Randomized Animal Study

Chronic Pregabalin Treatment Ameliorates Pain, but not Depressive-Like Behaviors, in a Reserpine-Induced Myalgia Model in Rats

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Disclaimer: Funded by Generalitat, Valenciana Conselleria d'Eduació, Investigació, Cultura I Esport, Grant/Award Number: GV/2018/049.

Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 02-04-2020
Revised manuscript received: 04-02-2020
Accepted for publication: 05-08-2020

Free full manuscript: www.painphysicianjournal.com

Background: Anticonvulsants are often prescribed as coanalgesics for pathologies presenting chronic pain, such as chronic neuropathic pain and fibromyalgia. These pathologies are associated with a wide range of comorbidities: chronic fatigue, cognitive impairment, sleep disturbances, and mood disorders. Pregabalin, an anticonvulsant used to treat fibromyalgia syndrome, has been proven to improve pain and fatigue symptoms. However, most studies have not considered the analytic effect of this drug on comorbid depressive-like symptoms in this syndrome.

Objectives: The main study objective was to examine the role of pregabalin in depressive symptomatology comorbid to chronic widespread pain using a reserpine-induced myalgia model.

Study Design: A randomized, controlled, animal study.

Setting: Research and data analyses were performed at the GESADA laboratory, Department of Human Anatomy and Embryology, University of Valencia, Spain.

Methods: Forty-six Sprague-Dawley male rats were used. Acute chronic pregabalin administration was tested for depressive-like behaviors (Forced Swimming and Novelty-Suppressed Feeding Tests) and for alteration of pain thresholds (tactile allodynia, Electronic Von Frey test; and mechanical hyperalgesia, Randall and Selitto test). The same procedures were followed with duloxetine as a positive control.

Results: Pregabalin significantly improved depressive-like behaviors in acute, but not chronic treatment, and significantly ameliorated pain thresholds.

Limitations: Lack of histological and electrophysiological tests.

Conclusions: Pregabalin is not effective in depressive-like symptoms associated with chronic pain but might play an acute antidepressive-like role given its antinociceptive effect.

Key words: Pregabalin, duloxetine, pain, chronic pain, comorbid depression, fibromyalgia, RIM, animal model

Pain Physician 2020: 23:E581-E590

Pain is one of the most frequent reasons why patients seek medical treatment and represents a major clinical and socioeconomic problem (1). Depression symptoms associated with chronic pain pathologies seem commoner than those associated with other chronic pathologies, such as cardiac diseases,

cancer, diabetes, and neurologic disorders. Besides, chronic pain patients with comorbid depression symptoms incur a higher health burden compared with pain patients without them (2,3).

Antiepileptic drugs, such as gabapentin and pregabalin (PGB), have been used as coanalgesics in many pathologies with chronic pain (4-7). These are voltage-gated calcium channel blocker anticonvulsant drugs that act by binding to the alpha-2-delta subunit of nervous system voltage-dependent calcium channels. Therefore they downregulate the ion flow in neurons and subsequently reduce the release of a variety of excitatory and inhibitory neurotransmitters (8-11).

Fibromyalgia syndrome (FMS) is a chronic pathology in which pain and depression coexist (12) and is also one of the chronic conditions in which PGB is effective. In 2007, PGB was the first pharmacologic therapy to be approved by the U.S. Food and Drug Administration to manage FMS (13,14). Both short- and long-term studies have shown significant improvements in pain, chronic fatigue, and sleep disturbances (13). In 2015, Arnold et al (15) analyzed the effects of PGB in synergy with antidepressants on FMS-related comorbid depressive symptomatology. However, their study did not consider the interaction of PGB with nonpharmacologic treatments and other drugs (15).

The main objective of this study was to examine the role of PGB in depressive symptomatology comorbid to chronic widespread pain using a reserpine-induced myalgia (RIM) model, which is an animal model of fibromyalgia in rat (16). The RIM has been the most robust model when it comes to reproducing the comorbid symptoms of FMS (17).

