disease, since they also complain of changes in bowel habit, bloating, abdominal pain/discomfort, nausea, and other irritable bowel syndrome-like symptoms (Hendrickson *et al.*, 2002). The pathophysiological mechanisms accounting for this complex array of symptoms have not been fully clarified. Changes in both intrinsic and extrinsic enteric nerves may contribute to altered motor function in the inflamed gut (Collins, 1996; Sharkey & Kroese, 2001). The present study demonstrates that the induction of colitis is associated with significant changes in the α_2 -adrenoceptor-mediated control of

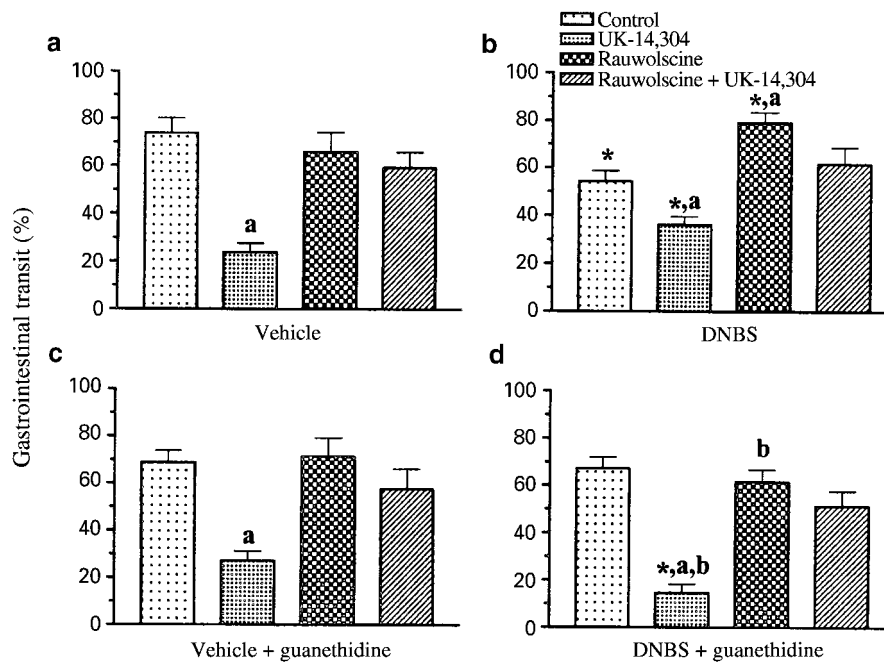


Figure 7 Effects of i.p. administrations of UK-14,304 ($0.2 \mu\text{mol kg}^{-1}$), rauwolscine ($1 \mu\text{mol kg}^{-1}$), or rauwolscine plus UK-14,304 on gastrointestinal transit in rats. Data displayed in panels a and b were obtained from animals in the absence or in the presence of DNBS-induced colitis, respectively. Data displayed in panels c and d refer to experiments where both vehicle-treated and DNBS-treated animals received guanethidine $70 \mu\text{mol kg}^{-1}$ i.p. daily for two consecutive days, before performing the gastrointestinal transit assay. Each value represents the mean of seven to ten experiments \pm s.e.m. (vertical bars). Significant difference from the respective values obtained in vehicle-treated animals: $^*P < 0.05$; significant difference from the respective control values: $^aP < 0.05$; significant difference from the respective values obtained in guanethidine-untreated animals: $^bP < 0.05$.

both enteric neurotransmission and digestive motility, not only in the colon but also at distant sites.

When applied to ileal or colonic preparations from rats treated with intrarectal vehicle (uninflamed), the agonist UK-14,304 inhibited the electrically induced cholinergic contractions with high potency and moderate efficacy, suggesting that the α_2 -adrenoceptors play a role in the prejunctional modulation of cholinergic transmission under standard conditions. In the presence of colitis, UK-14,304 decreased with higher efficacy the evoked cholinergic contractions at both colonic and ileal level. These results provide the first demonstration that colonic inflammation enhances the α_2 -adrenoceptor-mediated inhibitory control of enteric cholinergic transmission, and they further expand the available knowledge on neuroplastic changes occurring in the inflamed gut (De Giorgio *et al.*, 2001; Sharkey & Kroese, 2001). Of interest, the present findings are in keeping with previous data showing a significant decrease in electrically induced acetylcholine release from myenteric plexus preparations obtained from the small intestine of nematode-infected rats (Collins *et al.*, 1989).

