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Antinociceptive effects of oral clonidine and S12813-4 in acute colon inflammation in rats

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Abstract

Acute colonic inflammation was induced by perendoscopic injection of 50 μl of dilute formalin (5%) in the depth of the colonic wall (c.w.) in rats. Compared to saline injection, the procedure was followed by nociceptive behaviors from which visceral nociception was quantified. The α_2-adrenoceptor agonist, clonidine 2-[2,6-dichlorophenylamine]-2-imidazole hydrochloride (75, 150 and 300 mg/kg), administered orally 15 min after c.w. injection of formalin significantly reduced the nociceptive responses at the high dose only. However, when administered 30 min prior to nociceptive stimulation, the compound exhibited an antinociceptive effect at the three doses. A novel analgesic, the compound 'S12813-4' 3-[2-(4-phenylpiperazine-1-yl)-ethyl]-2-oxo-2,3-dihydro-oxazolo[*b*]pyridine, chlorydrate (10, 30 and 90 mg/kg), given orally displayed antinociceptive effects whatever the administration schedule, before or after c.w. injection of formalin. The antinociceptive effect of S12813-4 (30 mg/kg given orally) was prevented by subcutaneous (s.c.) injection of yohimbine or idazoxan (1 mg/kg). We conclude that visceral nociception elicited by formalin-induced colonic inflammation is attenuated by clonidine and S12813-4. The pharmacological profiles of the two compounds, and the inhibition of the antinociceptive effect of S12813-4 by yohimbine and idazoxan suggest that noradrenergic mechanisms are involved in the transmission and/or modulation of the nociceptive influx arising from the inflamed colon.

Keywords: Colonic inflammation; Visceral nociception; Clonidine; S12813-4; Antinociceptive effect; Noradrenergic mechanism

1. Introduction

Numerous animal studies have demonstrated that descending noradrenergic systems exert a tonic inhibitory effect on nociceptive transmission in the spinal cord (Fields and Basbaum, 1978; Yaksh, 1985). Intrathecal (i.t.) administration of noradrenaline or norepinephrine activates spinal α-adrenoceptors and reduces behavioral responses to noxious stimuli (Reddy et al., 1980; Howe et al., 1983). Adrenoceptors of both α_1 and α_2 subtypes seem to mediate these effects since the antinociceptive effect of i.t. noradrenaline is attenuated by both α_1 and α_2 subtype-selective antagonists (Bentley et al., 1977; Reddy et al., 1980; Yaksh, 1985). However, evidence indicates that the antinociception caused by the release of endogenous spinal cord noradrenaline appears to be mediated solely by activation of α_2-adrenoceptors (Jones and Gebhart, 1986). In the dorsal horn, α_2-adrenoceptors are located on neuronal elements intrinsic to the spinal cord and on nerve terminals of bulbospinal and/or primary afferent neurons (see Hamon et al., 1991 for a review). As expected from the latter location, the destruction by dorsal rhizotomy of primary afferent fibres results in a significant decrease in the density of α_2-adrenoceptor binding sites within the superficial layers of the dorsal horn (Howe et al., 1987). Thus, the possibility exists that α_2-adrenoceptor agonists exert their antinociceptive effects, at least partly, through presynaptic modulation of primary afferent fibres which convey the nociceptive messages to the spinal cord. Electrophysiological data provide direct support for this hypothesis since local application of α_2-adrenoceptor ligands produces significant alterations in the threshold of activation of primary

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afferent C fibres (Jeftinija et al., 1981; Calvillo and Ghignone, 1986; Carstens et al., 1987).

The α_2-adrenoceptor agonist, clonidine (2-[2,6-dichlorophenylamine]-2-imidazole hydrochloride), has been shown to produce antinociception in animals, mainly after i.t. administration (Solomon and Gebhart, 1988; Solomon et al., 1989; Ossipov et al., 1990). The suppression of C-fibre-evoked responses of spinal dorsal horn neurons following i.t. administration of clonidine is consistent with the involvement of a presynaptic (with respect to primary afferent fibres) mechanism in the analgesic action of this drug (Sullivan et al., 1987). However, few studies have addressed the question of antinociceptive effects of clonidine in experimental models of pain (Dennis et al., 1980; Hylden et al., 1991; Puke et al., 1991; Kayser et al., 1992a), particularly in experimental visceral pain (Danzebrink and Gebhart, 1990, 1991).

