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Evidence for mast cell, leukotriene and nitric oxide involvement in the regulation of the adrenoceptor number of inflamed small intestine in guinea pigs

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Abstract Changes in the populations of neurotransmitter receptors involved in the control of intestinal smooth muscle function have been associated with the altered motility of the inflamed gut. Thus, trinitrobenzenesulphonic acid (TNBS)-induced gut inflammation is accompanied by an increase in α - and a decrease in β -adrenoceptor numbers in guinea pig small intestine. In the present study, we investigated the effects of anti-inflammatory compounds (cyclooxygenase inhibitor indomethacin, lipooxygenase inhibitor MK-886, nitric oxide synthase inhibitor N^G -nitro-L-arginine methylester (L-NAME), mast cell stabilizer doxantrazole) on TNBS-induced adrenoceptor changes. Smooth muscle adrenoceptor populations, labelled by subtype-specific radioligands 6 days after TNBS, were significantly different from those of sham-treated controls: $\alpha 1$ - and $\alpha 2$ -adrenoceptor numbers increased by more than 50%, while β -adrenoceptor numbers decreased by more than 50%. These changes, associated with severe inflammation as assessed histologically and by myeloperoxidase assay, were prevented by doxantrazole or L-NAME, and only partly by MK-886. In contrast, indomethacin did not prevent these changes. It appears then that: (a) mast cell mediators, nitric oxide and leukotrienes are likely to contribute to TNBS-induced changes in adrenoceptor populations in the guinea pig inflamed intestine; (b) there is no evidence for prostanoïd involvement in this process. It was suggested that changes in smooth muscle adrenoceptor populations may be an important mechanism by which gut inflammation alters intestinal motility.

Keywords adrenoceptor, inflammation, intestinal motility, leukotriene, mast cell, nitric oxide.

INTRODUCTION

Intestinal inflammation is associated with altered intestinal motility both in the human¹ and animal species.² The mechanisms underlying these motility disturbances are poorly understood, but may follow alterations in intestinal smooth muscle receptors and/or innervation. Indeed, marked suppression of the norepinephrine release from the myenteric plexus has been described in the inflamed intestine of rats infected by *Trichinella spiralis*.³ We have previously reported changes in the concentrations of smooth muscle adrenoceptors in trinitrobenzenesulphonic (TNBS) acid-inflamed small intestine in guinea pigs.⁴ Nevertheless, the mechanisms underlying these changes in smooth muscle adrenoceptor number are not precisely known.

Infiltration of intestinal tissues by lymphocytes, eosinophils and neutrophils,^{5,6} as well as increased mast cell counts,^{7,8} are observed during gut inflammation. Moreover, prostaglandins (PGs) and leukotrienes (LTs), the concentrations of which are increased during inflammation, are probably involved in the course of intestinal inflammatory conditions.^{9,10} Nitric oxide (NO) is also strongly involved in intestinal inflammation, as evidenced by an enhanced release of NO in response to gut injury and the improvement of the inflammatory condition by treatment with NO synthase inhibitors.^{11,12}

Pharmacological tools with putative anti-inflammatory potential may prove useful in determining possible mechanisms: mast cell degranulation can be prevented by the mast cell stabilizer doxantrazole,¹³ NO generation can be blocked using the NO synthase inhibitor N^G -nitro-L-arginine methylester (L-NAME),¹² leukotrienes by the lipooxygenase inhibitor MK-886¹⁴

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and prostanoids by the prostaglandin synthase inhibitor indomethacin.¹⁵ With this specific aim, we performed [³H]prazosin, [³H]rauwolscine and [³H]dihydroalprenolol (DHA) binding in order to label α_1 -, α_2 - and β -adrenoceptors, respectively, in smooth muscle membranes from both 'sham-treated' and 'TNBS-treated' animals, which received these different pharmacological treatments. In parallel, the activity of the enzyme myeloperoxidase (MPO) was assessed and histological studies were performed in order to evaluate neutrophil activation and intestinal tissue damage associated with TNBS experimental inflammation.

METHODS

Animal preparation and experimental inflammation

The study was conducted in ten groups of six male guinea pigs (Interfauna, Loches, France) weighing 300–350 g. After a 24-h fast, the animals were submitted to abdominal laparotomy under aseptic conditions and deep anaesthesia (acepromazine, 2 mg kg⁻¹; ketamine 80 mg kg⁻¹; intraperitoneally, i.p.). Animals received intrajejunally at day 0 either 0.3 ml 0.9% NaCl (five 'sham-treated' groups), or 0.3 ml of a mixture of TNBS (80 mg kg⁻¹) in 50% ethanol solution (five 'TNBS-treated' groups). (Throughout the present paper 'TNBS' will stand for 'TNBS in 50% ethanol solution' which, both together, constitute the actual inflammatory compound.)

