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Research article

Interleukin-10 levels in rat models of nerve damage and neuropathic pain

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HIGHLIGHTS

- IL-10 plays an important role in the neuropathic pain following nerve injury.
- Chronic and partial nerve injury show decreased levels of IL-10 in DRG and sciatic nerve.
- Complete sciatic transection shows increased levels of IL-10.
- The role of IL-10 might be injury type specific.

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ABSTRACT

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that has been shown to play a role in inflammatory and autoimmune disorders as well as in neuropathic pain conditions. The objective of the present study was to assess the levels of IL-10 in rat's dorsal root ganglion (DRG) and the sciatic nerve following four different forms of sciatic nerve injury. The models used to induce the injury included two models of partial nerve injury: partial sciatic ligation (PSL) and chronic constriction injury (CCI), a model of complete sciatic transection (CST) and a model of perineural inflammation with minimal nerve damage (neuritis). Withdrawal responses for mechanical stimulus and withdrawal latency for thermal stimulation were used to measure mechanical and thermal hyperalgesia, respectively, and duration of the nociceptive withdrawal reflex to mechanical stimulus was used to measure mechanical hyperalgesia. The affected and contra-lateral nerves and the affected side DRG IL-10 levels were assessed by the means of enzyme-linked immunosorbent assay (ELISA), 3 and 8 days following the procedure and were compared to naïve rats' IL-10 levels. The rats exposed to CCI and neuritis developed significant mechanical and thermal hyperalgesia as well as mechanical hyperalgesia 3 and 8 days following the surgical procedure. Rats exposed to CST did not respond to mechanical stimulation and developed thermal hypoalgesia 3 and 8 days after the surgery. The DRG IL-10 levels were significantly reduced 3 and 8 days following CCI and PSL, significantly increased 3 and 8 days following CST, and remained unchanged following neuritis. The sciatic nerve IL-10 levels reduced significantly in both injured and contra-lateral nerves 3 and 8 days following CCI and PSL, elevated significantly in the injured but not in the contra-lateral nerve 3 and 8 days following CST and remained unchanged following neuritis. The results of this study suggest that IL-10's role in the neuropathic pain etiology may be specific to nerve injury type. Complete nerve transection increases while partial nerve injury reduces IL-10 levels in the involved nerve, and DRG. Perineural inflammation with minimal nerve damage has no effect on IL-10 levels.

1. Introduction

Neuropathic pain is defined as "Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" [1,2]. It is a complex, chronic pain state often associated with tissue damage, in which nerve fibers from both the peripheral

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and central nervous systems may be damaged, dysfunctional, or injured. It is estimated that 6.9–10% of the population suffer from pain with neuropathic pain characteristics [3]. The pain is usually of moderate to severe intensity, and can be continuous or episodic. Presently there is no effective cure for neuropathic pain, and the available treatments typically require a long-term pharmacotherapy which is often accompanied by significant side effects. Although neuropathic pain may be an idiopathic process reflecting abnormal sensory processing in the peripheral or central nervous system, it often appears following physical insult or disease affecting the peripheral or central nervous system. Occasionally, healed injury may leave the nervous system in a pathological state similar to that present in painful posttraumatic neuropathies.

Inflammation and immune response in the peripheral and central nervous systems have been shown to play an important role in the initiation and the maintenance of neuropathic pain [4]. It has been suggested that the imbalance between pro and anti-inflammatory cytokines might play a role in the development of chronic pain states [5].

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine with a broad spectrum of anti-inflammatory activity that has been shown to be involved in inflammatory and autoimmune disorders [6–8]. It is also known to play a role in neuropathic pain by decreasing the activity of nuclear factor kappa B (NFκB) and the synthesis of pro-inflammatory cytokines, including IL-1β and TNF-α [5]. IL-10 was also studied as a potential therapeutic agent for neuropathic pain [9]. The goal of the present study was to assess the levels of IL-10 in DRG, sciatic nerve and serum of rats exposed to four different forms of peripheral (sciatic) nerve injury and to relate the cytokine levels to the pain behaviors and the type of injury.

2. Materials and methods

Experiments were performed according to a protocol approved by the Rutgers Institutional Animal Care and Use Committee, Newark, NJ and in accordance with federal law, the regulations of the National Institute of Health, and the guidelines of the International Association for the Study of Pain [10] (Protocol number 10,077E1113). Adult male Sprague–Dawley rats weighing 250–300 grams at time of surgery were used.