On the other hand, a novel analgesic, the compound S12813-4 (3-[2-(4-phenylpiperazine-1-yl)-ethyl]-2-oxo-2,3-dihydro-oxazolo[*b*]pyridine, chlorydrate), has been shown to exert α_2-adrenoceptor agonist-like actions in animal models of chronic pain (Kayser et al., 1992b). Recently, it has been demonstrated that the antinociceptive effect of S12813-4 might involve some α_2-adrenoceptor-mediated control of substance P-like material release within the spinal cord (Collin et al., 1994). To our knowledge, no study of the antinociceptive effects of clonidine and of S12813-4 in visceral nociception has yet been reported. The aim of the present study was to evaluate these effects after oral administration in the model of inflammatory colonic pain induced by formalin (Miampamba et al., 1994). The experimental model of visceral nociception had been used to demonstrate the possible involvement of substance P in the transmission and/or modulation of nociceptive events arising from an inflamed colon (Miampamba et al., 1992). To evaluate whether the antinociceptive effects of S12813-4 are mediated by α_2-adrenoceptors (see Kayser et al., 1992b for details), yohimbine and idazoxan, two α_2-adrenoceptor antagonists (Ruffolo, 1990) were injected subcutaneously prior to nociceptive stimulation and drug administration. All procedures used in this study followed the IASP guidelines on the use of animals in pain research (Zimmermann, 1983).

2. Materials and methods

2.1. Animals

Adult female Sprague-Dawley rats (Iffa-Credo, l'Arbresle-France) weighing 240–260 g were used. The animals were housed in cages under controlled environmental conditions (23–24°C, light from 07:00 to 19:00 h, food pellets and water ad libitum) for at least five days before being tested. The animals were fasted twenty minutes before the beginning of the experiments.

2.2. Nociceptive stimulus

The stimulus used in the experiments was dilute formalin injected in the depth of the colonic wall (c.w.), or isotonic saline in control animals. The technique of c.w. injection has been reported in detail (Miampamba et al., 1994). Briefly the animal was anesthetized with a small amount of halothane (starting 3%, then 1.5% in a mixture of 2:3 nitrous oxide and 1:3 oxygen), which allowed a prompt return to consciousness. While anesthetized, the rat was suspended by its tail (less than 5 min), while a specially designed coloscope was inserted through the anus. The injection was performed in the depth of the c.w. at the sigmoid level, through a slot cut along the coloscope. It consisted of 50 μl of either 5% formalin solution or saline (0.9% NaCl) both supplemented with 1% Evans blue (w/v) to verify at the end of the test that (i) no luminal leakage had occurred at the time of injection, and (ii) the injection had not been trans-mural.

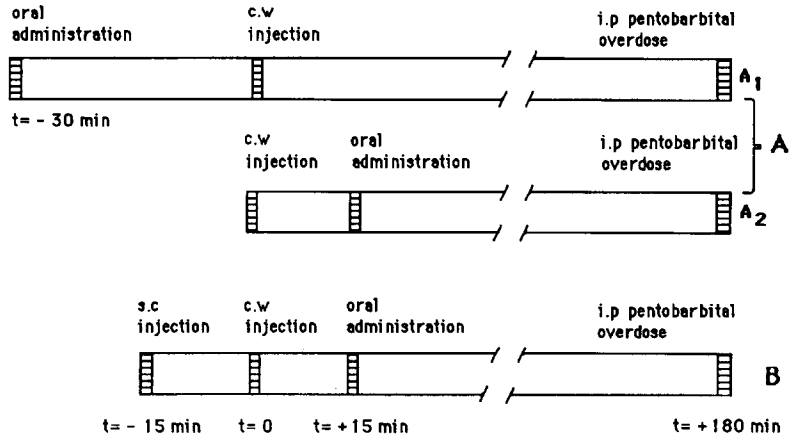

Fig. 1. Schedule of drug administration in the first (A; A_1 = before nociceptive stimulation; A_2 = after nociceptive stimulation) and the second (B) part of the study. Clonidine, S12813-4 and placebo were administered orally. c.w: colonic wall; i.p: intraperitoneal; s.c: subcutaneous.

2.3. Experimental protocol and drug administration

The study was conducted in two parts, each including control groups (i.e., c.w.-saline and formalin-injected rats without antinociceptive agent). The first part was designed to evaluate the antinociceptive effects of clonidine (75, 150 and 300 μg/kg) and of S12813-4 (10, 30 and 90 mg/kg) administered orally. The oral administration of drugs was chosen as a relatively painless procedure. In order to take into account possible variations of intestinal absorption of drugs, one group of rats received clonidine or S12813-4 30 min before the c.w. injection of dilute formalin, while in the second group, the drugs were administered 15 min after the c.w. injection of formalin. In the second part of the study, yohimbine or idazoxan was given subcutaneously at the dose of 1 mg/kg as a single pretreatment 30 min prior to the oral administration of S12813-4 (30 mg/kg). In this second set, each control rat received a subcutaneous (s.c.) injection of isotonic saline. The c.w. injection of dilute formalin or saline took place 15 min after s.c. injection. In both parts of the study, water was administered orally for controls.

Fig. 1 summarizes the administration schedule in the first and the second part of the study.

2.4. Nociceptive behavior assessment

The procedure for nociceptive behavior assessment was described previously (Miampamba et al., 1994). Weight-paired rats were used in each experimental session, conducted between 09:00 and 13:00 h, in a room slightly illuminated and protected from outer noise. One rat was observed on line, and the other was filmed with a video-tape recorder (Camescope VHS Panasonic NV-M25F) for subsequent analysis during full-time replay. Each animal was kept in an individual Plexiglas observation chamber (30 cm × 30 cm × 50 cm) with a layer of wood shavings on the floor. Plexiglas observation chambers were placed in such a way that visual interaction between animals was avoided. Drinking water was available. Before starting the session, each animal was placed in an observation chamber for 20 min to accommodate to its new environment.

