Antinociceptive Effect of Pregabalin in Septic Shock-Induced Rectal Hypersensitivity in Rats

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ABSTRACT

Pregabalin [S(-)-3-isobutylgaba] is a novel compound under development for its analgesic, anxiolytic, and anticonvulsant properties, and its interaction with the a,d-subunit of voltage-dependent Ca\(^{2+}\) channels. In this study, we investigate the antinociceptive activity of pregabalin in a rat model of delayed visceral hyperalgesia induced by i.p. lipopolysaccharide (LPS) administration. LPS (Escherichia coli, serotype O111:B4) leads to a delayed lowering threshold (9–12 h) of abdominal contractions in response to rectal distension (RD) in awake rats surgically prepared for electromyography of abdominal muscles. This allodynic effect of LPS was blocked by morphine (0.3 mg/kg s.c.), and the action of morphine was antagonized by naloxone (2.5 mg/kg s.c.). A single i.p. (10, 30 mg/kg) and oral (1, 3, 10 and 30 mg/kg) treatment of pregabalin dose dependently suppressed LPS-induced rectal hypersensitivity. When administered 2 h before RD (but preceded 12 h by LPS injection), the oral dose of 10 mg/kg was effective both in the allodynic response induced by LPS and in the intensity of the nociceptive response related to RD. Pretreatment by either naloxone or bicuculline (a GABAA antagonist, 0.5 mg/kg i.p.) did not affect the antiallodynic effect of pregabalin. We conclude that pregabalin is a therapeutic candidate in the treatment of gut hypersensitivity not acting through GABAA and opiate receptors.

Pregabalin [S(-)-3-isobutylgaba] is an anticonvulsant agent with greater efficacy than the related compound gabapentin (Neurontin, Parke-Davis) used in epileptic patients resistant to conventional therapy (Goa and Sorkin, 1993). Gabapentin, first described as a structural analog of \(\gamma\)-aminobutyric acid (GABA), which crosses the blood brain barrier, does not interact with either GABA\(_A\) or GABA\(_B\) receptor subtypes (Bartoszyk and Reimann, 1985). Recently, a molecular target for these compounds was purified and characterized as the a,d-subunit of a voltage Ca\(^{2+}\) channel (Gee et al., 1996).

Recently, pregabalin was shown to be effective in several models of neuropathic pain. Pregabalin effectively blocked the development and the maintenance of thermal hyperalgesia and/or mechanical allodynia caused by intrathecal injection of Substance P or N-methyl-D-aspartate (NMDA) (Partridge et al., 1998) for thermal injury (Jun and Yaksh, 1998). Moreover, systemic administration of pregabalin dramatically attenuates postoperative pain after surgical manipulations, although this effect is centrally mediated and naloxone resistant (Field et al., 1997a).

Morphine is a widely used opioid analgesic in the treatment of a range of pain symptoms. However, morphine produces side-effects such as sedation, nausea, vomiting, constipation, respiratory depression, and tolerance, limiting its use as a visceral analgesic drug (Foley and Inturrisi, 1987) and leading to the development of nonopioid analgesic agents, particularly for visceral pain.

Recently, a rat model of visceral hyperalgesia has been developed (Coelho et al., 1998, 2000) involving an i.p. administration of endotoxin [lipopolysaccharide (LPS) from Escherichia coli, serotype O111:B4] in rats surgically prepared for electromyography of abdominal muscles and submitted to a rectal distension (RD). The visceral nociceptive response observed is a delayed lowering threshold (9–12 h) to RD-induced abdominal contractions. This rectal allodynic response mimics the major symptom currently described in patients with irritable bowel syndrome. Thirty percent of these patients are also called patients with “postinfectious irritable bowel syndrome”. Indeed, one-third of patients with antecedents of bacterial gastroenteritis develop acute or chronic abdominal pain with lowered visceral sensory threshold to pain caused by balloon distension (Ritchie, 1973; Kullman and Fielding, 1981; Bergin et al., 1993).

