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The inhibitory effects of α_2-adrenoceptor agonists on gastrointestinal transit during croton oil-induced intestinal inflammation

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1 The peripheral effects of α_2-adrenoceptor agonists were investigated in a model of intestinal inflammation induced by intragastric administration of croton oil (CO). Our hypothesis was that inflammation would 'sensitize' adrenoceptors in peripheral and/or central terminals of myenteric and submucous plexus neurones, and enhance systemic effects of α_2-adrenoceptor agonists.

2 Male swiss CD-1 mice, received intragastrically CO (0.05 ml), castor oil (CA, 0.1 ml) or saline (SS) 3 h before the study: gastrointestinal transit (GIT) was evaluated 20 min afterwards with a charcoal meal. The presence of inflammation was assessed by electron microscopy.

3 The intragastric administration of CA or CO caused an increase in GIT and weight loss, but only CO induced an inflammatory response. Both clonidine (imidazoline$_1$/α_2-agonist) and UK-14304 (α_2-agonist) produced dose-related inhibitions of GIT in all groups. During inflammatory diarrhoea (CO), potencies of systemic (s.c.) clonidine and UK-14304 were significantly increased 3.5 and 2.1 times, respectively, while potencies remained unaltered in the presence of diarrhoea without inflammation (CA). The effects were reversed by administration (s.c.) of receptor-specific adrenoceptor antagonists, but not by naloxone.

4 Clonidine was 8.3 (SS) and 2.8 (CO) times more potent when administered intracerebroventricularly (i.c.v.), than when administered s.c. Inflammation of the gut did not alter the potency of i.c.v. clonidine, demonstrating that enhanced effects of s.c. clonidine are mediated by peripheral receptors. During inflammation, i.c.v. efaroxan did not antagonize low doses of s.c. clonidine (ED_{20} and ED_{50}s), but partially reversed ED_{80}s, further supporting the peripheral effects of the agonists in CO treated animals.

5 The results demonstrate that inflammation of the gut enhances the potency of α_2-adrenoceptor agonists by a peripheral mechanism. The results also suggest that the inflammatory response induces an up-regulation or sensitization of α_2-adrenoceptors and/or imidazoline receptors.

Keywords: α_2-Adrenoceptors; diarrhoea; gastrointestinal transit; intestine; peripheral inflammation

Introduction

α_2-Adrenoceptors mediate several physiological responses in the gastrointestinal tract, such as regulation of gastric and intestinal motility and fluid transport in small and large intestine (Ruffolo *et al.*, 1993). Several studies have shown that clonidine inhibits intestinal transit in rats (Ruwart *et al.*, 1980; Galligan & Burks, 1983; Jiang *et al.*, 1988), and mice (Jiang *et al.*, 1988; Ramabadran *et al.*, 1990); and has anti-diarrhoea effects in rats (Lal *et al.*, 1981; Spraggs & Bunce, 1983; Thollander *et al.*, 1991) and mice (Doherty & Hancok, 1983).

α_2-Agonists also block intestinal fluid secretion induced by prostaglandin E_2 (PGE_2), vasoactive intestinal peptide (VIP), dibutyryl adenosine 3': 5'-cyclic monophosphate (cyclic AMP), and cholera toxin in rat isolated jejunum (Nakaki *et al.*, 1982a,b), presumably by increasing net mucosal ion absorption; this effect is not species-dependent and occurs both in ileum and colon (Dharmsathaphorn *et al.*, 1984). It is accepted that the anti-secretory effects are mediated by α_2-receptors located on intestinal mucosal cells, demonstrated by radioligand binding studies (Nakaki *et al.*, 1983).

Peripheral inflammation and tissue injury induce an 'up regulation' or 'recruitment' of α_2-adrenoceptors (Sato & Perl, 1991) located on peripheral terminals of primary sensory neurones. Accordingly, inflammation increased the analgesic efficacy of systemically and intrathecally administered clonidine in a model of inflammatory arthritis in rats (Hylden *et al.*, 1991). In guinea-pigs, inflammation of the gut induced a simultaneous up-regulation of both α_1- and α_2-adrenoceptors (Martinolle *et al.*, 1993). However the effects of α_2-adrenoceptor agonists on intestinal motility during inflammation have not been investigated.

