Pregabalin Reduces Acute Inflammatory and Persistent Pain Associated with Nerve Injury and Cancer in Rat Models of Orofacial Pain

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Aims: To assess the analgesic effect of pregabalin in orofacial models of acute inflammatory pain and of persistent pain associated with nerve injury and cancer, and so determine its effectiveness in controlling orofacial pains having different underlying mechanisms. **Methods:** Orofacial capsaicin and formalin tests were employed in male Wistar rats to assess the influence of pregabalin (or vehicle) pretreatment in acute pain models, and the results from these experiments were analyzed by one-way analysis of variance (ANOVA) followed by Newman Keuls post-hoc test. Pregabalin (or vehicle) treatment was also tested on the facial heat hyperalgesia that was evaluated in rats receiving injection of the inflammatory irritant carrageenan into the upper lip, as well as after constriction of the infraorbital nerve (a model of trigeminal neuropathic pain), or after inoculation of tumor cells into the facial vibrissal pad; two-way repeated measures ANOVA followed by Newman-Keuls post-hoc test was used to analyze data from these experiments. **Results:** Facial grooming induced by capsaicin was abolished by pretreatment with pregabalin at 10 and 30 mg/kg. However, pregabalin failed to modify the first phase of the formalin response, but reduced the second phase at both doses (10 and 30 mg/kg). In addition, treatment of rats with pregabalin reduced the heat hyperalgesia induced by carrageenan, as well as by nerve injury and facial cancer. **Conclusion:** Pregabalin produced a marked antinociceptive effect in rat models of facial inflammatory pain as well as in facial neuropathic and cancer pain models, suggesting that it may represent an important agent for the clinical control of orofacial pain. *J Oral Facial Pain Headache 2014;28:350–359. doi: 10.11607/ofph.1317*

Key words: *cancer, inflammatory pain, infraorbital nerve constriction, pregabalin, trigeminal pain*

Pregabalin is an anticonvulsant that binds to the α2δ1 subunit of voltage-gated calcium channels expressed at presynaptic endings of neurons in the brain and spinal cord.[1,2] It is widely accepted that the main effect of calcium channel α2δ1 subunits is to increase the functional expression of these channels,[3–7] as a consequence of increased trafficking.[8] Thus, the analgesic action of pregabalin is proposed to be the result of impaired trafficking of the α2δ1 subunit with consequent diminished expression of functional calcium channels.[9] Pregabalin has been widely used clinically to treat neuropathic pain, including painful diabetic neuropathy, postherpetic neuralgia, and neuropathic pain due to spinal cord injury.[10–14] In addition, there is evidence that pregabalin can be effective in the treatment of trigeminal neuralgia.[15,16] In preclinical models of neuropathic pain conditions, increased expression of the α2δ1 subunit of voltage-gated calcium channels has been demonstrated, as has its correlation with the development of some nociceptive behaviors.[17–19] Thus, the analgesic efficacy of pregabalin in neuropathic pain models correlates well with its proposed mechanism. Nonetheless, there is growing evidence that pregabalin can produce marked analge-

sic effects also in acute pain models, as well as other persistent non-neuropathic pain conditions, both in preclinical models[20–24] and in clinical studies.[25–30] In this regard, it has recently been demonstrated that systemic pretreatment with pregabalin can attenuate sensorimotor responses and medullary glutamate release in an orofacial inflammatory model that involves the pulpal application of mustard oil.[31] This suggests that pregabalin, by binding to the $\alpha 2\delta 1$ subunits of voltage-gated calcium channels, may affect the release of neurotransmitters such as glutamate, which could contribute to its analgesic effect in acute and non-neuropathic pain conditions.

Given these earlier findings, the present study was initiated to assess the analgesic effect of pregabalin in orofacial models of acute inflammatory pain and of persistent pain associated with nerve injury and cancer, and so determine its effectiveness in controlling orofacial pains having different underlying mechanisms. The models used included the orofacial capsaicin test and the orofacial formalin test, as well as assessment of facial thermal hyperalgesia induced by carrageenan, infraorbital nerve (ION) constriction, and facial cancer. The orofacial capsaicin test is considered a valid and reliable method for studying trigeminal pain mechanisms and testing analgesic drugs. Facial subcutaneous injection of capsaicin provides a sustained noxious stimulation of primary afferents by a selective action on small sensory neurons; this results in hyperalgesia and central sensitization.[32] The orofacial formalin test represents a useful animal model of acute inflammatory nociception in the trigeminal region and consists of a biphasic response, in which the short-lasting first phase reflects the direct chemical stimulation of nociceptive afferent endings, whereas the second phase is characterized by inflammation and central sensitization.[33,34] The chronic constriction of the ION represents a trigeminal neuropathic pain model that results in the development of spontaneous pain-related behavior, mechanical allodynia, and thermal hyperalgesia.[35–37] Likewise, the facial cancer pain model is a persistent pain model, but it is considered to have inflammatory and neuropathic components that contribute to the development of spontaneous pain-related behavior, mechanical allodynia, and thermal hyperalgesia.[38,39]

