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Original experimental

Stimulation-induced expression of immediate early gene proteins in the dorsal horn is increased in neuropathy

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HIGHLIGHTS

- We studied the immunoreactivity of Arc, c-Fos and Zif268 after nerve ligation.
- Expression of Arc, Zif and Fos was not elevated in neuropathic animals before stimulation.
- Stimulus-induced immunoreactivity was clearly increased in neuropathy.
- Contralateral dorsal horn showed unchanged immunoreactivity after neuropathic treatment.
- The studied IEGP's may have a role in sensitization in neuropathic conditions.

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ABSTRACT

Background and aims: Peripheral neuropathic pain is described as a pain state caused by an injury or dysfunction of the nervous system, and could have clinical manifestations such as hyperalgesia, allodynia and spontaneous pain. The development of neuropathic pain may depend on long-term forms of neuronal plasticity in the spinal cord (SC). Expression of the immediate early gene proteins (IEGPs) Arc, Zif268, and c-Fos are implicated in establishment of long-term potentiation (LTP) induced by conditioning stimulation (CS) of primary afferent fibres. However, the impact of the neuropathic state (Bennett's model) on CS-induced expression of IEGPs has not been studied. The aim of this study was to compare the levels of Arc, c-Fos and Zif268 immunoreactivity prior to and after conditioning stimulation in animals with developed neuropathic pain, with sham operated, non-ligated controls.

Methods: Twenty-four animals were divided equally into the neuropathic and non-neuropathic groups. Neuropathic pain was induced in all animals by conducting a loose ligation of the sciatic nerve with Chromic Catgut 4.0 sutures 7 days prior to conditioning stimulation or sham operation. The loose ligation was performed by placing sutures around the sciatic nerve compressing the nerve slightly just enough to reduce but not completely diminish the perineural circulation. A state of neuropathy was confirmed by a significant decrease in mechanical withdrawal threshold measured by von Frey's fibres. Immunohistochemical analysis was performed on transverse sections obtained from the L3–L5 segments of the SC at 2 and 6 h post-CS and IEGP positive cells were counted in lamina I and II of the dorsal horn. During statistical analyses, the groups were compared by means of analysis of variance (univariate general linear model). If significant differences were found, each set of animals was compared with the sham group with post hoc Tukey's multiple comparison test.

Results: Strikingly, all IEGPs exhibited a significant increase in immunoreactivity at both time points compared to time-matched, sham operated controls. Maximal IEGP expression was found 2 h after CS in neuropathic rats, and there was a smaller but still significant increase 6 h after CS. The unstimulated side of the dorsal horn in stimulated animals did not show any significant change of the number of IEGP positive cells and was approximately at the same level as sham operated animals. The number of IEGP positive cells in sham operated controls (non-neuropathic and non-stimulated animals) showed same immunoreactivity in 2 and 6 h post sham operation.

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Abbreviations: NS, nociceptive specific neurons; WDR, wide dynamic range neurons; DAB, 3,3′-diaminobenzidine.
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Conclusions and implications: The neurophysiological process of neuropathic pain development is complex and needs to be studied further in order to clarify its nature and components. This present study is meant to reveal a step towards further understanding the role of Arc, c-Fos and Zif268 in neuropathic pain. Moreover, this study might contribute to the knowledge base for further research on better therapeutic possibilities for neuropathic pain.

1. Introduction

Neuropathic pain has been defined as a pain state caused by an injury or dysfunction of the nervous system, and has clinical manifestations such as hyperalgesia, allodynia and spontaneous pain [1,2]. Many etiological factors can lead to neuropathy as nerve compression or damage leading to partial or total nerve ligation. The nerve can be damaged on different locations between the CNS and the peripheral nerve ending [3].

However, there has been limited therapeutic success with drugs such as NSAIDS, opiates [4], anticonvulsants and antidepressants [5] against neuropathic pain. One of the best known animal models of neuropathic pain development is the loose sciatic nerve ligation described by Bennett [6], in which the sciatic nerve gets partially injured by light constriction of the sciatic nerve with a loosely tied thread.

Long-term potentiation (LTP) as a response to conditioning stimulation (CS) of afferent fibres is a common model of synaptic plasticity in the brain and the spinal cord [7]. Many hippocampal studies are used as a source on mechanisms of LTP formation, function and maintenance [8]. LTP has been divided into two phases, early and late, where the late phase has been proven to require de novo protein synthesis [9]. Immediate-early gene proteins (IEGP) such as early growth response protein 1 (Egr-1) (also known as Zif 268 – zinc finger protein 225) and activity regulated cytoskeletal protein (Arc) have been found to be required for the process of late-phase LTP establishment in the hippocampus [9,10]. In the spinal cord, the most effective type of stimulation to induce LTP has been shown to be a series of high-frequency (e.g. 100 Hz) trains of electric stimulation [11]. Previous studies have shown that peripheral stimulation of afferent fibres leads to an increase of expression of c-Fos mRNA in spinal cord neurons and synaptic Arc protein [12,13].