The aim of present study was to determine the mechanisms of the antitransit effects of α_2-agonists during inflammation of the gut. Our working hypothesis was that the effects of α_2-agonists would be enhanced in the presence of diarrhoea associated with intestinal inflammation, but not when transit was increased in the absence of inflammation. In order to achieve these experimental conditions, we have used two cathartic agents: castor oil (CA) and croton oil (CO). We have assessed the antitransit effects of several α_2-adrenoceptor agonists administered by the s.c. or intracerebroventricular (i.c.v.) routes and their reversibility by specific antagonists. In addition, due to the physiological interaction existing between the endogenous opioid and α_2-adrenoceptor systems, the ability of naloxone to reverse these effects was also evaluated. Experiments were performed in animals treated with CO, CA and saline (SS). I.c.v. administration of drugs was used in order to evaluate the peripheral component of the effects of α_2-agonists during intestinal inflammation. The drugs used in the present investigation were: clonidine, an imidazoline$_1$/α_2-agonist (Bricca *et al.*, 1994); UK-14304, an α_2-selective agonist (Cambridge, 1981); efaroxan, imidazoline$_1$/α_2-antagonist (Haxhiu *et al.*, 1995), idazoxan an α_2-selective antagonist (Shen *et al.*, 1990; Blandizzi *et al.*, 1991) and naloxone an opioid antagonist (Lord *et al.*, 1977; Magnam *et al.*, 1982).

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Methods

Animals

Male Swiss CD-1 mice weighing 20 to 25 g were used in all experiments. Animals were housed under 12 hour light/12 hour dark conditions in a room with controlled temperature (22°C) and humidity (60%). Mice had free access to food and water, and were used after a minimum of four days acclimatization to the housing conditions. All experiments were conducted between 09 h 00 min and 17 h 00 min. The International Association for the Study of Pain guidelines on ethical standards for investigations in animals were followed.

Diarrhoea

Prior to experiments, animals were fasted for 18 h, except for free access to water which was available for the duration of experiment. Mice were gavaged with 0.05 ml of croton oil, 0.1 ml of castor oil or saline, weighed and placed in separate cages. After three hours, animals were weighed again and the presence or absence of diarrhoea noted.

Histological examination.

Animals were killed three hours after p.o. administration of CO, CA or SS, and the small intestine rapidly excised. Samples of proximal jejunum were fixed with 2.5% glutaraldehyde in phosphate buffer (200–400 mOsm; pH 7.2–7.4) for 24 h, and processed either for optical or electron microscopy examination. For optical studies, samples were embedded in paraffin and 5 microns thick longitudinal and radial sections were obtained with a sliding microtome; dewaxed sections were stained with haematoxylin and eosin. For electron microscopic studies, small blocks were washed in phosphate buffer, and postfixed with 2% osmium tetroxide for 2 h; blocks were embedded in araldite, and ultrathin sections were obtained with a ultramicrotome; sections were stained with uranyl acetate and lead citrate. Five animals treated with CO, CA and SS were used for histological studies.

Gastrointestinal transit (GIT)

GIT was measured 3 h after treatment with CO, CA or SS. At this time, a charcoal meal (0.25 ml of 10% charcoal in 5% gum acacia) was administered intragastrically to assess GIT according to procedures previously described (Pol *et al.*, 1995; Ramabadran *et al.*, 1989). In these experiments two α_2-adrenoceptor agonists and vehicle were given 30 min before charcoal. The antagonists idazoxan and efaroxan were injected s.c. and naloxone intraperitoneally (i.p.), 40 and 15 min before the marker, respectively. In initial experiments, the animals were killed at 10 min intervals after the charcoal meal for a period of 40 min in order to assess GIT in CO, CA and SS treated animals. However, in subsequent experiments the effects of α_2-agonists on GIT were evaluated 20 min after administration of the marker. At this time, animals were killed and the small intestine separated from omentum avoiding stretching. The length of intestine from the pyloric sphincter to the ileocecal junction, and the distance travelled by charcoal meal, were measured. The effects of clonidine, UK-14304 and vehicle administered s.c. on GIT, in mice treated with CO, CA and SS were evaluated. Animals in control groups received s.c. vehicle injections (saline). All drugs were dissolved in pyrogen-free 0.9% sodium chloride just before use and injected in a volume of 10 ml kg^{-1}.