Materials and Methods

Animals

Experiments were conducted on male Wistar rats weighing 180 to 230 g provided by the Federal University of Paraná, housed five to a cage at 22°C ± 1°C on a 12-hour light/dark cycle (lights on at 07:00 hours) with free access to laboratory chow and tap water. A total of 179 rats were used in the study and were always randomly assigned to the experimental groups. They were acclimatized to the laboratory for at least 48 hours before use. All experiments were conducted under the ethical guidelines of the International Association for the Study of Pain,[40] and the experimental procedures were previously approved by the Committee on the Ethical Use of Animals of the Federal University of Paraná (authorization # 680), where the study was conducted.

Orofacial Capsaicin Test

The orofacial capsaicin test was performed as previously described, with minor modifications.[32] Briefly, animals (total 32 rats, 8 in each group) were placed individually in an observation chamber for 10 minutes to minimize any stress-related behavioral changes. The animals were gently held and then received a subcutaneous injection of vehicle (0.9% sodium chloride, 50 µL) or capsaicin (2 µg/50 µL) into the right upper lip and were returned immediately to the observation cage. The time each animal spent rubbing the injected area with its forepaws (facial grooming) was recorded cumulatively (using a stopwatch), in consecutive 3-minute intervals over a period of 30 minutes.

Orofacial Formalin Test

This test was conducted as previously described.[41] Briefly, each animal was placed in an individual plastic cage and left to adapt to the environment for at least 15 minutes. Subsequently, the animals (total 44, 8 to 10 in each group) were gently held and received a subcutaneous 50-µL injection of 2.5% formalin or vehicle (0.9% sodium chloride) into the right upper lip and were returned immediately to the observation cage. The time each animal spent rubbing the injected area with its forepaws was recorded in consecutive 3-minute intervals over a period of 30 minutes. The first and second phases of the formalin response were considered to be 0 to 3 minutes and 12 to 30 minutes after the formalin injection, respectively.

Evaluation of Orofacial Heat Hyperalgesia

Heat hyperalgesia on the face was measured as previously described.[37] On each occasion, the animal was temporarily removed from its home cage and gently held by the experimenter; a radiant heat source was positioned 1 cm from the surface of the vibrissal pad. The latency for the animal to display either head withdrawal or vigorous flicking of the snout was recorded (in seconds) using a stopwatch, and a 20-second cutoff time was used to prevent tissue damage. Reductions in the response latency to heat stimulation were considered to be indicative of heat hyperalgesia.

Carrageenan-Induced Orofacial Heat Hyperalgesia

After assessing initial basal responsiveness to the heat stimulus as described above, the animals (total 36 rats, 8 to 10 in each group) received a subcutaneous injection of vehicle (0.9% sodium chloride, 50 µL) or carrageenan (100 µg/50 µL) into the upper lip, and the heat stimulation was repeated every hour up to 6 hours.

Assessment of Heat Hyperalgesia in Trigeminal Nerve Injury Model

The method for producing ION injury was slightly different from that originally proposed by Vos et al.[35] Briefly, rats (total 36, 7 to 8 in each group) were anesthetized with an intraperitoneal injection of a mixture of ketamine and xylazine (50 and 10 mg/kg, respectively), and an incision was made in the skin of the snout, under the right eye, about 3 mm caudal to the vibrissal pads. The superior lip elevator and anterior superficial masseter muscles were bluntly dissected to expose the rostral end of the ION, as it emerged from the infraorbital fissure. Special care was taken not to damage the facial nerves. Two silk 4-0 ligatures were then tied loosely and 2 mm apart around the ION, and the wound was closed with additional silk sutures (4-0). Sham-operated rats were treated identically, but no ligatures were applied to the ION. After surgery, rats were maintained in a warm room until they recovered from anesthesia. The responsiveness to the heat stimulus was assessed, as described above, before the surgery (basal responsiveness) and on day 4 after the surgery, which is the peak of heat hyperalgesia after ION constriction.[37]

Assessment of Heat Hyperalgesia in a Facial Cancer Pain Model

Walker carcinoma 256B-cells were used to induce facial cancer in rats, as previously described but with minor modifications.[42,43] The cells were obtained by inoculating 1×10^7 (1 mL) tumor cells into the peritoneal cavity of the rats. Their maintenance was carried out by weekly passages through intraperitoneal inoculation. After 5 days, animals were submitted to euthanasia and their ascitic fluid was collected in a solution of ethylenediaminetetraacetic acid (EDTA) (0.5 M, pH 8.0, 1:1). The viability of tumor cells was assessed by the Trypan blue exclusion method in a Neubauer chamber.[44] Finally, approximately 2×10^6 cells per 100 µL were injected subcutaneously in the face (into the center of the right vibrissal pad). Control animals received the same volume of vehicle phosphate-buffered saline (PBS). A total of 31 rats were used in this experimental protocol (7 to 8 in each group). The responsiveness to the heat stimulus was assessed, as described above, before inoculation of the cells (basal responsiveness) and on day 6 after tumor induction.