Methods

Animals

Forty-six male Sprague-Dawley rats (Janvier Labs, Saint Berthevin, France) of consistent weight (300–400 g) were used. Water and food were given ad libitum (not to the animals used for the Novelty Suppressed-Feeding Test). After experimental procedures, animals were euthanized by a sodium pentobarbital overdose (120 mg/kg). All the experiments were approved by the ethics committee of the University of Valencia (Procedure numbers: A1359998202530; A1385127985666) and in accordance with International Association for the Study of Pain (IASP) ethical guidelines (18).

Reserpinization

Reserpine was administered after a 15-minute habituation process by a single daily subcutaneous injection of 1 mg/kg on 3 consecutive days. Reserpine (Sigma-Aldrich, crystallized, ≥ 99.0%; high performance liquid chromatography [HPLC]) (Sigma-Aldrich AG, Industriestrasse, Buchs, Switzerland) was diluted in glacial acetic acid to a final concentration of 0.5% acetic acid in distilled water (vehicle) (17). All the animals received reserpine.

Drug Regime

Animals were randomly allocated to the experimental groups. On the one hand, a group of 16 animals orally received PGB (Lyrica; Pfizer, Madrid, Spain) at a single daily dose of 30 mg/kg. A group of 16 animals were orally given duloxetine (DLX, Cymbalta; Eli Lilly and Company, Fresno, CA) at a single daily dose of 30 mg/kg as an antidepressant control. A group of 14 animals were orally given tap water as a vehicle at a single daily dose. These treatments were maintained for 15 days. It has been established that acute treatment is a single 30 mg/kg oral dose, whereas chronic treatment is a 15-day treatment of the drug at a single daily dose of 30 mg/kg.

Depressive-Like Behaviors Measurement

Forced Swimming Test

The modified Forced Swimming Test (FST) was performed as previously described by Slattery and Cryan (19). On days 4 and 5 after the last reserpine dose, animals were exposed to the FST in 2 stages, a pretest and test, respectively. In the first stage (pretest), rats were individually placed inside a 40-cm depth x 20 cm-diameter methacrylate cylinder filled with water (25ºC) at a height of 30 cm for 15 minutes. Then rats were administered a single dose of drug or vehicle. After 24 hours (test), rats were administered a second single dose of drug or vehicle and were exposed to the same test conditions for 5 minutes. This test involves scoring active (swimming and climbing) and passive (immobility) behaviors while forcing rodents to swim in a cylinder from which there is no escape. Immobility behavior reflects failure to persist with directed escape attempts (learned helplessness). Therefore this behavior is related to clinical depression. It has been proposed that swimming behavior is mediated by serotonergic action, whereas climbing behavior is norepinephrine-mediated, and an increase in these behaviors spells a

direct decrease in immobility (19). FST has become the most widely used model to evaluate the antidepressant effect of new-generation drugs in animals, and it has also been shown to be sensitive to both the acute effect of antidepressant treatments and depressive states induced by various factors. The sample size (N) for this procedure was 24 male rats: 8 treated with PGB, 9 with DLX, and 7 with the vehicle.

Novelty-Suppressed Feeding Test

The whole procedure was performed as previously described by Blasco-Serra et al (20) in 2017. The open field was 100 x 100 x 40 cm, and the center of the test was well-lit (1,000 lumens) with 800 lumens intensity in the periphery, whereas the surrounding environment remained in the dark. A Petri dish containing a small amount of high palatability food was placed in the center of the field on a white platform. The whole procedure was video-recorded. Food deprivation was carried out by a 3-phase food deprivation method (20). The Novelty-Suppressed Feeding Test (NSFT) is based on the phenomenon of hyponeophagy, which is inhibition of intake produced by exposure to a novelty, for example, an unknown environment or new food. In this paradigm, rodents face a conflict between avoiding an unknown and highly lit environment (due to their innate fear of new open spaces) and their need to eat. Thus latency to eat is measured as depressive-like behavior. The use of NSFT as a validated depressive-like behavior test has increased in the last decade (20). The sample size (N) for this procedure was 22 male rats: 8 treated with PGB, 7 with DLX, and 7 with the vehicle.