Intracerebroventricular injections

Drugs were delivered i.c.v. in a volume of 5 μl, by a Hamilton microlitre syringe (Microdispenser Socorex, PANREAC S.A.) fitted with a 26-gauge needle, by the method of Haley & McCormick (1957). The site of injection was 2 mm caudal and 2 mm lateral to bregma, and 3 mm in depth from the skull surface.

Drugs

The drugs used were: clonidine and idazoxan (Research Biochemicals Incorporated, U.S.A.); UK-14304 (S-Bromo-N (4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine) a gift from Dr Bostock (Pfizer, England); efaroxan and naloxone (Sigma Chemical, Co, U.S.A.).

Data analysis

For each animal, GIT was calculated as the percentage (%) of distance travelled by the charcoal, relative to the total length of the small intestine (% of GIT). The inhibitory effects of adrenoceptor agonists on GIT are expressed as a percentage of inhibition of the transit in drug-treated animal (test GIT) when compared with the mean transit measured in a group of vehicle-treated mice ($n=20$).

$$\%\text{inhibition} = [(\text{vehicle GIT} - \text{test GIT})(\text{vehicle GIT})] \times 100$$

The data are expressed as a group mean ± s.e. All statistical calculations were performed as described by Tallarida and Murray (1986). ED_{50} ± s.e. (dose which produced a 50% effect) values were determined by linear regression analysis of dose-response relations based on at least ten mice per dose. Tests for parallelism and validity of the test were estimated by parallel line assay. Statistical analysis for significant differences between two groups were obtained by Student's t test; when multiple groups were compared, this was by one or two way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test, whenever applicable. When variances between groups were different, a non-parametric ANOVA was applied (Kruskall-Wallis) followed by a Mann-Whitney U test. A value of $P<0.05$ was considered as significant.

Results

Effects of castor (CA) or croton oil (CO) on the gut

The p.o. administration of CO or CA produced loose watery faeces (diarrhoea) accompanied by a significant decrease in body weight. Control animals receiving p.o. saline (SS) lost 4% body weight during the experiment (3 h) and no diarrhoea was observed. Weight loss in animals treated with CO or CA was 11.2% and 10.4%, respectively. One way ANOVA revealed a significant decrease in body weight in CO and CA groups ($P<0.05$; $n=10$ for each treatment), when compared to SS animals.

GIT was assessed in mice treated with CO, CA or SS (all p.o.). In these experiments a charcoal meal was given 3 h after treatment, and animals killed at 10 min intervals for a period of 40 min (n = 10 for each time point). When compared to SS treated animals, both CO and CA mice showed a significant increase of GIT which was observed 20 and 30 min after the marker ($P<0.05$, Student's t test; Figure 1); no significant differences were obtained between the CA and CO groups. Maximal differences between groups were seen at 20 min, and consequently, all subsequent experiments were carried out at this time point; absolute values of % GIT were: 48.8 ± 1.5% in SS, 60.4 ± 2.1% in CA and 68.7 ± 2.2% in CO-treated animals.

The histological examination by light microscopy of intestinal preparations from proximal jejunum of mice treated with CO, CA or SS did not show morphological changes in any of the groups. However, when similar tissue samples were examined under electron microscopy ($n=5$ each group), CO treated animals showed an increased number of clear vesicles, enlarged cisterns of the endoplasmic reticulum and Golgi complex in the cytoplasm of epithelial cells together with enlarged spaces with fine granular material in

extravascular compartment of the villi. In CA treated animals, we could not demonstrate the presence of inflammation of the gut by electron microscopy (Figure 2).

These results show that both CO and CA produce diarrhoea, weight loss and a significant increase in GIT. However, only animals treated with CO presented histological changes that demonstrate the presence of inflammation.