Drugs and Reagents

The following drugs were used: pregabalin (Lyrica, Pfizer), carrageenan (Sigma), formalin (Vetec), capsaicin (Sigma), morphine (Dimorf, Cristália), xilazine (Dopaser, Calier SA), and ketamine (Ketamin-S, Cristália). Carrageenan and formalin were dissolved in 0.9% sodium chloride just before use. Capsaicin was prepared in a stock solution containing 10% polysorbate 80 and 10% ethanol in PBS (pH 7.4), maintained at –18°C and dissolved in PBS just before use. The concentration of ethanol in the final solution never exceeded 0.5% and did not cause any effect per se. Pregabalin was prepared daily in a suspension of 0.5% carboxymethylcellulose in 0.9% sodium chloride. Morphine was administered subcutaneously at 2.5 mg/kg and dissolved in 0.9% sodium chloride. The doses of both drugs were selected based on a previous study by the authors that demonstrated their analgesic efficacy when used at these doses and routes of administration without interfering with the motor coordination of rats.[45] The choice of pregabalin doses was also based on previous studies that have shown its efficacy in different pain models.[20,23,31] Doses higher than 30 mg/kg (ie, 60 or 100 mg/kg) were not used in the present study because they have been reported to cause motor deficits in rats.[20] Maximum plasma concentration as well as antinociceptive effects of pregabalin have been observed within 1 hour after its oral administration.[46,47] Based on this information, treatment was initiated 1 hour prior to the behavioral tests.

Experimental Protocols

Pregabalin (10 and 30 mg/kg) was administered by oral gavage 1 hour before the local injection of capsaicin, formalin, or carrageenan. Control animals received a systemic injection of the vehicle (1 mL/kg, by oral gavage) of pregabalin followed by a local injection of 0.9% sodium chloride (50 µL), which was the vehicle of capsaicin, formalin, and carrageenan; thus, control animals are indicated as vehicle plus vehicle group in Figs 1 to 5. In addition, in the formalin test, a different group of animals was treated with morphine (2.5 mg/kg, subcutaneously), which was used as a positive control. In the nerve injury and cancer pain models, pregabalin (10 and 30 mg/kg) or its vehicle (1 mL/kg) was administered by oral gavage on day 4 or 6 after the procedures for induction of the nociceptive behaviors, after the basal responsiveness to heat stimulation was assessed. In these latter experiments, heat hyperalgesia was also assessed at 1-hour intervals up to 6 hours after the treatments. All experiments were carried out by an observer blind to the drug treatments.

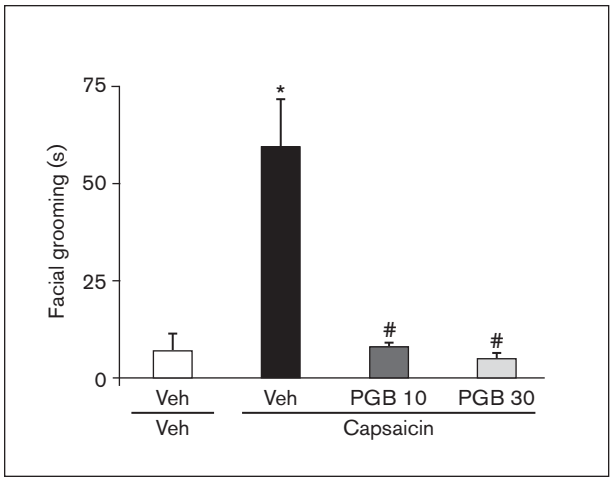


Fig 1 Influence of pregabalin in capsaicin-induced facial grooming. Rats were treated with vehicle (Veh, 1 mL/kg, orally) or pregabalin (PGB, 10 or 30 mg/kg, orally), and 1 hour later received a local injection (subcutaneously into the upper lip) of vehicle (50 µL) or capsaicin (2 µg/50 µL); the facial grooming time was recorded over the next 30 minutes. Values represent mean ± SE of 8 rats per group. *,# indicate $P < .05$ when compared to corresponding values of Vehicle+Vehicle and Vehicle+Capsaicin groups, respectively (one-way ANOVA followed by Newman-Keuls test).