As soon as the c.w. injection was completed, the rat was placed in its observation chamber and within 1–2 min had recovered from the anesthesia, at which time nociceptive scoring started. All selected behaviors related to visceral nociception (i.e., licking and nibbling of abdomen or perineal area (L), body stretching (B), contraction of the flanks (C) and whole body contraction (W), listed here in increasing order of nociceptive intensity, were recorded along with their duration for 3 h. The latter behavior (W) was further graded according to the duration of the given episode: W_1 for less than 30 s, W_2 between 30 s and 1 min and W_3 for more than 1 min. The nociceptive response (S) was then calculated for each of the successive 15-min periods, using the formula: $S = 1L + 2B + 3C + 4W_1 + 5W_2 + 6W_3$. The resulting nociceptive response drawn from the relative frequency of each behavior was taken as an index of nociception (see Miampamba et al., 1994 for details). In all experiments animal behaviors were recorded

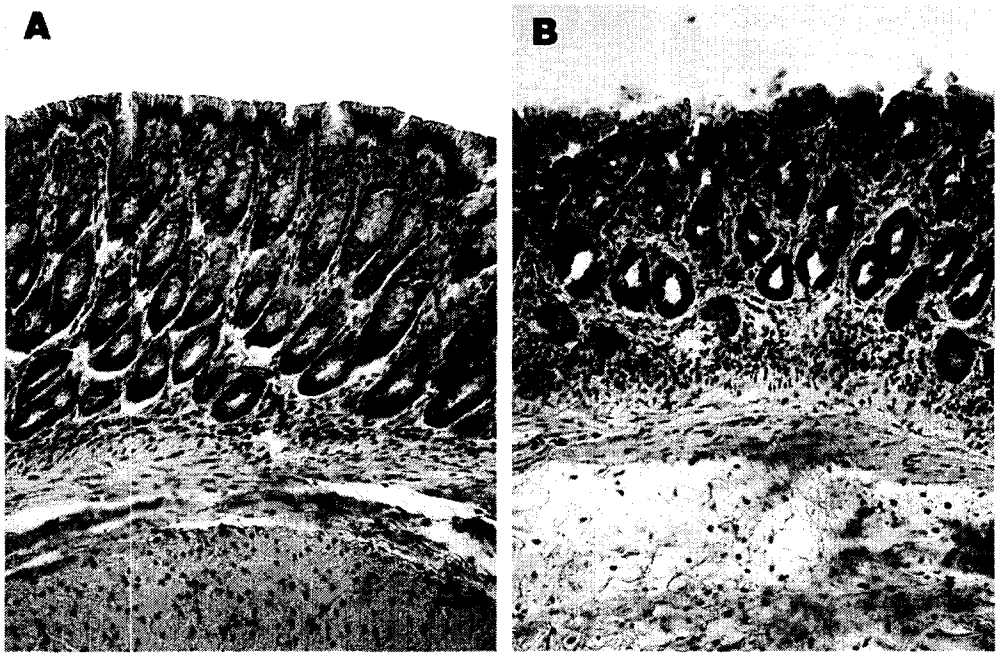

Fig. 2. (A) Histological appearance of full-thickness sections of colonic wall in rats subjected to c.w injection of saline. No significant histologic modification was observed in the tissue exposed to saline. (B) Three hours after formalin the epithelial surface was undergoing erosion and was covered by an inflammatory exudate with polymorphonuclear cells. The architecture of the mucosa was disorganized and the loss of crypt epithelial cells can be seen. Mild cellular infiltration in lamina propria is also noted. These features are accompanied by marked submucosal edema (magnification ×160).

