Research report

Temporal and spatial dynamics of peripheral afferent-evoked activity in the dorsal horn recorded in rat spinal cord slices

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ABSTRACT

In the present study, multi-electrode array recording was used to examine dorsal horn activity following stimulation of primary afferents in a rat dorsal root attached-spinal cord slice preparation. The multi-electrode array probe was placed under the dorsal horn slice and local field potentials evoked by stimulation on the dorsal root were analyzed. Three kinds of dorsal root-evoked responses were identified. In lamina II_o, local field potentials exhibited P1 (peak latency 1.46 ± 0.08 ms), N1 (2.77 ± 0.18 ms, n = 12), N2 (7.31 ± 0.48 ms), N3 (12.12 ± 0.73 ms) and P2(18.30 ± 0.80 ms) waves. In lamina II_i local field potentials exhibited P (1.99 ± 0.10 ms), N1 (3.35 ± 0.17 ms) and N2 (8.58 ± 0.44 ms) waves. In laminae III–VI, local field potentials exhibited P1 (3.01 ± 0.07 ms), P2 (7.02 ± 0.21 ms) and N waves (22.57 ± 0.79 ms). Sweep spread was calculated by two dimensional current source density (2D-CSD) analysis. Both α-amino-3-hydroxy-5-methylisoxazole-4-propionic a/kainate and N-methyl-D-aspartate-type glutamate receptors participated in this neuronal circuitry. Morphine diminished local field potentials. Gabapentin diminished the negative components in lamina II and P2 component in lamina II_o, but increased the positive components in lamina II_i and laminae III–VI. The present study revealed that functional dorsal horn activity was preserved in the spinal cord slice preparation. Glutamatergic synapses were crucially involved in information processing. Opioid interneurons and gabapentin may play a modulatory role in regulating signal flows in the dorsal horn. Taken together, these results identify a spatio-temporal profile of dorsal horn activity evoked by dorsal root stimulation, and implicate glutamatergic and opioidergic receptors and gabapentin in this activity.

1. Introduction

The dorsal horn of spinal cord is the major receiving and transmitting zone for nociceptive messages (Bourane et al., 2015; Braz et al., 2014). Dorsal horn neurons are divided into projection cells, which make up a small proportion of spinal cord neurons, and interneurons, which form the majority of the spinal cord neuronal population (Chung et al., 1984; Ganley et al., 2015). Interneurons transmit regulatory information to projection neurons for relay to several pain-related brain areas. Neuronal circuits formed by propriospinal neurons involved in nociceptive processing include interneuronal components, meaning that this type of neuronal circuit has a greater effect than merely acting as a relay station. Recent studies showed that the neuronal circuit of segmental dorsal horn played a key role in spinal cord stimulation (SCS)

and pain relief (Smits et al., 2012; Terashima et al., 2011). However the nociceptive circuits in the dorsal horn linking incoming primary afferents to projection neurons in pain related sensory processing are still uncharacterized. Elucidating the functional circuitry of the spinal dorsal horn that processes nociceptive messages activated by synaptic inputs from peripheral primary afferent fibers is essential.

Local field potentials (LFPs) of dorsal horn neurons are induced by dorsal root stimulation (Lidierth, 2006). Dorsal root stimuli by a single electrode evokes glutamate-mediated excitatory postsynaptic potentials (EPSPs) in these neurons (Schouenborg, 1984). But these methods could not record neuronal electric activity across the whole dorsal horn at once. It is important to answer the basic question as to how neuronal signals are related to nociception flow in dorsal horn circuits after primary afferent input, and how these signals are − regulated.

The goal of this study was to investigate the spatial–temporal processes of nociception-related neuronal circuits activated by dorsal root inputs in rats. Recordings that cover activity across the dorsal horn are therefore necessary to identify this spatially distributed dynamic profile. However, in view of the previously extensive literature about dorsal horn neuronal circuits (Zhang et al., 2015; Zhang et al., 2006), the mechanisms of sensory information processing in the dorsal horn are still poorly understood. This is not only because the great diversity of dorsal horn neurons has made it difficult to develop a comprehensive classification scheme for either the interneurons or the projection neurons, but also because neuroimaging results provide a view of only macroscopic network changes rather than cellular origins and neuronal circuit configuration. Our laboratory has previously described the spatial-temporal characteristics of pain related neuronal circuits in the hippocampus, primary somatosensory cortex (S1 area) and anterior cingulate cortex (ACC) using the multi-electrode array technique (Zhao et al., 2009; Wang et al., 2010; Lyu et al., 2013). In the present study, LFPs in dorsal root-attached spinal cord slices were investigated with MEA to illustrate the spatial-temporal characteristics in the nociceptive neuronal circuits of dorsal horn.

In addition, we investigated the role of morphine and gabapentin, two drugs widely used for pain relief, on LFPs evoked by dorsal root stimulation. Morphine is widely used for pain relief in the clinic, and it activates opioid receptors (Turnaturi et al., 2016; Convertino et al., 2015). Early experiments showed three subtypes of opioid receptors concentrated predominantly in the superficial laminae (laminae I-II) of the dorsal horn (Turnaturi et al., 2016). Opioid receptor activation led to the suppression of pain related neuronal activity (Narita et al., 2008). Neuropathic pain is unique in that opioid analgesics are not always effective agents. Gabapentin is a medication used to treat neuropathic pain, and is recommended as a first line treatment for neuropathic pain in diabetic neuropathy, post-herpetic neuralgia, and central neuropathic pain (Kalso et al., 2013). Our previous work has reported that gabapentin had a dose-dependent anti-allodynic effect for intractable central post-stroke pain in the rat (Yang et al., 2014). Therefore the effects of morphine and gabapentin on the LFPs evoked by dorsal root stimulation were also assessed in this study.