Statistical Analyses

All data were presented as mean ± SEM (standard error of the mean) of 7 to 10 rats per group, as indicated in the figure legends. Results from capsaicin and formalin tests were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test. Two-way repeated measures ANOVA was used to analyze all data from animals receiving heat stimulation, with drug treatment as the independent factor and the different evaluation time points of nociceptive behavior as the repeated measure. In case of significant differences with the independent factor or with the interaction between the independent and repeated factors, one-way ANOVA followed by the Newman-Keuls post-hoc test was performed. In all statistical analyses, *P* values less than .05 were considered significant.

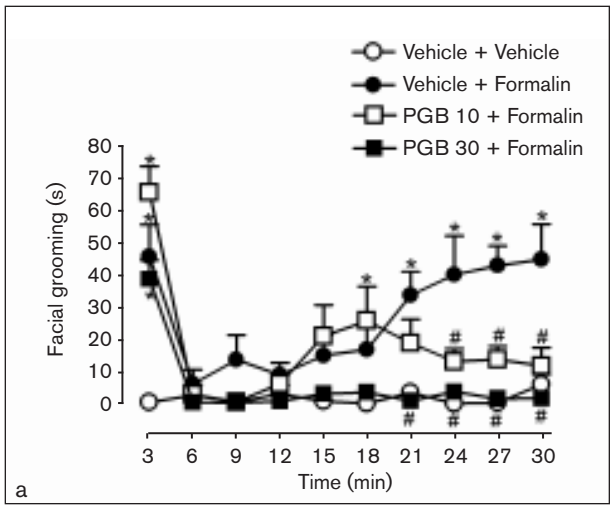
Results

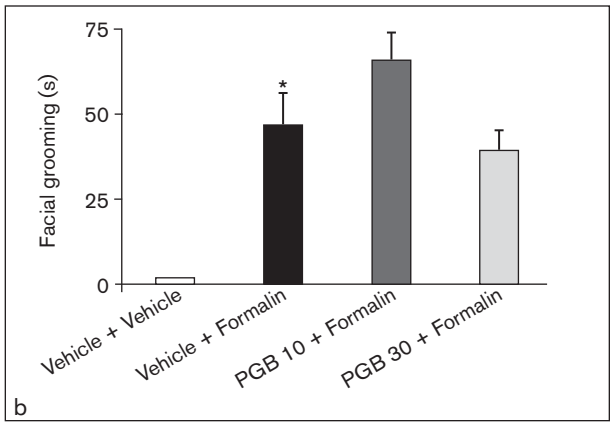
Injection of capsaicin into the upper lip induced significant facial grooming (performed with both forepaws) compared to vehicle (59.1 ± 11.7 and 6.7±4.4 seconds, respectively). Pretreatment (1 hour beforehand) with pregabalin at 10 and 30 mg/kg (but not vehicle) abolished the facial grooming induced by capsaicin (Fig 1).

Injection of formalin induced a significant increase in the facial grooming time compared to vehicle in the first phase (45.8 ± 9.8 and 0.7 ± 0.4 seconds, respectively), as well as in the second phase of the response (207.4 ± 18.8 and 13.25 ± 6.1 seconds, respectively). Pregabalin (also at 10 and 30 mg/kg, orally) failed to modify the first phase of the formalin response (Figs 2a and 2b). However, oral administration of pregabalin at the lowest dose (10 mg/kg, 1 hour beforehand) caused a significant reduction (ie, by 43%) of the second phase of the formalin response, while pregabalin at the highest dose (30 mg/kg) abolished the second phase of formalin response; vehicle was ineffective (Figs 2a and 2c). Since pregabalin did not show any antinociceptive effect in the first phase of the formalin response, the opioid analgesic morphine was used as a positive control in this experiment. Systemic pretreatment of animals with morphine (2.5 mg/kg, subcutaneously) but not vehicle resulted in inhibition of the first and second phases of the formalin response by 58% and 44%, respectively (data not shown). The oral route of administration was not used for morphine, as for pregabalin, because of the low bioavailability of oral morphine.[48]

Pretreatment of rats with vehicle was ineffective, but pregabalin (10 and 30 mg/kg, orally, 1 hour beforehand) reduced the heat hyperalgesia induced by carrageenan from 2 up to 6 hours after its injection into the upper lip (Fig 3). The injection of vehicle into the upper lip did not modify the response latency to the heat stimulus compared to the pretreatment values (Fig 3).

Pregabalin was also effective in reducing heat hyperalgesia in the nerve injury model. Four days after ION ligation, the oral treatment of rats with pregabalin caused an increase in the response latency to the heat stimulus that was significant from 3 to 4 hours after the treatment with the lowest dose and from 2 to 5 hours with the highest dose compared to vehicle-treated rats (Fig 4). Sham-operated animals treated with vehicle did not show any significant changes in the heat hyperalgesia during the test compared to their presurgery values (Fig 4).