In this study, we determined the influence of an oral treatment of pregabalin on the basal nociceptive response evoked...
by RD. Using the animal model of endotoxin-induced delayed visceral hyperalgesia, we also examined the antinociceptive activities of pregabalin administered by both i.p. and oral routes. To elucidate its mechanisms of action, we also determined whether naloxone (an opiate receptor antagonist) and bicuculline (a GABA<sub>A</sub> receptor antagonist) were able to antagonize the antinociceptive effect of pregabalin on LPS-induced visceral hyperalgesia.

**Materials and Methods**

**Animal Preparation.** Animals were surgically prepared for electromyography according to Ruuskabusch and Fioramonti (1975). Rats were anesthetized by i.p. injection of acepromazine (0.6 mg/kg; Calmivet, Vetiquinol, Lure, France) and ketamine (120 mg/kg; Imalgene 1000, Rhone Merieux, Lyon, France). Three groups of three electrodes were implanted in the abdominal external oblique musculature, just superior to the inguinal ligament. Electrodes were exteriorized on the back of the neck and protected by a glass tube attached to the skin. Animals were individually housed in polypropylene cages and kept in a temperature-controlled room (21°C). Food (UAR pellets, Epinay, France) and water were provided ad libitum.

**Electromyographic Recording.** Electromyographic recordings began 5 days after surgery. The electrical activity of abdominal striated muscles was recorded with an electromyograph machine (Mini VIII Alvar, Paris, France) using a short time constant (0.03 s) to remove low-frequency signals (<3 Hz) and a paper speed of 3.6 cm/min. Spike bursts were recorded as an index of abdominal contractions.

**Distension Procedure.** Rats were placed in plastic tunnels (6-cm diameter × 25-cm length), where they could not move, escape, or turn around, to prevent damage to the balloon. Animals were accustomed to this procedure for 4 days before RD to minimize stress reactions during experiments. The balloon used for distension was an arterial embolectomy catheter (Fogarty, Edwards Laboratories, Inc.). RD was performed by insertion of the balloon (2-mm diameter × 2 cm length) into the rectum at 1 cm from the anus. The catheter was fixed at the base of the tail. It was inflated progressively with tepid water by 0.4-ml steps, from 0 to 1.2 ml, each step lasting 5 min. To detect possible leakage, the volume of water introduced in the balloon was checked by complete removal with a syringe at the end of the distension period.

**Experimental Protocol.** Four series of experiments with groups of eight male Wistar rats (250–300 g) were conducted. In a first series of experiments, five groups of rats were used. Two groups of rats were injected i.p. with LPS (1 mg/kg) or its vehicle, and RD with concomitant electromyographic recording of abdominal contractions was performed 9 and 12 h after this administration. In four other groups, systemic pretreatment with morphine sulfate (0.3 and 3 mg/kg s.c.), naloxone (2.5 mg/kg s.c.), or naloxone plus morphine was performed 30 min before RD, which was preceded (12 h) by LPS administration.

Using five groups of rats, a second series of experiments was performed to determine the antinociceptive properties of pregabalin in basal nociceptive conditions evoked by RD. Pregabalin (1, 3, 10, and 30 mg/kg) or vehicle was administered p.o. 2 h before RD.

To determine the antinociceptive effect of pregabalin in hyperalgesia conditions, a third series of experiments was performed using eight groups of rats. In three groups, pregabalin or vehicle (NaCl 0.9%, 0.3 ml/rat) was administered i.p. at 10 and 30 mg/kg 30 min before RD, but preceded (12 h) by injection of LPS (1 mg/kg i.p.). In the last five groups of rats, pregabalin (1–30 mg/kg) or vehicle (NaCl 0.9%, 1 ml/rat) was administered p.o. 2 h before RD, also preceded (12 h) by i.p. LPS administration.

A last series of experiments performed on four groups of animals was aimed at determining the ability of opiate and GABA receptor antagonists to reverse the antinociceptive effect of pregabalin on LPS-induced visceral hyperalgesia. In two groups of rats, naloxone (2.5 mg/kg s.c.) or bicuculline (0.5 mg/kg i.p.) was administered 10 min before pregabalin (30 mg/kg i.p.) and 40 min before RD, also preceded (12 h) by LPS injection. The effect of naloxone and bicuculline per se was evaluated in two other groups of rats.