Three genetically distinct α_2 -adrenoceptor subtypes, named α_{2A} , α_{2B} and α_{2C} , have been identified in mammals (Hieble *et al.*, 1997). In the present investigation, an interaction with α_{2A} -adrenoceptor subtypes seems to account for the inhibitory actions of UK-14,304 on cholinergic motility in both uninflamed and inflamed rats. Indeed, in both ileal and colonic tissues, phentolamine was more potent than rauwolscine in antagonizing the inhibitory effects of UK-14,304. This pharmacological pattern has been previously reported for rat, guinea-pig, and mouse α_{2A} -adrenoceptors, and is not compatible with affinity profiles of α_{2B} - or α_{2C} -adrenoceptors

(Simonneaux *et al.*, 1991; Trendelenburg *et al.*, 1997). Consistent with this view, recent experiments, performed on α_{2A} -adrenoceptor knockout mice, have provided conclusive evidence that enteric cholinergic neurons are subjected to presynaptic modulation by α_{2A} -adrenoceptor subtypes (Scheibner *et al.*, 2002).

Beside the participation of intrinsic cholinergic pathways in the processes of remodelling and adaptation of enteric neurons in intestinal inflammation (Collins *et al.*, 1989; Hosseini *et al.*, 1999), several lines of evidence suggest that the extrinsic sympathetic nerves supplying the gut are involved (Collins, 1996; Sharkey & Kroese, 2001). Substantial changes in the evoked noradrenaline release have also been shown in experimental gut inflammation (Swain *et al.*, 1991) both at inflamed and distant noninflamed sites (Jacobson *et al.*, 1995). In keeping with these studies, the evoked tritium outflow in the present ileal preparations, preincubated with [^3H]noradrenaline, was significantly lower in rats with colitis, as compared to uninflamed animals. Furthermore, our data demonstrate that the application of UK-14,304 to ileal preparations from animals with or without colitis significantly depresses the electrically evoked tritium efflux, whereas phentolamine or rauwolscine exerts potentiating effects. The latter observation might appear inconsistent with data obtained from *in vitro* experiments on muscle contraction, where both phentolamine and rauwolscine *per se* failed to affect the electrically induced cholinergic responses. This apparent discrepancy may be explained considering that muscle contraction experiments were performed in the presence of guanethidine, which, because of its action on noradrenergic fibres, prevented the enhancing effect of α_2 -adrenoceptor antagonists.

The most interesting observation is that the α_2 -adrenoceptor ligands act on the evoked [3 H]noradrenaline with different degrees of efficacy, when assayed on ileal preparations retrieved from animals with or without colitis. A comparison of the concentration–response curves, constructed for UK-14,304, clearly indicates that this agonist inhibits with greater efficacy the release of [3 H]noradrenaline in ileal tissues from inflamed animals. Likewise, the stimulant actions of rauwolscine or phentolamine on the evoked tritium output are further enhanced when these antagonists are tested on ileal preparations from animals with colitis. These findings suggest that, after induction of colitis, the sympathetic pathways supplying the gut undergo an enhanced prejunctional modulation, mediated by α_2 -adrenoceptors located on noradrenergic nerve endings.

Three major mechanisms may be hypothesized to account for the enhanced inhibitory control mediated by α_2 -adrenoceptors on cholinergic and noradrenergic transmissions in animals with colitis: (1) increased receptor affinity; (2) increased receptor density and/or recruitment and (3) changes in transduction pathways. In the present study, both RT–PCR and Western blot assays provide consistent evidence that the induction of colitis is associated with a significant increase in α_2 -adrenoceptor mRNA and protein expression in the muscle layers. These findings suggest that colonic inflammation promotes an increment of α_2 -adrenoceptor density within the neuromuscular compartments of both inflamed and noninflamed gut. In support of this view, the inhibitory effects of UK-14,304 on cholinergic motility and [3 H]noradrenaline release in animals with colitis are characterized by higher E_{\max} , but similar EC_{50} values, than in uninflamed animals. These results expand previous radioligand binding data, showing that intestinal inflammation is associated with an upregulation of α_2 -adrenoceptors in gut tissues (Martinolle *et al.*, 1993). However, the possibility that our results might be interpreted in terms of changes in the efficiency of receptor–effector coupling mechanisms cannot be ruled out, and this issue should be addressed in separate studies. Although the mechanisms underlying the enhancement of α_2 -adrenoceptor expression and function at noninflamed gut sites were not specifically investigated in the present study, two main hypotheses can be proposed to explain these findings: (1) changes at distant sites might depend on systemic effects elicited by inflammatory/immune mediators released from colonic inflammatory lesions; this mechanism has been previously suggested by Jacobson *et al.* (1995) to explain the reduced noradrenaline release from preparations of non-inflamed transverse colon and ileum in rat models of colitis induced by 2,4,6-trinitrobenzenesulphonic acid (TNBS) or nematodes; (2) neural and receptor changes at remote sites might be related to nerve reflex pathways, as proposed by Moreels *et al.* (2001) for rat TNBS-induced ileitis.