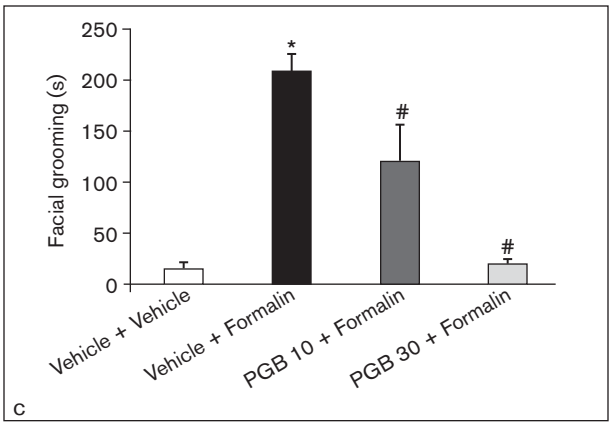


Fig 2 Influence of pregabalin in orofacial nociception induced by formalin. Rats were treated with vehicle (1 mL/kg, orally) or pregabalin (PGB, 10 or 30 mg/kg, orally), and 1 hour later received a local injection (subcutaneiously into the upper lip) of vehicle (50 µL) or formalin (2.5%/50 µL); the facial grooming time was recorded over the next 30 minutes. **(a)** The time course of formalin-induced nociceptive behaviors compared to vehicle and the effect of pregabalin throughout the observation period. **(b and c)** The cumulative grooming response in the first 3 minutes after injection (first phase) and from 12 to 30 minutes after the injection (second phase), respectively. Values represent mean ± SE of 8 to 10 rats per group. *,# indicate $P < .05$ when compared to the corresponding value of Vehicle+Vehicle and Vehicle+Formalin groups, respectively (one-way ANOVA followed by Newman-Keuls test).

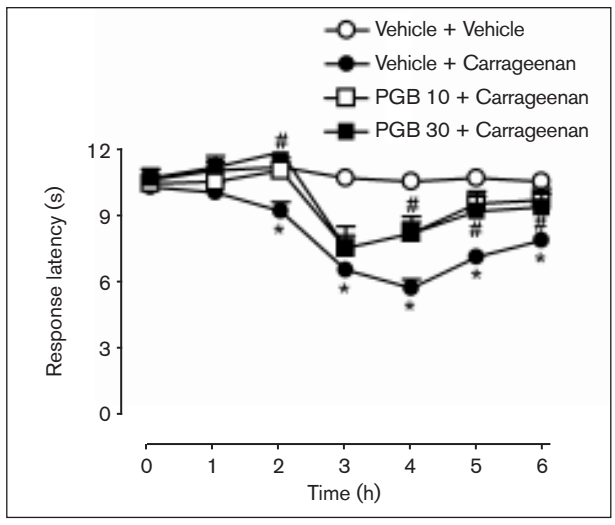


Fig 3 Influence of pregabalin in carrageenan-induced heat hyperalgesia. Rats were treated with vehicle (1 mL/kg, orally) or pregabalin (PGB, 10 or 30 mg/kg, orally) and 1 hour later they received a local injection (subcutaneously into the upper lip) of vehicle (50 µL) or carrageenan (100 µg/50 µL). Heat hyperalgesia was assessed before the treatments (time 0) and at 1-hour intervals up to 6 hours. Values represent mean ± SE of 8 to 10 rats per group. *,# indicate $P < .05$ when compared to corresponding value of Vehicle+Vehicle and Vehicle+Carrageenan groups, respectively (two-way repeated measures ANOVA followed by Newman-Keuls test).

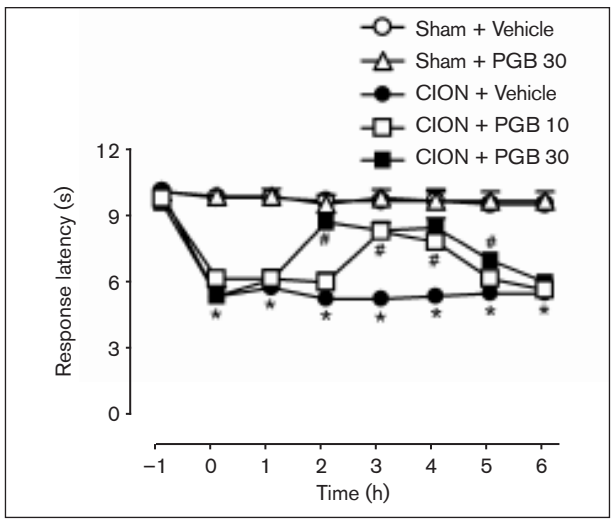


Fig 4 Influence of pregabalin on heat hyperalgesia in trigeminal nerve injury model. Basal response latency was assessed before infraorbital nerve constriction (ION) (time –1) and then again on day 4 after surgery before any treatment (time 0). Rats then received vehicle (1 mL/kg, orally) or pregabalin (PGB, 10 or 30 mg/kg, orally), and heat hyperalgesia was assessed at 1-hour intervals up to 6 hours. Values represent mean ± SE of 7 to 8 rats. *,# indicate $P < .05$ when compared to corresponding value of sham- and ION-operated rats treated with vehicle, respectively (two-way repeated measures ANOVA followed by Newman-Keuls test).

