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EFFECTS OF PROGESTERONE ON NEUROPATHIC PAIN RESPONSES IN AN EXPERIMENTAL ANIMAL MODEL FOR PERIPHERAL NEUROPATHY IN THE RAT: A BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDY

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Abstract—Progesterone (PROG) is promising as an important protective agent against various injuries to the nervous system. The present study was designed to investigate whether starting PROG administration, when symptomatology is already established, would alleviate the expression of nociceptive behaviors (mechanical allodynia and thermal hyperalgesia) and electrophysiological changes in a chronic constriction injury (CCI) model of neuropathic pain in rats. Male rats were given PROG (1.5, 3, 6 and 12 mg/kg, i.p.) 12 days after CCI induction, and dosing continued daily until day 26. Behavioral tests were done immediately before surgery (day 0) and on days 12, 26, 28, and 35 post-CCI, and were followed by electrophysiological measurements in the last day. PROG at doses of 6 or 12 mg/kg reduced both the thermal hyperalgesia and mechanical allodynia induced by CCI. Electrophysiological data indicated that CCI-induced animals had a remarkable decrement of both compound muscle and nerve action potential amplitudes recorded in the gastrocnemius muscle and sural nerve, respectively. CCI also caused a significant reduction in motor and sensory conduction velocities measured in the sural and tibial nerves, respectively. PROG at doses of 6 or 12 mg/kg induced a significant recovery of all electrophysiological changes. Our data indicated that starting PROG therapy when symptomatology is already established, and continuing it for a sufficient period of time, may have a therapeutic effect. This suggests that PROG may offer new strategies for the treatment of neuropathic pain.

Key words: neuropathic pain, peripheral neuropathy, progesterone, neuroprotection, therapeutic effects.

INTRODUCTION

Peripheral neuropathy is a term used to describe damage to nerves of the peripheral nervous system which may be caused either by diseases of or trauma to the nerve. Prevalence of peripheral neuropathy is about 2.4% and increases up to 8% with age (Martyn and Hughes, 1997). Neuropathy may be associated with varying combinations of weakness and autonomic and sensory nervous system changes. The most important symptoms of peripheral neuropathy are pain, abnormal responses to painful (hyperalgesia) and painless (allodynia) stimuli, muscle weakness and atrophy, and autonomic dysfunction (Roglio et al., 2008b). Unfortunately, many treatment strategies for peripheral neuropathy are symptomatic, which actions are not satisfactory (Baastrup and Finnerup, 2008; Colleoni and Sacerdote, 2010).

Recent findings suggest that neuroactive steroids may be promising factors in treatment studies as their protective effects on the nerve have shown their efficacy in the treatment of neurological disorders, physical injuries, aging, or inherited demyelinating diseases (Colleoni and Sacerdote, 2010). Progesterone (PROG) is one of the most important neuroactive steroids. It is well known that PROG is produced in the central and peripheral nervous systems, and its receptors are located on the neurons and supporting cells in the nervous system (Inoue et al., 2002; Schumacher et al., 2012). Previous studies have shown the neurotrophic and neuroprotective effects of exogenous PROG. Administration of PROG and its derivate has been demonstrated to be an effective treatment for traumatic brain injury, stroke (Roof et al., 1994, 1996), and peripheral neuropathy in experimental animals (Azcoitia et al., 2003; Melcangi and Garcia-Segura, 2006). PROG also reduces neuronal cell death in neurons of the caudate nucleus (Cervantes et al., 2002) and induces neuroprotective effects following brain and spinal cord injuries (Jiang et al., 1996; Thomas et al., 1999). These results suggest that PROG or some of its metabolites can be successfully used to treat traumatic brain and spinal cord injury, as well as ischemic stroke.

There are some reports suggesting the involvement of PROG in the modulation of pain. For example, a recent study showed that PROG levels were significantly reduced in STZ-induced diabetic rats, and one-month chronic treatment with PROG prevented a reduction in

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Abbreviations: BW, body weight; CCI, chronic constriction injury; CMAPs, compound muscle action potentials; CV, conduction velocity; MNCV, motor nerve conduction velocity; mtPTP, mitochondrial permeability transition pore; NCV, nerve conduction velocity; PROG, progesterone; SNAP, sensory nerve action potentials; SNCV, sensory nerve conduction velocity; VEH, vehicle.