**Drugs.** Pregabalin (PD-144723–0000) was synthesized at Parke Davis Research Laboratories (Ann Arbor, MI). Morphine sulfate was obtained from Sanofi Francopia (Gentilly, France). LPS (E. coli, serotype O111:B4), naloxone, and bicuculline were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All compounds were dissolved in sterile NaCl (0.9% isotonic saline) immediately before use. The different doses of the antagonists used in this study (naloxone and bicuculline) were selected according to the literature.

**Statistics.** Statistical analysis of the number of abdominal contractions occurring during each period of RD was performed by one-way ANOVA followed by parametric Student’s unpaired t test.

**Results**

**LPS Rectal Hyperalgesia and Morphine.** Under basal conditions, gradual RD induced abdominal bursts of spikes indicating the occurrence of abdominal contractions in a volume-dependent manner. This effect became significant (P < .05) when the distension volume reached 0.8 ml. In contrast, 9 and 12 h after its administration, LPS (1 mg/kg i.p.) significantly increased the number of abdominal contractions for the lowest volume of distension, i.e., 0.4 ml (Fig. 1). At other RD volumes (0.8 and 1.2 ml), the abdominal responses were not affected by LPS, compared with saline (Fig. 1A). The time point of 12 h post-LPS administration was chosen to perform RD for subsequent pharmacological investigations.

Systemic administration of morphine sulfate (0.3 mg/kg s.c.) abolished the increase in the number of contractions from the volume 0 to 0.4 ml induced by LPS. In the presence of naloxone (2.5 mg/kg s.c.), this increase was similar to that observed with LPS alone (Fig. 1B). At 3 mg/kg s.c., morphine sulfate significantly suppressed not only the allodynic effect of LPS but also the nociceptive response to RD recorded for the volumes 0.8 and 1.2 ml. Naloxone administered alone had no effect per se on LPS-induced hyperalgesia.

**Per Os Pregabalin on Basal Abdominal Response to RD.** Oral administration of pregabalin at 1, 3, 10, and 30 mg/kg, 2 h before RD, decreased the abdominal response to RD in a dose-related manner (Table 1), reducing by 6, 16, 25, and 72%, respectively, the number of abdominal contractions for the volume of 0.8 ml. The 30 mg/kg p.o. dose of pregabalin was the most efficient dose tested to block the nociceptive response under basal conditions (Table 1).

**Intraperitoneal versus p.o. Pregabalin on LPS-Induced Rectal Hyperalgesia.** Intraperitoneal administration of pregabalin 30 min before RD at 30 mg/kg suppressed the enhancement of abdominal contractions induced by LPS in response to RD at 0.4, 0.8, and 1.2 ml (Fig. 2A). When administered at 10 mg/kg i.p. 30 min before RD, pregabalin had no effect on LPS-induced visceral hyperalgesia.

When given orally as a single treatment, pregabalin administered at 1, 3, 10, and 30 mg/kg, 2 h before RD, dose-dependently inhibited the enhancement of abdominal response to RD caused by LPS (Fig. 2B), with an inhibitory effect of 24, 77, 76, and 83%, respectively, for the volume of 0.4 ml. Like morphine (3 mg/kg s.c.), the dose of 10 mg/kg p.o. of pregabalin was also effective at significantly reducing the nociceptive effect of RD for volumes of 0.8 and 1.2 ml (Fig. 2B).
out pharmacological studies on the mechanisms of action of pregabalin in LPS-induced visceral allosthesia.

Antagonism of the Antihyperalgesic Effects of Pregabalin. Treatment with bicuculline (0.5 mg/kg i.p.) failed to antagonize the antinociceptive effect of pregabalin (30 mg/kg i.p.) on LPS-induced visceral hyperalgesia (Fig. 3). At a dose of 30 mg/kg i.p., pregabalin suppressed the increase in the number of contractions from the volume 0 to 0.4 ml induced by LPS. This effect was not modified by previous treatment with naloxone (2.5 mg/kg s.c.) (Fig. 3). Pregabalin also significantly attenuated the increase in the number of contractions observed at 0.8 and 1.2 ml, and this effect was not significantly reduced \((P < .05)\) by naloxone with 25 and 28% inhibition of the effect of pregabalin alone, respectively (Fig. 3).