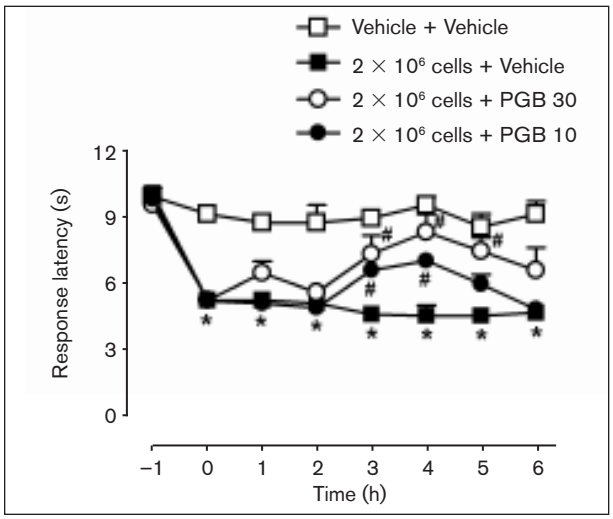


Fig 5 Influence of pregabalin on heat hyperalgesia on a facial cancer pain model. Basal response latency was assessed before the inoculation of tumor cells (time –1) and then again on day 6 after the inoculation before any treatment (time 0). Rats then received vehicle (1 mL/kg, orally) or pregabalin (PGB, 30 mg/kg, orally), and heat hyperalgesia was assessed at 1-hour intervals up to 6 hours. Values represent mean ± SE of 8 rats. *,# indicate $P < .05$ when compared to corresponding value of control and facial tumor rats treated with vehicle, respectively (two-way repeated measures ANOVA followed by Newman-Keuls test).

Inoculation of tumor cells caused a significant facial heat hyperalgesia that peaked on day 6 after the procedure. Treatment of animals with pregabalin on day 6 after inoculation showed a significant antihyperalgesic effect that persisted for 1 and 2 hours, respectively, at doses of 10 and 30 mg/kg, but the effect did not start until 3 hours after the treatment; pretreatment with vehicle was ineffective. Control animals injected with vehicle did not show any significant differences in the response latency before and after the inoculation procedure (Fig 5).

Discussion

In this study, pregabalin produced marked antinociceptive effects in orofacial models of acute inflammatory and persistent pain associated with nerve injury and cancer, corroborating some previous clinical and preclinical evidence that pregabalin presents analgesic efficacy in neuropathic and non-neuropathic conditions.[20–31]

It has been widely demonstrated that subcutaneous or intradermal capsaicin injection provides a sustained noxious stimulation. Capsaicin elicits nociceptive behavior by activating transient receptor potential vanilloid (TRPV1) receptors, which are expressed almost exclusively on small-diameter peptidergic and nonpeptidergic trigeminal neurons.[49] When injected into the upper lip of rats, capsaicin evokes an increase in the facial grooming time, which is attenuated by morphine at doses known to be effective in other pain behavior tests.[32] In humans, capsaicin induces spontaneous pain, flare, primary and secondary hyperalgesia, and allodynia. Therefore, the capsaicin test is considered an important method for an early screening of novel analgesic compounds.[27] The present study showed that pregabalin pretreatment was able to abolish capsaicin-evoked responses in the orofacial region. This finding corroborates the study of Narita and collaborators, who reported an analgesic effect of pregabalin on orofacial nociceptive responses evoked by application of the small-fiber excitant and inflammatory irritant mustard oil to the dental pulp.[31] In sharp contrast, it has been demonstrated that pregabalin could not modify the capsaicin-evoked release of the neuropeptides substance P and calcitonin gene-related peptide (CGRP) from rat spinal cord slices in vitro, but it could attenuate the neuropeptides' release when the paw was previously sensitized with complete Freund's adjuvant.[50] Thus, it can be suggested that pregabalin plays a role in presynaptic transmitter release only after previous sensitization of the hind paw, but in the orofacial region it can modulate acute nociceptive responses. However, it is important to point out that in a model of acute heat nociception in mice (ie, the hot plate test), pregabalin pretreatment did cause dose-dependent antinociceptive effects.[22]

