Pregabalin (CI-1008) Inhibits the Trinitrobenzene Sulfonic Acid-Induced Chronic Colonic Allodynia in the Rat

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ABSTRACT

In human, digestive disorders are often associated with visceral pain. In these pathologies, visceral pain threshold is decreased indicating a visceral hypersensitivity. Pregabalin [CI-1008; S-(+)-3-isobutylgaba] presents antihyperalgesic actions in inflammatory somatic pain models. This study was designed to evaluate 1) the effect of injection of TNBS into the colon on visceral pain threshold, and 2) the antihyperalgesic effect of pregabalin on TNBS-induced chronic colonic allodynia. A significant decrease in the colonic pain threshold was observed in trinitrobenzene sulfonic acid (TNBS)-treated animals (17.8 ± 1.27 versus 43.4 ± 1.98 mm Hg). Pregabalin (30–200 mg/kg s.c.) and morphine (0.1–1 mg/kg s.c.) showed a dose-related inhibition of TNBS-induced colonic allodynia. Pregabalin did not inhibit the colonic inflammatory effect of TNBS. In normal conditions (control animals), morphine (0.3 mg/kg s.c.) significantly increased the colonic pain threshold, whereas pregabalin (200 mg/kg s.c.) did not modify the colonic pain threshold. Pregabalin suppressed the TNBS-induced colonic allodynia but did not modify the colonic threshold in normal conditions. The ability of pregabalin to block the chronic colonic allodynia indicates that it is effective in abnormal colonic hypersensitivity, suggesting a possible effect in chronic pain in irritable bowel syndrome.

The irritable bowel syndrome (IBS) is one of the most common disorders in gastroenterology (Camilleri, 2001). Altered motility, psychosocial factors, and hypersensitivity are major mechanisms that interact in IBS disorders. The mainstays of the current therapeutic approach continue to be stress management strategies, dietary modification entailing addition of dietary fiber, and pharmacotherapy (Camilleri, 2001). Pharmacological therapy is still limited to treating symptoms such as diarrhea or constipation, bloating, and discomfort. Antidiarrheal agents seem to treat the diarrhea in IBS patients (Cann et al., 1984). Synthetic opioids acting on peripheral μ-opioid receptors reduced gut motility and secretion in gastrointestinal tract. Whether the diarrhea seems to improve, the abdominal pain was not changed in diarrhea-predominant IBS. Patients with alternating bowel habits had no improvement, whereas the constipation-predominant IBS patients have worsening of their symptoms (Hovdenak, 1987). Prokinetics agents have no significant efficacy in the treatment of IBS (Mayer et al., 1998). Some antispasmodic agents are thought to relieve abdominal symptoms but become less effective with long term. Antidepressants present clinical benefits in pain-predominant IBS patients (Clouse et al., 1994). These activities are due to their analgesic actions and/or antidepressant effects. However, the side-effect profile and the tolerance limit the long-term use in IBS patients. Current conventional therapies do not address pain in IBS. Better understanding of the brain-gut axis is key to the development of effective therapies for IBS.

In human, digestive disorders are often associated with visceral pain. In these pathologies, the visceral pain threshold is decreased, indicating a visceral hypersensitivity (Mayer and Gebhart, 1994). Indeed, lower visceral sensory thresholds to colorectal distension have been found in patients suffering from irritable bowel syndrome. Some patients with functional bowel disorders have pain or discomfort at pressures producing normal internal sensations. Thus, normally nonpainful distension is sensed as painful (mechanical allodynia) and pain threshold/response magnitude is altered (hyperalgesia). Colonic distension in human produces reliable autonomic and visceromotor responses as well as reliable reports of pain (Ness et al., 1990). The behavioral, autonomic, and motor responses induced by colonic distension have been also observed in rats (Ness and Gebhart, 1990). Inflammation of the colon can change the threshold to colonic distension, demonstrating a visceral hypersensitivity (Morteau et al., 1994). Indeed, after TNBS-induced rectocolitis, colonic and abdominal responses were observed at a lower diameter of distension than in control animals (Morteau et al., 1994). In our hands, intracolonic administration of acetic acid induced the sensitization of nociceptive

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ABBREVIATIONS: IBS, irritable bowel syndrome; TNBS, trinitrobenzene sulfonic acid; GABA, γ-aminobutyric acid; MPO, myeloperoxidase; HTAB, hexadecyltrimethylammonium bromide; EtOH, ethanol; ANOVA, analysis of variance.

visceral afferents, which in turn produced colonic hypersensitivity (Langlois et al., 1994). However, the time course and the chronicity of the development of these hyperalgesia and allodynia are poorly understood.

Focusing on hyperalgesia, the antagonists of GABA$_A$ and N-methyl-D-aspartate receptors have demonstrated activity in these models (Yaksh, 1989; Woolf and Thompson, 1991; Sivilotti and Woolf, 1994; Woolf, 1994). The hyperalgesia and allodynia are mediated by a recruitment of silent afferents at the spinal and supraspinal levels (Coderre et al., 1993). Gabapentin is an antiepileptic agent currently on the market as add-on therapy in patients with partial seizures resistant to conventional therapies (for review, see Goa and Sorkin, 1993). Although gabapentin was originally designed as a GABA analog that would penetrate into the central nervous system, it does not interact with either GABA$_A$ or GABA$_B$ receptors (Bartoszyk and Reimann, 1985). More recently, the gabapentin recognition site was identified as the two subunits of voltage-dependent calcium channels, $\alpha_2\delta$ (Gee et al., 1996). In binding studies, gabapentin and (R,S)-3-isobutylgaba were the most active compounds identified for this site. (R,S)-3-Isobutylgaba stereoselectively inhibited [^{3}H]gabapentin binding to brain membranes with (S)-$(+)$-3-isobutylgaba (pregabalin), showing similar affinity as gabapentin, whereas the corresponding (R)-$(-)$-enantiomer was found to be 10 times weaker (Taylor et al., 1993). Pharmacological studies have also shown the stereospecific effect of pregabalin in neuropathic or inflammatory pain models (Field et al., 1997b; Chen et al., 2001). Recent studies have shown that gabapentin possesses antihyperalgesic actions in animal models of inflammatory and neuropathic pains (Field et al., 1997a,b, 1999). For instance, it has been reported that gabapentin and pregabalin selectively block the second phase of the formalin response and carrageenan-induced thermal and mechanical hyperalgesia (Field et al., 1997; Houghton et al., 1998). Other studies have shown that these compounds can also block hyperalgesia and allodynia in rat models of neuropathic pain or postoperative pain (Xiao and Bennett, 1995; Field et al., 1997; Hwang and Yaksh, 1997). It is also interesting to note that there is an up-regulation of the $\alpha_2\delta$ subunit mRNA in the model of neuropathic pain (Philp et al., 1999) and an increase in [^{3}H]gabapentin binding sites in dorsal horn of spinal cord in chronic constriction injury model (Field et al., 2000). In the present study, we have investigated the time course of the induction of colonic inflammation induced by TNBS and the changes induced on the visceral sensitivity. In addition, we have compared the activities of pregabalin and morphine in this chronic model of colonic allodynia.