Animal treatments

In addition to the intrajejunal instillation of saline at day 0, animals of the so-called 'sham-treated' groups were given twice a day during 7 days (i.e. from day -1 to day 5) the following treatments. 'Control group': saline alone (0.3 ml NaCl 0.9%, i.p.); 'DOX group': the mast cell stabilizer doxanzazole (5 mg kg⁻¹, i.p.) in 0.3 ml saline,¹³ 'MK group': the lipoxygenase inhibitor MK-886 (5 mg kg⁻¹, orally) in 0.3 ml saline,¹⁴ 'NAME group': the NO synthase inhibitor L-NAME (10 mg kg⁻¹, i.p.) in 0.3 ml saline,¹² 'INDO group': the cyclooxygenase inhibitor indomethacin (1 mg kg⁻¹, i.m.) in 0.3 ml 5% sodium bicarbonate solution.¹⁵

Similarly, in addition to the intrajejunal instillation of 0.3 ml TNBS solution at day 0, animals of the so-called 'TNBS-treated' groups were given twice a day during 7 days (i.e. from day -1 to day 5) treatments similar to those described above, to constitute the following experimental groups: 'TNBS', 'TNBS-DOX', 'TNBS-INDO', 'TNBS-MK' and 'TNBS-NAME', respectively.

Animals were killed 6 days after intrajejunal injection of saline or TNBS. Before being killed by exsanguination animals were anaesthetized with urethane (100 mg kg⁻¹, i.p.). The small intestine was rapidly collected and thoroughly rinsed in ice-cooled physiological saline solution. All procedures were approved by the local INRA Animal Care and Use Committee.

Histology

For each animal, five pieces of ileum (about 0.5 cm each) were randomly collected for histological studies from 20 to 10 cm oral to the ileo-cecal junction, and rapidly immersed in 10% buffered formalin for 24 h in an attempt to evaluate the extent of inflammatory damages as well as the effects of the various treatments described above. After dehydration through graded strengths of ethanol, intestinal samples were cleared in xylene and imbedded in Histomed[®] standard (Labo Moderne, Paris, France). Transverse sections were cut at a thickness of 5 μ m, deparaffinized in xylene and rehydrated through an ethanol series. Thereafter, slides were routinely stained with haemalum and eosine.

Myeloperoxidase activity assay

The activity of intestinal myeloperoxidase (MPO), a specific enzyme marker of polymorphonuclear neutrophil primary granules, was measured in samples prepared from full thickness ileal wall fractions according to the method of Bradley *et al.*¹⁶ with slight modifications. A small piece of ileum (about 0.5 cm) was suspended in hexadecyl trimethylammonium bromide buffer (HTAB 0.5% in 50 mM phosphate buffer, pH 6.0), a detergent that releases MPO from the primary granules. Intestinal tissues were minced with scissors, homogenized using a Polytron (20 sec, at maximal setting) and then filtered through two layers of surgical gauze. The remaining filtrate was sonicated on ice (10 sec) and centrifuged at 40 000 \times g for 15 min at 4°C. The pellet was discarded and the supernatant was assayed spectrophotometrically for MPO activity: 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer (pH 6.0), containing 0.167 mg ml⁻¹ O-dianisidine hydrochloride and 0.0005% hydrogen peroxide. The kinetics of changes in absorbance at 460 nm were measured with an Uvikon 860 spectrophotometer (Kontron Instruments, St Quentin-en-Yvelines, France). The protein concentration was determined by the method of Lowry (Bio-Rad Detergent Compatible Protein Assay, Bio-Rad, Ivry, France) and MPO activity expressed as IU mg⁻¹ of protein. One IU of MPO activity was defined as that

degrading 1 nM of peroxide per minute at 25°C. The assay sensitivity was 0.1 IU ml⁻¹ and the mean intra-assay coefficient of variation was 4%.