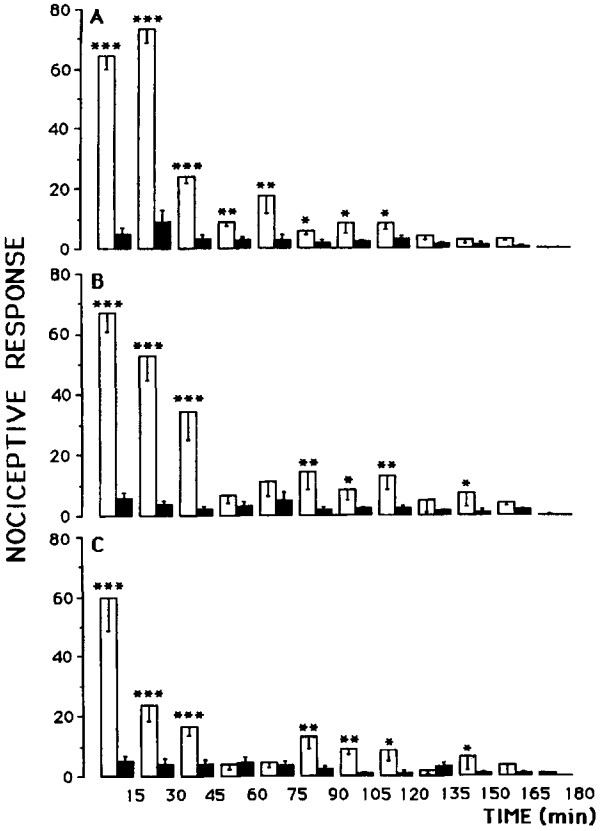

Fig. 3. Time course of nociceptive responses of rats following the c.w. injection of 50 μl of 5% formalin (open bars) or 50 μl of isotonic saline (closed bars). These data indicate the noxious effect of formalin in each experimental set, i.e. when clonidine and S12813-4 were administered before (A) or after (B) the c.w. injection of formalin, and during the test of the yohimbine and idazoxan effect on antinociception by S12813-4 (C). $n = 8$ in each experimental group. Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.005$, Wilcoxon test.

blind as regarded control or treated status.

The ability of the drugs (i.e., clonidine and S12813-4) under test to decrease the number and the duration of the selected behaviors related to visceral nociception was taken as a measure of their antinociceptive action.

2.5. Assessment of colon inflammation

At the end of the session, rats were killed by an intraperitoneal (i.p.) pentobarbital overdose. The colon was excised and opened longitudinally. The quality of the injection and the spread of inflammation (in formalin-injected rats) could then be evaluated by the diffusion of Evans blue around the injection site. If the injection site was not accurately limited to the depth of the c.w., the viscera around the colon sigmoid (inside the abdominal cavity) were stained by Evans blue. In this case, the data obtained from the animal were excluded from the study. Histological assessment was then performed with a segment of colon including the injection site. The tissue was removed, fixed overnight by immersion in Bouin's solution, dehydrated, embedded in paraffin and stained with haematoxylin and eosin.

2.6. Drugs and chemicals

The drugs and chemicals used were: formaldehyde aqueous solution min. 37% w/v (Prolabo, France), clonidine and idazoxan hydrochloride (Sigma St Louis, MO, USA), S12813-4 (Institut de Recherches Internationales Servier, I.R.I.S., Courbevoie, France), yohimbine hydrochloride (Aldrich Milwaukee, USA) and Evans blue (Rhône-Poulenc, France). All drugs were freshly dissolved in sterile saline (0.9% NaCl) on the day of the experiment, with the exception of clonidine and S12813-4 which were dissolved in water.

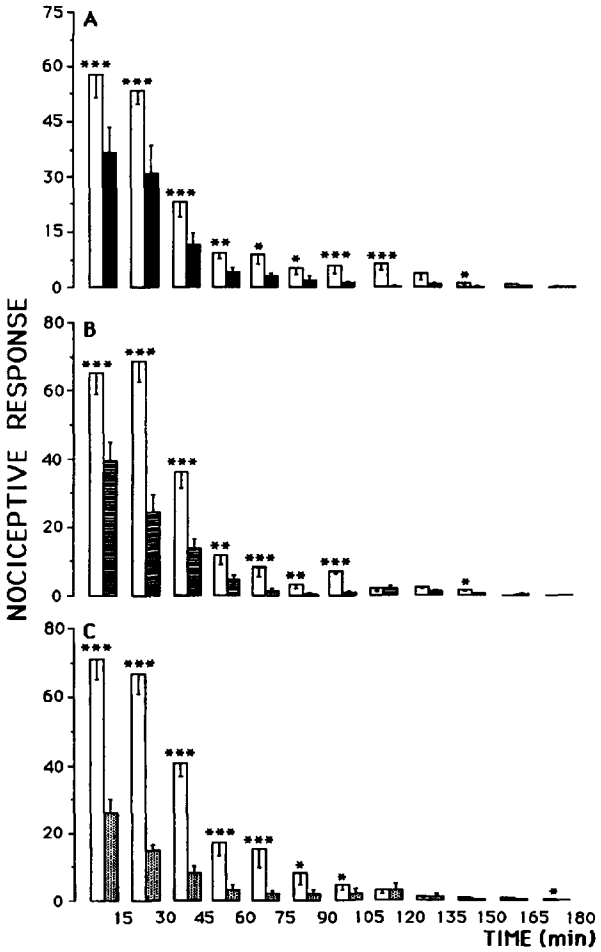

Fig. 4. Time course of nociceptive responses of rats following the c.w. injection of 50 μl of 5% formalin 30 min after administration of the three dosages of clonidine ({75 μg/kg, A, closed bars, $n = 6$}, {150 μg/kg, B, hatched bars, $n = 8$} and {300 μg/kg, C, dotted bars, $n = 6$}) compared to that of the nociception control group responses (water given orally followed by the c.w. injection of formalin, A,B,C, open bars). Numbers of nociception control and of clonidine-treated rats were similar in each group. Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$, Wilcoxon test.

