available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Spinal ventral root after-discharges as a pain index: Involvement of NK-1 and NMDA receptors

*Shohei Yamamoto, Motoko Honda, Mitsuo Tanabe, Hideki Ono**

Laboratory of CNS Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

ARTICLE INFO

Article history:
Accepted 25 January 2006
Available online 28 February 2006

Keywords:
After-discharge
Ezlopitant
Ketamine
Resiniferatoxin
Spinal cord
Ventral root

ABSTRACT

Nociceptive signals are transmitted to the spinal dorsal horn via primary afferent fibers, and the signals induce withdrawal reflexes by activating spinal motoneurons in the ventral horn. Therefore, nociceptive stimuli increase motoneuronal firing and ventral root discharges. This study was aimed to develop a method for the study of pain mechanisms and analgesics by recording ventral root discharges. Spinalized rats were laminectomized in the lumbo-sacral region. The fifth lumbar ventral root was sectioned and placed on a pair of wire electrodes. Multi unit efferent discharges from the ventral root were increased by mechanical stimulation using a von Frey hair applied to the plantar surface of the hindpaw. The low-intensity mechanical stimuli increased the discharges during stimulation (during-discharges) without increasing the discharges after cessation of stimulation (after-discharges), and the high-intensity mechanical stimuli increased both during- and after-discharges. Pretreatment with resiniferatoxin, an ultrapotent analogue of capsaicin, halved during-discharges and eliminated after-discharges, suggesting that after-discharges are generated by heat- and mechanosensitive polymodal nociceptors. Ezlopitant, a neurokinin-1 (NK-1) receptor antagonist, but not its inactive enantiomer, selectively reduced the after-discharges. Ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, preferentially reduced the after-discharges, demonstrating that NK-1 and NMDA receptors mediate the after-discharges. Morphine reduced the after-discharges without affecting during-discharges. By contrast, mephenesin, a centrally acting muscle relaxant, reduced both during- and after-discharges. There results suggest that simultaneous recordings of during- and after-discharges are useful to study pain mechanisms and analgesics as well as to discriminate the analgesic effects from the side effects such as muscle relaxant effects.

1. Introduction

In view of established importance of the spinal cord in the study of pain mechanisms and analgesics (Dickenson, 1995; Morris et al., 2004), several in vivo and in vitro electrophysiological methods have been developed and used to evaluate spinal cord function in nociceptive transmission. For example, intra- or extracellular recordings from dorsal horn neurons are conducted using anesthetized animals and slice preparations. However, long-term recordings with in vivo preparations require a sophisticated technique (Gjerstad et al., 2001; You and Arendt-Nielsen, 2005; You et al., 2003, 2004), and intra-

* *Corresponding author*. Fax: +81 52 836 3676.
E-mail address: hiono@phar.nagoya-cu.ac.jp (H. Ono).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.01.097

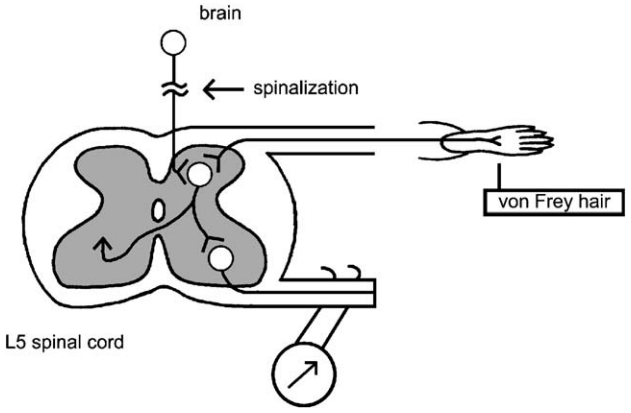

Fig. 1 – Schematic representation of measurement of ventral root discharges and neuronal organization of the lumbar spinal cord.

spinal and peripheral connections are often disrupted in in vitro preparations. Although some studies have recorded the nociceptive reflex responses from the spinal cord retaining connections to the tail (Otsuka and Yanagisawa, 1988) or hindlimb (King et al., 1990) of neonatal or young animals, there are several differences in neuronal connections (Nakatsuka et al., 2000) and the expression of neurotransmitter receptors (Rahman et al., 1998; Urch et al., 2001) between neonatal and adult animals. Since behavioral studies are performed in adult animals, it is important to use adult animals for electrophysiological studies of spinal nociceptive mechanisms as studied by You and Arendt-Nielsen (2005) and You et al. (2003, 2004).