Nociceptive information in the spinal cord is acquired, processed and transmitted mainly by nociceptive specific neurons (NS) and wide dynamic range neurons (WDR). The NS neurons are most abundant in Lamina I and II and respond to intense stimuli [14,15] while in deeper in laminae V and VI, WDR neurons are most abundant. Our research focused on Lamina I and II whose borders were previously defined in the research of Molander et al. [16]. Furthermore, it was shown that WDR neurons react in a graded manner to gentle touch, stronger mechanical and noxious stimulations [17].

Previous studies have shown elevated c-Fos, Zif268 and Arc immunoreactivity in neuropathic rats where neuropathy was induced by means of various methods such as the Bennett protocol [18] and Kim and Chung protocol [19]. However, it is not known whether CS-induced expression of IEGPs in the spinal cord dorsal horn differs between neuropathic and non-neuropathic states. Here, we compared CS-induced of IEGPs in the Bennett model of neuropathic pain relative to shame operated, non-ligated controls. The present study contributes to the clarification of the highly complex process of neuropathic pain formation. The protocol of sciatic nerve ligation represents a model of constriction nerve injury, such as a nerve injury present during spinal nerve root constriction caused by disc prolapse, or nerve constriction in the carpal tunnel syndrome.

2. Materials and methods

2.1. Animals and surgery

Female Sprague-Dawley rats, 2–3 months of age, weighing 240–300 g were used (NTac:SD, Taconic Europe, Ejby, Denmark). The animals had free access to food and water and were held on a 12/12-h light/dark cycle.

Twenty-four animals were divided in two groups of twelve animals, the neuropathic and non-neuropathic groups. All animals underwent a surgical intervention where the non-neuropathic animals were sham operated while the sciatic nerve of the neuropathic rats was ligated. All animals were operated under brief Isoflurane anaesthesia. Neuropathic pain was induced by means of surgical procedures previously described [6]. During this intervention, the common sciatic nerve was revealed by a blunt dissection through the biceps femoris muscle. Around 10 mm of the nerve was freed of adhering tissue and 4 ligatures with 4/0 chromic catgut (Chromic Catgut 1/2 circle, 4.0 round bodied; from KRUUSE Norge) were tied loosely around it with 1– 2 mm spacing between them and constricted to a degree previously described [6] to reduce the diameter of the nerve by a just noticeable amount [5]. The sutures were placed on the caudal part of the sciatic nerve, leaving the upper, cranial part free from sutures. The cranial part will further on be the location for stimulation electrode placement.

To check the level of allodynia as a clinical indication of neuropathic pain, the mechanical withdrawal thresholds were tested first at baseline before surgery and afterwards from the 4th post-surgery day with von Frey's filaments of varying thickness. Before testing, the animal was placed in an elevated Plexiglas cage with a wire mesh floor and allowed to adapt for 10 min. The mid-plantar surface of the rats' hind paws was stimulated with von Frey's filaments through the wire mesh floor until the filament bent slightly. During allodynia testing we used 14 von Frey filaments, numbers 1–20 (Somedic Sverige) with a calibrated stiffness corresponding to 0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2, 4, 6, 8, 10, 15, 26, 60, 100, 180 and 200 g. The filaments are presumed to roughly represent a logarithmic scale of applied force and a linear scale of perceived force (information provided by the manufacturer: North Coast Medical Inc.). As previously described [20] the filaments were applied to each hind paw in an order of increasing stiffness and the withdrawal threshold of each individual hind paw was defined as the force (in grams) of the filament that induced three of five positive responses (brisk withdrawal). The non-lesioned side served as a control, displaying no effect of the contralateral loose nerve ligation [20].

The baseline response thresholds before surgical intervention in the non-affected side were >60–70 g pressure [20]. Allodynia was defined as severe if the response was positive to the filaments with a stiffness corresponding to 0.16–1.0 (g) (four filaments 0.16; 0.40, 0.60 and 1.0 g). Allodynia was defined as moderate if the animals responded to filaments with a stiffness corresponding to 1.4–6.0 (g) (four filaments 1.4, 2.0, 4.0 and 6.0). Mild allodynia was positive if the animals responded to the filaments with a stiffness corresponding to 8.0–26 (g) (four filaments 8.0, 10, 15 and 26).

Both the neuropathic and non-neuropathic group was divided in 2 sub-groups, the two and six-hour groups with six animals