Membrane preparation

A portion of about 50 cm of jejunum ending at 10 cm oral to the ileo-cecal junction was excised, cut open lengthwise and rinsed with ice-cooled physiological saline. The mucosa was removed under a stereomicroscope, so that only smooth muscle layers with the inherent enteric nerves remained. The tissue was minced with scissors, crushed in an electric mill, and homogenized with a Polytron (three bursts of 10 sec at maximal setting, interspersed with 20-sec cooling intervals) in 10 volumes of ice-cold physiological buffer solution (pH 7.4) consisting of (mM): KCl, 4.6; MgSO₄, 1.16; CaCl₂, 2.5; NaH₂PO₄, 1.16; NaCl, 115.5; NaHCO₃, 21.9 and glucose, 11.1. The homogenate was centrifuged twice at 1000 × g for 10 min at 4°C, with intermediate rinsing and resuspension in ice cold buffer using a glass/glass Potter (Braun, Melsungen, Germany). The supernatants were again centrifuged twice at 10 000 × g for 10 min at 4°C; the pellets were discarded while the resulting supernatants were centrifuged at 100 000 × g for 30 min at 4°C. The final supernatant was discarded and the pelleted membranes were weighted and resuspended in a buffer volume adequate to get a final protein concentration of 1–3 mg ml⁻¹ (Bio-Rad Protein Assay, Bio-Rad, Ivry, France). This subcellular fraction, contained mainly myocyte (i.e. post-junctional) plasma membranes but also, some innermost components of myenteric innervation (i.e. pre-junctional synaptosomes).^{17–19}

Binding assay

Adrenoceptor subtypes were characterized by binding of tritiated radioligands, [³H]prazosin, [³H]rauwolscine and [³H]DHA labelling α₁-, α₂- and β-adrenoceptors, respectively. Binding conditions for the three radioligands (i.e. buffers, time and temperature of incubation) were previously described.⁴ Buffers (pH 7.4) were: 50 mM TRIS-HCl, 10 mM MgCl₂ for [³H]prazosin and [³H]DHA binding, and 20 mM MOPS-Na, 0.5 mM MgCl₂ for [³H]rauwolscine binding. Incubations were performed in duplicate for 30 min at 30°C. The total volume of the reaction mixture was 400 μl and contained 85–120 μg of membrane protein. For α₁-adrenoceptor saturation studies, 0.0125–2 nM [³H]prazosin were added; non-specific binding was measured in the presence of 20 μM of unlabelled prazosin. For α₂-adrenoceptor saturation studies, 0.25–15 nM [³H]rauwolscine were added; non-

specific binding was measured in the presence of 20 μM of unlabelled yohimbine. For β-adrenoceptor saturation studies, 0.25–15 nM [³H]DHA were added and non-specific binding was measured in the presence of 20 μM alprenolol. Incubation was stopped by adding 4 ml ice-cold incubation buffer to the tubes. Bound radioactivity was separated by filtration through Whatman GF/C glass-fibre filters, presoaked in buffer containing 0.3% polyethylenimine (used to reduce the amount of radioactivity unspecifically adsorbed to the filter). Filters were washed twice with 10 ml ice-cold buffer, dried under vacuum, and placed into 4 ml scintillation solution (Pico-fluor, Packard, Rungis, France). Radioactivity was measured with a liquid scintillation counter (Beta-matic V, Kontron Instruments, St Quentin-en-Yvelines, France) with an efficiency of 50–52%.

Chemicals

[³H]prazosin (24 Ci mmol⁻¹), [³H]rauwolscine (70–85 Ci mol⁻¹) and [³H]DHA (70–85 Ci mol⁻¹) were purchased from Amersham International plc (Amersham, Bucks., UK). Unlabelled prazosin was a gift from Pfizer France (Paris, France), doxantrazole from The Wellcome Research Laboratories (Beckenham, UK) and MK-886 from Merck Sharp and Dohme (St Louis, MO, USA). Yohimbine, alprenolol and indomethacin were from Sigma (St Quentin Fallavier, France). Trinitrobenzenesulphonic acid was from Fluka (St Quentin Fallavier, France). Other chemicals were of high analytical grade.

Statistical and binding data analysis

Data were expressed as the mean ± SEM for each experimental group, with at least three determinations performed in duplicate per group. Data from binding saturation studies were analysed on a personal micro-computer with the nonlinear regression fitting program EBDA/LIGAND. All data were analysed using models for one and two sites of binding. Statistical differences were determined using non-parametric analysis of variance (Kruskal–Wallis test) and Mann–Whitney *U* test.