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nerve conduction velocity (NCV) and impairment of thermal threshold (Leonelli et al., 2007). Also, it was shown that PROG and its derivative dihydroprogesterone counteracted biochemical alterations and nociception impairment in an experimental model of nerve crush injury (Roglio et al., 2008a). Another recent study showed that daily administration of PROG (16 mg/kg) prevented the development of mechanical allodynia and reduced painful responses to cold stimulation in rats who were subjected to a sciatic nerve single ligature constriction (Coronel et al., 2011). PROG (8 and 16 mg/kg/day) was also shown to produce antinociceptive effects through neuroprotective action in animals with lysophosphatidic acid-induced trigeminal neuropathic pain (Kim et al., 2012). Although these observations suggest that PROG may play a role in controlling pain in pathologic conditions, further studies using different models and approaches are necessary to verify such role and particularly the therapeutic effects of PROG.

The well-established chronic constriction injury (CCI) model of neuropathic pain is a valuable experimental tool as it demonstrates that, after experiencing a painful neuropathy, animals exhibit behavioral changes in the perception of sensations, such as hyperalgesia and allodynia, which mirror the changes that are observed in human pain states (Bennett and Xie, 1988; Wang and Wang, 2003). Therefore, we examined whether starting PROG treatment when symptomatology is already established would be effective against the neuropathic pain in the rat CCI model. For this propose, different doses of PROG were administrated 12 days after CCI induction and continued daily until day 26. Behavioral tests (mechanical and then thermal pain testing) were done immediately before surgery (day 0) and on days 12, 26, 28, and 35 post-CCI. Electrophysiological measurements were performed on day 35 following behavioral tests. PROG at doses of 6 or 12 mg/kg was able to alleviate CCI-induced nociceptive behaviors and to restore the resulting electrophysiological changes in both motor (tibial) and sensory (sural) peripheral nerves, indicating that this steroid may have a therapeutic effect against neuropathic pain.

EXPERIMENTAL PROCEDURE

Animals

Adult male Wistar rats (250 ± 10 g) were individually housed in cages (50 × 26 × 25 cm) and kept on a 12-h light/dark cycle (6 am lights on–6 pm lights off) with food and water available *ad libitum*. The ambient environment was maintained at a constant temperature (22 ± 2 °C) and relative humidity (50–60%). The experimental protocol was approved by the Ethical Review Board of Semnan and Kerman Universities of Medical Sciences (Iran). All of the experimental trials were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. In addition, care was taken to minimize the number of animals that were used in each experiment. Three days before any behavioral testing, the rats were kept in their testing room for about two hours each day. During this time, they were alternatively handled by the investigator and were kept in the testing chamber for about 15 min.

Drugs

PROG (Sigma–Aldrich) was dissolved in propylene glycol as a vehicle (VEH), and doses of 1.5, 3, 6, and 12 mg/kg were prepared and injected intraperitoneally at a volume of 2 ml/kg. All injections were administered after behavioral tests on different experiment days.

CCI model

CCI was produced by loose ligation of the sciatic nerve as previously described (Bennett and Xie, 1988). Procedures were performed under a mixture of ketamine and xylazine (Sigma–Aldrich) (60 and 8 mg/kg, respectively) anesthesia and included right sciatic nerve exposition at mid-thigh level by blunt dissection through the biceps femoris muscle. Four constrictive ligatures (4–0 chromic gut suture) were loosely tied around the nerve at distances of about 1 mm. Muscle and skin were closed in layers. In the sham group, operations were performed to expose and mobilize the nerve, but there was no ligation. All operations were done by the same person to minimize differences in method.

Behavioral tests

All behavioral tests (mechanical and then thermal pain testing) were performed between the hours of 10:00 and 14:00, immediately before surgery (day 0), and on days 12, 26, 28, and 35 post-CCI. Body weight (BW) of the animals was measured on days 0, 12, and 28 after surgery.

Mechanical allodynia (von-Frey filament testing). The animals were placed in a Plexiglas box (30 × 30 × 30 cm) with a mesh floor based on the method described by Pitcher et al. (1999) in their 1999 study. Each rat was placed in the testing chamber and permitted to acclimatize for 30 min prior to testing. von Frey filaments (Stoelting Co, Wood Dale, IL, USA) in scores of 2, 4, 6, 8, 10, 15, 26, and 60 gr were applied to the plantar soft tissue of the hind paw to determine the withdrawal threshold. The first filament applied matched to a force of 2 gr. Each filament was applied three times, each for three seconds, and at intervals of three seconds. If a negative response (no movement) was seen, the filament exerting the next greater force was used. If two of the observed responses were positive (paw withdrawal), the obtained score was considered a response threshold. If the animal did not respond to the score of 60 gr, that score was considered a threshold response.

Thermal hyperalgesia. For thermal hyperalgesia, paw-withdrawal latency to a thermal nociceptive stimulus was assessed as described elsewhere (Hargreaves et al., 1988). Rats were placed in a Plexiglas box