2.7. Data analysis and statistics

Firstly, the nociceptive effect of formalin was assessed by comparing nociceptive responses obtained in formalin and in saline-injected rats, without antinociceptive agents. Secondly, the effects of drugs were evaluated by comparing nociceptive responses obtained in treated rats (c.w. injection of formalin preceded or followed by the administration of antinociceptive agent) and in controls for nociception (c.w. injection of formalin without antinociceptive agent). The results were expressed as means ± S.E.M. nociceptive responses for each 15-min period, and the data were analysed using an analysis of variance (ANOVA) (Friedman) and the Wilcoxon rank test. Significance was set at the $P < 0.05$ level.

3. Results

3.1. Formalin-induced inflammation

Gross inspection of the colon after intramural injection of saline showed an apparently normal colon. There were minimal changes, and the c.w. was histologically normal as seen in Fig. 2A. However, after c.w. injection of formalin, the colon showed a grossly thickened, egg-shaped, inflamed area (about 2 cm^2) around the injection site. The histologic appearance of the sigmoid segment including the injection site showed marked exfoliation of the epithelium and exulceration covered by an exudate with polymorphonuclear cells. Disorganization of the mucosal architecture with a loss of crypts could be noted. A few polymorphonuclear cells were also observed in the lamina propria, while the muscularis mucosae was normal. These features were associated with marked submucosal edema (Fig. 2B).

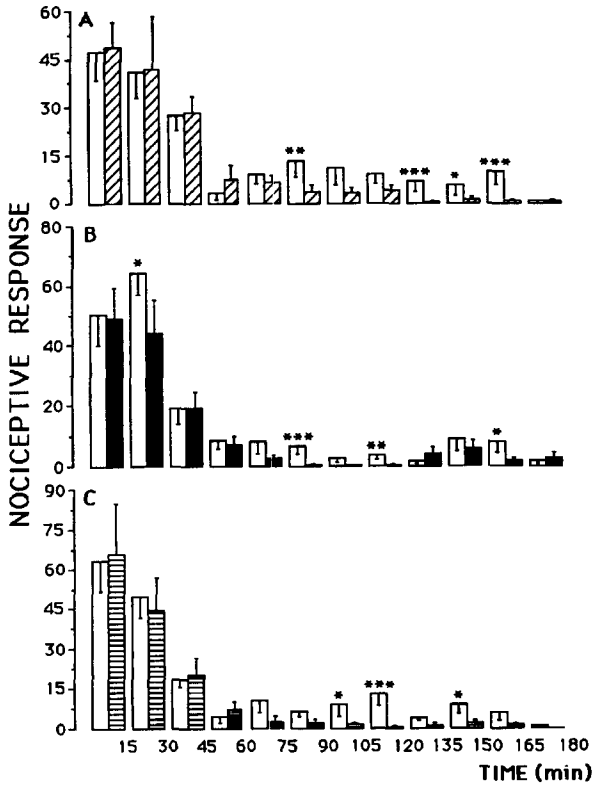

Fig. 5. Time course of nociceptive responses following the c.w. injection of 50 μl of 5% formalin followed 15 min later by one of the three dosages of clonidine ({75 μg/kg, A, hatched bars, $n = 9$}, {150 μg/kg, B, closed bars, $n = 9$} and {300 μg/kg, C, hatched bars, $n = 8$}) compared to that of the nociceptive control group responses (c.w. injection of formalin followed by oral administration of water, A,B,C, open bars). Numbers of nociception control and of clonidine-treated rats were similar in each group. Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$, Wilcoxon test.