RESULTS

Histological study

Inflammatory sites were not detected macroscopically or microscopically in intestinal tissue samples from any of the 'sham-treated' groups (Fig. 1a). These were animals in which saline (but not TNBS) was instilled intrajejunally, and that were further given either saline,



Figure 1 Transverse section of guinea pig ileum from: (a) 'Control group', i.e. animals given 0.9% NaCl saline intraperitoneally: normal aspect of the full thickness of the ileal wall; (b) 'TNBS group', i.e. animals killed 6 days after being given TNBS intrajejunally: note the dramatic enlargement of the lymphatic central capillary of the villi, and that the interglandular spaces of the lamina propria are oedematous. (Original magnification $\times 95$).

doxantrazole, indomethacin, MK-886 or L-NAME. In contrast, critical inflammation was observed in intestinal tissue samples taken from animals which received TNBS into the jejunum and saline systemically ('TNBS group'). It was characterized by severe transmural inflammation, extended randomly from the injection site in the jejunum, down to the distal ileum. Macroscopically, there were areas of grossly visible bowel wall thickening, associated with major sites of inflammation and/or mucosal ulceration. Microscopically, an enlargement of the central lymphatic capillary in the core of the villi, and an oedema of the lamina propria were observed (Fig. 1b). In some areas, ulcerative foci extended through the mucosa, and the lamina propria was infiltrated by mononucleated cells (mast cells and macrophages), and scattered eosinophils and neutrophils. In the 'TNBS-INDO' group (Fig. 2a), the histological profile was similar, thus highlighting the absence of beneficial effects after blockade of prostaglandin synthesis. In contrast, in the 'TNBS-MK' group (Fig. 2b), as well as in the 'TNBS-DOX' and 'TNBS-NAME' groups, healing patterns were observed. These latter were characterized macroscopically by the almost normal appearance of the major part

of the intestinal length, with focal hyperemia and rare ulcerative foci, microscopically, only oedema and vascular stasis were detected.

Myeloperoxidase activity

The effect of TNBS on MPO activity, assayed in full thickness ileal wall preparations, is summarized in Table 1. Intrajejunal instillation of TNBS produced a significant, four-fold increase in MPO activity in the ileum ($P < 0.05$, compared to 'Control group'). In the 'TNBS-treated' groups, this increase in MPO activity was nearly halved ($P < 0.05$), compared to 'TNBS group', by daily treatment with either doxantrazole, MK-886 or L-NAME, while indomethacin had no effect. In contrast, there were no significant differences in MPO activity values between any of the 'sham-treated' groups (Table 1).

Binding saturation studies

The characteristics of [3 H]prazosin, [3 H]rauwolscine and [3 H]DHA, binding to intestinal smooth muscle mem-