2. Materials and methods

2.1. Animals

The present study was performed on male albino Sprague Dawley rats (3–4 weeks old, 80–120 g, n = 32) obtained from Laboratory Animal Center of Fourth Military Medical University (FMMU, PR China). Animals were housed individually under standard laboratory conditions (12:12 h day/night cycle, 22–26 °C, humidity 55–60%), with free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of FMMU (Permit number: SCXK2007-007). The number of animals used and their suffering were minimized as much as possible.

2.2. Slice preparation

The general procedures for obtaining slice preparations of the rat spinal cord were similar to those described previously (Yoshimura and Nishi, 1993; Nakatsuka et al., 1999). Briefly, rats were anesthetized with 4% sodium pentobarbital (40 mg/kg, i.p.), and then a lumbosacral laminectomy was performed. The lumbar spinal cord (S1-L1) with the ventral and dorsal roots attached was quickly isolated, dissected and submerged into cold pre-oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) containing 124 mmol/L NaCl, 3.3 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 2.4 mmol/L $MgSO_4$, 2.5 mmol/L $CaCl_2$, 26 mmol/L $NaHCO_3$ and 10 mmol/L glucose (pH 7.35-7.45). Rats were then immediately sacrificed by exsanguination.

After removing the dura mater, all ventral and dorsal roots near the root entry zone were cut except the lateral L4 and L5 dorsal root. The pia and arachnoid maters were also removed except for the area around the entry of the preserved dorsal roots. The spinal cord segment containing L4 and L5 was fixed on an agar stage with cyanoacrylate glue. The agar stage with spinal cord segment mounted on a Vibratome (Dosaka, DTK-1000), and transverse 400 µm slices were cut, with the dorsal roots remaining attached. Slices were placed on nylon mesh in a chamber continuously perfused with oxygenated ACSF at room temperature, and were allowed to recover for at least 2 h before electrophysiological recording.

2.3. Multichannel recording and extracellular stimuli

A multi-electrode (64 channels) dish system (MED-64, Alpha-Med Scientific, Japan) was used for extracellular stimuli and field potential recordings. Procedures for MEA were similar as previously described (Lyu et al., 2013; Wang et al., 2010; Zhao et al., 2009). A single electrode was 50 × 50 µm (P515A), and all 64 electrodes were arranged in an 8 × 8 square with interelectrode distances of 150 µm to cover the whole spinal dorsal horn. Care was taken to ensure that several electrodes were positioned to cover the dorsal root for extracellular stimuli. The surface of the new MED-64 probe (Alpha-Med Scientific, Japan) was treated with 0.1% polyethyleneimine (Sigma, St. Louis, MO; P-3143) in 25 mmol/L borate buffer (pH 8.4) overnight at room temperature before use, and was rinsed with deionized water before recording. At the end of each recording, the probe was cleaned and wet sealed with deionized water. Each MED-64 probe was used for approximately 15 recording sessions with a mean duration of 4–6 h for each time.

The spinal cord slice was placed on the MED-64 probe, and the relative position of MED-64 probe was such that it was covered by all laminae of the dorsal horn and dorsal root when viewed on an inverted microscope (IX71, Olympus, Japan). When the relative position of slice was settled on the probes, a nylon mesh anchor (SHD-22L, Harvard, USA) was attached to the slice to ensure stability during perfusion and electrophysiological recording. The slice was continuously perfused with a peristaltic pump (PERI-STARTM, PI, USA) and oxygenated ASCF at a flow rate of 2 ml/min at room temperature. The probe was connected to a 64-channel amplifier. Each electrode of the probe corresponded to an amplifier channel and could deliver electrical stimuli and record electrophysiological signals. The high-pass filter had a 1 Hz cutoff and the low-pass filter had a 10 kHz cutoff. One electrode on the dorsal root was selected for electrical stimulus while the other 63 electrodes were used for recording. A biphasic square-wave pulse (0.1 Hz, 0.2 ms, 10 − 199 µA) was delivered to evoke local field potentials (LFPs) over the entire dorsal horn. The graphical data was displayed on the monitor screen and stored on hard disk of computer.

2.4. Drug application

The slice was perfused with ACSF at least 30 min, and baseline recordings were performed every 10 min until amplitude of waves became stable. The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainite receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 µM, Sigma-Aldrich, USA), the N-methyI-D-aspartate (NMDA) receptor antagonist DL-2- amino-5-phosphonopentanoic acid (APV, 50 µM, Sigma-Aldrich, USA) and tetrodotoxin (TTX, 1 µM, Sigma-Aldrich, USA) were perfused during recording to identify synaptic mediation of the dorsal horn neural circuit. When the drug exposure was complete, the slice was perfused with ACSF for at least 40 min until the wave form was stable, indicating a return to baseline. The modulatory effects of morphine (10 µM) and gabapentin (10 µM) on dorsal horn neural circuit activity were also tested. All drugs were perfused for 15–30 min, and recordings were performed every 5 min until the drug effect became stable.