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Progesterone Prevents Allodynia After Experimental Spinal Cord Injury

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Abstract: Chronic pain after spinal cord injury represents a therapeutic challenge. Progesterone, a neuroprotective steroid, has been shown to modulate nociceptive thresholds, whereas its effect on neuropathic pain needs to be further explored. In this study, we evaluated whether progesterone could ameliorate pain-associated behaviors in animals subjected to a spinal cord hemisection. The development of mechanical and cold allodynia was assessed in injured male rats treated with daily injections of progesterone or vehicle. The expression of N-methyl-D-aspartate receptor (NMDAR) subunits, protein kinase C gamma (PKCγ), preprodynorphin (ppD), and kappa opioid receptor (KOR), key players in chronic pain mechanisms, was determined in the dorsal spinal cord. Twenty-eight days after injury, all vehicle-treated animals presented allodynic behaviors and a marked increase in NMDAR subunits, PKCγ, and ppD mRNA levels, with no changes in KOR mRNA levels. Progesterone prevented the development of mechanical allodynia and reduced the painful responses to cold stimulation. In correlation with the attenuation of pain behaviors, the steroid prevented NMDAR subunits and PKCγ mRNAs upregulation, did not modify the elevated ppD mRNA levels, but increased KOR expression. In conclusion, progesterone modulates neuropathic pain after spinal cord injury, creating a favorable molecular environment that may decrease spinal nociceptive signaling.

***Perspective:** The present study suggests that progesterone administration could represent an interesting strategy to modulate neuropathic pain circuits after spinal cord injury. Further studies are needed to investigate the potential progesterone receptors involved in these actions.*

***Key words:** Chronic pain, protein kinase C, N-methyl-D-aspartate receptor, preprodynorphin, kappa opioid receptors.*

Chronic pain is a major concern for patients with spinal cord injury, with an estimated incidence that ranges from 40 to 60%.[3,9] These patients, already burdened with the disability of paralysis, emotional trauma and spasticity, must contend with severe unrelenting pain.[9,24] Below-level mechanical and thermal allodynia, 2 neuropathic pain-associated behaviors, are commonly observed after spinal cord injury in humans.[9,24] Unfortunately, most of the currently available drugs for the treatment of neuropathic pain are relatively ineffective.[3]

Although the precise mechanisms underlying chronic pain after spinal cord injury remain elusive, several maladaptive molecular events are known to contribute to the observed pain-related behaviors following injury.[17,49,97] In this regard, both the increased expression and/or the activity of the N-methyl-D-aspartate receptor (NMDAR) play a critical role in the development and maintenance of chronic pain.[6,37]

The functional NMDAR contains an obligatory NR1 subunit in combination with at least 1 of the 4 NR2 subunit family members, of which NR2A and NR2B are the most abundant in the adult rat dorsal horn.[60,66] Previous studies have found that several conditions such as diabetes,[88] excitotoxic[10] or traumatic[30] spinal

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cord injuries, or morphine tolerance[52] alter the expression of NMDAR subunits in the spinal cord contributing to abnormal pain processing. Moreover, increased phosphorylation of the NR1 subunit correlates with the presence of neuropathic pain behaviors after excitotoxic spinal lesions[10] and peripheral nerve injuries.[26,89]

Furthermore, dynorphin and the gamma isoform of the protein kinase C (PKCγ), both key players in neuropathic pain signaling, have been shown to amplify the NMDAR-mediated circuit by either direct or indirect activation of this receptor.[48,50,58]

A recent report from our laboratory indicates that spinal cord hemisection induces a time-dependent upregulation of NMDAR subunits, PKCγ and preprodynorphin (ppD), the dynorphin precursor peptide, in the dorsal horn of animals exhibiting allodynic behaviors.[43] Therefore, drugs that could either prevent or attenuate the maladaptive molecular changes that arise after injury could represent an effective treatment for central neuropathic pain.