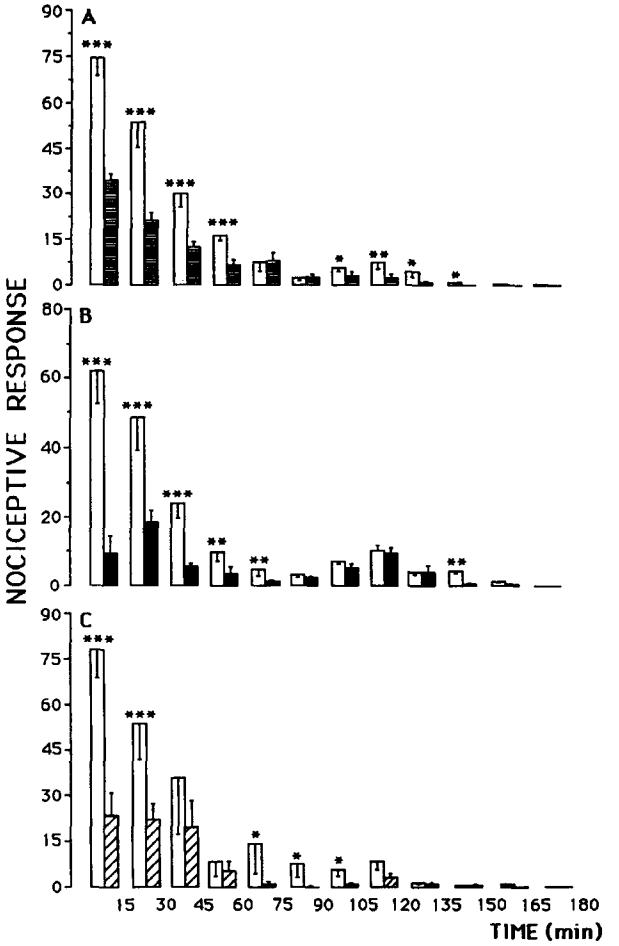

Fig. 6. Time course of nociceptive responses following the c.w. injection of 50 μl of 5% formalin 30 min after administration of one of the three dosages of S12813-4 ({10 mg/kg, A, hatched bars, $n = 6$}, {30 mg/kg, B, closed bars, $n = 7$} and {90 mg/kg, C, hatched bars, $n = 7$}) compared to that of the nociception control group responses (water given orally followed by the c.w injection of formalin, A,B,C, open bars). Numbers of nociception control and of S12813-4-treated rats were similar in each group. Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$, Wilcoxon test.

3.2. Nociceptive responses after c.w. injection of formalin or saline

The effects of formalin or saline injected into the c.w., in the first and the second part of the study, respectively,

are illustrated in Fig. 3A,B,C. Both saline and formalin injections were followed by measurable variations of the nociceptive response, which followed roughly parallel patterns. A first phase occurred just after the injection and lasted for about 45 min, while a second phase took place between 75 and 120 min. For both phases, nociceptive responses in formalin-injected animals were significantly higher than the values in saline-injected rats, confirming the noxious effect of formalin (Friedman ANOVA).

3.3. Effects of clonidine

The α_2-adrenoceptor agonist, clonidine, reduced the nociceptive responses at the three doses tested (75, 150, 300 μg/kg) when administered 30 min before noxious stimulation. The reduction of nociceptive responses was observed in both phases of nociception (Fig. 4A,B,C). Although the high doses of clonidine appeared to be more potent, the variability of nociceptive responses in control rats (Fig. 4A,B,C, open columns), precluded the finding of any clear dose-response effect of clonidine. In contrast,

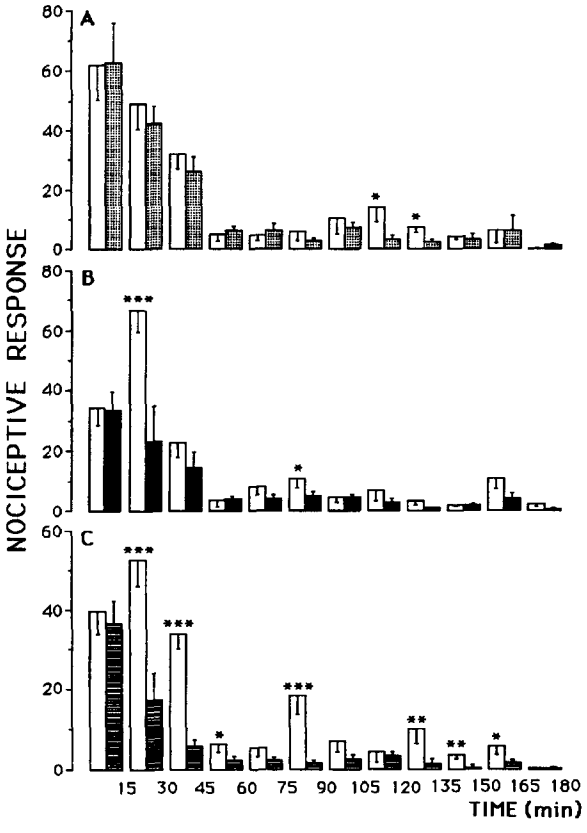

Fig. 7. Time course of nociceptive responses after the c.w. injection of 50 μl of 5% formalin followed 15 min later by one of the three dosages of S12813-4 ({10 mg/kg, A, dotted bars, $n = 8$}, {30 mg/kg, B, closed bars, $n = 11$} and {90 mg/kg, C, hatched bars, $n = 11$}) compared to that of the pain control group responses (c.w injection of formalin followed by oral administration of water, A,B,C, open bars). Numbers of nociception control and of S12813-4-treated rats were similar in each group. Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$, Wilcoxon test.