To verify whether changes in α_2 -adrenoceptor expression, evoked by gut inflammation, might reflect variations of *in vivo* intestinal motility, the effects of UK-14,304 and rauwolscine have been tested in a model of gastrointestinal transit. Data obtained from these experiments refer only to transit in the small intestine, and therefore a correlation between *in vitro* and *in vivo* results was not made for colonic motility. In the absence of colitis, UK-14,304 causes a rauwolscine-sensitive inhibition of small intestinal transit. This finding is in line with previous reports suggesting that the activation of α_2 -adrenoceptors may lead to a decreased intestinal motor activity, via the inhibition

of enteric cholinergic neurons (De Ponti *et al.*, 1996; Stebbing *et al.*, 2001). During colitis, gastrointestinal transit was reduced and, at variance with data obtained from *in vitro* experiments, UK-14,304 exerted a moderate inhibitory effect. In this setting, rauwolscine completely restored the propulsive activity, indicating that, in the presence of colonic inflammation, the sympathetic nerve pathways mediate a tonic inhibitory control on bowel motility through the activation of α_2 -adrenoceptors. Such hypothesis was tested in animals treated with guanethidine, to induce a pharmacological ablation of sympathetic pathways. In this case, the stimulant action of rauwolscine on gastrointestinal transit of inflamed animals no longer occurred, and UK-14,304 decreased the charcoal propulsion to a greater extent than in uninflamed rats. These findings, taken together with data from the present experiments on *in vitro* cholinergic motility, suggest that the induction of colonic inflammation causes a tonic activation of noradrenergic sympathetic nerves and a concomitant increase of α_2 -adrenoceptor expression, resulting in an enhanced inhibitory control of digestive motor activity. On the other hand, the reduced noradrenaline release, observed in our *in vitro* experiments, might reflect the activation of counterregulatory mechanisms against the increase of sympathetic tone promoted by intestinal inflammation. In line with this view, evidence of altered sympathetic activity was previously obtained both in patients with inflammatory bowel disease (Lindgren *et al.*, 1991) and in preclinical models of intestinal inflammation (Sharkey & Kroese, 2001), whereas *in vitro* experiments on intestinal tissues showed a decrease in noradrenaline release at inflamed and noninflamed sites (Collins, 1996).

The use of guanethidine as an adrenergic neuron blocker deserves some comments. We are aware that guanethidine has been suggested to act also as a nicotinic receptor blocker at the level of enteric neurons (Blommaart *et al.*, 1999). Therefore, when interpreting the present results from guanethidine-treated animals, we took into account the possibility that nicotinic transmission might be implicated in the reduction of intestinal transit elicited by colitis. However, the putative antagonism of guanethidine on nicotinic receptors seems to occur only at high concentrations ($>10\ \mu\text{M}$), and was not substantiated by radioligand binding experiments (Blommaart *et al.*, 1999). Furthermore, the colitis-induced reduction of intestinal transit was fully reversed by rauwolscine, suggesting the presence of a sympathetic inhibitory tone on digestive motility, driven via activation of α_2 -adrenoceptors. On this basis, it appears unlikely that guanethidine affected the intestinal transit in inflamed animals via an interaction with nicotinic receptors.

In conclusion, the results obtained in the present study indicate that, in the presence of intestinal inflammation, prejunctional α_2 -adrenoceptors contribute significantly to pathophysiological changes characterized by an enhanced inhibitory control of cholinergic and noradrenergic neurotransmission both at inflamed and non-inflamed distant sites. Direct evidence was also obtained that an increased expression of α_{2A} -adrenoceptors within the enteric nervous system may contribute to such modulatory actions.

The present work was supported in part by a grant for scientific research issued from the Italian Ministry of Education, University and Research (COFIN 1999, Project number 9905222532).

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(Received October 8, 2002

Revised December 11, 2002

Accepted February 21, 2003)