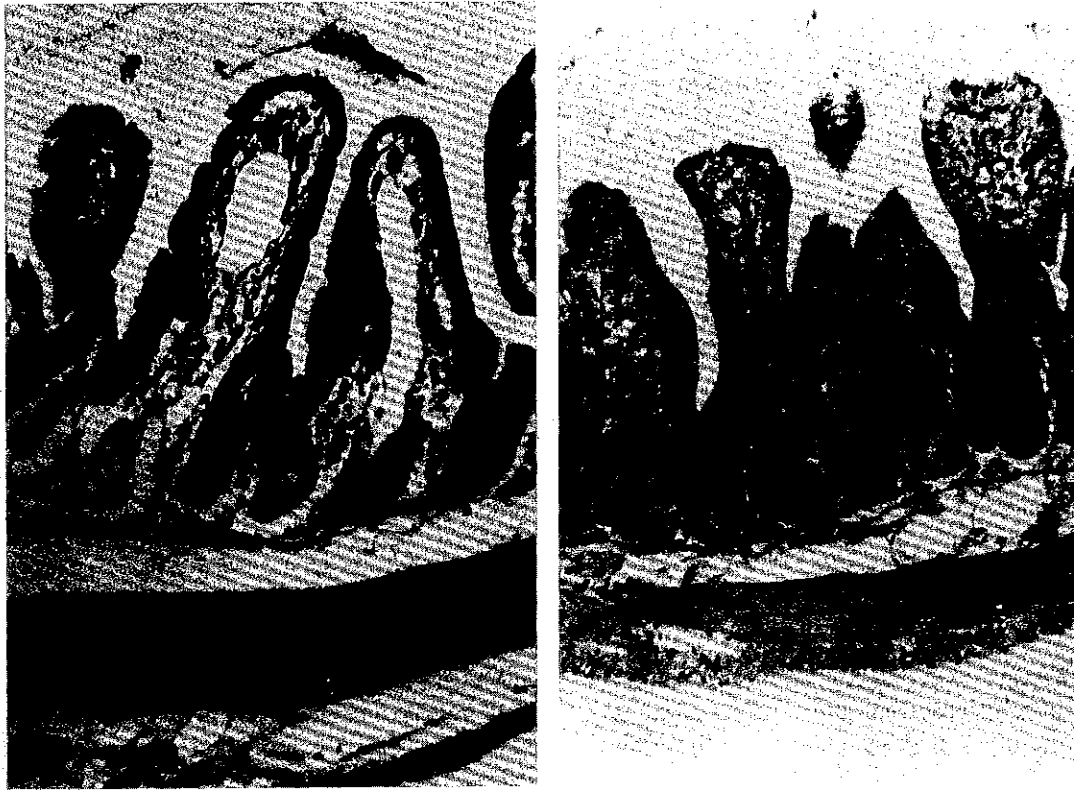


Figure 2 Transverse section of guinea pig ileum from: (a) 'TNBS-INDO group' at day 6 after intraluminal instillation of TNBS; note that the central lymphatic capillary in the core of the villi is distended; (b) 'TNBS-MK group': note the normal appearance of intestinal villi, lamina propria and muscular layers. (Original magnification $\times 95$).

branes from each group under study, are reported in Table 2. For the different experimental groups, specific binding of either radioligand was a saturable process of high affinity. Non-specific binding, determined using unlabelled ligands, increased linearly with radioligand concentrations. For each group and each radioligand, saturation data analysed by the EBDA/LIGAND program were better fitted by monoexponential equations, consistent with interactions at only one class of binding sites. Scatchard analysis, which yielded single straight lines (Fig. 3), and Hill coefficients, which were close to unity for each group (data not shown), confirmed that a

single class of non-interacting binding sites was labelled by either radioligand. Adrenoceptor subtype affinities, expressed as dissociation constants (K_D) for each radioligand, showed no significant differences ($P > 0.05$) between groups. In contrast, adrenoceptor subtype densities or numbers, expressed as maximal binding capacities (B_{max}), varied between groups. Indeed, B_{max} values for both α -adrenoceptor subtypes were significantly ($P < 0.05$) increased at day 6 after TNBS injection in the 'TNBS group', compared to the 'Control group'. Conversely, B_{max} values for β -adrenoceptors were decreased ($P < 0.05$) at the same time.

Table 1 MPO activity in 'sham-treated' and 'TNBS-treated' groups in full thickness ileal tissue samples (see text for definition of experimental groups). MPO activity is expressed for each group as the mean \pm SEM (IU mg^{-1} of protein) of six experiments assayed in duplicate. Superscripts indicate statistical differences ($P < 0.05$) either from the corresponding 'sham-treated' (^a) or from the TNBS group (^b)

| | Saline | Doxantrazole | MK-886 | Indomethacin | L-NAME |
|--------------|-----------------|---------------------|---------------------|-----------------|---------------------|
| Sham-treated | 0.9 ± 0.2 | 1.3 ± 0.7 | 0.6 ± 0.2 | 0.8 ± 0.2 | 0.9 ± 0.4 |
| TNBS-treated | 4.2 ± 0.3^a | $2.6 \pm 0.2^{a,b}$ | $2.2 \pm 0.4^{a,b}$ | 5.0 ± 0.5^a | $1.6 \pm 0.3^{a,b}$ |

Table 2 Binding saturation parameters (B_{\max} and K_D) for each radioligand in smooth muscle cell membranes from 'sham-treated' and 'TNBS-treated' groups (see text for definition of experimental groups). Binding parameters were calculated by the EBDA/LIGAND program. Each value was the mean \pm SEM of at least three determinations performed in duplicate per group. The affinities of α_1 -, α_2 - and β -adrenoceptor subtypes for their respective radioligands were expressed as equilibrium dissociation constants (K_D in nM) and adrenoceptor densities as maximal binding capacities (B_{\max} in fmol mg^{-1} of protein). Superscripts indicate statistical differences from either the corresponding 'sham-treated' (^a) or the TNBS group (^b) ($P < 0.05$).