On the other hand, in line with previous observations in the mouse and rat hind paw,[24,51,52] pretreatment with pregabalin in the present study caused a significant reduction only in the second phase of the formalin response. Formalin typically produces a biphasic pain response, with the first phase thought to be mediated by direct activation of nociceptors and the second phase mediated by the development of central sensitization due to the ongoing afferent fiber activity and localized inflammation.[53] Opioids have been extensively demonstrated to affect both phases of the formalin response, [34,54] while the second phase is most sensitive to anti-inflammatory agents.[34,41,54,55] Altogether, the results obtained with the paw and the facial formalin tests suggest that pregabalin is only effective in reducing the inflammatory phase of formalin-induced nociceptive behaviors, and it presents

a different antinociceptive profile from opioids. Unlike opioids, pregabalin seems not to be able to prevent the activation of nociceptors, which could explain its lack of effect in the first phase of formalin-induced nociceptive behavior in the present study. However, its ability to modulate the function of calcium channels, and consequently the release of neurotransmitters in the brainstem and spinal cord, could explain pregabalin's antinociceptive effect in the second phase of the formalin response.[31,56]

In line with these observations, this study demonstrated that heat hyperalgesia induced by carrageenan is significantly reduced by pregabalin pretreatment. Carrageenan-induced orofacial heat hyperalgesia clearly results from inflammation, as it is markedly suppressed by either nonsteroidal (indomethacin and celecoxib) or steroidal (dexamethasone) anti-inflammatory drugs.[36] Using an inflammatory dental pain model, Narita and colleagues[31] have recently shown that systemic pretreatment with pregabalin attenuated sensorimotor responses elicited by injection of mustard oil into the dental pulp. The authors proposed that the mechanism involved in the analgesic action of pregabalin is the inhibition of glutamate release in the medullary dorsal horn.[31] Furthermore, other mechanisms have been suggested to contribute to the analgesic effects of pregabalin in inflammatory pain models, such as the activation of adenosine triphosphate (ATP)–dependent potassium channels.[24] However, the involvement and contribution of each of these proposed mechanisms to pregabalin antinociceptive effects in the models evaluated in the present study remain to be investigated. In this regard, it is important to point out that there is no evidence of correlation between an increase in the expression of $\alpha 2\delta 1$ calcium channels subunits and pain behavior after capsaicin, formalin, or carrageenan injection, but it is widely accepted that a common characteristic of all these models is the induction of central sensitization.[32,53,57–60] Thus, it can be suggested that pregabalin's effect in these models is related to its ability to attenuate central sensitization by mechanisms that still need to be elucidated. Indeed, according to Bannister and colleagues,[56] the inhibitory action of pregabalin in non-neuropathic conditions is facilitated in the presence of a sensitized state in the spinal cord, which may be related to modulation of the function of, but not to the upregulation of, voltage-gated calcium channel subunits, as is observed in neuropathy. Although the sensitization that develops as a consequence of tissue injury is generally reversible, this phenomenon contributes to abnormal responsiveness to noxious and innocuous stimuli and a spread of tenderness beyond the sites of injury in several pathologic pain states.[61]

The above-mentioned pathologic pain states include a wide diversity of neuropathic pain conditions, where the efficacy of pregabalin has been most explored. In the case of trigeminal neuropathic pain, Kumar and colleagues have recently reported that pregabalin is able to suppress both facial mechanical hypersensitivity and evoked glutamate release in the medullary dorsal horn after ION transection in rodents.[62] Additionally, Cao and collaborators have shown that after partial ION transection in rats, pregabalin can attenuate mechanical hyperalgesia as well as central sensitization in nociceptive neurons of the medullary dorsal horn, reflected in reversal of their reduced activation threshold, increased responses to pinch/pressure stimuli, and enhanced stimulus-response function.[63] Consistent with this previous evidence, the present study demonstrated that pregabalin is also able to reduce orofacial heat hyperalgesia induced by ION constriction in rats. Altogether, these results provide evidence in support of the few clinical studies that favor the use of pregabalin for managing trigeminal neuropathic pain.[15,16]

In spite of the accumulating evidence supporting the use of pregabalin in neuropathic pain, its effectiveness in cancer pain has only recently been addressed. In a systematic review of the literature, Bennett and collaborators concluded that due to limited published data reporting safety and efficacy of pregabalin in cancer pain, as well as limitations within the studies included in the review, further studies are required to determine the usefulness of pregabalin to treat cancer pain.[64]