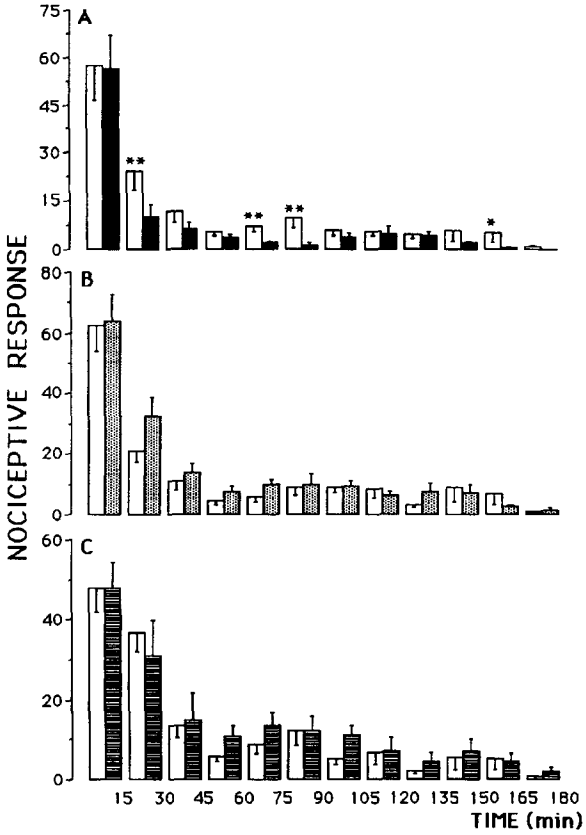

Fig. 8. Time course of nociceptive responses after the c.w. injection of 50 μl of 5% formalin followed by peroral administration of placebo (A,B,C, open bars) or of 30 mg/kg of S12813-4 (A, closed bars; B, dotted bars and C, hatched bars). 15 min before the c.w. injection of formalin, the rats received a subcutaneous injection of (i) saline (1 ml/kg, A, closed bars and A,B,C, open bars), (ii) yohimbine (1 mg/kg, B, dotted bars) or (iii) idazoxan (1 mg/kg, C, hatched bars). Numbers of nociception control and treated rats were similar in each group ($n = 9$ in A and C; $n = 11$ in B). Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.01$, Wilcoxon test.

there was a slight reduction of nociceptive responses when the drug was administered 15 min after the c.w. injection of formalin (Fig. 5A,B,C). In that case, the antinociceptive effect was restricted to the second phase (from 90 min on).

3.4. Effects of S12813-4

Compound S12813-4, administered orally, reduced nociceptive responses induced by c.w. injection of formalin. When given 30 min before the c.w. injection of formalin, the drug exerted significant antinociceptive effects on both phases of nociception (Fig. 6A,B,C). When S12813-4 was given 15 min after c.w. injection of formalin, no decrease of nociceptive responses was observed at the dose of 10 mg/kg (Fig. 7A). The intermediate dose (30 mg/kg) slightly reduced the nociceptive responses during the early phase (Fig. 7B), while the larger dose (90 mg/kg) reduced nociceptive responses in the early and the late phases (Fig. 7C).

3.5. Effects of yohimbine and idazoxan on antinociception by S12813-4

In the control group (i.e., c.w. injection of formalin followed by oral administration of S12813-4, 30 mg/kg), the drug reduced nociceptive responses, although the variation reached significance at 30 and 90 min only (Fig. 8A). No such effect was observed when yohimbine (Fig. 8B) or idazoxan (Fig. 8C) was subcutaneously injected before S12813-4 administration. In these cases, the nociceptive responses were closely similar to those of the control groups for nociception (i.e., c.w. injection of formalin without antinociceptive agent) throughout the study period.

4. Discussion

The present results confirmed that formalin injected into the c.w. provokes visceral nociception, as reflected by behavioral responses such as abdominal contraction, stretching and licking of the perineal area (Miampamba et al., 1994). These behaviors were also reported for other visceral pain models, e.g. writhing test (Koster et al., 1959) and urinary bladder pain model (Abelli et al., 1989).

We have evaluated the antinociceptive effects of clonidine and of S12813-4, and the α_2-adrenoceptor agonist-like actions of S12813-4 using this model of inflammatory colon pain. To our knowledge, this is the first application of formalin as noxious stimulus injected into the c.w., to assess the antinociceptive effects of peroral clonidine and S12813-4 in acute colonic inflammation.