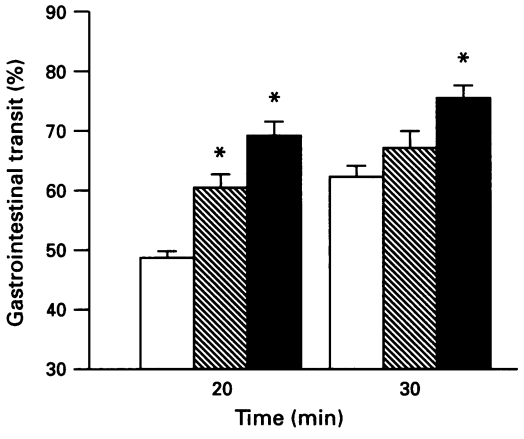

Figure 1 Effects of p.o. saline (open columns), castor oil (hatched columns) or croton oil (solid columns) on the gastrointestinal transit (GIT) of a charcoal meal. Animals were killed 20 or 30 min after the marker. Each point represents the mean value ± s.e. of at least 10 mice. $*P<0.05$, when compared to SS; no statistically significant differences were observed between CA and CO (Student's t test).

Inhibitory effects of s.c. administration of α_2-adrenoceptor agonists on GIT

The effects of s.c. administration of clonidine (imidazoline$_1$/α_2) and UK-14304 (α_2-selective) on GIT were determined in CO-, CA- and SS-treated animals. Both clonidine and UK-14304 (Figure 3) produced dose-related inhibition of transit in CO-, CA- and SS-treated animals. In all instances, analysis of the dose-response lines showed coefficients of co rrelation close to 1 and no significant differences in the slopes. In animals treated with CO, dose-response curves to both agonists were shifted to the left, demonstrating an increased response in these experimental conditions. From the dose-response curves, ED_{50} values for clonidine and UK-14304 were obtained in three groups of study (Table 1). The ED_{50} of clonidine was similar in CA and SS groups, but was significantly decreased in CO treated animals, demonstrating that inflammation increased the potency of clonidine by approximately 3.5 times. Similarly, the ED_{50} of UK-14304 was decreased 2.1 times in animals primed with CO, while it was unaltered in CA group.

For each drug, the effects of treatment (CO, CA, SS) and dose, were analysed by 2-way ANOVA. Our results show that each factor (treatment and dose) had a significant effect on % inhibition of GIT ($P<0.001$; $n=10$ for each time point); however, no significant interaction could be demonstrated. Comparison with one-way ANOVA showed, that for both agonists (clonidine and UK-14304) the effect of treatment is related to CO, since this agent significantly increased the inhibition of GIT when compared to CA or SS ($P<0.02$; $n=10$ for each time point). From these results we conclude that inflammation induced by administration of CO, enhances the antitransit effects of α_2-adrenoceptor agonists.