In many types of cancer, the associated pain is not only nociceptive but also neuropathic, which can make the pain refractory to several different treatments.[65,66] However, especially in these cases, two recent studies have reported that pregabalin may function as an important adjuvant in the control of cancer-related neuropathic pain. The first study concluded that pregabalin may lead to better control of the neuropathic component of the cancer pain.[67] The second study showed that the combination of low-dose pregabalin and antidepressants with opioids was effective in the management of painful bone metastases.[66] In line with these observations, the present study has shown for the first time that pregabalin alleviates heat hyperalgesia in a facial model of cancer pain. This model was proposed in 2007 by Nagahata and collaborators,[42] and since then it has been established as a cancer pain model with nociceptive and neuropathic components and with a symptom profile similar to that of orofacial cancer patients.[38,39,43,68] Thus, the marked antihyperalgesic effect of pregabalin in the facial cancer model reinforces the need for further clinical studies to examine the potential use of pregabalin to control pain related to different cancer types.

Orofacial pain may be classified clinically into three categories: acute pain after injury, chronic inflammatory pain (eg, some forms of temporomandibular disorders), and neuropathic pain (eg, trigeminal neuralgia).[69,70] The orofacial capsaicin and formalin tests both mimic acute post-injury inflammatory pain in humans, although with different underlying mechanisms, while carrageenan-induced hyperalgesia can be used to assess enhanced pain sensitivity due to peripheral inflammation. Sustained activity of peripheral trigeminal afferents can cause central sensitization, a phenomenon that has been described after capsaicin local injection, in the second phase of the formalin response, as well as in carrageenan-induced hyperalgesia.[32,53,58,59] Based on the inhibitory action of pregabalin in these acute pain models, it can be surmised that it has potential utility against central sensitization–mediated types of inflammatory orofacial pain.

Moreover, pregabalin showed marked antihyperalgesic effects in the ION constriction model, which reproduces important aspects of human trigeminal neuropathic pain states such as trigeminal neuralgia, including signs of abnormal spontaneous pain-related behavior, mechanical allodynia, and thermal hyperalgesia.[35–37] Treatment of trigeminal neuralgia continues to represent a therapeutic challenge, and the present data strongly suggest that the potential therapeutic action of pregabalin should be further explored in this condition.

Finally, as already pointed out, cancer pain is considered to have both inflammatory and neuropathic components, and as for neuropathic pain, cancer pain represents still a clinical challenge. It is estimated that 45% to 80% of all cancer patients have inadequate pain management.[71,72] The present study has provided the first preclinical demonstration that pregabalin is effective in controlling hyperalgesia associated with facial cancer and suggests that the $\alpha 2\delta 1$ subunit might constitute a new target for treatment of this disorder.

It is also important to mention that while pregabalin was used in a prophylactic regimen in the acute inflammatory orofacial pain models in the present study and a previous study,[31] it was only used in the present study after the development of hyperalgesia in the persistent pain models (ie, ION constriction and facial cancer pain). The present findings are consistent with other studies that also evaluated the influence of pregabalin in trigeminal neurpathic pain models and showed that pregabalin interferes with already-developed hyperalgesia.[45,62,63] However, in a model of chronic constriction injury of the sciatic nerve, pregabalin as a pretreatment successfully decreased mechanical and cold hypersensitivity but was less effective at suppressing cold hyperalgesia when administered as a posttreatment.[73] Moreover, a recent study that employed the spared spinal nerve injury model demonstrated that continuous intrathecal infusion of pregabalin (ie, for 28 days) resulted in attenuation of mechanical and cold hyperalgesia (measured in the rat hind paw), which persisted for a brief period after the infusion was terminated, but it did not suppress the activation of spinal microglia or prevent the increase in the spinal $\alpha 2\delta 1$ subunit.[74] Thus, the limited data on this topic suggests that pregabalin may be more effective when given as a pretreatment than as a posttreatment.

Although there are some differences between the trigeminal region and the spinal innervated areas, including their sensitivity to different drug treatments,[45] effects of pregabalin on pain evaluated in these two territories seem to be quite similar. This observation is based on effects of pregabalin in the formalin test, in which it significantly reduced only the second phase, both in the paw and in the face, as well as in the nerve injury models, in the present and previous studies.[24,51,73,75] In models of trigeminal neuropathic pain, pregabalin is reported to attenuate mechanical hyperalgesia[62,63] and heat hyperalgesia (present study), which corroborates the reported effects of pregabalin in spinal nerve injury models.[47,75] Indeed, in the studies of Kumar and collaborators, it was demonstrated that similar doses of pregabalin resulted in the reduction of mechanical hypersensitivity in the rodent face and paw, as well as reduction of evoked glutamate release both in the medullary and spinal dorsal horns.[62,73]

In conclusion, pregabalin produced a marked antinociceptive effect in rat models of facial inflammatory pain as well as in facial neuropathic and cancer pain models, suggesting that it may represent an important agent for the clinical control of orofacial pain.

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