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THE ROSTROVENTROMEDIAL MEDULLA IS ENGAGED IN THE EFFECTS OF SPINAL CORD STIMULATION IN A RODENT MODEL OF NEUROPATHIC PAIN

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Abstract—The neurobiological mechanisms underlying the suppression of neuropathic pain by spinal cord stimulation (SCS) are still incompletely known. The present study aims at exploring whether the descending pain control system in the rostroventromedial medulla (RVM) exerts a role in the attenuation of neuropathic pain by SCS. Experiments were performed in the rat spared nerve injury (SNI) pain model. The effects of SCS on neuronal activity of pronociceptive ON-like, antinociceptive OFF-like, and neutral cells, including 5-HT-like cells, in the RVM were analyzed in SCS responding and SCS non-responding SNI animals as well as in naïve controls. Decreased spontaneous activities in OFF-like cells and increased spontaneous activities in ON-like cells were observed in SNI animals, whereas the spontaneous activities of 5-HT-like and neutral cells were unchanged. SCS produced a prominent increase in the discharge of OFF- and 5-HT-like cells in SCS responding, but not in non-responding SNI animals or controls. Discharge rates of ON-like and neutral cell were not affected by SCS. In awake SNI animals, microinjection of a $GABA_A$ receptor agonist, muscimol, into the RVM significantly attenuated the antihypersensitivity effect induced by SCS while a non-selective opioid receptor antagonist, naltrexone, was ineffective. It is concluded that SCS may shift the reciprocal inhibitory and facilitatory pain modulation balance controlled by the RVM in favor of inhibition. This increase in the descending antinociceptive effect operates in concert with segmental spinal mechanisms in producing pain relief.

Key words: neuropathic pain, spinal cord stimulation, rostroventromedial medulla, 5-HT.

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Abbreviations: ANOVA, analysis of variance; AUC, area under curve; DLF, dorsolateral funiculus; MT, motor threshold; RVM, rostroventromedial medulla; SCS, spinal cord stimulation; SD, standard deviation; SNI, spared nerve injury; WT, withdrawal thresholds.

INTRODUCTION

Spinal cord stimulation (SCS) has developed into an indispensable treatment modality in the management of certain forms of neuropathic pain. Until recently, experimental SCS research has focused on spinal segmental mechanisms (Yakhnitsa et al., 1999; Guan et al., 2010; Smits et al., 2012) in spite of the fact that supraspinal mechanisms of SCS were postulated already in the mid 1980s (Saade et al., 1986). In intact animals, SCS was found to produce an augmented spinal release of serotonin (Linderoth et al., 1992) and a decreased release of GABA in the periaqueductal gray (Stiller et al., 1995). Moreover, SCS applied rostrally to chronic dorsal column lesions may still have an effect on neuropathic manifestations (El-Khoury et al., 2002; Barchini et al., 2012). A further support to the involvement of supraspinal regions is the SCS-induced increase of c-fos expression in the rostral ventromedial medulla (RVM) (Maeda et al., 2009).

In patients, SCS evokes paresthesiae suggesting that the pain-relieving effect is associated also with orthodromic activity in the dorsal columns. In line with this, human brain imaging studies show SCS-induced activation of the medial primary sensorimotor cortex, the ipsilateral secondary somatosensory cortex and the contralateral insula (Stancak et al., 2008). The involvement of serotonergic mechanisms in the SCS effects is substantiated by increased spinal serotonin content and enhanced staining of serotonin-positive terminals in the superficial dorsal horn following SCS (Linderoth et al., 1992; Song et al., 2009). At the spinal level the pain-relieving effect appears to be predominantly related to the activation of $5\text{-HT}_{2,3,4}$ receptors (Song et al., 2011b). In line with this, intrathecal administration of some antidepressants enhanced the SCS effect (Song et al., 2011a). Since there are very few serotonergic neuronal cell bodies in the spinal cord, these findings indicate the engagement of brain stem centers, notably the RVM, the main source of descending serotoninergic innervation (Newton and Hamill, 1988; Pertovaara, 2000; Suzuki et al., 2004; Vera-Portocarrero et al., 2006). The RVM contains several well characterized cell types which project to the dorsal horn and are directly related to the modulation of pain. The so-called ON cells facilitate and OFF cells inhibit the transmission of nociceptive signals at a spinal segmental level. Other cell types have been referred to as 5-HT-like cells and neutral cells

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depending on their firing pattern and responsiveness to external stimuli (Fields, 2004).

Here we explored the role of the RVM focusing on the descending control in the pain-relieving effect induced by SCS in a rat model of neuropathic pain. Therefore, we assessed discharge rates in different types of RVM cells following SCS both in SCS responding as well as in SCS non-responding neuropathic animals and in sham controls. Additionally, the SCS-induced antihypersensitivity effect was assessed behaviorally following microinjections of a $GABA_A$ receptor agonist or an opioid receptor antagonist into the RVM.