| | | | Vehicle | Doxantrazole | MK-886 | Indomethacin | L-NAME |
|----------------------------|--------------|------------|-------------------------------|--------------------------------|-------------------------------|------------------------------|-------------------------------|
| ³ H]prazosin | Sham-treated | B_{\max} | 28.0 \pm 1.9 | 29.0 \pm 5.1 | 34.1 \pm 5.0 | 24.9 \pm 8.1 | 25.9 \pm 6.3 |
| | | K_D | 0.52 \pm 0.1 | 0.4 \pm 0.1 | 0.5 \pm 0.3 | 0.7 \pm 0.3 | 0.5 \pm 0.3 |
| | TNBS-treated | B_{\max} | 50.5 ^a \pm 7.0 | 33.7 ^b \pm 2.5 | 51.3 ^a \pm 4.7 | 51.4 ^a \pm 5.7 | 32.1 ^b \pm 3.7 |
| | | K_D | 0.74 \pm 0.1 | 0.73 \pm 0.3 | 0.6 \pm 0.3 | 0.7 \pm 0.1 | 0.4 \pm 0.2 |
| ³ H]rauwolscine | Sham-treated | B_{\max} | 30.7 \pm 1.7 | 26.3 \pm 5.6 | 36.1 \pm 4.8 | 36.7 \pm 4.2 | 28.6 \pm 5.5 |
| | | K_D | 2.2 \pm 0.3 | 2.6 \pm 1.0 | 1.9 \pm 0.4 | 1.8 \pm 0.7 | 1.7 \pm 0.9 |
| | TNBS-treated | B_{\max} | 68.5 ^a \pm 12.4 | 37.1 ^b \pm 3.9 | 55.8 ^a \pm 4.8 | 53.9 ^a \pm 3.4 | 26.2 ^b \pm 2.4 |
| | | K_D | 2.5 \pm 1.2 | 2.4 \pm 0.3 | 2.5 \pm 1.0 | 2.5 \pm 0.8 | 2.0 \pm 0.6 |
| ³ H]DHA | Sham-treated | B_{\max} | 326.6 \pm 14.2 | 310.9 \pm 37.5 | 335.8 \pm 20.9 | 358.9 \pm 47.3 | 335.6 \pm 29.9 |
| | | K_D | 2.2 \pm 0.6 | 2.0 \pm 0.6 | 1.7 \pm 0.5 | 3.5 \pm 1.7 | 4.1 \pm 1.8 |
| | TNBS-treated | B_{\max} | 116.5 ^a \pm 23.0 | 256.2 ^{ab} \pm 27.5 | 323.3 ^b \pm 12.5 | 145.8 ^a \pm 4.0 | 258.0 ^b \pm 37.6 |
| | | K_D | 2.4 \pm 0.8 | 2.9 \pm 1.1 | 4.0 \pm 1.1 | 3.1 \pm 0.9 | 3.4 \pm 1.1 |

We next examined whether the adrenoceptor numbers modifications, observed after TNBS administration, could be antagonized by treatment with various drugs of putative anti-inflammatory potential. Changes in the B_{\max} values for the α_1 -, α_2 - and β -subtypes, respectively, were partly prevented in 'TNBS-treated' groups, by a daily treatment with either doxantrazole, or L-NAME ($P < 0.05$, compared to TNBS alone; Table 2, Fig. 3). In both these latter groups, the increase in α -adrenoceptor number was abolished, while the decrease in β -adrenoceptor number was only prevented by about 60–70%. Daily treatment with MK-886 abolished the β -adrenoceptor number decrease ($P < 0.05$, compared to TNBS alone), but not the increase in α_1 - and α_2 -adrenoceptor density. Treatment with indomethacin did not reduce the TNBS-induced adrenoceptor changes (Table 2, Fig. 3). On the other hand, B_{\max} values for all adrenoceptor subtypes were not affected by any of the treatments in the 'sham-treated' groups (Table 2). Likewise, K_D values obtained for each radioligand in the ten experimental groups were not significantly different from each other (Table 2).

DISCUSSION

The present study confirms that the instillation of TNBS into guinea pig small intestine induces an alteration of smooth muscle membrane adrenoceptor number. It consisted of an up-regulation of both α_1 - and α_2 -adrenoceptors, contrasting with a down-regulation of β -adrenoceptors, as reported in a previous work.⁴ These modifications of adrenoceptor number are associated

with histologically observable alterations of small intestinal mucosa characteristic of inflammation, and an infiltration of polymorphonuclear neutrophils, evidenced by a rise in tissue MPO activity as reported by others in the TNBS-induced model of colitis⁶ or ileitis.¹²

