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BEHAVIORAL, PHARMACOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE SAPHENOUS NERVE PARTIAL LIGATION: A NEW MODEL OF NEUROPATHIC PAIN

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Abstract—The saphenous partial ligation (SPL) model is a new, easily performed, rodent model of neuropathic pain that consists of a unilateral partial injury to the saphenous nerve. The present study describes behavioral, pharmacological and molecular properties of this model. Starting between 3 and 5 days after surgery, depending on the modality tested, animals developed clear behaviors indicative of neuropathic pain such as cold and mechanical allodynia, and thermal and mechanical hyperalgesia compared with naive and sham animals. These pain behaviors were still present at 1 month. Signs of allodynia also extended to the sciatic nerve territory. No evidence of autotomy or bodyweight loss was observed. Cold and mechanical allodynia but not thermal and mechanical hyperalgesia was reversed by morphine (4 mg/kg i.p.). The cannabinoid receptor agonist WIN 55,212-2 (5 mg/kg i.p.) improved signs of allodynia and hyperalgesia tested except for mechanical hyperalgesia. Gabapentin (50 mg/kg i.p.) was effective against cold and mechanical allodynia but not hyperalgesia. Finally, amitriptyline (10 mg/kg i.p.) failed to reverse allodynia and hyperalgesia and its administration even led to hyperesthesia. Neurobiological studies looking at the expression of μ opioid receptor (MOR), cannabinoid CB_1 and CB_2 receptors showed a significant increase for all three receptors in ipsilateral paw skin, L3–L4 dorsal root ganglia and spinal cord of neuropathic rats compared with naive and sham animals. These changes in MOR, CB_1 and CB_2 receptor expression are compatible with what is observed in other neuropathic pain models and may explain the analgesia produced by morphine and WIN 55,212-2 administrations. In conclusion, we have shown that the SPL is an adequate model that will provide a new tool for clarifying peripheral mechanisms of neuropathic pain in an exclusive sensory nerve. © 2005 Published by Elsevier Ltd on behalf of IBRO.

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Abbreviations: CCI, chronic constriction injury; DMSO, dimethylsulfoxyde; DRG, dorsal root ganglia; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MOR, μ opioid receptor; PMSF, phenyl methyl sulfonyl fluoride; PSL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation; SPL, saphenous nerve partial ligation.

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Diseases or trauma affecting peripheral nerves often induces the development of neuropathic pain, which is characterized clinically by hyperalgesia and allodynia. In recent decades, animal models have been developed in order to have a better understanding of the pathophysiology of neuropathic pain and to test new drugs. Many models of physical nerve injury are targeted at the sciatic nerve. They impart a partial injury of the nerve using different techniques such as chronic constriction injury (CCI; Bennett and Xie, 1988), partial sciatic nerve ligation (PSL; Seltzer et al., 1990), spinal nerve ligation (SNL; Kim and Chung, 1992), spared nerve injury (SNI; Decosterd and Woolf, 2000) and tibial nerve injury (Hofmann et al., 2003). Despite the advances that these models have brought us in terms of understanding the mechanisms of neuropathic pain, many questions remain unanswered.

In order to approach the complex pathophysiology of neuropathic pain from a different angle, we decided to develop a neuropathic pain model based on the saphenous nerve. One of the first experiments showing an implication of the saphenous nerve in neuropathic pain was described by Wall et al. (1979). The authors showed that a complete ligation of the saphenous nerve increased autotomy (a sign of anesthesia dolorosa) induced by ligation of the sciatic nerve. Later, the participation of the saphenous nerve in mechanical allodynia in the medial territory of the paw, following a CCI, was demonstrated (Tal and Bennett, 1994). In that case, transection of the saphenous nerve was used as a complementary explanation for the phenomenon that occurred on the sciatic nerve.

We have also developed a new model of neuropathic pain by partial ligation of the saphenous nerve for the following reasons: first, this model is less traumatic for the animal and easier to perform because of the superficial localization of the saphenous nerve on the anterior part of the thigh. In addition, this model is performed on an exclusively sensory nerve so that it is possible to discern any effect on motor fibers, as observed with the sciatic nerve which is a mixed one. Finally, this new model is an ideal one to use in the well-known skin-nerve preparation (Reeh, 1986) for gaining a better understanding of the peripheral mechanisms of neuropathic pain.

We describe the development of the neuropathy after saphenous nerve partial ligation (SPL). To better characterize this new model, we studied the effects of standard

analgesic drugs used in the treatment of neuropathic pain such as morphine (opioid agonist), WIN 55,212-2 (cannabinoid agonist, only used in animal studies), and the adjuvant drugs gabapentin and amitriptyline. In addition to behavioral and pharmacological experiments, Western blot analysis was performed to evaluate if changes in the expression of the μ opioid receptor (MOR) and of the cannabinoid CB_1 and CB_2 receptors occurred at the paw skin innervated by the saphenous nerve, at the L3–L4 dorsal root ganglia (DRG) and at the spinal cord.

