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Pregabalin Suppresses Nociceptive Behavior and Central Sensitization in a Rat Trigeminal Neuropathic Pain Model

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Abstract: The aim of this study was to determine whether pregabalin affects nociceptive behavior and central sensitization in a trigeminal neuropathic pain model. A partial infraorbital nerve transection (p-IONX) or sham operation was performed in adult male rats. Nociceptive withdrawal thresholds were tested with von Frey filaments applied to the bilateral vibrissal pads pre- and postoperatively. On postoperative day 7, the behavioral assessment was conducted before and at 30, 60, 120, and 180 minutes after and 24 hours after pregabalin (.1, 1, 10, 100 mg/kg intraperitoneally) or saline injection. The effects of pregabalin or saline were also examined on the mechanoreceptive field and response properties of nociceptive neurons recorded in the medullary dorsal horn at postoperative days 7 to 10. Reduced withdrawal thresholds reflecting bilateral mechanical allodynia were observed in p-IONX rats until postoperative day 28, but not in sham-operated rats. At postoperative day 7, pregabalin significantly and dose-dependently reversed the reduced mechanical withdrawal thresholds in p-IONX rats. Pregabalin also attenuated central sensitization of the neurons, as reflected in reversal of their reduced activation threshold, increased responses to pinch/pressure, and enhanced stimulus-response function. This study provides the first documentation that pregabalin attenuates the mechanical allodynia and central sensitization that characterize this trigeminal neuropathic pain model, and supports its clinical use for treating craniofacial neuropathic pain.

***Perspective:** Trigeminal nerve injury in rats produced facial mechanical hypersensitivity and trigeminal central sensitization of medullary dorsal horn neurons that were markedly attenuated by systemically administered pregabalin, suggesting its potential clinical utility for orofacial neuropathic pain.*

***Key words:** Pregabalin, allodynia, central sensitization, trigeminal neuropathic pain.*

Neuropathic pain is defined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system.[47] Neuropathic pain patients may suffer from spontaneous or evoked pain, and treatment of the pain has often been inadequate.[32,54] The gabapentinoid pregabalin has recently been shown to be efficacious in many neuropathic pain states. Pregabalin is a ligand to the $a_2\delta$ subunit of the voltage-gated calcium channel, and binding at this site attenuates calcium influx at nerve terminals that reduces the release of several neurotransmitters involved in nociceptive transmission, such as glutamate and substance P.[12,18,27,30,33] Recent animal studies suggest that pregabalin may impair the development or maintenance of spinal central sensitization underlying the hyperalgesic state.[3,53] Pregabalin is effective not only in several types of neuropathic pain in humans[14,19,32,41,45] but also in rat models of neuropathic pain[5,17,27,34] and in other pain models.[21,35,57] A related gabapentinoid, gabapentin, has also been shown to be effective clinically and in animal pain models.[2,10,15,32] Other than reports on its clinical effectiveness specific for trigeminal neuralgia,[35,38] there have been no detailed clinical investigations of the effectiveness of pregabalin in human orofacial neuropathic pain states.

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Such findings bearing on the clinical efficacy of gabapentin and pregabalin and their possible underlying mechanisms in animal pain models have come almost exclusively from studies of spine-related pain. There is very limited evidence of their actions and mechanisms in craniofacial pain states, even though chronic as well as acute craniofacial pain conditions are very common.[28,29] Nociceptive behavior accompanied by central sensitization of nociceptive neurons in the medullary dorsal horn (also termed the trigeminal subnucleus caudalis)[13,43] can be produced in animal models of craniofacial neuropathic pain.[23,24,36] Although gabapentin has been shown to be effective in rat behavioral models of craniofacial inflammatory and neuropathic pain,[9,20] pregabalin has not been tested on both nociceptive behavior and central sensitization in animal models of craniofacial neuropathic pain. Therefore, the aim of the present study was to use a craniofacial neuropathic pain model involving partial transection of the infraorbital nerve[31] to determine whether pregabalin affects craniofacial nociceptive behavior and central sensitization in functionally identified nociceptive neurons in the rat's medullary dorsal horn. Data have been partly reported in abstract form.[4]