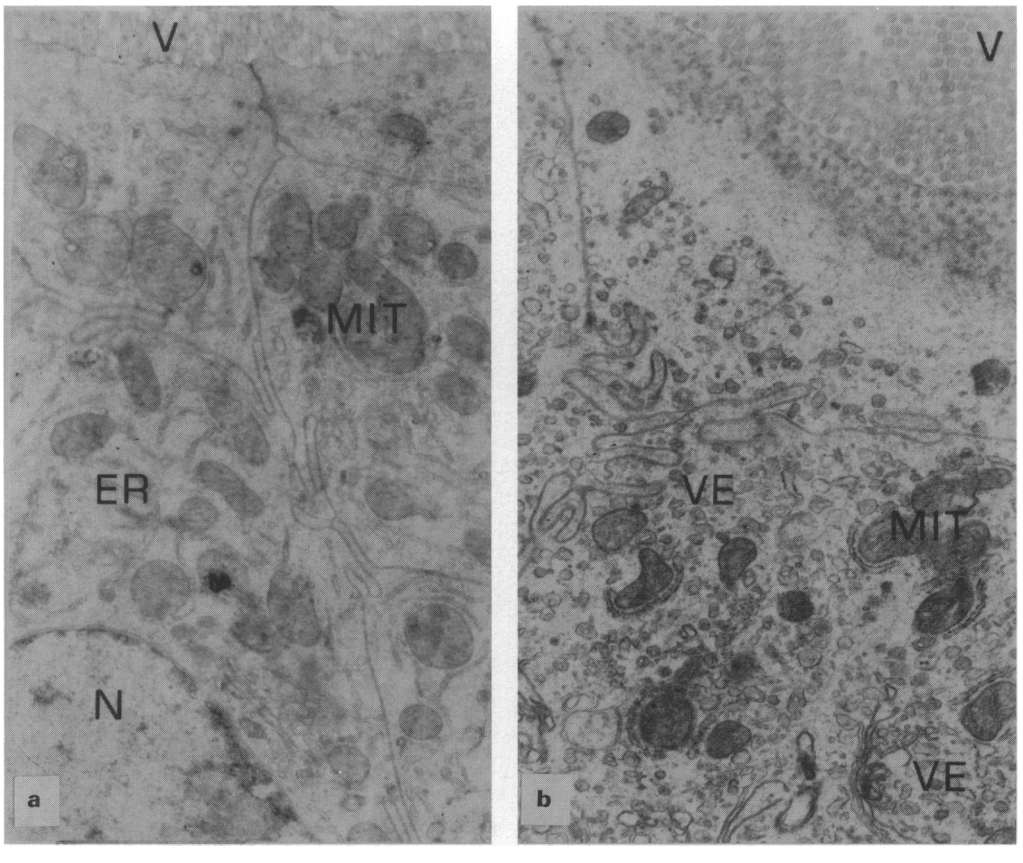

Figure 2 Electron microscopical examination of intestinal preparations obtained from the proximal jejunum. Epithelial cells at the luminal border of the villi 3 h after p.o. administration of castor oil (a) or croton oil (b). Increased number of clear vesicles (VE), and enlarged cisterns of the endoplasmic reticulum and Golgi complex are seen in croton oil treated animals. V: villi; MIT: mitochondria; ER: endoplasmic reticulum; N: nucleus. Magnification: × 16,000.

Attenuation of the antitransit effects of s.c. α_2-adrenoceptor agonists by antagonists

In order to evaluate the specificity of observed responses in the presence of inflammatory diarrhoea, the actions of clonidine and UK-14304 were assessed after administration of naloxone, idazoxan and efaroxan. Naloxone, was administered at a fixed dose of 0.1 mg kg^{-1} (6.87 μmol) which has been shown to antagonize μ-, δ- and κ-opioid receptor mediated effects (Lord *et al.*, 1977; Magnam *et al.*, 1982). Figure 4 shows % inhibition of GIT induced by 2.4 in SS and 0.7 nmol in CO of clonidine (Figure 4a) and 1.7 in SS and 0.8 nmol in CO of UK-14304 (Figure 4b) alone, and in the presence of naloxone (6.87 μmol), idazoxan (10.38 μmol) or efaroxan (9.89 μmol). These experiments were performed in SS and CO treated animals; in each experimental condition results were evaluated by analysis of variance. A non-parametric 1-way ANOVA revealed that the antitransit effects of clonidine (2.4 nmol) were antagonized by administration of efaroxan and idazoxan, both in SS- and CO-treated animals ($P<0.001$, Mann-Whitney U-test; $n=10$ animals for each treatment). Similarly, the antitransit effects of UK-14304 (1.7 nmol) in all experimental conditions, were prevented by administration of idazoxan and efaroxan ($P<0.001$; $n=10$ animals for each treatment). However, naloxone, did not alter the antitransit effects of α_2-agonists in SS- or CO-treated animals. Thus, the enhanced antitransit effects of s.c. clonidine and UK 14,304 in CO were antagonized by their specific antagonists, while the response was not significantly altered in the presence of naloxone, demonstrating the participation of α_2-adrenoceptors in the observed responses.

The antitransit effects of α_2-adrenoceptor agonists in CA-treated animals were also antagonized by administration of idazoxan and efaroxan (data not shown).