Pain Thresholds Measurements

Pain thresholds were measured by an Electronic Von Frey test (EVF) for tactile allodynia and the Randall and Selitto test (R&S) for mechanical hyperalgesia. The whole procedure was performed as previously described (21). Briefly, the EVF animals were placed on an elevated grid floor inside a 20-cm diameter and 30-cm high clear methacrylate cylinder. An electronic Von Frey Tester (IITC Inc., Woodland Hills, CA) was used with an attached 0.8-mm diameter rigid tip. Pain thresholds were acquired by applying increasing pressure to the right hind paw sole until the animal withdrew its limb. To perform the R&S, the center section of the animal's body was held with an elevated fabric support, and hind paws were slipped through holes in the fabric. Increasing pressure was applied to the midsection of the gastrocnemius muscle of the right hind paw using an adapted R&S Tester (IITC Inc., Woodland Hills, CA, USA) until the animal withdrew its paw. Three measurements were taken by applying a 30-second interstimulus range. Data were obtained on treatment days 1, 3, 5, 7, 9, 11, 13, and 15. Pain threshold measures were taken in all the study animals.

Statistics

Data were analyzed and represented on graphs using SPSS Version 22 (IBM Corporation, Armonk, NY). They were expressed as the mean ± standard error mean and were analyzed for normality (Kolmogorov–Smirnov test) and variance homogeneity (Levène test). A value of $P < 0.05$ was considered statistically significant for all the comparisons. The most adequate statistical test was used for each experimental procedure.

Results

Acute PGB Treatment Ameliorates Depressive-Like Behaviors in the RIM Model

At an acute orally dose of 30 mg/kg, PGB significantly cut the immobility time of the animals subjected to the RIM model versus the vehicle-treated animals ($P < 0.05$), similarly to DLX. Moreover, DLX treatment proved more effective in reducing the immobility time than PGB treatment. Likewise, this acute PGB dose significantly prolonged the swimming time of the animals subjected to the RIM model ($P < 0.05$), as shown by DLX. Otherwise, PGB had no effect on climbing behavior ($P > 0.05$), unlike the effect of DLX. A graphic representation of the results are provided in Fig. 1.

Chronic PGB Treatment Does Not Substantially Ameliorate Depressive-Like Behaviors in the RIM Model

Chronic PGB administration did not reduce latency to eat from the center compared with the vehicle-treated group ($P > 0.05$). No differences were found in the times per minute when animals smelled or touched food ($P > 0.05$). However, a significant increase compared with the vehicle-treated animals was noted in the times per minute when animals approached food. The chronically DLX-treated animals significantly improved for all the parameters: decreased latency in eating ($P < 0.05$), prolonged times per minute that animals approached food ($P < 0.05$), and times per minute that animals smelled/touched food ($P < 0.05$). The results are graphically represented in Figs. 2 and 3.

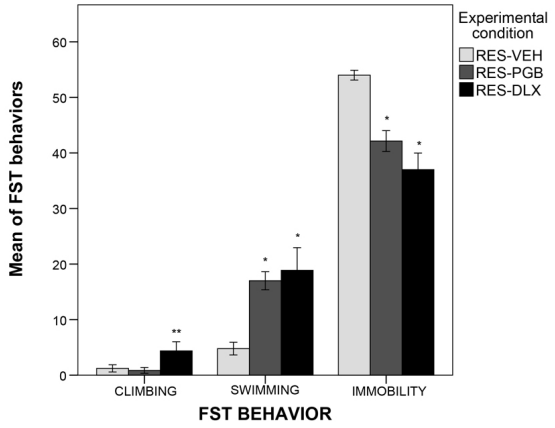

Fig. 1. *Means of each of the behaviors studied in FST (climbing, swimming and immobility). There are statistically significant differences of RES-DLX with respect to RES-VEH in all behaviors. Statistically significant differences were found between RES-PGB and RES-VEH in swimming and immobility behaviors. Statistically significant differences were found between RES-=DLX and RES-PGB groups in climbing. *Significance P < 0.05 with respect to RES-VEH. **Significance P < 0.05 of RES-DLX with respect to RES-PGB and RES-VEH. All data are presented as the mean ± SEM.*