Several reports support the crucial role of neuroactive steroids in the modulation of pain sensation.[62] Progesterone, in particular, mediates gestational antinociception,[29] contributes to sex-related differences in pain,[29,45,54] and reduces pain sensitivity in intact rats.[25] In addition, progesterone attenuates neuropathic pain-associated behaviors in animals displaying a peripheral nerve injury[82] or diabetic neuropathy.[51]

Interestingly, several neuroactive effects of progesterone involve the recruitment of the opioid system,[20,29,74] the modulation of dynorphin levels,[84] and the activity of NMDAR[78] and PKC,[5] suggesting that this steroid could have the ability to influence pain sensitivity through the modulation of these molecules.

The present study was designed to examine the role of progesterone on the onset of chronic pain in male rats subjected to a spinal cord hemisection, a well-recognized model of central neuropathic pain.[14] Furthermore, we investigated the spinal expression of NMDAR subunits, PKCγ, ppD, and KOR, all key players in the process of central sensitization, trying to provide insight into the potential molecular mechanisms involved in progesterone analgesic effects. Our current investigations suggest further applications for progesterone-based therapies and may open new avenues for the treatment of chronic pain after central injuries.

Methods

Spinal Cord Injury and Progesterone Administration

All experimental procedures were reviewed by the local Animal Care and Use Committee (Assurance Certificate N A5072-01, Instituto de Biología y Medicina Experimental) and followed the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). Care was taken to minimize animal discomfort and to limit the number of animals used. Male Sprague-Dawley rats (200–220 g), bred at the colony of the Instituto de Biología y Medicina Experimental (Buenos Aires, Argentina), were deeply anesthetized with chloral hydrate (400 mg/kg, ip). In a group of rats, the spinal cord was exposed and unilaterally hemisected at thoracic T13 level (n = 32),[43] as originally described by Christensen et al.[14] In hemisected animals, the ipsilateral hindlimb is acutely paralyzed while the contralateral limb is unimpaired.[14] For this reason, all animals presenting acute contralateral hindlimb involvement after the surgery—a demonstration of overhemisection or ischemic complication—were excluded from the study. Postoperative care included control of body temperature and antibiotic administration.[42] Injured animals received daily subcutaneous injections of natural progesterone (16 mg/kg/day, n = 12, HX+PG; Proluton, Schering Laboratories, Buenos Aires, Argentina), vehicle (Ricine oil; Ewe, Sanitas, AR; n = 10), or none (n = 10, HX). This protocol of progesterone administration has been shown to prevent oedema and neuronal loss and improve cognitive responses following brain-contusion injury,[18] and to induce oligodendrogenesis and remyelination after spinal cord injury.[44] Since progesterone may exert sex-based divergent analgesic properties,[46,54] further studies are in progress in order to analyze the effects of long-term progesterone administration and the influence of the oestrous cycle in female rats with spinal cord injury. Control groups included sham-operated rats (n = 32) receiving either progesterone (n = 12), vehicle (n = 10), or none (n = 10), and intact animals (n = 26) receiving either progesterone (n = 10), vehicle (n = 8), or none (n = 8).

Behavioral Assessment

Behavioral testing was performed in all animals before surgery (day 0) and at different time points after spinal cord hemisection or sham operation, as previously described.[16,43] Briefly, the animals were placed in their acrylic testing chambers for 15 minutes for adaptation, and mechanical sensitivity was assessed with von Frey hairs (Stoelting, Wood Dale, IL). The hairs were applied in ascending order (1, 2, 4, 6, 8, 10, 15, 26 g) onto the plantar surface of both ipsilateral and contralateral hindpaws.[11] Each hair was delivered 3 times with 5-second intervals. The lowest force at which application elicited a brisk paw withdrawal was taken as the mechanical response threshold. A paw withdrawal reflex obtained with 6 g or less was considered as an allodynic response. Cold sensitivity of the hindpaw to acetone (Choi test)[13] was quantified by foot withdrawal frequency. Thus, 100 μl of acetone was applied to the plantar surface of the paw using a plastic tubule connected to a 1 mL syringe. Acetone was applied 5 times to each paw at an interval of at least 5 minutes. The number of brisk foot withdrawals was recorded. Only rats showing normal responses to mechanical and thermal stimulation before surgery were included in the experiments. After spinal cord hemisection, the ipsilateral hindlimb was acutely paralyzed but presented a considerable return to motor function by 5 days postsurgery, when the animals entered an early phase of recovery with return of paw placement and frequent-to-consistent weight