Materials and Methods

Animals and Surgery. Male Sprague-Dawley rats (Janvier, Le Genest-St-Isle, France) weighing 340 to 400 g were used in this study. The animals were housed three per cage in a regulated environment (20 $\pm$ 1°C, 50 $\pm$ 5% humidity, with light from 7:00 AM to 7:00 PM). Under anesthesia (ketamine, 80 mg/kg i.p.; acepromazine, 12 mg/kg i.p.), the injections of TNBS (50 mg/kg; 1.5 ml/kg) or saline (1.5 ml/kg) were performed into the proximal colon (1 cm from the cecum). After surgery, animals were individually housed in polypropylene cages and kept in a regulated environment (20 $\pm$ 1°C, 50 $\pm$ 5% humidity, with light from 7:00 AM to 7:00 PM).

Distension Procedure. The balloon (5 cm in length) was inserted through the anus and kept in position (tip of balloon 5 cm from the anus) by taping the catheter to the base of the tail. The animals were individually placed without restraint in polypropylene cages for distension session. The balloon was progressively inflated by step of 5 mm Hg, from 0 to 75 mm Hg, each step of inflation lasting 30 s. Each cycle of colonic distension was controlled by a standard barostat (ABS, St-Dié, France). The pain threshold corresponds to the pressure that produced the first abdominal contraction. The abdominal contraction corresponds to waves of contraction of oblique musculature with inward turning of the hindlimb, or to humpbacked position, or to squashing of the lower abdomen against the floor (Wesselmann et al., 1998). To determine the colonic threshold, four cycles of distension were performed on the same animal with an interval of 10 min. Data are analyzed by comparing test compound-treated group with TNBS-treated group and control group. Mean and S.E.M. are calculated for each group. The antiallodynic activity of the compound is calculated as follows: % activity = [(group C − group T)/(group A − group T)] × 100, where group C is mean of the test compound-treated group, group T is mean of the TNBS-treated group, and group A is mean of the control group.

Assessment of Colonic Damage. Seven days after administration of TNBS/ethanol, the animals were sacrificed by cervical dislocation, and the total colon was removed, opened, rinsed in saline, pinned out on a flat surface, and digitized with an image analyzer station. Immediately after digitization, the colon was divided in half by a longitudinal cut. The two parts of the colon were weighed, frozen in liquid nitrogen, and stored at −80°C until used for MPO assay. The colonic damage was evaluated by means of computerized morphometric analysis and MPO measurement as detailed subsequently.

Computerized Morphometric Analysis. The sample to be digitized was placed on a flat surface and uniformly illuminated by four lamps that projected the light tangentially. A color video camera (Sony XC777P), which was set up at a fixed distance from the sample and connected to a PC computer, was used to transmit the image of the colon to the computer. The digitized image was saved in an electronic file and stored onto an optical disk. The system was automatically calibrated with calibration factors previously determined by x- and y-coordinate measurements of rules placed in the video field. Subsequently, the image of the colon was retrieved and converted into a gray scale image for morphometric analysis. A standard square object of known size (100 mm^2) was included with each colon image and was used to verify proper system calibration before morphometric analysis of each colon image. The computer calculated the areas of the different regions and expressed the extent of such areas in square millimeters. TNBS induced necrotic and hyperemic areas in the colon. To minimize the differences in measurement caused by the preparation of the sample to be digitized, necrotic and hyperemic areas were expressed as the percentage of the total colon area calculated by dividing both the areas of necrosis and hyperemia by the total area of the colon specimen used.

MPO Assay. MPO activity was measured spectrophotometrically, with 0.0005% hydrogen peroxide as the substrate, by a modification of the method described by Krawisz et al. (1984) and Grisham et al. (1990). The frozen colon samples were cut into 3- to 5-mm^2 pieces and added to 50-ml plastic centrifuge tubes, which contained 1 ml of 0.5% hexadecyltrimethylammonium bromide (HTAB) per 100 mg of colon tissue. The minced tissue was homogenized for approximately 15 s. The homogenate was separated into 1-ml aliquots in Microfuge tubes and stored at −80°C. At the time of the assay, 1-ml aliquots of the homogenate were frozen and thawed twice. After the second thaw, the samples were spun at 19,000g for 15 min in a Sigma (Bioblock, Illkirch, France) centrifuge. The supernatant was discarded and the pellet was resuspended in 500 μl of 0.5% HTAB solution. It was sonicated for 10 s with a Vibra Cell (Bioblock) and respun at 19,000g for 15 min. MPO activity was determined in 80 μl of the supernatant to which was added 120 μl/ml potassium phosphate buffer (50 mM,
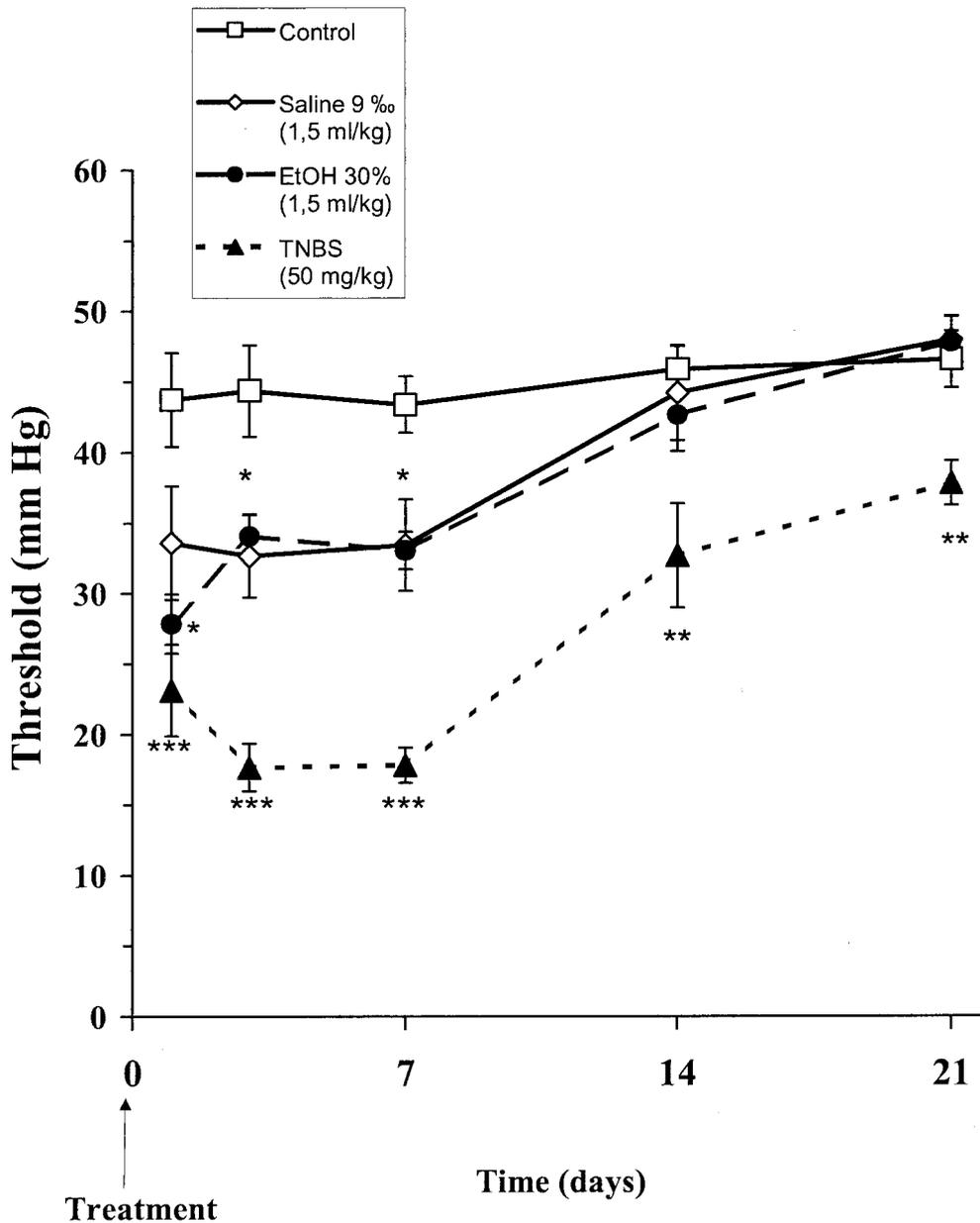
Fig. 1. Effect of injection of TNBS on colonic threshold. Pain threshold (pressure of distention inducing the first abdominal contraction) after distal colonic distention was determined at days 1, 3, 7, 14, and 21 in four groups of awake rats: control animals (□), sham animals (◇), 30% EtOH-treated animals (●), and TNBS-treated animals (▲). Results are expressed as mean of colonic threshold ± S.E.M. (mm Hg) (n = 7–8/group). Statistical significance between each group was determined by using a one-way ANOVA followed by Student's unpaired t test. Differences were considered statistically significant at $p < 0.05$ (⋆, $p < 0.05$; ⋆⋆, $p < 0.01$; ⋆⋆⋆, $p < 0.001$ versus control).