Neither naloxone nor bicuculline administration modified the abdominal electromyographic response to RD in LPS-treated animals.

Discussion

Our results provide evidence that pregabalin, when administered 2 h before RD, has potent antihyperalgesic effects on rectal allodynia to distension induced by LPS at doses lower than that affecting the normalgesic response. They also indicate that the visceral antiallodynic effect of pregabalin is not mediated by an opiate or a GABAergic mechanism. These results extend the analgesic properties of pregabalin, already found in somatic pain, to the digestive tract.

Recently, Coelho et al. (1998) pointed out a delayed (9–12 h) lowered threshold of RD-induced nociceptive reactions subsequent to peripheral LPS administration. The authors explain this phenomenon as changes in sensitivity resulting from both sensitization of high threshold nociceptors with the gut and facilitation of synaptic transmission at dorsal horn level involving cytokines (interleukin-1β and tumor necrosis factor-α) (Coelho et al., 2000). Hyperalgesia, subsequent to a local inflammation, is a result of changes in the sensitivity of high threshold nociceptors (Reeh, 1994) and in the excitability of the second-order spinal neurons (McMahon et al., 1993). These changes result in the activation of chemosensitive nociceptors by proinflammatory and/or proalgesic mediators (Ferreira et al., 1993; Reeh, 1994; Dray, 1995). Moreover, in this experimental model, animals injected with LPS also showed different signs of illness, such as piloerection and inactivity (Coelho et al., 2000).

Pregabalin is a new compound with a high affinity for the \(\alpha_2\delta\)-subunit of the L-type \(\text{Ca}^{2+}\) channel. This compound possesses several pharmacological properties, such as antiepileptic activity. We have shown that pregabalin antagonizes LPS-induced visceral hyperalgesia when administered by both peripheral and oral routes. Moreover, we have found that pregabalin is active at lower doses to reduce hyperalgesia than those required to influence rectal sensitivity under basal conditions.

This study is the first to illustrate an orally antinociceptive effect of pregabalin against LPS-induced visceral hyperalgesia. These results are in agreement with Field et al. (1997a) who reported that systemic administration of pregabalin was very effective in reducing nociceptive behaviors in the formalin test. Moreover, in a rat model of postoperative pain, it has been shown that pregabalin is more effective than the structurally related compound gabapentin in blocking the main-
tenance of hyperalgesia and allodynia (Field et al., 1997b). This antinoceptive effect may be optimal when this compound is administered during and after surgery to provide a maximal effect (Field et al., 1997b). Under basal conditions, when orally administered 2, 4, and 6 h before the nociceptive stimulus (RD), pregabalin at 10 mg/kg p.o. significantly reduced abdominal contractions induced by RD (data not shown), with a maximal efficacy at 2 h. Previous results have established a half-life of between 4 and 5 h in a rat anticonvulsion model, with a maximal effect at 2 to 3 h after administration of pregabalin (Taylor et al., 1993). Tissue distribution studies have shown that this delay (2 h) corresponds to the maximal brain concentrations, suggesting that this effect is centrally mediated. This result may also explain that in our study a lower antinoceptive efficacy was seen for i.p. route at 30 min than for oral route at 2 h. The central origin of the visceral antinoceptive effect of pregabalin is in agreement with previous studies showing that gabapentin administered into the spinal cord attenuates nociceptive behaviors in an acute arthritis model in rats and also reduces nociceptive behaviors during the tonic phase of the formalin test (Shimoyama et al., 1997; Lu and Westlund, 1999), which is thought to reflect central sensitization (Coderre et al., 1990). Similarly, pregabalin blocks the maintenance of carrageenan-induced sensitization of dorsal horns in the joint acute arthritis model (Houghton et al., 1998). All these data suggest that pregabalin may act directly or indirectly on dorsal horn neurons to block their activation and thereby to suppress central sensitization.