All the rats were ordered from a single lab (Charles laboratory) and were housed in the animal facility, under veterinary supervision. During the entire period the rats were maintained on standard mouse chow, reverse osmotically treated water and also maintained on a 12-hour day and night cycle.

2.1. Behavioral assays

All behavioral testing were performed at the rat's mid-plantar hind paw. Tactile allodynia, heat and mechanical hyperalgesia were tested prior to surgery (baseline), 3 and 8 days following the surgery. All rats were habituated pre-operatively by allowing them 15–20 min in the sensory-testing apparatus for four consecutive days. During that time, the rats' hind paws were tested with the testing apparatus with von Frey filaments, heat stimuli and a blunt acupuncture needle. During the entire study the examiner was blind to the study groups.

2.2. Tactile allodynia

Tactile allodynia was tested with von Frey monofilaments as described previously [11] by using Semmes–Weinstein monofilaments (Stoelting Co., Wood Dale, IL, USA). Three different monofilaments applying a force of 8 g, 16 g, and 26 g were utilized. The rat was placed on a perforated floor and the monofilaments were gently applied 5 times to the mid-plantar area of the hind paw with one second inter-stimulus interval. The number of times the rat withdrew its paw was recorded and converted to a percentage response. A higher withdrawal percentage represented increased tactile allodynia. The data is expressed as percentage response.

2.3. Thermal hyperalgesia

The rats were placed on a glass floor of an elevated platform. A high intensity, movable radiant heat source, was placed underneath the glass and aimed at the plantar surface of one hind paw [12]. Care was taken to initiate the test when the animal is at rest, not walking and the hind paw is in contact with the glass floor of the testing apparatus. Stimulus onset activated a timer that was controlled by a photocell. The hind paw withdrawal reflex was interrupted by the photocell's light and automatically stopped the timer. Latencies of the reflex were measured from the onset of radiant heat until hind paw withdrawal to the nearest 0.1 s. Each hind paw was tested three times at intervals of 5 min. The light intensity was adjusted at the beginning of the experiment in order to produce latencies of approximately 20 s and held constant thereafter.

2.4. Mechanical hyperalgesia

Mechanical hyperalgesia was evaluated by measuring the duration of paw withdrawal from a blunted acupuncture needle in a pinprick test. The rat was placed on an elevated, perforated floor and the tip of a 0.2 mm diameter blunted acupuncture needle was pricked against the mid-plantar hind paw, until the needle slightly bent (the skin was dimpled but not penetrated). The duration of paw nursing subsequent to the pinprick-evoked nociceptive withdrawal reflex was timed for up to 15 s by a calibrated investigator. Normal responses of very short duration, too quick to time accurately were assigned a duration of 0.5 s. An increase in the withdrawal duration was interpreted as an increased pain level. Data is presented as withdrawal duration in seconds.

2.5. Surgeries

2.5.1. Anesthesia

For surgical procedures, rats were anesthetized with ketamine (50 mg/kg) and xylazine (7.5 mg/kg) solution administered intraperitoneally (i.p). Following verification of the anesthesia, the area of surgery was shaved and subsequently sterilized with betadine and alcohol wipe. The eyes were lubricated and the rat was placed on warming pad to maintain constancy of body temperature during surgery. A single calibrated investigator performed the surgeries.

2.6. Chronic constriction injury

CCI surgery ($n = 10$) was performed based on the original developed procedure [12]. In brief, the left common sciatic nerve was exposed at the mid-thigh level. The nerve was gently freed from surrounding tissue and ligated loosely with three chromic gut ligatures (4.0 Ethicon Chromic Catgut, Ethicon US, LLC) 1–1.5 mm apart. During surgery the ligatures were placed such that the nerve is barely constricted, and the circulation through the superficial epineurial vasculature was not arrested; slight movement of the ligature on the nerve was possible.

2.7. Partial sciatic ligation (PSL)

PSL ($n = 10$) surgery was performed, based on the original description [13]. The dorsum of the nerve was gently separated from the surrounding tissue near the trochanter, distal to the point at which the posterior biceps semitendinosus nerve branches off