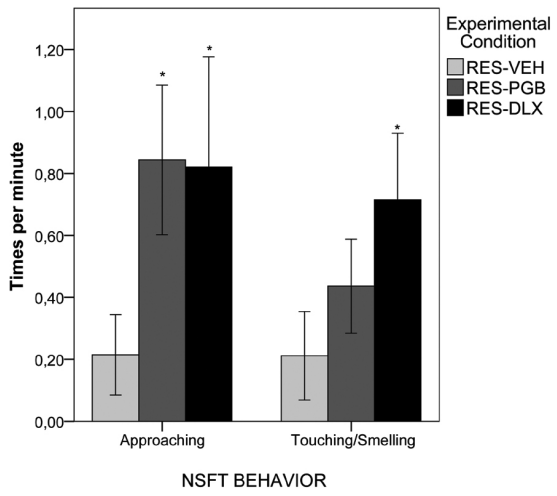

Fig. 2. *Figure shows a significant increase in the number of times per minute that the animal approaches the center of the test in the RES-DLX and RES-PGB conditions. there is a significant increase in the number of times per minute tha the animal touches/smells the food in the RES-DLX. *Significance P < 0.05 with respect to RES-VEH. All data are presented as the ± SEM.*

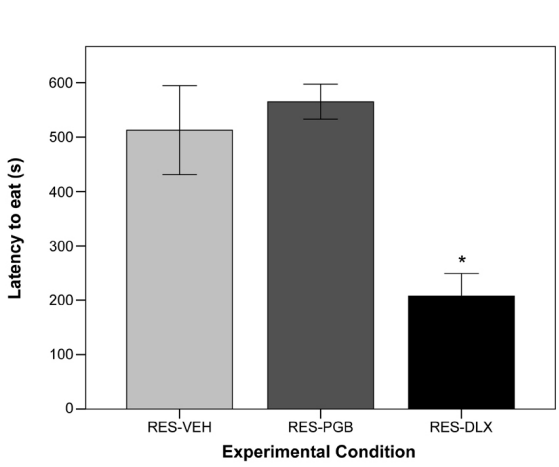

Fig. 3. *Figure shows a significant decrease in the latency to eat of rats treated with duloxetine (RES-DLX). *Significance P < 0.05 with respect to rats treated with pregabalin (RES-PGB) and rats treated with vehicle (RES-VEH). All data are presented as the ± SEM.*

PGB Ameliorates Tactile Allodynia in the RIM Model

All the animals displayed signs of tactile allodynia after reserpine administration. The results showed that the 15-day PGB treatment given at a single oral daily dose of 30 mg/kg diminished tactile allodynia in the rats subjected to the model compared with the vehicle-treated animals ($P < 0.05$), and similarly to DLX treatment. Both acute doses of DLX and PGB had amelioration effects on the alteration to tactile allodynia processing compared with the vehicle-treated animals ($P < 0.05$) (Fig. 4).

PGB Ameliorates Mechanical Hyperalgesia in the RIM Model

All the animals showed signs of mechanical hyperalgesia after reserpine administration. The results showed that the 15-day PGB treatment given at a single oral daily dose of 30 mg/kg diminished mechanical hyperalgesia in the rats subjected to the RIM model compared with the vehicle-treated animals ($P < 0.05$). Chronic DLX treatment increased the pain thresholds to higher levels than the baseline (Fig. 5).