pH 6.0), 0.0005% o-dianisidine dihydrochloride, and 0.1% hydrogen peroxide. The total volume in each well was 200 μl. After 1 min the reaction was stopped by addition of 25 μl of catalase (180 μg/ml). The wells containing 225 μl of sample or MPO standard were read at 450 nm on an iEMS (Labsytems, Helsinki, Finland) spectrophotometer. One unit of MPO activity was defined as that which would produce an increase in absorbance (Δ A450 nm) of 1.0 unit/min at pH 7.0 and 25°C, calculated from the initial rate of reaction with peroxide (1 μM) as the substrate. The results are expressed as MPO units per milligram of tissue.

Histological Study. For each animal, one segment of the proximal colon and one of the distal part were removed, sectioned transversely, in their entirety, into 5- to 8-mm cross-sections, and immersion-fixed overnight in Carnoy (ethanol, 6 volumes; chloroform, 3 volumes; and acetic acid, 1 volume). The fixed tissues were processed into paraffin, cut into 5-μm sections, stained with hematoxylin-eosin, and examined with light microscopy.

Experimental Protocol. Nine series of experiments were conducted. In the first series of experiments, pain threshold (pressure of distention inducing the first abdominal contraction) after distal

colonic distention was determined at day 1, 3, 7, 14, and 21 in four groups (n = 7–8 animals) of awake rats: control animals, sham animals, 30% EtOH-treated animals, and TNBS-treated animals. The control animals had no treatment and no surgery. The sham animals received an injection of saline (1.5 ml/kg) into the proximal colon (1 cm from the cecum). The 30% EtOH-treated animals and TNBS-treated animals received the injections of 30% EtOH or TNBS (50 mg/kg) into the proximal colon, respectively. In the second series, the inflammatory parameters (colon weight, area of hyperemia and necrosis, and colonic myeloperoxidase content) have been measured at day 3 and day 7 in four groups of animals (n = 6–13 animals): control animals, sham animals, 30% EtOH-treated animals, and TNBS-treated animals. For all following pharmacological experiments with pregabalin and morphine, the experiments were performed at day 7. To determine the antinociceptive effect of pregabalin, a third series of experiments was performed using six groups (n = 7–8) of rats. Control groups received s.c. injection of 0.9% saline 30 min before colonic distension series. TNBS-treated rats received either s.c. injection of 0.9% saline or pregabalin (30, 60, 100, and 200 mg/kg s.c.) 30 min

A

B
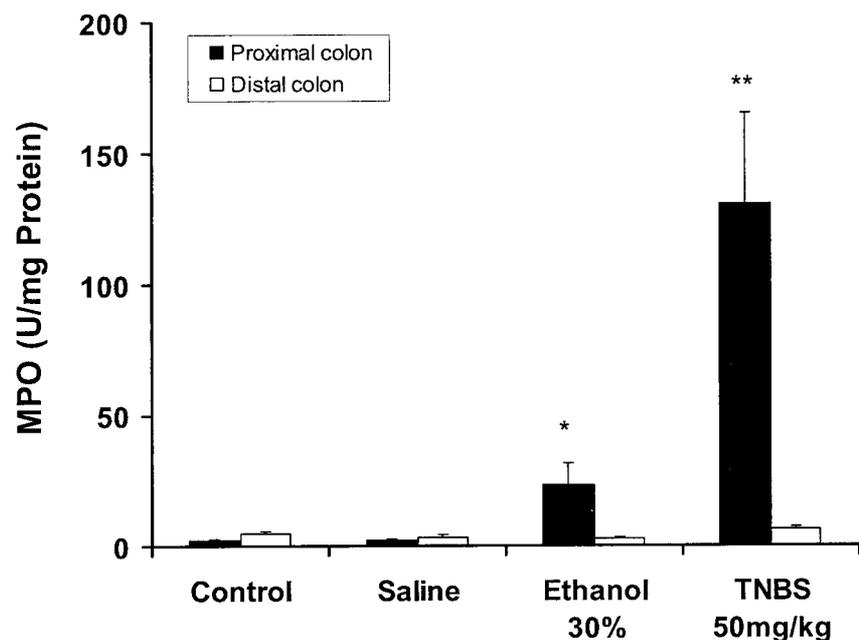
Fig. 2. Effect of TNBS on MOP activity in the proximal (■) and distal (□) colon at day 3 (A) and day 7 (B). One unit of MPO activity was defined as that which would produce an increase in absorbance (Δ A450 nm) of 1.0 unit/min at pH 7.0 and 25°C, calculated from the initial rate of reaction with peroxide (1 μM) as the substrate. The results are expressed as MPO units per milligram of protein (mean $\pm$ S.E.M.; n = 6–13/group).
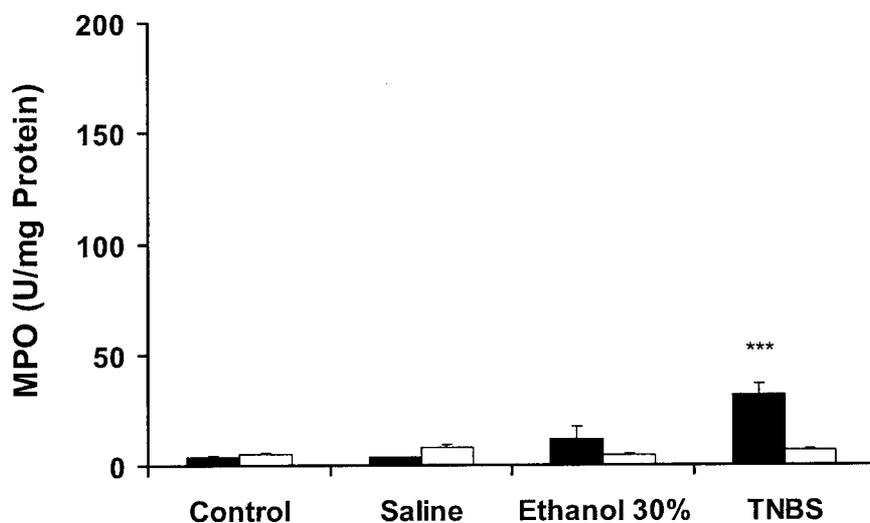
before the colonic distension series. In the fourth series of experiments, seven groups (n = 7–16) of rats were used. Control groups received s.c. injection of 0.9% saline 30 min before colonic distension series. TNBS-treated rats received either s.c. injection of 0.9% saline or morphine (0.01, 0.03, 0.1, 0.3, and 1 mg/kg s.c.) 30 min before colonic distension series. In the fifth series of experiments, the effects of s.c. pregabalin and morphine on colonic threshold in normal rats were evaluated. Two groups (n = 6–8 rats) received s.c. injection of 0.9% saline or pregabalin (200 mg/kg s.c.) 30 min before colonic distension series. Five groups (n = 5–13 rats) received s.c. injection of 0.9% saline or morphine (0.01, 0.1, 0.3, and 1 mg/kg s.c.) 30 min before colonic distension series. To study the effect of naloxone on pregabalin- and morphine-induced