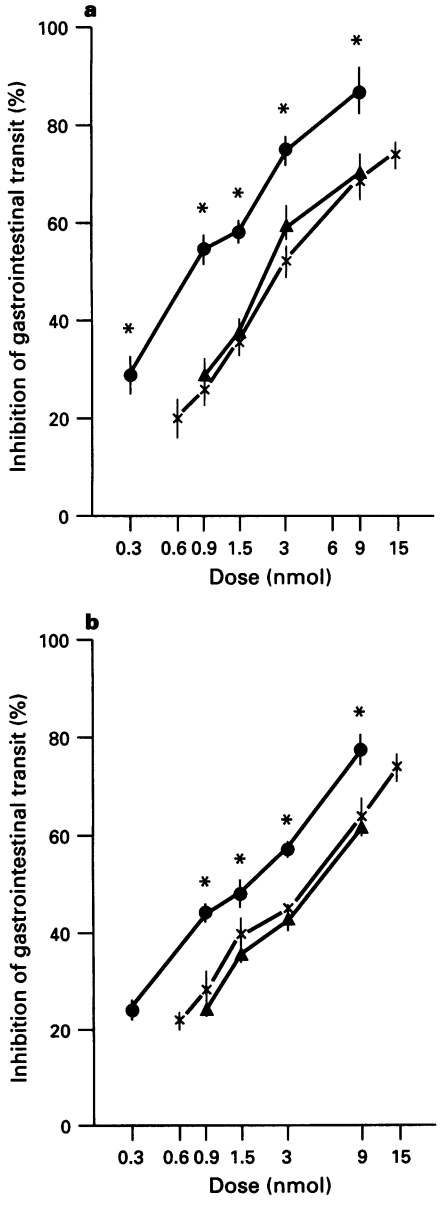

Figure 3 Dose-related inhibition of gastrointestinal transit (GIT) induced by s.c. clonidine (a) and UK-14304 (b) in animals primed with saline (SS, ×), castor oil (CA, ▲) or croton oil (CO, ●). GIT was evaluated 20 min after the administration of the charcoal. Each point represents the mean ± s.e. of 10 or more mice. *$P<0.02$, when compared CO to CA and SS (Student-Newman-Keuls test).

Table 1 Potencies (ED_{50}) of clonidine and UK-14304 on % inhibition of gastro-intestinal transit (GIT) in mice treated with saline (SS), castor (CA) or croton oil (CO)

Clonidine	*ED_{50} ± s.e. (nmol)*	*Ratio*	
SS	2.41 ± 0.04	SS/SS	1.00
CA	2.24 ± 0.03	SS/CA	1.07
CO	0.69 ± 0.04	SS/CO	3.49
UK-14304	ED_{50} ± s.e. (nmol)	Ratio	
SS	1.71 ± 0.04	SS/SS	1.00
CA	1.76 ± 0.04	SS/CA	0.97
CO	0.80 ± 0.02	SS/CO	2.13

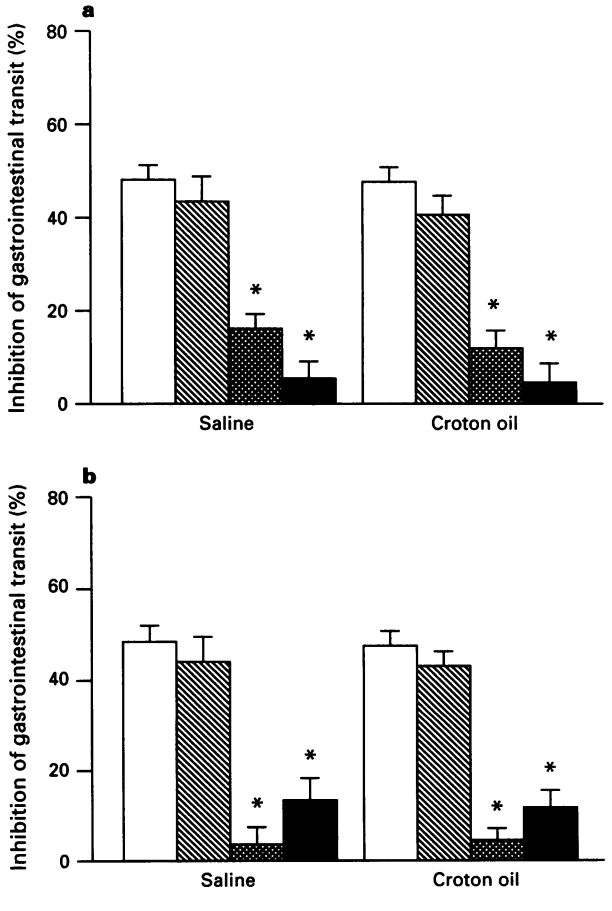

Figure 4 Inhibition of GIT induced by (a) clonidine, 2.4 nmol in saline primed (SS) and 0.7 nmol in croton oil primed (CO) animals and (b) UK-14304, 1.7 nmol in SS and 0.8 nmol in CO animals, alone (open columns) and in the presence of naloxone (6.87 μmol; hatched columns), idazoxan (10.38 μmol; cross-hatched columns) or efaroxan (9.89 μmol; solid columns). *$P<0.001$ (Mann-Whitney U-test).