We have shown that clonidine administered orally reduced the nociceptive responses observed after the c.w. injection of formalin. Consistent antinociception was observed when the drug was given 30 min prior to nociceptive stimulation. When clonidine was administered 15 min after formalin injection, the antinociception was observed on the second phase only. These different effects of clonidine were probably related to its mechanisms of action. Clonidine has been shown to produce antinociceptive effects in several different procedures presumed to induce pain in laboratory animals (see Fielding et al., 1981 for details), including the inhibition of writhing (Fielding et al., 1978; Bentley et al., 1977) which has some similarities with the model of visceral nociception used in the present study. In several reports, the analgesic effectiveness of clonidine has been evaluated in relation to its interaction with noradrenergic mechanisms and its activity in morphine-sensitive systems. The hypothesis that clonidine reduces nociceptive responses through an α-adrenergic mechanism in association with morphine-sensitive sites is based on the premise of ascending and descending projections in pain-associated pathways (Fielding et al., 1981). Adrenergic projections from the locus coeruleus have been found in many central areas implicated in analgesia (Ader et al., 1979). Clonidine can promote analgesia through a central action at both supraspinal (Holman et al., 1971; Paalzow, 1974) and spinal levels (Spaulding et al., 1979; Yaksh and Reddy, 1981; Hare and Franz, 1983), and the inhibitory effect that clonidine has on noradrenergic neurons appears to result from presynaptic activation of α-receptors (Andén et al., 1970; Starke, 1972; Svensson et al., 1975). In addition, it was shown that other α-sympathomimetics of the imidazoline series that do not cross the blood-brain barrier have analgesic activity only when administered intracerebroventricularly (Schmitt et al., 1974). In writhing induced by i.p. injection of phenylquinone, s.c administration of 0.05 mg/kg of clonidine almost completely abolishes the abdominal contractions at the peak of antinociception, evaluated at 15 min (Fielding et al., 1981). This antinoceptive effect could be mediated centrally as mentioned above. However, some data indicate that when pain is evoked by an inflammatory stimulus such as in the phenylquinone writhing test, clonidine as well as several other α-adrenoceptor agonists, including those which do not cross the blood-brain barrier, also promote analgesia (Bentley et al., 1977). This suggests that, in addition to their well known centrally mediated analgesic actions, clonidine-like drugs can also promote antinociception through a peripheral mechanism of action. It is postulated that the antinocicepive action of the α-adrenoceptor agonists is due to an effect on α-adrenoceptors located on sensory nerve endings in the peritoneum, whose affinities for the α-adrenoceptor agonists and antagonists are different from those shown by either pre- or postsynaptic α-adrenoceptors (Bentley et al., 1977). Some data suggest that drugs such as clonidine could induce peripheral antinociception by an α_2-adrenoceptor-mediated local release of enkephalin-like substances (Nakamura and Ferreira, 1988). Consequently, local clonidine seems to have a beneficial effect in nociception from inflamed or irritated tissues via a mechanism different from those described for the central level. In theory, clonidine administered orally could act on α-adrenoceptors located on sensory nerve endings in the sigmoid colon.

However, we speculate that the antinoceptive effect of clonidine might be mediated at the central level, after intestinal absorption of the drug. The weak effect of the drug is indeed consistent with limited bioavaibility upon oral administration. Since antinociceptive activity was measured in intact animals, factors such as sequestration in other tissues and reduction of the concentration by metabolic processes could decrease the potency of the drug. It was demonstrated that the ability of systemic clonidine (0.05 mg/kg) to alter significantly the writhing occurred 15 min after its administration (Fielding et al., 1981). By analogy, the time lag to the peak antinociceptive effect of clonidine given orally was probably longer than 15 min, due to gastric emptying and intestinal absorption. The hypothesis of a central action is supported by the fact that the drug seemed to be more potent when given 30 min before the noxious stimulus. In other visceral pain models such as colon distension, the effective analgesic effect of

clonidine was obtained immediately after i.t. administration (Danzebrink and Gebhart, 1990, 1991). When clonidine was given by the intracisternal route, its antinociceptive effect on writhing was considerably greater than when it was given subcutaneously (Bentley et al., 1977). According to these data, the route of administration of this drug is a critical factor for maximum antinociceptive effect. Another possible explanation is that formalin injected into the c.w. induced severe damage of the colonic tissue. Possibly the potency of clonidine was not sufficient to strongly reduce the nociception associated with inflammation and/or injury in the viscera. Nevertheless, the behavioral results of the present study suggest the possible involvement of noradrenergic pathways in the transmission and/or modulation of a nociceptive influx, as previously reported from several studies (Sullivan et al., 1987; Hylden et al., 1991; Takano et al., 1992; Kayser et al., 1992a).

S12813-4 is a new compound whose antinociceptive effect appears to be mediated by noradrenergic pathways (Kayser et al., 1992b). Administered orally before or after c.w. injection of formalin, S12813-4 displayed antinociceptive effects in both phases of nociception. In addition, the antinociceptive effect of S12813-4 (30 mg/kg) administered orally was prevented by yohimbine and idazoxan. Interestingly, Kayser et al. (1992b) noted that S12813-4, like clonidine, caused significant and dose-dependent antinociceptive effects in two experimental models of inflammatory and neuropathic pain in rats, and that these effects were prevented by yombine and idazoxan, as expected from their mediation through the stimulation of α_2-adrenoceptors. Recently, it was demonstrated that S12813-4 inhibits the release of substance P-like material in the spinal cord. In addition, the inhibitory effect of S12813-4 on substance P-like material release could be prevented by the α_2-adrenoceptor antagonist, idazoxan (Collin et al., 1994). In view of the well established role of substance P as neurotransmitter of nociceptive fibres entering the dorsal horn of the spinal cord (Weihe, 1992), the antinociceptive effect of S12813-4 might result from an inhibitory influence on these fibres. The suppression of the antinociceptive effect of S12813-4 by yohimbine and idazoxan observed in the present study confirms that the compound probably modulates nociceptive events from the inflamed colon via the α_2-adrenergic system.

In conclusion, formalin-induced colonic inflammation evoked visceral nociception. The two α_2-adrenoceptor agonists used here reduced the nociceptive responses, suggesting that noradrenergic pathways are involved in the transmission and/or modulation of the nociceptive influx from inflamed colon.

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