Morphine is well known to act both at spinal and supraspinal levels to inhibit pain sensation and nociceptive reflexes (Yaksh, 1986). Intrathecal administration of μ- and δ-opioid, but not κ-receptor agonists, significantly attenuates the transmission of visceral pain nociception in response to colorectal distension (Diop et al., 1994; Danzebrink et al., 1995). In the present study, blockade by morphine of the allodynic response to RD suggests that LPS facilitates spinal transmission of nociceptive messages resulting from primary activation of nociceptors by inflammatory mediators within the peritoneum. Naloxone, at the dose required to block the effect of morphine, does not affect the antiallodynic (0.4 ml) effect of pregabalin. This result suggests that pregabalin does not act on LPS allodynia by an opiate mechanism. However, naloxone reduced but did not block the efficacy of pregabalin effect for the highest volume of RD (0.8 and 1.2 ml). This observation suggests that in contrast to the antiallodynic effect of pregabalin not sensitive to naloxone, its antinoceptive effects are partially dependent on an opiate mechanism. In addition, gabapentin enhances the antinociceptive effect of spinal morphine in the rat tail-flick test, indicating that they act in synergy (Shimoyama et al., 1997). These authors also hypothesized that spinal gabapentin enhances the antinociceptive effects of spinal morphine by blocking a spinal antinoci-}

### Table 1

<table>
<thead>
<tr>
<th>RD Volume (ml)</th>
<th>NaCl 0.9%</th>
<th>Pregabalin 1 mg/kg p.o.</th>
<th>Pregabalin 3 mg/kg p.o.</th>
<th>Pregabalin 10 mg/kg p.o.</th>
<th>Pregabalin 30 mg/kg p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.7 ± 0.9</td>
<td>2.7 ± 2.9</td>
<td>1.6 ± 0.4</td>
<td>2.9 ± 1.1</td>
<td>1.1 ± 1.4</td>
</tr>
<tr>
<td>0.4</td>
<td>4.5 ± 1.8</td>
<td>6.1 ± 2.8</td>
<td>3.7 ± 1.8</td>
<td>5.6 ± 3.2</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>0.8</td>
<td>28.4 ± 3.4</td>
<td>26.9 ± 2.5</td>
<td>24.3 ± 2.0</td>
<td>20.1 ± 3.7</td>
<td>5.0 ± 1.5**</td>
</tr>
<tr>
<td>1.2</td>
<td>38.6 ± 3.5</td>
<td>36.4 ± 2.3</td>
<td>34.1 ± 2.8</td>
<td>25.7 ± 4.2**</td>
<td>13.4 ± 4.0**</td>
</tr>
</tbody>
</table>

*P < .05, **P < .01.

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Pregabalin (1–30 mg/kg p.o.) was administered 2 h before RD. Note that the maximal effect of pregabalin (i.e., blockade of basal abdominal response to RD for the volume of 0.8 and 1.2 ml) was observed for the dose of 30 mg/kg p.o. Results are expressed as mean ± S.E. of eight animals per group (ANOVA followed by Student’s t test for unpaired data).

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Influence of pregabalin administered p.o. on basal abdominal response to RD

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In the present study, blockade by morphine of the allodynic response to RD suggests that LPS facilitates spinal transmission of nociceptive messages resulting from primary activation of nociceptors by inflammatory mediators within the peritoneum. Naloxone, at the dose required to block the effect of morphine, does not affect the antiallodynic (0.4 ml) effect of pregabalin. This result suggests that pregabalin does not act on LPS allodynia by an opiate mechanism. However, naloxone reduced but did not block the efficacy of pregabalin effect for the highest volume of RD (0.8 and 1.2 ml). This observation suggests that in contrast to the antiallodynic effect of pregabalin not sensitive to naloxone, its antinoceptive effects are partially dependent on an opiate mechanism. In addition, gabapentin enhances the antinociceptive effect of spinal morphine in the rat tail-flick test, indicating that they act in synergy (Shimoyama et al., 1997). These authors also hypothesized that spinal gabapentin enhances the antinociceptive effects of spinal morphine by blocking a spinal antinociceptive system. Thus, we can suggest that for high volume of RD (0.8 and 1.2 ml) pregabalin may indirectly activate some opioid system at the spinal cord level, modulating indirectly the intensity for highly painful stimuli.