antiallodynia, seven groups (n = 7–8) were used. Control groups received s.c. injection of 0.9% saline. TNBS-treated rats received either s.c. injection of 0.9% saline or naloxone (1 mg/kg s.c.) 35 min before colonic distension series. TNBS-treated rats received morphine (1 mg/kg s.c.) alone or morphine + naloxone. TNBS-treated rats received pregabalin (200 mg/kg s.c.) alone or pregabalin + naloxone. The eighth series of experiments was aimed at determining the effect of p.o. administration of pregabalin. Six groups (n = 7–8) of rats were used. Control groups received p.o. administration of water 1 h before colonic distension series. TNBS-treated rats received either p.o. administration of water or pregabalin (30, 60, 100, and 200 mg/kg p.o.) 1 h before colonic distension series. In the last series of experiments, the time course
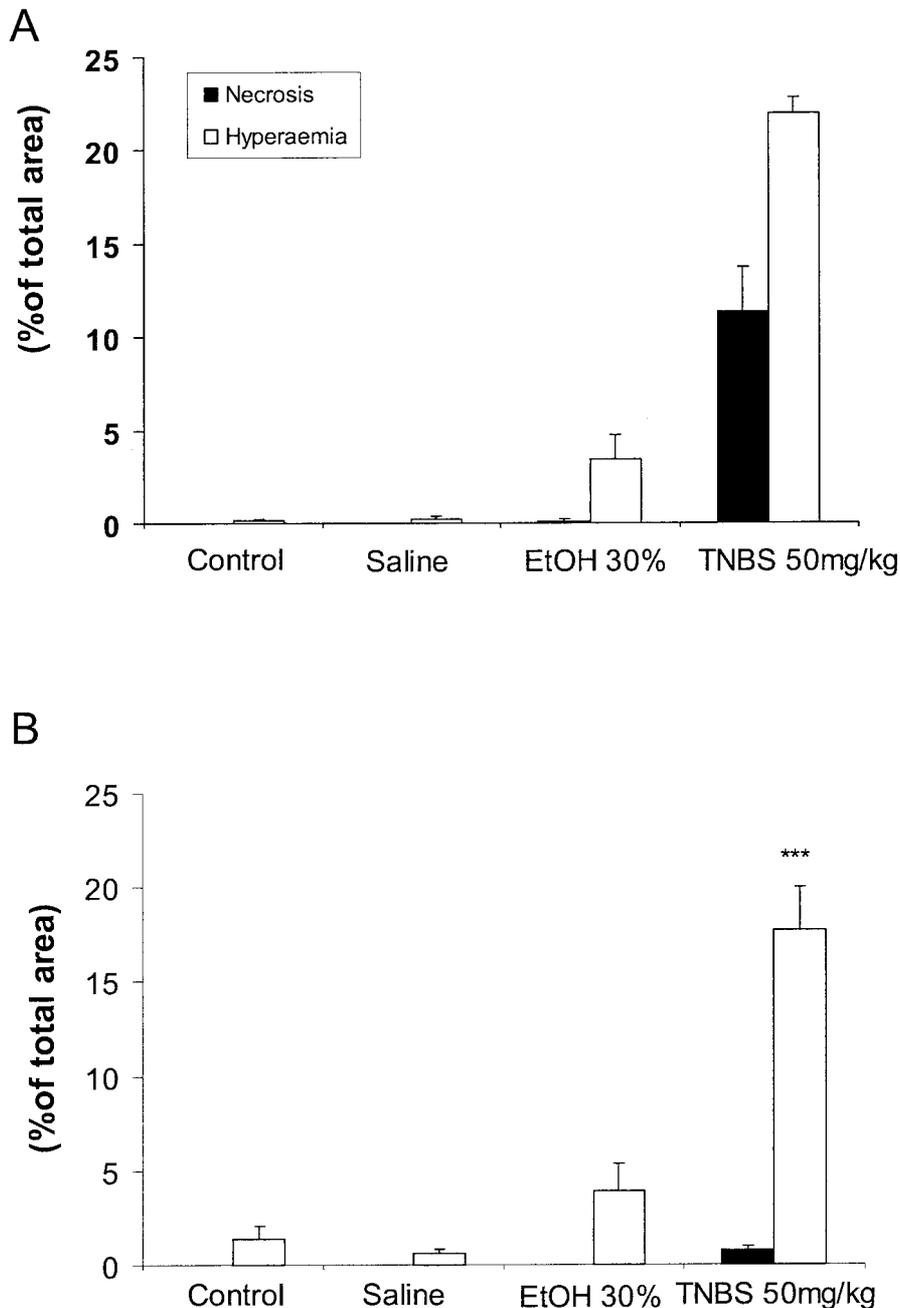
Fig. 3. Percentage of necrosis (■) and hyperemic (□) areas induced by TNBS at day 3 (A) and at day 7 (B). Necrotic and hyperemic areas were expressed as the percentage of the total colon area calculated by dividing both the areas of necrosis and hyperemia by the total area of the colon specimen used. Results were expressed as the mean ± S.E.M. (n = 6–13/group).

of effect of pregabalin (200 mg/kg p.o.) was studied in TNBS-treated rats (n = 7–8 rats) at different times.

Compounds. Pregabalin was synthesized at Pfizer Global Research & Development (Ann Arbor, MI). TNBS was dissolved in 30% EtOH, whereas all other compounds were dissolved in saline. Subcutaneous injection of vehicle was given in a volume of 2 ml/kg. HTAB, TNBS, and morphine were purchased from Sigma-Aldrich (St. Louis, MO), Fluka (Buchs, Switzerland), and Francopia (Gentilly, France), respectively. The other compounds were performed from Sigma-Aldrich.

