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Morphine sensitivity of spinal neurons in the chronic constriction injury neuropathic rat pain model

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HIGHLIGHTS

- Morphine reduces spinal neuronal activity in the CCI rat pain model.
- Morphine sensitivity of WDR neurons is similar across pain models.
- Neuropathic pathology rather than the animal model determines morphine effectiveness.

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ABSTRACT

Opioid analgesia involves suppression of neuronal activity in central sensory pathways. We show that the classic opioid morphine reduces spinal neuronal spontaneous and evoked activity after induction of neuropathy by chronic constriction injury of the sciatic nerve in rats. The minimal effective dose of morphine was 0.3 mg/kg for most response parameters tested. Morphine sensitivity of spinal cord neurons is similar across neuropathic pain models. We therefore conclude that nerve damage per se rather than the experimental model determines the effectiveness of opioids in general and investigate several pain measurement endpoints which might be important to clinically determine morphine's efficacy in neuropathic pain.

1. Introduction

Opioids in the treatment of neuropathic pain, a pain arising as a direct consequence of a lesion or disease affecting the somatosensory system [1], were recently recommended as second-line treatments that can be considered for first-line use in certain clinical circumstances [2]. Combination pharmacotherapy including opioids is considered to be beneficial for the treatment of neuropathy in adults [3,4]. Besides adverse effects, such as tolerance in chronic use [5] or opioid-induced hyperalgesia [6,7] opioids are still supported for the treatment of moderate to severe chronic pain [8], particularly in cancer pain [9] or older patients [10]. However, many studies assessing their effectiveness revealed only small benefits after spinal cord injury [11]. To improve patient outcomes, a thorough understanding of pain mechanisms is requested [12]. We therefore studied morphine effectiveness at the electrophysiological level in an animal model of neuropathic pain.

Animal models were developed to study the pathophysiological mechanisms underlying neuropathic pain symptoms. Three models of neuropathic pain in the rat are the most commonly used: the chronic constriction injury (CCI), the partial sciatic ligation (PNL), and the spinal nerve ligation (SNL) model [13]. Their predictive power for humans has not differentially been studied. They share an induced neuropathy by surgical damage of the sciatic nerve. However, the site of nerve injury is different as are some behavioural consequences [14]. Morphine analgesia was reported with effective doses of 1–3 mg/kg for the CCI and SNL model [15,16]. Morphine suppresses mechanical and cold hypersensitivity in CCI and SNL [17]. The underlying basis of neuropathic pain may therefore be similar irrespective of the site of nerve injury. 6 mg/kg morphine reduced deficits in place escape [18], cold allodynia [19], mechanical allodynia and weight bearing [20] in CCI. Electrophysiological data on spinal neuronal morphine sensitivity exist for SNL animals [21–23]. Here, we examine wide dynamic range (WDR) neuronal morphine sensitivity in the CCI model. According to the behavioural

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studies, the CCI model shall resemble the SNL model in morphine effectiveness for most response parameters. Reflecting the clinical situation, co-administration of morphine with a second analgesic drug was recently shown to enhance antinociception in CCI in a behavioural assay [24].

2. Materials and methods

2.1. Animals

Male Wistar rats (300–400 g body weight, Charles River Laboratories) were housed in group cages under a 12 h alternating light/dark cycle with food and water ad libitum in the institutional animal facilities. All housing conditions and experimental procedures were approved by the German federal government and supervised by the institution's department of animal welfare. Experiments followed the guidelines of the German Animal Welfare Act 2006. All efforts were made to minimize animal suffering and to reduce the number of animals used.

To induce neuropathy, animals were subjected to surgery for chronic constriction injury (CCI) with four loosely constrictive ligatures of chrome catgut placed around the left sciatic nerve [25] or a sham surgery. Surgeries and electrophysiological experiments were done under anaesthesia, controlled by the animal anaesthesiologist of the institution.

All animals were behaviourally tested on paw withdrawals to mechanical and thermal stimuli. Animals subjected to electrophysiology had shown characteristic pain behaviour with paw withdrawal thresholds of <30 g EvF for CCI and <30 g EvF for sham. Likewise paw withdrawal thresholds were <42 °C for CCI and >42 °C for sham animals. 20 CCI and 30 sham animals were included in data analysis. Only one neuron was recorded in each animal to avoid possible interactions with previous morphine injections. The experimental design of this study was aligned with the methods previously used to study morphine efficacy in SNL animals [22,23] to allow for a comparison.