Whether post- or pre-junctionally located, intestinal adrenoceptors, however, are an interesting basis from which to tentatively explain the intestinal motility disturbances associated with gut inflammation.^{1,2,20} Indeed, though smooth-muscle β -adrenoceptors have been demonstrated to relax the intestine,²¹ the role of the α_1 - and α_2 -adrenoceptor subtypes is not well documented. Even the precise, pre- or post-junctional, location of intestinal α -adrenoceptors remains an open issue.^{18,21} Nevertheless, previous works have suggested a contractility-stimulating function for both α -adrenoceptor subtypes.^{22,23} Therefore, the inverse regulation, occurring at the intestinal level, could be considered as a highly favourable situation to the general motility enhancement observed during intestinal inflammatory processes.^{1,2} Thus, gut inflammation is often associated with structural damage to myenteric plexus neurons, as seen in patients with either inflammatory bowel disease (IBD)²⁴ or autonomic neuropathy.²⁵ Loss of noradrenergic nerve supply to the gut has been reported in connection with inflammation, such as that of the intestine of rats infected by *Trichinella spiralis*.³ This functional denervation is likely to explain, at least in part, the observed increase in α -adrenoceptor numbers in the inflamed tissue, as a result of a drop of the circulating agonist noradrenaline. Nevertheless, it hardly explains the parallel decrease in β -adrenoceptors,

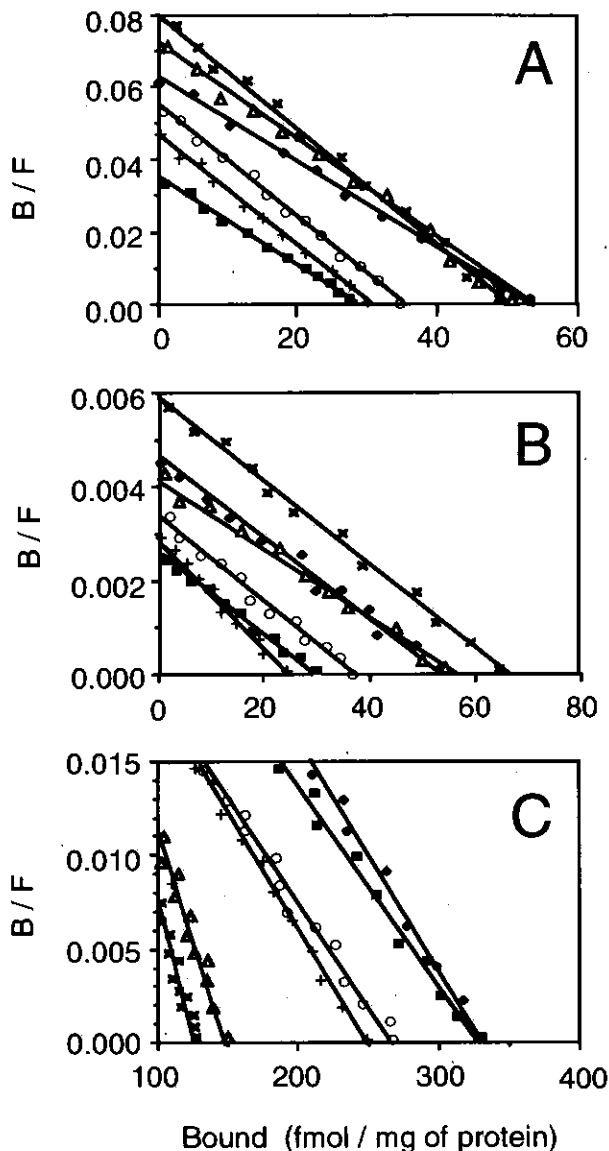


Figure 3 Scatchard plots of adrenoceptor saturation binding data for the 'Control group' and all 'TNBS-treated' groups. The ratio of bound to free (B/F) is expressed as a function of bound [³H]prazosin (α_1 -adrenoceptors; A), [³H]rauwolscine (α_2 -adrenoceptors; B) and [³H]DHA (β -adrenoceptors; C). Each plot is representative of at least three determinations performed in duplicate [see Methods section for definition of experimental groups]. \square Control, \times TNBS, \triangle TNBS-INDO, \blacklozenge TNBS-MK, $+$ TNBS-NAME, \circ TNBS-DOX.

which is probably controlled by a different mechanism. On the other hand, gut inflammation has been associated with hyperplasia and hypertrophy of the intestinal muscle layers.²⁶ Nevertheless thickening of intestinal wall is also unlikely to account for the observed changes, as receptor number is normalized to protein contents in the present study.

According to the present data, it seems that intestinal adrenoceptor density modifications could also result from the enhanced release of inflammatory mediators. These mediators could well be directly responsible for the intestinal motility disturbances, but, by modifying the number of α - and β -adrenoceptors, they exert an additional influence on smooth muscle contractility.