Statistical Analysis. For the kinetics of the allodynic and inflammatory effect of TNBS, statistical significance between each group (TNBS-treated group versus control) was determined using a one-way ANOVA followed by Student's unpaired t test. Differences were considered statistically significant at $p < 0.05$.

For the drug-response curve, statistical significance between each group (compound + TNBS-treated group versus TNBS-treated

group) was determined by using Dunnett's test. Differences were considered statistically significant at $p < 0.05$.

Results

TNBS-Induced Colonic Allodynia. In anesthetized rats, TNBS (50 mg/kg in 30% ethanol) or saline (1.5 ml/kg) was injected into the proximal colon. Pain threshold (pressure of distention inducing the first abdominal contraction) after distal colonic distention was determined at day 1, 3, 7, 14, and 21 in four groups of awake rats: control animals, sham animals, 30% EtOH-treated animals, and TNBS-treated animals. At day 1, no significant change of colonic threshold was observed between the four groups of animals (Fig. 1). At day 3 and 7, a significant decrease in the pain threshold was observed in TNBS-treated animals
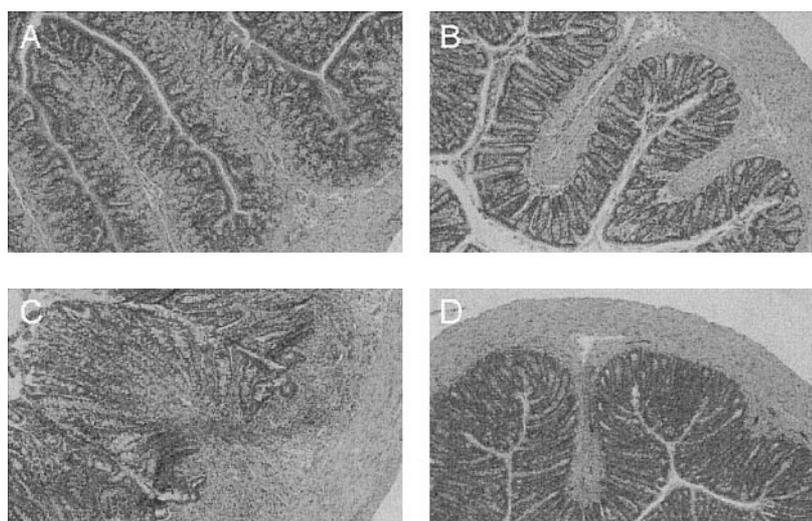
Fig. 4. Light micrographs of the proximal colon (A) and of the distal colon (B) of sham rats (saline-treated rats) at day 7. The proximal colon (C) and of the distal colon (D) of TNBS-treated rats at day 7.
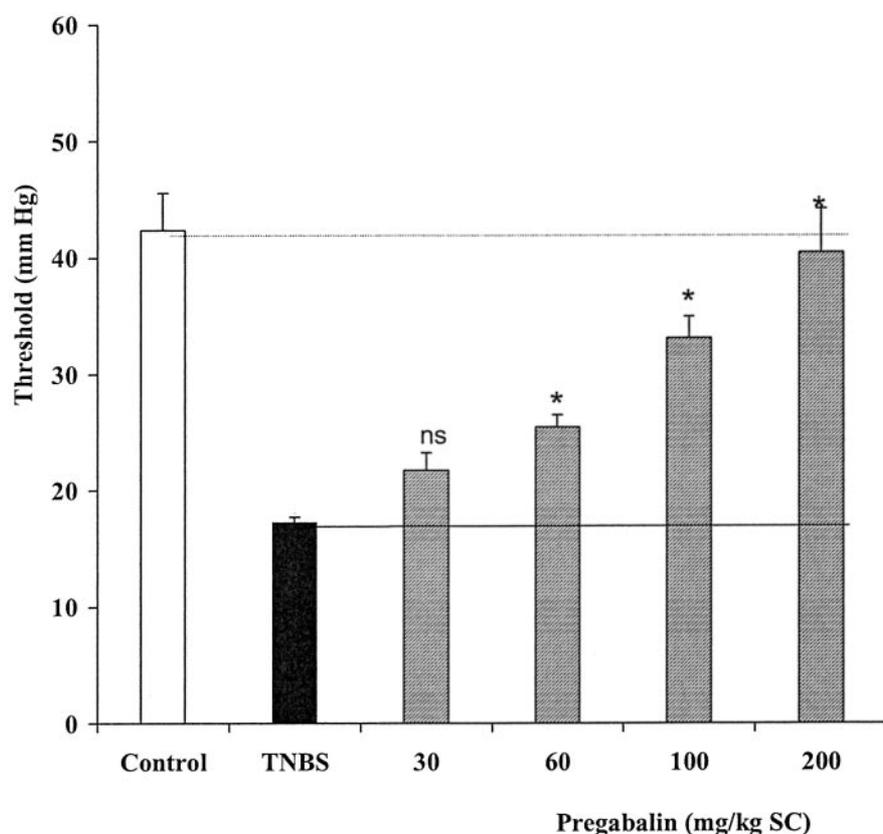
Fig. 5. Effect of pregabalin on TNBS-induced colonic allodynia in the rat. Pregabalin was administered by s.c. injection 30 min before colonic distension series. Results are expressed as mean of colonic threshold $\pm$ S.E.M. (mm Hg) ($n = 7$–8/group). Statistical significance between each group (TNBS versus compound) was determined by using a Dunnett's test. Differences were considered statistically significant at $p < 0.05$ ($\star$, $p < 0.05$ versus control); ns, not significant versus control.

but also in sham animals and 30% EtOH-treated animals (Fig. 1). At day 14 and day 21, only the TNBS-treated animals presented a decrease of colonic threshold (Fig. 1).

TNBS-Induced Colonic Inflammation. MPO activity was maximal at day 3 in the proximal colon, whereas MPO activity was not increased in the distal colon (Fig. 2). In the EtOH-treated animals, MPO was increased in the proximal colon. At day 7, MPO was significantly increased in the proximal colon of TNBS-treated animals (Fig. 2).

Inflammation defined by morphometric analysis (area of hyperemia and necrosis) was measured in the proximal colon at day 3 and 7 after TNBS treatment. At day 3, the colon of TNBS-treated rats presented important areas of necrosis and

hyperemia (Fig. 3). Only the EtOH-treated animals presented small areas of hyperemia. At day 7, hyperemia was significantly increased in TNBS-treated and EtOH-treated animals. None of the groups were presenting necrosis in the proximal colon.