EXPERIMENTAL PROCEDURES

All procedures conformed to the Canadian Council on Animal Care guidelines and we received authorization from the animal ethics committee of the Université de Montréal. Care was taken to minimize the number of animals used and their suffering. The animals were housed two per cage with a bedding of wood sawdust (8–16 mm; Pro Chip, Canada) and maintained on 12-h light/dark cycle with free access to food (18.0% protein, 4.5% fat, 5.5% fiber, 7.0% ash, 2.5% added minerals; Charles River, Canada) and water. During periods of testing, rats were brought to the testing room 1 day before testing and there, they were kept on natural daylight. Thirty-six male Wistar rats (175–200 g) were used to assess the development of the neuropathy and were dispatched in either SPL, sham or naive groups of 12 animals each. Eighty-four rats were used to test standard pharmacological compounds and 24 were used for Western blot analysis. During sensory testing, animals were placed in elevated Plexiglas boxes (21×17×14 cm) with a 0.7 cm diameter mesh floor, except for thermal hyperalgesia testing where the boxes had a dry glass floor. In testing boxes, rats were allowed to acclimatize for 15 min or until exploratory behavior ceased. On a given day, the sequence of behavioral testing for each animal was: cold allodynia (acetone), mechanical allodynia (von Frey hairs), mechanical hyperalgesia (pinprick) and thermal hyperalgesia (plantar test). The rationale was that the sequence of testing should start with the least stressful tests and finish with the most disturbing ones, in order to minimize the influence of one test on other ones. Therefore, mechanical and thermal hyperalgesia testing was performed last. Acetone, von Frey hairs and pinprick techniques are performed on a mesh floor, so the plantar test was performed last because it minimized handling, thus stress (one change of environment instead of two). The time to complete one battery of tests was approximately 45 min.

Surgery

Under gaseous anesthesia with isoflurane (1–2%) in oxygen, a small incision (6–7 mm) was made on the anterior surface of the right thigh at a distance of 1 cm medial to the anterior superior iliac spine, the skin incision being transversal to the saphenous nerve. At that point, the saphenous vein is easily identified through the skin of a shaved animal, running parallel and medial to the nerve. The nerve was gently exposed under microscope magnification (20×).

In the operated group, 30–50% of the nerve was trapped in a tight ligature made with an 8.0 nylon suture, whereas in the sham-operated group, the nerve was left intact. For the two groups, the wound was closed with two skin staples.

Sensory testing

Cold allodynia. Cold allodynia was assessed using the acetone drop application technique (Choi et al., 1994). Briefly, a drop of acetone was gently applied on the medial part of the plantar surface of the paw from the tip of a 1 ml syringe without touching the skin. The acetone quickly spreads over the plantar face and the medial hairy skin innervated by the saphenous nerve. A response was considered positive if the animal withdrew his paw following application. The acetone was applied five times with 1–2 min between each test and a percentage of positive responses was calculated.

Mechanical allodynia. Mechanical allodynia was tested with von Frey hairs (Senselab aesthesiometer; Somedic, Sweden). The medial plantar surface of the paw was stimulated with a series of von Frey filaments of ascending forces (with a range comprised between 0.63 and 235 mN). For each filament, the stimulus was repeated five times with an interval of 1–2 s between each stimulation. The threshold was determined as the lower force that evoked a withdrawal response to one of the five stimuli (Tal and Bennett, 1994).

Mechanical hyperalgesia. Mechanical hyperalgesia was evaluated using a safety pin: the medial plantar surface of the hind paw was briefly stimulated at an intensity sufficient to indent but not to penetrate the skin. The duration of paw withdrawal was recorded: a brief normal response was considered to be 0.5 s and the maximum time recording was arbitrarily stopped at 10 s. Each result is expressed as the mean of three measurements. Intervals between tests were of at least 3 min (Decosterd and Woolf, 2000).

Thermal hyperalgesia. Thermal hyperalgesia was assessed using an infrared noxious heat stimulus (Plantar test; Ugo Basile, Italy). The center of a focused beam of radiant heat was applied to the medial plantar surface of the hind paw and the withdrawal latency time recorded. Results of each test are expressed as the mean of three withdrawal latencies (s). Three minutes was allowed between each test (Hargreaves et al., 1988).

Development of the neuropathy

The time-course of expression of the neuropathy was assessed by testing animals 3 days prior and every 2 days after the surgery during 2 weeks, then every week until 1 month postsurgery. Each test was made on both the operated and the contralateral hind paws.

Effects of SPL on sciatic nerve territory

After having evaluated baseline sensory threshold of 20 rats on the lateral plantar face of the paw (sciatic nerve territory), 10 of them had a SPL and 10 remained sham rats. Two weeks after surgery, mechanical allodynia and hyperalgesia were assessed in each group.

Pharmacological interventions

Sensory thresholds were assessed preoperatively and 14 days postoperatively. The day after, standard drugs were tested to characterize the model. Animals were randomized into five groups and received either morphine (4.0 mg/kg i.p.) (Hofmann et al., 2003) in 0.9% saline solvent, a synthetic cannabinoid CB_1 and CB_2 agonist (WIN 55,212-2; 5.0 mg/kg i.p.) in 0.9% saline+40% dimethylsulfoxyde (DMSO) solvent (Bridges et al., 2001), gabapentin (50 mg/kg i.p.; Abdi et al., 1998; Hofmann et al., 2003; De Vry et al., 2004) and amitriptyline (10 mg/kg i.p.; Idänpään-Heikkilä and Guilbaud, 1999; Esser and Sawynok, 1999) both in 0.9% saline. The fifth group only received 0.9% saline+40% DMSO solvent as a control. Each drug was administered 45 min before testing in a volume of 2 ml/kg. The experimenter was blinded to the drug administered. In order to test if i.p. injection would change pain behavior, 12 of the SPL and six of the naive rats received an injection of 0.9% saline (2 ml/kg) and were used as control for the following experiments. Indeed, in order to evaluate if any of the drugs injected had an effect per se, 24 naive rats were divided into four groups of six animals and received either morphine, WIN 55,212-2, gabapentin or amitriptyline in the same solvents and at the same concentrations as SPL animals. Naive animals followed the same testing protocol as SPL rats with first baseline testing, then 2 weeks later, an assessment following no injection, vehicle or