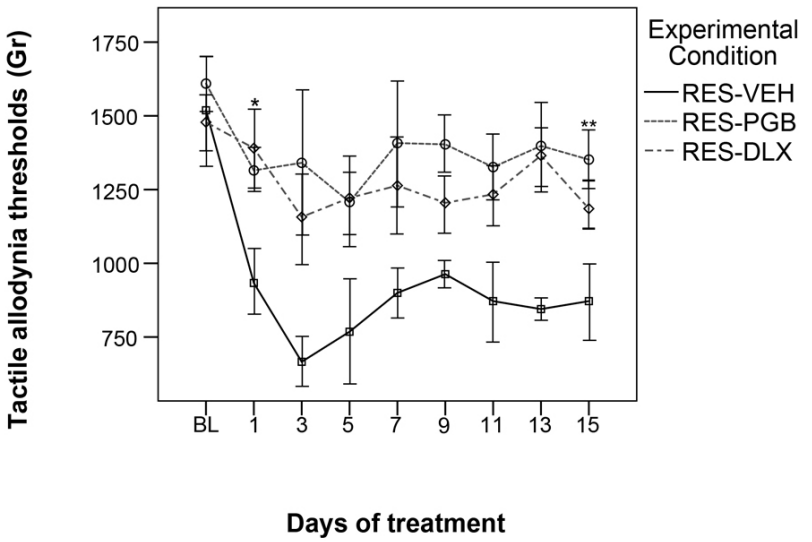

Fig. 4. *Evolution of tactile allodynia levels during the whole procedure. A sharp decrease in allodynia thresholds, without a significant improvement, in animals treated with vehicle is observed. Animals treated with duloxetine or pregabalin show improvement since the first dose. No differences were observed in the fifteenth day post-treatment between the two experimental groups (P > 0.05). RES-VEH: resperine-vehicle treatment; RES-PGB: resperine-pregabalin treatment. RES-DLX: resperine-duloxetine treatment. □ VEH measurement point. ◊ DLX measurement point. ○ PGB measurement point. * and ** P < 0.05 in respect to RES-VEH. All data are presented as the mean +/- SEM. BL: baseline.*

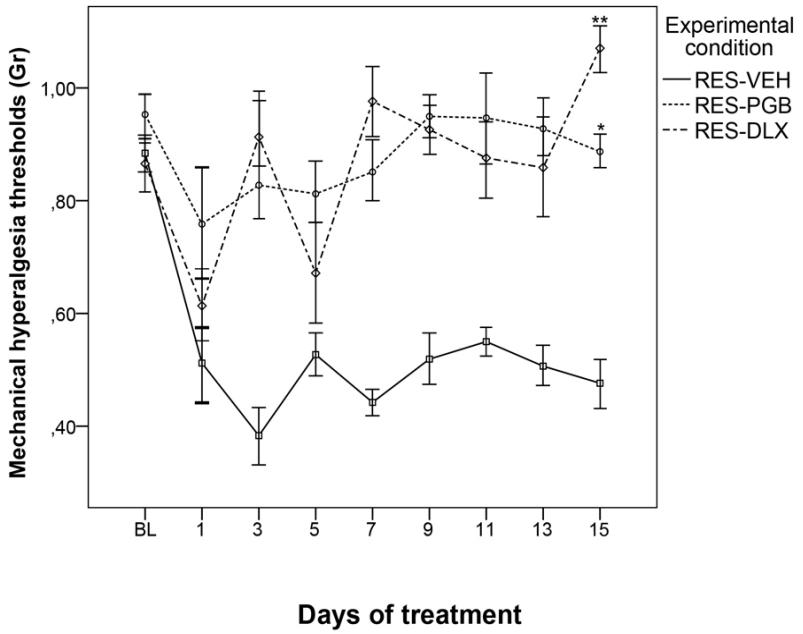

Fig. 5. *Evolution of mechanical hyperalgesia levels during the whole procedure. A sharp decrease in hyperalgesia thresholds, without a significant improvement, in animals treated with vehicle is observed. Animals treated with duloxetine or pregabalin show improvement since the first dose. RES-VEH: resperine-vehicle treatment; RES-PGB: resperine-pregabalin treatment. RES-DLX: resperine-duloxetine treatment. □ VEH measurement point. ◊ DLX measurement point. ○ PGB measurement point. * and ** P < 0.05 in respect to RES-VEH. All data are presented as the mean +/- SEM. BL: baseline.*

Discussion

This study explored the role that PGB might play in depressive-like behaviors in the RIM model. The analyses revealed that: (1) the acute PGB treatment ameliorated the depressive-like behaviors caused by the RIM model; (2) the chronic PGB treatment did not ameliorate the depressive-like behaviors caused by the RIM model; and (3) PGB had analgesic effects on both the tactile allodynia and mechanical hyperalgesia caused by the RIM model.

Acute PGB or DLX treatments (30 mg/kg) are capable of reversing the depressive symptomatology caused by the RIM model during the FST.