Methods

Animals

Experiments were performed on adult male Sprague Dawley rats weighing 280 to 400 g (n = 84; Charles River, Saint-Constant, QC, Canada). The animals were housed in a temperature and humidity controlled environment on a 12-hour light/dark cycle. Food and water were freely available. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

Infraorbital Nerve Transection

Under isoflurane anesthesia (5% induction, 2–2.5% maintenance), an incision was made in the right maxillary gingivo-buccal groove of the rats. The infraorbital nerve was exposed and dissected free from the surrounding tissue. As previously described by Miyamoto et al[31] and Xu et al,[56] the lateral half of the nerve was lifted from the maxillary bone and cut with scissors in some rats to produce a partial transection of the nerve (p-IONX); care was taken not to damage facial nerve branches. In other rats, a sham operation was performed but without any nerve injury. After the surgery, the wounds were closed by sutures. The animals were returned to their home cages and fed with mash and chow.

Drug Treatments

Pregabalin was dissolved in sterilized saline and administered intraperitoneally (i.p.) at 2 mL/kg in the behavioral testing experiments (see below). p-IONX rats displaying mechanical allodynia (see below) following baseline behavioral testing were randomly placed into 5 different groups (n = 6/group) and received a single administration of pregabalin (.1, 1, 10, 100 mg/kg) or vehicle (saline) at postoperative day 7. This dose range of pregabalin was guided by our recent study showing that pregabalin at doses between 1 and 100 mg/kg was effective in dose-dependently attenuating orofacial electromyography activity and medullary release of glutamate evoked by noxious orofacial stimulation.[33] The group of sham-operated rats (n = 6) received a single administration of 10 mg/kg pregabalin at postoperative day 7. Since 1 mg/kg was the minimum effective dose in the behavioral testing (see Results) during the neuronal recording experiments, pregabalin at a dose of 3 mg/kg (or saline) was selected to be delivered after the baseline properties of each neuron were obtained (see below). The neuronal properties were also assessed at 30 and 60 minutes after drug administration, based on the time course reported in previous electrophysiological studies using systemically administered pregabalin.[5,53]

Behavioral Testing

The testing was similar to that used in previous studies.[48,49] Briefly, 36 rats were first trained daily prior to surgery to remain in a plastic container and place their noses through a hole in the container. Mechanical withdrawal thresholds were determined at 5 locations bilaterally on the vibrissal pad by using von Frey filaments (North Coast Medical, Inc, Gilroy, CA). The lowest stimulus intensity, in ascending order, to evoke an escape response (ie, head aversion and scratching) was taken as the withdrawal threshold. Filaments exerting forces greater than 15 g could not be used because their application moved the head before the hair bent, so 15 g was used as the cutoff value. In the group of p-IONX rats treated with saline (n = 6) and the sham-operated group treated with pregabalin (n = 6), behavioral tests were conducted at preoperative days 1, 2, and 3 and at postoperative days 1, 3, 5, 7, 10, 14, 21, 28, and 35. In the groups of p-IONX rats treated with different doses of pregabalin (n = 24), behavioral tests were conducted at preoperative days 1, 2, and 3 and at postoperative days 1, 3, 5, 7. On postoperative day 7, the time at which p-IONX rats displayed significant mechanical allodynia (see Results), the behavioral assessments were conducted in all the rats (n = 36) from the p-IONX and sham-operated groups before and at 30, 60, 120, and 180 minutes after and 24 hours after injection of pregabalin or saline.

Neuron Recording and Stimulation Procedures

The methods used for animal preparation, stimulation, neuronal recording, and classification were similar to those described previously in detail[6,7,55] and so will only be briefly outlined here. As well as the rats used in the behavioral testing experiments outlined above, additional rats were used to provide a total of 48 rats (24 p-IONX rats, 24 sham-operated rats) that were used for the neuronal recording experiments at postoperative days 7 to 10 (shown in the