2.2. Spinal cord electrophysiology

Electrophysiological studies were conducted three weeks post CCI surgery. Animals were anesthetized with pentobarbital: 90 mg/kg intraperitoneal initially, then kept on a 30 mg/kg/h intravenious infusion rate during the whole experiment. One of each arteria carotis, vena jugularis and vena femoralis were cannulated for blood pressure control (120 ± 10 mmHg systolic blood pressure), anaesthesia and drug application respectively. A tracheal tube was used for artificial respiration (UNO Micro Ventilator, UNO Roestvaststaal BV, Zevenaar,NL). The body temperature of the rat, measured rectally, was maintained at 37 °C using a heated plate and infrared light. By laminectomy the L4-6 segmental region of the spinal cord receiving afferent input from the hind paw was exposed. Extracellular recordings of deep wide dynamic range (WDR) neurons (300–600 μm) with defined receptive fields in the hind paw were performed using a tungsten electrode (6MOhm, FHC, Bowdoinham, ME, USA), a differential amplifier (DAM80, WPI, Sarasota, FL, USA) and a data capturing interface with software (Notocord-hem 3.5, Notocord Systems, Croissy-sur-Seine, France). All neurons responded to light touch and pinch, to cold (4 °C) and dynamic (brush) stimuli. Spontaneous activity (frequency of action potentials) was quantified over a period of 40 s before stimulation. Action potential shape and size was the criterion to make sure that only one neuron was recorded.

2.3. Mechanical and thermal stimulations

Electronic von Frey (EvF) filaments (Somedic, Hoerby, Sweden) of 1.0 mm tip diameter were applied to the plantar surface of the hind paw with a stimulus interval of 10 min and resulting neuronal activity was recorded at three stimulation strengths: innocuous (10 g EvF, 5 s), intermediate (30 g EvF, 5 s) and noxious (100 g EvF, 5 s) stimuli. The stimulation strength of 30 g EvF of the intermediate stimulus was noxious for CCI animals but innocuous for sham controls in the behavioural testing. Frequencies of action potentials were measured on plateau phases of stimuli. During onset and at the abrupt end of a mechanical stimulus ON- and OFF-responses were detected and excluded from analysis by counting action potentials only during the five second plateau phase of each stimulus where the stimulus strength was held constant. 76 ± 12 Hz and 80 ± 13 Hz were mean frequencies ± SEM of WDR neurons responses to noxious mechanical stimulation in CCI and sham respectively. Water of 4, 37, 42 and 47 °C was poured over the hind paw with a stimulus interval of 10 min and resulting neuronal activity was recorded over 5 s. Similarly to the neuronal response characteristics to mechanical stimuli, also the onset and end of the constant water flow caused non-constant firing and was excluded from data analysis by counting action potentials only during the five second constant thermal stimulus with water poured at a constant flow rate. Dynamic stimuli were applied by five brush strokes within a test stimulus with an interval of 10 min. Action potential frequencies were averaged over five strokes. All mechanical and thermal stimuli were applied consecutively to each neuron, and this group of stimuli was repeated every 10 min.

Control responses before drug application were recorded at least three times and, if variation was less than 10%, the mean of the three responses recorded before drug application was normalized to 100%. Drug effects were calculated as % of control response. The first three responses at 5, 15 and 25 min after drug application were averaged because peak drug effect occurred within the first half hour after drug administration. If no baseline stability could be obtained for a recording, no data were collected from this particular neuron. Baseline activity of recorded neurons was 11.9 Hz ± 2.9 Hz for CCI animals and 6.4 Hz ± 2.3 Hz for sham animals (n.s., $p > 0.05$). Silent neurons were not included in this study. This is congruent with the literature on morphine efficacy in the SNL neuropathic pain model. These studies are based on similar selection criteria of recorded neurons and report similar spontaneous frequencies of WDR neurons in SNL animals [23].

2.4. Drug application

Morphine hydrochloride TriHydrate (Caesar & Lorentz, Hilden) was dissolved in 0.9% NaCl and applied intraveniously (i.v.) by bolus injection. Doses were 0.1, 0.3 and 1.0 mg/kg. Drug effects were followed over one hour until recovery of firing frequencies. Neuronal response parameters were determined every 10 min.

2.5. Statistics

For statistical analysis Prism5 (GraphPad Software, La Jolla, CA, USA) was used with its Mann–Whitney and Student's *t*-tests. Significance levels were taken to be $^{*}p < 0.05$ and $^{**}p < 0.01$.

3. Results

3.1. Spinal neurons of CCI animals are sensitive to 0.3 mg/kg morphine

WDR neuronal response parameters were recorded before and after bolus intravenious morphine application. Upon