The acute PGB treatment results demonstrated not only a significant increase in swimming behavior, but also a significant reduction in the time animals spent floating immobile in the test phase compared with the vehicle-treated rats. As previously mentioned, PGB has been proven effective in the FMS, but its role in depressive-like symptoms has not yet been analytically studied (6,7,13,22-25). A common link apparently exists between the different indications in which antiepileptic drugs are used: they are prescribed in pathologies with underlying states of hyperexcitability, which may be manifested as sleep disorders, mood swings, or impulsivity (e.g., social anxiety disorders, FMS, or chronic neuropathic pain). An effect of PGB has been reported, which is sometimes observed as a side effect (26-28). Some authors point out that an anxiolytic and acute antidepressant effect can be found in these drugs, which can be explained, at least in part, by the acute modulation of serotonin (5-HT), dopamine (DA), noradrenaline (NA), and GABAergic action of γ-aminobutyric acid (GABA) (29,30). In contrast, a study carried out in mice has shown that the serotoninergic system does not modulate the antinociceptive effect of PGB but, conversely, the opioidergic pathway seems to participate in this effect (31). One hypothesis about our results is that this "acute antidepressant effect" is due to an acute modulation of monoamines and is also owing to the antinociceptive effect of PGB, with which animals would note an early improvement of symptoms, which would motivate active escape behaviors during the test.

There is also evidence to suggest that DLX has an early acute effect and is very efficient in treating physical symptoms of depression, and this effect is dependent on an early acute increase in 5-HT and NA levels in the central nervous system (CNS) (32,33). Although this was not a main objective of our work, the results herein obtained showed a possible effect on the behaviors regulated by 5-HT and NA, for example swimming and climbing, respectively, and in behavioral terms. These results are also consistent with those found in neuropathic pain models that present depression (34), which would reinforce evidence of pain and depression sharing the pathways regulated by biogenic amines (35-37). In human studies, however, some reviews have revealed that DLX does not seem to offer any significant advantage of its efficacy over other antidepressant agents for the acute phase treatment of major depression (38).

The chronic treatment results obtained with PGB did not improve the depressive-like symptoms produced by the RIM model, whereas chronic DLX treatment effectively reversed depressive-like symptoms.

With PGB, the behaviors related to approaching and contacting food increased, but animals' latency to eat did not lower compared with the vehicle-treated group. To the best of the authors' knowledge, PGB has not been tested for chronic antidepressant effects in animal models presenting pain. It should be noted that PGB is not an antidepressant drug, although a wide range of comorbid symptoms deriving from pain improvement under chronic conditions has been ameliorated (39,40). It is worth stressing that the increase in the behaviors of approaching and contacting food without eating was probably caused by the rapid anxiolytic effect of PGB, which has been previously described in humans (41). In the same vein, some works have found an antidepressant effect of subacute PGB treatment on depression symptoms associated with generalized anxiety disorder, and interesting differences have been found between male and female patients (42). Clinical research data about the effect of PGB on depressive-like behaviors of FMS patients suggest that this is no direct effect and, if we rely on Cohen's categories, the effect of PGB versus a placebo on depression is not substantial (43). Experimental data suggest that PGB activates the supraspinal noradrenergic system of descending inhibitory pain control. Moreover, all the hypotheses posed about a modulatory effect of central monoamines by PGB refer to an acute effect, with no evidence yet available for a chronic effect (29,30,44,45). Finally, in regard to different comorbid symptoms, this drug has been shown to have a specific benefit for sleep disorders, fatigue, and anxiety (46-49).

With the chronic DLX treatment, animals approached, smelled, and touched food more times per minute compared with the vehicle-treated group, and their latency to eat diminished. Although the chronic effects of DLX on the RIM model were not exhaustively analyzed, these results are consistent with those reported in humans after treatments lasting approximately 3 months. In fact, it has been observed that the effect of DLX on FMS is stronger on depressive comorbid symptoms than on painful symptomatology (13,50,51). Several studies have evidenced that DLX is more efficient against other serotonin–norepinephrine reuptake inhibitors (SNRIs) in treating FMS with both painful symptomatology and depressive-like behaviors,

and even with certain aspects of cognitive symptoms. Similarly, DLX is frequently used and is the first-choice drug for FMS patients in whom depression predominates as a comorbid symptom (52-56). Some authors have emphasized that, although DLX is effective, it is necessary to improve the efficacy of pharmacologic treatments to substantially enhance this efficacy, encompass more aspects of comorbid symptoms, and reduce adverse effects (57).

