Brain Research Bulletin 130 (2017) 112–117

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Peripheral nerve injury potentiates excitatory synaptic transmission in locus coeruleus neurons

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ARTICLE INFO

Article history:
Received 6 December 2016
Received in revised form 9 January 2017
Accepted 11 January 2017
Available online 16 January 2017

Keywords:
Peripheral nerve injury
Neuropathic pain
Locus coeruleus
Excitatory synaptic transmission

ABSTRACT

Peripheral nerve injury (PNI) is believed to cause maladaptive changes at synaptic level, leading to neuropathic pain which is difficult to treat with common analgesic drugs. Noradrenergic locus coeruleus (LC) neurons have a crucial role in neuropathic pain modulation. In this study we examined whether chronic constriction injury (CCI) could affect glutamatergic synaptic transmission in LC neurons.

CCI was performed on P10 to P12 Sprague Dawley pups. Seven days after CCI, horizontal slices of brainstem (300 μm thick) were prepared and whole-cell patch clamp recording was performed. Evoked and spontaneous excitatory postsynaptic currents (eEPSC and sEPSC) were recorded from LC neurons at a holding potential of −70 mV, in the presence of bicuculline (20 μM).

The sEPSCs recorded from LC neurons of neuropathic rats showed a significant increase in amplitude, but not in frequency. The eEPSC amplitude in neurons of rats under gone CCI was significantly increased compared to the control group ($P < 0.05$). The paired pulse ratio (PPR) elicited with different inter-stimulus intervals (50–250 ms) did not show any difference between neurons of CCI and control pups.

This study shows that PNI increases excitatory synaptic transmission in LC neurons 7 days after chronic constriction injury. The observed synaptic potentiation is mainly due to postsynaptic mechanisms.

1. Introduction

Neuropathic pain is caused by damage or lesion to those parts of the peripheral and central nervous system that conduct or process somatosensory information. Neuropathic pain is a chronic medical condition that is generally resistant to nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics (Havelin et al., 2016). Although long-term potentiation (LTP) is known as the cellular mechanism of learning and memory in the brain, many studies support the involvement of the same mechanism in chronic pain conditions such as peripheral neuropathies (Ji et al., 2003). Noxious stimuli and painful experiences could provoke plastic changes in different spinal and supra-spinal structures. The afferent barrage provoked by primary lesions generally leads to development of hypersensitivity and neuronal hyperexcitability. The most studied site for this phenomenon is the spinal dorsal horn (SDH), where the intense activity of peripheral afferents leads to synaptic strengthening following persistent inflammatory (Ikeda et al., 2006) or neuropathic pain (Woolf and Salter 2000; Ikeda et al., 2003; Taves et al., 2013) through induction of molecular changes at synaptic level, which is known as the mechanism of central sensitization (Ji et al., 2003; Boadas-Vaello et al., 2016). There are also several evidences indicating that the same changes in synaptic strength could develop in supra-spinal structures involved in pain modulation and perception; such as anterior cingulated cortex (ACC) (Xu et al., 2008), insular cortex (Qiu et al., 2013), nucleus accumbens (Xu et al., 2015), rostroventral medulla (RVM) (Robinson et al., 2002) and amygdala (Nakao et al., 2012).

Locus coeruleus (LC) is the main source of noradrenaline in the CNS and its descending inhibitory projections to superficial layers of SDH cause antinociception. This system is more effective in persistent pain conditions (West et al., 1993; Tsuruoka et al., 2004). It has been shown that tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH) immunoreactivity in spinal cord as well as the noradrenergic innervation to SDH increases following peripheral nerve injury (PNI) (Ma and Eisenach 2003). The ascending LC projections to the medial thalamus can also exert antinociceptive effects (Voisin et al., 2005). On the other hand, the first-line drugs recommended for the treatment of neuropathic pain include

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http://dx.doi.org/10.1016/j.brainresbull.2017.01.012
0361-9230/

serotonin-noradrenaline reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) (Attal et al., 2010). The main mechanism proposed for the analgesic action of these antidepressants is the inhibition of noradrenaline (NA) and serotonin (5HT) reuptake. Studies have revealed that the necessary component of the analgesic effect of TCAs on neuropathic pain is the reinforcement of noradrenergic system (Suzuki et al., 2008; Arsenault and Sawynok 2009; Bohren et al., 2013) and as their effect appears after a chronic treatment, the possible involvement of neuronal plasticity in this system could be supposed (Kremer et al., 2016).