Histological examination of proximal colon of TNBS-treated rats showed a severe loss of the mucosa architecture (Fig. 4C), a submucosal edema, erosion, partial necrosis, and extensive inflammatory cell infiltration (polymorphonuclear cells). In contrast, no microscopic damage was observed in the distal colon of TNBS-treated rats (Fig. 4D). Similarly, no abnormality was observed in the proximal and distal colon of sham rats (saline-treated rats; Fig. 4, A and B).
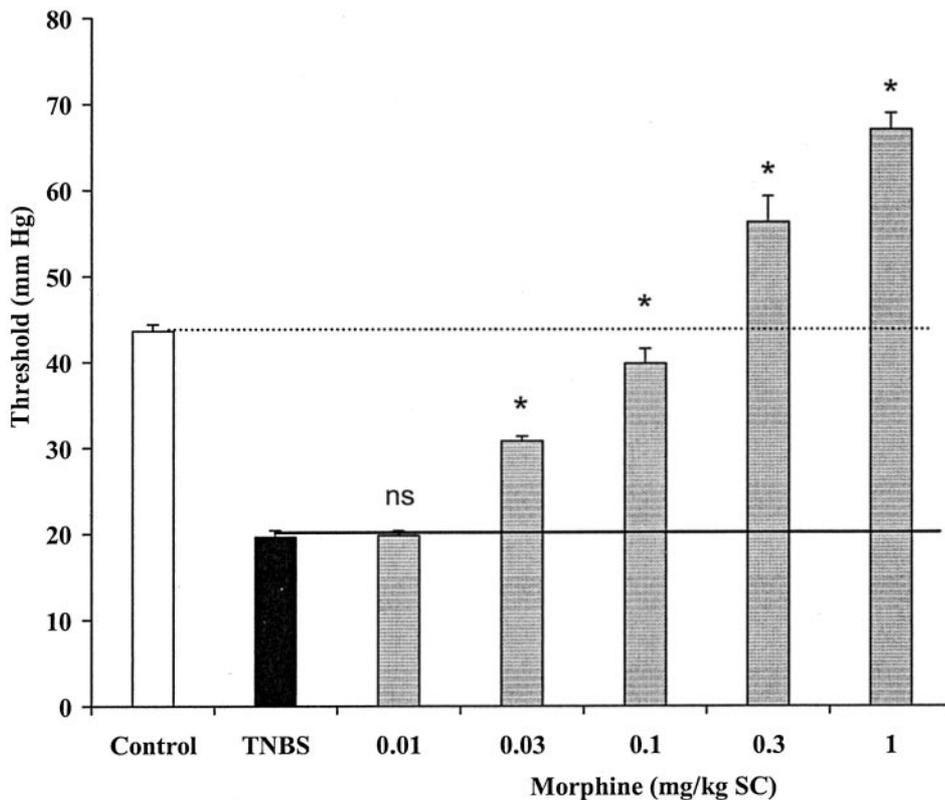
Fig. 6. Effect of morphine on TNBS-induced colonic allodynia in the rat. Morphine was administered by s.c. injection 30 min before colonic distension series. Results are expressed as mean of colonic threshold ± S.E.M. (mm Hg) (n = 7–8/group). Statistical significance between each group (TNBS versus compound) was determined by using a Dunnett's test. Differences were considered statistically significant at $p < 0.05$ (⋆, $p < 0.05$ versus control); ns, not significant versus control.

TABLE 1

Effect of pregabalin and morphine on colonic threshold in normal rats

Pregabalin (200 mg/kg s.c.) and morphine (0.01–1 mg/kg s.c.) were administered 30 min before colonic distension session. Results are expressed as mean of colonic threshold ± S.E.M. (mm Hg) (n = 7–8/per group). Statistical significance between each group (control versus compound) was determined by using a Dunnett's test. Differences were considered statistically significant at $p < 0.05$.

Treatment	Colonic Threshold		S.E.M.	n	p
	mm Hg				
Control	43.33	±	1.23	6	
Pregabalin					
200 mg/kg s.c.	46.41	±	2.26	8	N.S.
Control	51.73	±	2.65	13	
Morphine					
0.01 mg/kg s.c.	52.92	±	1.86	6	N.S.
0.1 mg/kg s.c.	60.00	±	4.64	5	N.S.
0.3 mg/kg s.c.	67.50	±	2.52	6	*
1 mg/kg s.c.	71.88	±	1.40	6	*

N.S., not significant versus control.
* $p < 0.05$ versus control.

Subcutaneous Pregabalin and Morphine on TNBS-Induced Colonic Allodynia. Pregabalin (30, 60, 100, and 200 mg/kg s.c.) and morphine (0.01, 0.03, 0.1, 0.3, and 1 mg/kg s.c.) were administered 30 min before colonic distension. Pregabalin showed a dose-related inhibition of the decrease in pain threshold (Fig. 5). At 200 mg/kg s.c., pregabalin completely suppressed the allodynia induced by TNBS. Pregabalin had an ED_{50} value of 79 mg/kg s.c. (51.1–127.4). Morphine (0.1 mg/kg s.c.) completely suppressed the TNBS-induced decrease in pain threshold after colonic distension (Fig. 6). Morphine had an ED_{50} value of 32 µg/kg s.c. (19.5–54.3).

Subcutaneous Pregabalin and Morphine on Colonic Threshold in Normal Rats. In normal conditions (control animals), morphine (0.3 mg/kg s.c.) significantly increased

the colonic pain threshold, whereas in the same conditions pregabalin (200 mg/kg s.c.) did not modify the colonic pain threshold (Table 1).

Effect of Naloxone on Pregabalin- and Morphine-Induced Antiallodynia. The antihyperalgesic activity produced by pregabalin (200 mg/kg s.c.) was not modified by pretreatment with naloxone at 1 mg/kg s.c. (Fig. 7). At this dose, naloxone suppressed the antiallodynic activity of morphine in this model.

Subcutaneous Pregabalin on TNBS-Induced Colonic Inflammation. Although pregabalin (200 mg/kg s.c.) blocked the TNBS-induced colonic allodynia, it was inactive on TNBS-induced inflammation as measured by the colonic weight, MPO activity, and percentage of hyperemia and necrosis (Table 2).

Subcutaneous and p.o. Pregabalin on TNBS-Induced Colonic Allodynia. Oral administration of pregabalin at 30, 60, 100, and 200 mg/kg, 1 h before colonic distension, reduced in a dose-related manner the TNBS-induced colonic allodynia. Similarly, s.c. injection of pregabalin reversed the effect of TNBS on colonic threshold (Fig. 8). The ED_{50} values were very similar: 79 (51.1–127.4) and 63 (41.7–108.2) mg/kg for s.c. and p.o. administration, respectively.

Time Course of p.o. Pregabalin on TNBS-Induced Colonic Allodynia. The effect of pregabalin peaked within 2 h in TNBS-induced colonic allodynia and was maintained for the ensuing 1 h (Fig. 9). After the 3rd h, pregabalin-induced antiallodynic activity decreased and the colonic threshold returned to values of TNBS-treated animals.