At the spinal level, nociceptive signals are transmitted from the dorsal horn to the motoneurons in the ventral horn, and withdrawal reflexes are induced via motoneurons to avoid noxious stimuli (Clarke and Harris, 2004). Therefore, nociceptive responses can be evaluated by measuring ventral root activities. In our laboratory, spinal reflex potentials have been measured from anesthetized adult rats (Ono and Fukuda, 1995). With this approach, the dorsal root is stimulated electrically and then mono- and polysynaptic reflex potentials are recorded from the ipsilateral ventral root. In the present study, nociceptive mechanical stimulation was applied to a hindpaw instead of applying electrical stimulation to the dorsal root, and the multi unit discharges of the whole ventral root were recorded (Fig. 1). The effects of the neurokinin-1 (NK-1) receptor antagonist ezlopitant (Tsuchiya et al., 2005), the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine (Kronenberg, 2002) and morphine were determined to confirm that this recording of ventral root discharges is suitable for studies of pain mechanisms and analgesics.

2. Results

2.1. *Ventral root during- and after-discharges evoked by mechanical stimuli*

As the responses to a high-intensity mechanical stimulus for 3 s consisted of high-frequency initial firings for about 3 s and a long-lasting firing (about 60 s) (Fig. 2A(b)), discharges were counted for 3 s and 60 s, respectively. Any firings for 3 s or 60 s before mechanical stimulation were counted as spontaneous firings. In this study, the firing ("initial 3 s firings" minus "spontaneous 3 s firings") during mechanical stimulation was termed "during-discharges", and the firing ("subsequent 60 s firings" minus "spontaneous 60 s firings") after mechanical stimulation was termed "after-discharges".

The relationship between stimulus intensity and evoked discharges was examined. The most sensitive point of the plantar surface was marked and stimulated. Nine von Frey hairs (2.0, 3.6, 5.5, 8.5, 11.7, 15.1, 28.8, 75.9 and 125.9 g) were applied to the plantar surface of the hindpaw in randomized order, and evoked firings were recorded from the ipsilateral ventral root. The measurements were performed twice in each animal, and the firing produced by each mechanical stimulus was averaged and expressed as the frequency (Hz). Discharges increased according to the strength of the mechanical stimuli. Low-intensity mechanical stimuli evoked during-discharges (Fig. 2A(a), 11.7 g). High-intensity mechanical stimuli provoked discharges not only during stimulation (during-discharges) but also after stimulation (after-discharges) (Fig. 2A(b), 75.9 g). These intensities (11.7 and 75.9 g) were innocuous and noxious stimuli in conscious rats, respectively, and used for the similar experiments in anesthetized rats (Condes-Lara et al., 2005; Cuellar et al., 2004; Zhuo and Gebhart, 2002). The stimulus–discharge relationships of during- and after-discharges are shown in Fig. 2B. Additionally, high-intensity stimulation to the peroneal nerve or plantar surface (30 V, 2 ms duration, 10 Hz, 3 s) but not low-intensity stimulation (3 V) evoked after-discharges in the ventral root (not shown).

2.2. *Effects of resiniferatoxin pretreatment on during- and after-discharges*

Resiniferatoxin (RTX) is an ultrapotent analog of capsaicin (Szallasi and Blumberg, 1989), and a single injection of RTX desensitizes capsaicin-sensitive primary afferent fibers (Szallasi and Blumberg, 1992; Xu et al., 1997). RTX was administered at 200 μg/kg subcutaneously (s.c.) 1 week before testing. The desensitization of capsaicin-sensitive fibers was confirmed by lack of eye-scratching evoked by instillation of 1% capsaicin solution into the right eye, and an increase of paw withdrawal latency from 12.8 ± 1.0 s (n = 5) to 22.7 ± 1.7 s 1 week after RTX treatment in the plantar test in accordance with Pan et al. (2003).

The relationship between stimulus intensity and evoked discharges was compared in RTX-pretreated rats (Fig. 3). Although during-discharges increased in a stimulus intensity-dependent manner in both RTX-pretreated and control rats, RTX significantly reduced during-discharges upon application of the high-intensity mechanical stimuli (Fig. 3C left, 75.9 g: vehicle-pretreated 391.1 ± 40.3 Hz; RTX-pretreated 156.8 ± 40.0 Hz). On the other hand, the development of after-discharges was inhibited significantly in RTX-pretreated rats (Fig. 3C right); at 75.9 g, after-discharges after pretreatment with vehicle were 76.7 ± 15.3 Hz and those after pretreatment with RTX were 3.5 ± 1.4 Hz. Raw traces upon application of the low-intensity (11.7 g) and high-intensity mechanical stimuli (75.9 g) recorded in a RTX-pretreated rat are shown in Fig. 3B. The after-discharge evoked by the high-intensity mechanical