Antitransit effects of clonidine after intracerebroventricular administration

The effects of clonidine on the % inhibition of GIT were also assessed after i.c.v. administration. These experiments were carried out in order to evaluate the relevance of intestinal α_2-receptors in the enhanced response to clonidine during inflammation. Due to the fact that specific peripherally acting α_2-adrenoceptor drugs are not available, we studied the antitransit effects of clonidine after i.c.v. administration, assuming that centrally located (brain) α_2-receptors would be unaltered by intestinal inflammation.

Dose-response curves to i.c.v. clonidine were performed in mice treated with SS or CO. The resulting lines were superimposed, had coefficients of correlation of 0.999 and 0.997, and their slopes did not differ significantly from each other (SS, 39.1 ± 1.8 and CO, 37.2 ± 1.5). In SS-treated animals, the ED_{50} values of s.c. and i.c.v. clonidine were 2.41 ± 0.04 and 0.29 ± 0.007 nmol, respectively; during inflammation (CO) the ED_{50} values were 0.69 ± 0.04 nmol (s.c.) and 0.24 ± 0.014 nmol (i.c.v.). Thus our results show that i.c.v. clonidine is approximately eight and three times more potent than s.c. clonidine, in SS and CO animals, respectively. The results also illustrate that the potency of s.c. clonidine increases 3.5 times in the presence of inflammation, while it is not altered when the drug is administered by the i.c.v. route. The results suggest that the enhanced effects of clonidine are mediated by peripheral α_2-adrenoceptors 'sensitized' or 'up regulated' by the inflammatory response.

Attenuation of the antitransit effects of i.c.v. clonidine by the α_2-adrenoceptor antagonist efaroxan

The ED_{50} doses of clonidine obtained in different experimental conditions were tested in the presence of i.c.v. efaroxan (0.02 nmol). In these experiments a fixed dose of antagonist was given in all instances by the i.c.v. route, while the agonist was administered either by i.c.v. or s.c. routes. Thus, the ED_{20}, ED_{50}, and ED_{80} doses (calculated from the dose-response curves) obtained in the different experimental conditions were used in these experiments.

The results show that the antitransit effects of the ED_{50} doses of clonidine, i.c.v. (0.29 and 0.24 nmol) were completely antagonized by efaroxan, both in SS and CO treated animals. Efaroxan was unable to reverse the effects of the ED_{20} dose or ED_{50} dose of s.c. clonidine in SS or CO animals, demonstrating that the antitransit effects of low doses of systemic clonidine are mediated by peripheral α_2-adrenoceptors. However, the effect produced by an ED_{80} dose of clonidine, s.c., (14.7 and 4.3 nmol) in SS and CO animals, was reduced to 50 and 70%, respectively, after the same dose of efaroxan.

Discussion

The intragastric administration of either CA or CO to fasted mice 3 h before the charcoal meal, had several effects: (a) weight loss suggesting fluid and electrolyte hypersecretion; (b) a maximal increase in GIT of approximately 12 (CA) and 20% (CO), respectively; and (c) electron microscopy evidence of inflammation in CO but not in CA animals. Therefore we concluded that only CO induces diarrhoea associated with intestinal inflammation.

The cathartic effects of CA (Stewart *et al.*, 1975b; Niemegeers *et al.*, 1984; Sinar *et al.*, 1986) had been attributed to ricinoleic acid, the active component of CA, which alters fluid and electrolyte transport and produces an hypersecretory response (Bright-Asare & Binder, 1973; Ammon *et al.*, 1974); thus, diarrhoea induced by CA is not related to an increase in smooth muscle contractility. (Stewart *et al.*, 1975a; Gaginella *et al.*, 1975). However, CO is an irritant that produces inflammation in different tissues, specially skin and mucosae (Nishiki *et al.*, 1988; Colorado *et al.*, 1991) and induces diarrhoea associated with intestinal inflammation in mice (Pol *et al.*, 1994; 1995).