Discussion

Alterations of visceral sensory threshold have been shown in the majority of IBS patients (Naliboff et al., 1998). This
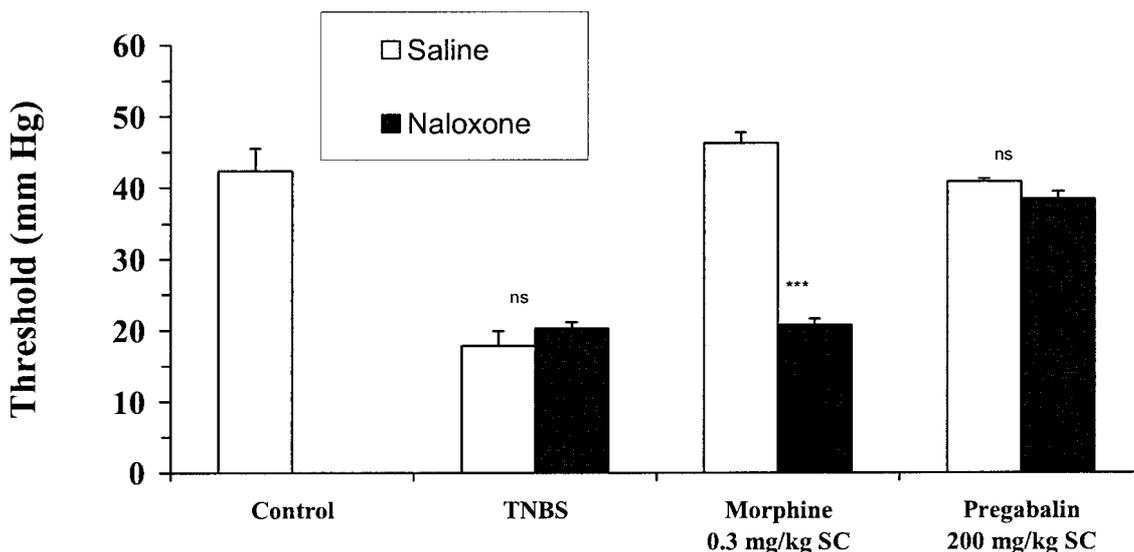
Fig. 7. Effect of naloxone (1 mg/kg s.c.) on pregabalin and morphine-induced antiallodynic activity in TNBS-induced colonic allodynia. Naloxone was administered subcutaneously 35 min before colonic distension series. Morphine (0.3 mg/kg s.c.) and pregabalin (200 mg/kg s.c.) were administered by s.c. injection 30 min before colonic distension series. Results are expressed as mean of colonic threshold $\pm$ S.E.M. (mm Hg) (n = 7–8/group). Statistical significance between each group (compound versus compound + naloxone) was determined by using a one-way ANOVA followed by Student's unpaired t test. Differences were considered statistically significant at $p < 0.05$; ★★★, $p < 0.001$ versus control; ns, not significant versus control.

TABLE 2
Effect of pregabalin (200 mg/kg s.c.) on the inflammatory parameters in the proximal colon at day 7

One unit of MPO activity was defined as that which would produce an increase in absorbance (Δ A450 nm) of 1.0 unit/min at pH 7.0 and 25°C, calculated from the initial rate of reaction with peroxide (1 μM) as the substrate. The results are expressed as MPO units per milligram of protein (mean $\pm$ S.E.M.; n = 6–8/group).

	Weight	MPO Content	Hyperemia and Necrosis
	g	U/mg of protein	% of colonic area
Saline	0.62 $\pm$ 0.02 (8)	4.04 $\pm$ 0.94 (8)	0.4 $\pm$ 0.1 (8)
TNBS	1.39 $\pm$ 0.10 (6)	34.45 $\pm$ 8.31 (6)	31.1 $\pm$ 6.0 (6)
Pregabalin (200 mg/kg s.c.)	1.50 $\pm$ 0.09 (8)	43.87 $\pm$ 5.56 (8)	28.4 $\pm$ 5.1 (8)

visceral hypersensitivity seems to be the consequence of previous sensitization of visceral afferent pathways localized at peripheral and/or central levels. Balloon distension of hollow organs has extensively been used to evaluate the viscerosensitivity in humans and animals (Ness and Gebhart, 1990; Mayer and Gebhart, 1994). In animals, inflammation of the colon can change colonic threshold to distension, indicating a visceral hypersensitivity. Indeed, TNBS-induced rectocolitis produces a colonic allodynia (Morteau et al., 1994). In our laboratory, we have demonstrated that colonic hypersensitivity can also be induced by intracolonic instillation of acetic acid (Langlois et al., 1994). This effect was observed during 1 day. However, the time course of the development of these allodynia and hyperalgesia are poorly understood. Research carried out on these experimental models has shown that the role of primary sensory afferents in the transmission of nociceptive messages is much more complex than fist thought. It seems that these afferents are not simple pathways for conducting peripheral messages toward the central nervous structures. This complexity resides partly in the diversity of the mediators expressed by the primary afferents, and also in the existence of bidirectional relationships between the primary sensory afferents and the immune system. In the present study, we demonstrated that proximal injection of
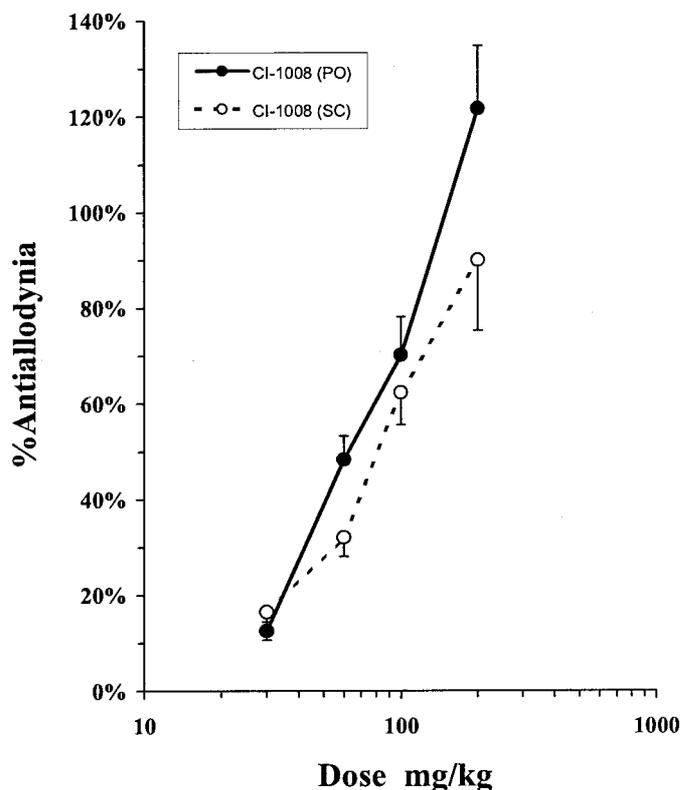
Fig. 8. Effect of s.c. and p.o. administration of pregabalin on TNBS-induced colonic allodynia. Pregabalin was administered 30 min before colonic distension series for s.c. injection and 1 h before for p.o. administration. Data are analyzed by comparing test compound-treated group with TNBS-treated group and control group. Mean and S.E.M. are calculated for each group. The antiallodynic activity of the compound is calculated as follows. % Activity = [(test compound-treated group – TNBS-treated group) × 100]/(control group – TNBS-treated group).