Previous studies (Gotz et al., 1993; Petroff et al., 1996) have suggested that α2δ-binding compounds like gabapentin favor GABA release or reduce its turnover; we consequently administered bicuculline, a GABA<sub>δ</sub> receptor antagonist that acts competitively at postsynaptic GABA<sub>A</sub> receptors (Beitz and Larson, 1985). This previous treatment did not alter the antinociceptive effect of pregabalin, suggesting that this effect of pregabalin does not involve GABA<sub>δ</sub> receptor activation or an increase in GABA release. In the rat tail-flick test, gabapentin-induced analgesia was not prevented nor reversed by bicuculline (Shimoyama et al., 1997). Moreover, Rock et al. (1993) reported that, at therapeutic doses, gabapentin had no effect on inhibitory GABA and glycine or excitatory NMDA and non-NMDA receptor-mediated antinociceptive responses. In contrast to these data, recent studies have shown that gabapentin may potentiate GABAAergic inhibitory modulation via indirect mechanisms that do not involve a direct action of the drug on GABA<sub>A</sub> or GABA<sub>B</sub> receptors (Taylor, 1995; Petroff et al., 1996). Nevertheless all of these discrepancies may be related to the use of a different experimental animal model of hyperalgesia (acute versus chronic). Indeed, there is evidence that the activity of bulbo-scapal pain modulatory pathways is increased during the development of acute inflammation (Schaible et al., 1991) secondarily associated with an excitability of second-order spinal neurons (McMahon et al., 1993). Accordingly, some studies suggest that there is an important release of GABA and an activation of GABA<sub>A</sub> receptors in the spinal cord in various rat models, i.e., during the late phase of the formalin test (Kaneko and Hammond, 1997), or after induction of an inflammation by injection of Freund’s adjuvant (Castro-Lopez et al., 1992). It is likely that, according to the nature of afferent stimulation, the amounts of GABA released are either sufficient or not sufficient to cause hyperpolarization of dorsal horn neurons, thereby hindering or preventing the activation of NMDA receptors.

Recently, a high-affinity binding protein for pregabalin has been isolated from pig cerebral cortex membranes and characterized as an α2δ-subunit of a voltage-dependent calcium channel.
these voltage-dependent channels appears to be involved in the development of hyperalgesia induced by LPS administration (Neugebauer et al. 1996). In contrast, blockade of voltage-dependent calcium channels with naloxone failed to reverse the antinociceptive effect of pregabalin. Results are expressed as mean ± S.E. of eight animals per group (ANOVA followed by Student’s t test for unpaired data). *P < .05, **P < .01, ***P < .001, significantly different from LPS + vehicle. *P < .05, significantly different from pregabalin alone. LPS (1 mg/kg i.p.) + NaCl 0.9%; LPS + pregabalin 30 mg/kg i.p.; ⊙, LPS + bicuculline + pregabalin 30 mg/kg i.p.; □, LPS + naloxone + pregabalin 30 mg/kg i.p.

Consequently, pregabalin could modulate more than one type of voltage-dependent calcium channel, including those located at the periphery on the nociceptors through an interaction with the α_δ-subunit and diminish or prevent Ca^{2+}-induced currents in the membrane, ultimately preventing or attenuating the generation of action potentials. However, in cultured rodent neurons, Rock et al. (1993) were unable to show an effect of gabapentin on voltage-dependent calcium channel currents. Finally, further investigations are necessary to elucidate whether the α_δ Ca^{2+}-channel subunit mediates the effects of pregabalin on visceral hyperalgesia or whether there is a link between these effects and NMDA receptor activation.

In conclusion, the present study provides evidence for an antinoceptive property of pregabalin in behavioral responses to visceral pain produced herein by LPS administration. This indicates a therapeutic interest of this compound in the treatment of a large number of patients consulting in gastroenterology for visceral hypersensitivity. The allodynic response to colorectal distension is the major symptom currently reported in these patients.

Acknowledgments

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References