Our results show that the s.c. administration of clonidine and UK-14304 inhibited GIT in a dose-related manner and thus ED_{50} values could be calculated. In the presence of inflammation (CO), the potencies of clonidine and UK-14304 were significantly increased; however, treatment with CA did not alter the ED_{50} values of the agonists, demonstrating that inflammation of the gut is required in order to enhance the inhibitory effects of systemically administered α_2-adrenoceptor agonists. These results show that the inflammatory process 'sensitizes' the gut to the effects of α_2-agonists; this could be related to an 'up regulation' of α_2-receptors in the gut, during inflammation. Our results support the findings of other investigators who have demonstrated an increased response to α_2-agonists in articular vessels of the knee in rabbits with acute inflammation induced by carrageenin (Gray & Ferrell, 1992).

The effects of α_2-agonists in the gut have not been completely characterized. Clonidine, a partial α_2-agonist, also binds to α_1 (relative potency 300:1) and imidazoline$_1$ receptors. Due to the high affinity of clonidine for the imidazoline binding sites, we also studied the inhibitory effects of UK-14304 a selective α_2-agonist (Cambridge, 1981) in the presence of inflammation. Our results show that the effects of both drugs were enhanced by CO, but the increase in potency was more prominent for clonidine (1.4 times), suggesting that binding to I_1 or α_1-receptors could be responsible for the difference. However, the antagonism of the effects of clonidine by efaroxan (imidazoline$_1$/α_2-antagonist) and UK-14304 by idazoxan (α_2-selective antagonist) suggest that the 'up-regulation' of α_1-adrenoceptors is of little relevance in mediating the enhanced effects of α_2-adrenoceptor agonists during acute inflammation of the gut. Data regarding changes in the population of adrenoceptors during peripheral inflammation are inconsistent. Thus, the number of α_1-adrenoceptors has been found to be increased during intestinal inflammation (Martinolle *et al.*, 1993), and decreased in a model of arthritis induced by carrageenan (Gray & Ferrell, 1992). However, β-adrenoceptors in the guinea-pig small intestine are decreased during inflammation (Martinolle *et al.*, 1993; 1995).

In order to be able to compare (approximately) the potency of clonidine administered by s.c. and i.c.v. routes, we present our results in nmol instead of the more conventional mg kg^{-1}. Since the effects of peripherally acting α_2-agonists have not been described, we used the i.c.v. route to demonstrate the peripheral effects of clonidine during inflammation. A peripheral site of action of clonidine was previously suggested in the literature by identical antitransit effects of s.c. clonidine in normal and in spinally transected mice (Jiang *et al.*, 1988). Our initial experiments also suggested that the enhanced effects of s.c. clonidine were mediated by peripheral receptors, and thus we hypothesized that the potency of i.c.v. clonidine in CO animals would be unaltered. Clonidine was eight and two times more potent by the i.c.v. than s.c. route, in SS and CO animals. In addition, the ratio of the ED_{50} values of SS/CO was 3.5 and 1.2 for s.c. and i.c.v. clonidine, demonstrating the validity of our hypothesis.

The antitransit effects of systemic clonidine and UK-14304 were antagonized by s.c. efaroxan and idazoxan, both in SS and CO treated animals. Due to the specificity of the antagonists, the results demonstrate that during inflammation, the enhanced effects of the agonists are mediated by α_2/imidazoline$_1$ receptors. The results also show that naloxone, an opioid antagonist, did not alter the inhibitory response to α_2-agonists, demonstrating the absence of interaction between these receptors.

When efaroxan was administered i.c.v., the partial reversal of the effect of an ED_{80} dose clonidine, s.c., could be related to the large doses needed to produce an 80% inhibition in SS (14.7 nmol) and CO (4.3 nmol) animals; consequently a higher proportion of the drug would reach the CNS where its effects could be antagonized by i.c.v. efaroxan. This is supported by the different % reversal induced by efaroxan of the ED_{80} doses of clonidine in SS (30%) and CO (10%) animals.

In conclusion, our results demonstrate that inflammation of the gut enhances the potency of α_2-adrenoceptor agonists by a peripheral mechanism. The results also suggest that the inflammatory response induces an up-regulation or sensitization of α_2-adrenoceptors and/or imidazoline receptors.

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