TNBS produced at distance a colonic allodynia after the inflammation of the proximal colon. The colonic hypersensitivity was maximal at day 3 and 7 and lasted 21 days. The
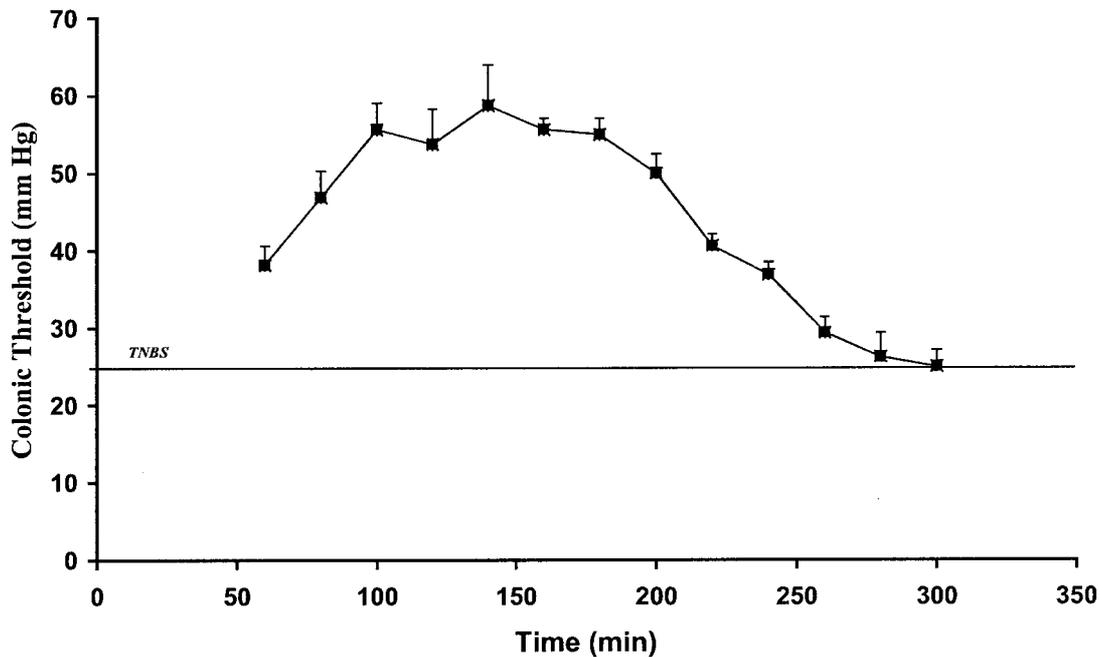
Fig. 9. Time course of p.o. administration of pregabalin on TNBS-induced colonic allodynia. Results are expressed as mean of colonic threshold ± S.E.M. (mm Hg) (n = 7–8/group).

inflammation was maximal at day 3 and localized only in the proximal colon. Indeed, the MPO activity after injection of TNBS was very low into the distal colon where the colonic distension was applied, suggesting that TNBS did not induce an inflammation in the distal colon. This colonic allodynia may be due to the facilitation of spinal transmission of nociceptive messages resulting from primary activation of nociceptors during the development of inflammation. TNBS produces an increase of leukotriene B4, platelet activating factor, and prostaglandins E_2 and $F_{2\alpha}$ in gastrointestinal tissues (Wallace et al., 1989). TNBS also increased colonic inducible nitric-oxide synthase activity (Kiss et al., 1997). The maximal responses of inflammatory parameters occurred at day 3, which then subsequently subsided in the following week. Moreover, Sengupta et al. (1999) have reported a greater number and activity of fibers in the pelvic nerve of TNBS-treated rat colons in response to colonic distension. It is not known whether this higher frequency of nerve discharges corresponded to the colonic allodynia. This mechanism of fiber hyperactivity could play a role to maintain the low threshold observed in TNBS-treated rats.

Pregabalin produces an antiallodynic activity in a dose-related manner. The ED_{50} value was 79 mg/kg s.c. By oral route, the activity of pregabalin was similar with ED_{50} value of 60 mg/kg p.o. The potency of pregabalin seems to be similar for s.c. route at 30 min and for oral route at 1 h. Further study is needed to compare the kinetics and/or the potency of pregabalin by s.c. and p.o. route in TNBS-induced colonic allodynia. The antiallodynic effect of pregabalin is consistent with other pharmacological studies performed in somatic pain. Singh et al. (1996) demonstrated that systemic administration of pregabalin and gabapentin reduced thermal hyperalgesia after carrageenan-induced inflammation of the paw. In neuropathic pain models, gabapentin blocked the mechanical allodynia in rats. More recently, Field et al. (1997a,b, 1999) demonstrated that pregabalin is antihyper-

algesic in the formalin test, the carrageenan-induced inflammatory and postoperative pain models. The antiallodynic properties of pregabalin are thought to be centrally mediated. Indeed, Stanfa et al. (1997) demonstrated that carrageenan-induced sensitization of dorsal horn neurons can be blocked by gabapentin. In normal condition (without inflammation), gabapentin is inactive on neurons of the dorsal horn. Behavioral study demonstrated that gabapentin injected intrathecally blocked allodynia, whereas a same dose injected peripherally was inactive. In our laboratory, we have recently shown the intraventricular or intrathecal injections of pregabalin were more potent than the systemic administration in an inflammatory colonic pain model in rats (Diop et al., 1999). Taken together, these data suggest that the antiallodynic activity of pregabalin may be centrally mediated.

Morphine is well known to produce an antinociceptive activity via spinal and supraspinal pathways to inhibit pain and nociceptive reflexes (Yaksh, 1987). The μ-opioid agonists have been demonstrated active in visceral pain induced by colonic distension (Diop et al., 1994; Danzebrink et al., 1995). Moreover, intrathecal administration of μ- and δ-opioid agonists but not κ-agonists inhibited the colonic pain (Diop et al., 1994). In the present study, morphine presents a potent activity on TNBS-induced colonic allodynia. The fact that morphine increased the colonic threshold above normal threshold in TNBS-treated animals shows its powerful analgesic action. However, the undesirable effects of morphine, e.g., respiratory depression, nausea, and decrease of intestinal motility, prohibit its use in IBS patients to treat visceral pain. The dose of naloxone that blocked the morphine-induced antiallodynic effect was unable to attenuate the antinociception of pregabalin, suggesting that its action is not mediated through opioid pathways. The profile of action of pregabalin is very different from morphine because pregabalin restores the normal visceral sensibility and is inactive in normal conditions. Taken together, the present results indi-

cate that the blockade of colonic allodynia by pregabalin may represent an effective treatment of visceral hypersensitivity.

The mechanism of action of pregabalin remains unclear. In vitro binding study showed that pregabalin binds to the $\alpha2\delta$ subunit of voltage-dependent calcium channels. The $\alpha2\delta$ subunit seems to be common to all voltage-dependent calcium channels. However, the physiological role of $\alpha2\delta$ subunit of a voltage-dependent Ca^{2+} channel is not well understood. It has been reported that there are at least three subtypes of $\alpha2\delta$ subunits (Klugbauer et al., 1999). As demonstrated in the somatic pain models, the antiallodynic properties of pregabalin on TNBS-induced colonic allodynia may be mediated by several types of calcium channels. Indeed, N- and L-type voltage-dependent calcium channels are involved in the phenomena of hyperalgesia (Neugebauer et al., 1996). Because voltage-dependent calcium channels can mediate the release of neuromediators, pregabalin may modify the release of transmitters such as substance P and glutamate in the mechanisms of hypersensitivity in visceral pain. Therefore, further pharmacological and electrophysiological investigations will be necessary to elucidate whether the interaction of pregabalin with $\alpha2\delta$ subunit mediates its antinociceptive effects on visceral allodynia.

In conclusion, the present study demonstrated the potent antiallodynic activity of pregabalin in TNBS-induced colonic allodynia. The ability of pregabalin to block the chronic colonic allodynia indicates that it may be are effective in abnormal colonic hypersensitivity observed in the chronic pain in IBS.

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