The results about pain thresholds show that administering DLX or PGB as an acute or a 15-day continuous oral treatment reverses both the mechanical hyperalgesia and allodynia produced by the RIM model induced in rats. Likewise, an acute DLX dose has proven to be more effective in reversing tactile allodynia than PGB.

Our results are similar to those reported by Nagakura et al (17). In the RIM model, they tested the effect of an acute dose of 3, 10, and 30 mg/kg of PGB or DLX on day 5 after the last injection of reserpine (17). They obtained a recovery of the pain thresholds for the 10 and 30 mg/kg PGB doses for muscular hyperalgesia and for the 30 mg/kg dose for tactile allodynia. Although this group found no improvement of tactile allodynia, improvement appeared in mechanical hyperalgesia at an acute DLX dose of 30 mg/kg on day 5 after the last administration of reserpine. It should be noted that our results are not completely comparable to those of Nagakura et al (17) because their group tested the effect of an acute drug dose on day 5 after setting up the model, whereas our research group did so on day 1.

We found no more current studies that have tested for anticonvulsants in pain thresholds in this model. However, some studies have focused on using PGB in animal models of neuropathic pain, showing that improvement in pain thresholds can be maintained from day 3 of continuous oral administration, which is consistent with our results (58). In the last decade, the use of PGB as a coanalgesic has spread worldwide, and it has been approved for various pathologies presenting pain, such as neuropathic pain associated with diabetic peripheral neuropathy, postherpetic neuralgia, central and peripheral neuropathic pain, and FMS (59-61).

Recent studies about the RIM model have focused on new SNRIs given their stronger effect on the monoaminergic system. Murai et al (62) reported a significant improvement in mechanical hyperalgesia after oral acute DLX doses of 10 and 30 mg/kg on day 5 after the last administration of reserpine. Zhang et al (63) found an improvement in tactile allodynia after an acute DLX dose of 50 mg/kg on day 5 after the last administration of reserpine. The results of the present study allow us to hypothesize that a combined increase of 5-HT and NA could be more beneficial for persistent pain attenuation than an increase in either of the 2 agents alone. Our results are partially consistent with those of Zhang et al (63) and support the evidence of SNRIs showing antiallodynic effects on the RIM model. Our results are also consistent with studies conducted in humans in recent decades, which reveal a significant improvement in painful symptoms after chronic DLX treatment at a dose of 60/120 mg/day (64).

Our study limitations involve lack of histological and electrophysiological studies, which would open a door to more objective data, and it is doubtlessly a good future research line. One aspect worth mentioning is that this study only included male rats as it has been classically postulated that FMS is a predominant syndrome in women. Recent studies have shown that, thanks to correctly applying the 2011 American College of Rheumatology (ACR) diagnostic criteria, the prevalence between men and women is not as high as historically described (65). Besides, studies of pain and mood disorders have been historically, and are more commonly, conducted in men given the influence that sex hormones and hormonal cycles have on these systems in women (66,67). It would be interesting to start conducting such studies in women as a future research line and, in turn, to compare these results to those already found in men.

A valid animal model capable of reproducing disease implies an accelerated advance in experimental research, which would also be a basic research approach to clinical practice and would provide material to work in translational medicine. In this work, we detected major and interesting changes in the functionality of the CNS linked to the pharmacologic treatment of FMS in both the short and long terms, which could open a door to develop new therapeutic strategies to treat FMS.

Conclusions

Acute PGB treatment proved efficient in ameliorating depressive-like behaviors in this model. This can be explained by an acute 5-HT, DA, NA, and GABA. On the one hand, this acute monoamine modulation could also indicate an antinociceptive effect by placing animals in a state of early improved symptoms, which would motivate active escape behaviors during the test. However, chronic PGB treatment improves

pain thresholds, but not depressive-like behavior, in the RIM model. These results led us to raise a question about the acute and chronic effects of PGB on supraspinal systems, which relate to anxiety, pain, and depression pathways, and suggest the need to further explore in-depth the implication of anticonvulsants in neuronal circuits related to pain and depressive-like behaviors to find new treatment targets.

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