An increased synthesis of cyclooxygenase metabolites has been reported during the inflammatory response of the gut.⁹ However, our attempt to inhibit the cyclooxygenase pathway with indomethacin failed to revert, or attenuate, changes in adrenoceptor numbers, thus suggesting that prostanoids do not play a major role in this process. In our study, indomethacin treatment neither improves nor exacerbates TNBS-induced intestinal injury, as evidenced by the histological picture obtained (Fig. 2a), and the level of intestinal tissue MPO activity, which remains high. The present results are consistent with investigations with the TNBS model, showing that indomethacin, though effective in inhibiting prostanoid synthesis without effect on 5-lipoxygenase products, did not reduce the severity of intestinal inflammation.²⁷ However, treatments with prostaglandin analogues have shown a reduction of the extent of gut damage, induced by experimental inflammation.²⁸ Therefore, further experiments will be required to precise the role of cyclooxygenase products, in the overall intestinal response to TNBS inflammation.

The lipoxygenase synthesis inhibitor MK-886¹⁴ prevented the decrease in β -adrenoceptor density but was apparently unable to prevent the increase in α -adrenoceptor number. Although the mechanisms whereby α - and β -adrenoceptor subtypes were differently affected by MK-886 treatment remain to be elucidated, this observation illustrates the fact that changes in α - and β -adrenoceptor subtypes certainly involve different mechanisms, with leukotrienes being implicated in the regulation solely of the β -adrenoceptor number. Our data also show that MK-886 significantly reduces MPO activity in TNBS-treated animals, in agreement with results supporting a role for LTs, particularly LTB₄ and LTD₄, in the pathogenesis of intestinal inflammation.^{9,10,29} They are also consistent with a chemotactic role for LTs on polymorphonuclear neutrophils, as similar doses of MK-886 accelerate healing in TNBS-induced colitis,²⁹ and are effective in inhibiting LTB₄ synthesis for more than 24 h.³⁰

Daily treatment with the NO-synthase inhibitor L-NAME prevented the changes in smooth muscle adrenoceptor number, as well as the mucosal injury and the rise in intestinal MPO activity observed after TNBS treatment. These findings support the involvement of NO in TNBS-induced intestinal inflammation, which

agrees with the increased concentrations of nitrites reported either in patients with ulcerative colitis,³¹ or in experimental models of chronic inflammation.¹¹ Interestingly, Miller *et al.*¹² recently reported an increased MPO activity in intestinal tissues of healthy guinea pigs treated with L-NAME, and an improvement of TNBS-induced ileitis by treatment with this NO synthase inhibitor. In comparison, in our study we used a three-fold lower dose of L-NAME which was without effect in 'sham-treated' animals, while it efficiently reduced TNBS-induced tissue injury and enhancement of MPO activity when administered to TNBS-treated animals. However, we have no data on the mechanisms whereby NO could act to directly or indirectly modulate intestinal smooth muscle adrenoceptors during the inflammatory process. TNBS is thought to release radicals, as it decomposes in the mucosa, and these may interact with NO.³² It is possible that the beneficial effects of L-NAME on inflammatory damages, and the prevention of adrenoceptor changes, could result from avoiding interactions between NO, which is not generated owing to the action of L-NAME, and TNBS-released free radicals.

Finally, daily treatment with doxantrazole, a mast cell stabilizer acting on the mast cell populations of both intestinal mucosa and connective tissue,¹³ prevented TNBS-induced changes in smooth muscle adrenoceptors, and reduced the increase in MPO activity, therefore suggesting the involvement of mast cells in the intestinal response to TNBS. In other inflammatory models, the release of mast cell mediators has been associated with mucosal injury.³³ In addition, others have shown that mast cell hyperplasia and activation occur during intestinal inflammation,^{7,8} and that mast cell stabilizers reduce the severity of inflammatory damage.^{34,35} Moreover, studies demonstrating the close apposition of nerves and mast cells in the intestinal mucosa,³⁶ as well as an alteration of the autonomic nervous supply associated with mast cell degranulation,³⁷ suggest that products of mast cell degranulation, such as LTS, NO or cytokines, could be the regulatory mediators tentatively involved in both TNBS-induced intestinal adrenoceptor changes, and tissue damage.^{33,38}

In conclusion, the present work brings new evidence that mast cell degranulation, nitric oxide generation and leukotriene production could be major mechanisms involved in the intestinal smooth muscle adrenoceptor regulation observed in the guinea pig during TNBS-induced gut inflammation. In contrast, evidence for the involvement of prostanoids has not been obtained. Secondly, our results point out a special relationship between LTs and β -adrenoceptor down-regulation, whereas mast cells and NO are involved in the regu-

lation of both α - and β -subtypes. Finally, our work brings additional evidence for the existence of an extended network relating inflammatory cells, intestinal smooth muscle cells and the autonomic nervous system, especially its adrenergic component.

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