EXPERIMENTAL PROCEDURES

Animals and anesthesia

The experiments were performed on male Wistar rats (Harlan, Horst, The Netherlands), weighing 250–350 g, in accordance with protocols approved by the Local Swedish Animal Welfare Agency (N 385/10).

The surgical procedures were performed under general anesthesia delivered through an open mask system. Anesthesia was induced by 4% Isoflurane (Forene® – Abbott, Solna, Sweden) and maintained with 1–2% in a 1:1 mixture of air and oxygen at a flow-rate of 2 L/min. During surgery (for creation of nerve lesions, implantation of SCS electrodes and brain stem cannulas), the body temperature was maintained at 37 ± 0.5 °C by an automatic heating pad (CMA/150, CMA Microdialysis AB, Stockholm, Sweden). Postoperative analgesia was provided by s.c. injection of 5 mg/kg carprofen (Rimadyl®, Pfizer, New York, NY, USA). For the electrophysiological recordings, the anesthesia was induced by pentobarbitone at a dose of 50 mg/kg i.p. Anesthesia was maintained by infusing pentobarbitone (15–20 mg/kg/h). The level of anesthesia was frequently monitored by assessing the size of the pupils, general muscle tone and by assessing withdrawal responses to noxious stimulation. Supplemental doses of sodium pentobarbital were given as required. The rats were spontaneously breathing and the body temperature was kept within physiological range with a warming blanket. At the completion of the recordings, the rats were given a lethal dose of sodium pentobarbital and the brains were removed for histological verification of the recording sites in the medulla.

Spared nerve injury

A spared nerve injury (SNI) rat model was created as described in detail earlier (Decosterd and Woolf, 2000). In short, the skin of the lateral surface of the thigh was incised and the biceps femoris muscle was divided, exposing the sciatic nerve and its three terminal branches. Following ligation and removal of 2–4 mm of the distal nerve stumps of the tibial and common peroneal nerves, muscle and skin were closed in two layers. In sham-operated animals, the surgical procedure was identical, except for that the tibial and common peroneal nerves were left intact.

Assessment of pain related behavior

The behavioral studies were carried out under standardized conditions in a separate quiet room 2 weeks after the induction of nerve injury. For the tactile sensitivity tests, the rat was placed in a circular observation plexiglass cage equipped with a metal mesh floor and allowed to acclimatize to the environment for at least 15 min before the beginning of the experiments.

Tactile sensitivity was assessed using regularly calibrated von Frey filaments (MARSTOCKnervtest, Schriesheim, Germany) with stiffness corresponding to 0.5, 0.8, 1.5, 2.6, 4.0, 4.5, 5.5, 7, 8.5, 10, 12.5, 15, 18.5, 20, 22, 26 and 30-g bending force in order to quantify the sensitivity. The filaments were applied to the mid-plantar surface of the hind paw until the filament gently bent. A brisk withdrawal of the hind limb was considered a positive response. As a control, the withdrawal thresholds (WT) were also determined for the same part of contralateral, intact paw. In order to avoid nociceptive sensitization the test was always started with the softest filament and continued in ascending order of stiffness. The softest filament that produced a brisk withdrawal to at least three out of five applications determined the WT. In this study, only rats that had developed tactile hypersensitivity, similar to tactile ‘‘allodynia’’ observed in patients and defined as a response to a filament corresponding to 7 g or less, were included in the subsequent experiments. The filament corresponding to 30 g was selected as the cut-off level.

Implantation of a spinal cord stimulation system

Rats that developed tactile hypersensitivity, as defined above, were under general anesthesia subjected to implantation of an electrode system for SCS. The cathode, a solid rectangular platinum–iridium plate, 3 × 1.5 mm, thickness 0.25 mm, was placed in the dorsal epidural space via a small laminectomy at the T11 level. The anode, a platinum–iridium disc Ø = 6 mm, was implanted in the subcutaneous tissue on the left chest wall. This technique has been described in detail earlier (Meyerson et al., 1995). The two poles were connected via insulated stainless-steel wires (Medtronic Inc., Minneapolis, MN, USA) tunneled subcutaneously to microcontacts fixed to the neck skin.

After the electrode implantation the rats were allowed to recover for at least 48 h before starting further experiments. They were kept in separate cages in order to avoid damage to the microcontacts caused by other rats. Rats with signs of neurological sequelae after the surgery were excluded from the subsequent experiments. SCS lead migration or malfunction was carefully controlled by daily inspections, double-checks of motor threshold and by post mortem examination. Data from animals in which the SCS lead had migrated or was malfunctioning were excluded from the study.