Since neuropathic pain results at least in part, from maladaptive changes in synaptic transmission at SDH and regarding the involvement of LC as the main noradrenergic site in modulation of chronic pain, we examined whether peripheral nerve chronic constriction injury (CCI) could affect excitatory synaptic transmission in LC neurons as a compensation for pain modulation.

2. Materials and methods

2.1. Animals

All animal experiments were carried out in accordance with the "Ethical Committee of Faculty of Medical Sciences, Tarbiat Modares University". Wistar rats of either sex (17–19 days old) were deeply anaesthetized by ether and decapitated. The brain was quickly removed from the skull and trimmed in ice-cold (1–4 °C) low-calcium, sucrose based artificial cerebrospinal fluid (sucrose-aCSF) containing (mM): sucrose 213, KCl 2.6, $CaCl_2$ 0.1, $MgCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.23, L-Ascorbic Acid 0.4, D-glucose 2 (290–310 mOsmol/L, pH 7.3–7.4 when bubbled with 95% O_2, 5% CO_2).

2.2. Slice preparation

A block of brainstem tissue was glued to the cutting stage of a vibratome (1000 Plus, Vibratome, USA) with the dorsal side up. 2–3 horizontal slices of 300 μm thickness containing the LC nuclei were prepared at 1–4 °C. The slices were first incubated in a holding chamber with standard aCSF (mM): NaCl 125, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 25, $CaCl_2$ 2, $MgCl_2$ 1.3, L-Ascorbic Acid 0.4 and D-Glucose 10, saturated with 95% O_2 and 5% CO_2, for 30–40 min at 35 °C. Osmolarity was maintained between 290 and 310 mOsmol/L and pH between 7.3 and 7.4. The slices were then kept at room temperature (20–25 °C) in the same chamber until the recording. The slice was transferred to a recording chamber mounted on a Zeiss Axioskop. It was fixed in the chamber under nylon strings attached to a U-shaped platinum frame and continuously perfused with the standard aCSF at a flow rate of 1–2 ml/min. Cells were visualized with an upright microscope (Axioskop2, Zeiss, Germany) using infrared differential interference contrast (IR-DIC) illumination system with a 10X or 40X water immersion objective lens (IR-1000, USA).

2.3. Whole-cell recording

Whole cell recording in LC neurons was performed as has been previously described in details (Kaeidi et al., 2015) and is briefly reviewed here. The patch electrodes were made from borosilicate glass pipettes of 1.5 mm outer diameter (GC150F-10, Harvard Apparatus, USA) with a programmable puller (P-97, Sutter instruments, USA). The tip resistance of the electrode was 3–7 MΩ when filled with intracellular solution of the following composition (mM): $CsMeSO_3$ 120, NaCl 5, MgATP 4, NaGTP 0.3, EGTA 1.1, HEPES hemisodium 10, TEA-Cl 10, QX314 5 (pH 7.3 as adjusted with CsOH; osmolarity was approximately 285 mOsmol/L). The LC neurons were preselected according to the IR-DIC image. After establishment of the cell attached configuration (2–10 GΩ seal resistance), the whole cell mode was established with a brief negative pressure pulse (before rupture, holding potential was put on −70 mV). Recording was started at least 5 min after the rupture of the patch membrane to stabilize the intracellular milieu. During this time, the membrane potential was held at −70 mV. Neurons showing unstable or large (>50 pA) holding current were rejected. Access resistance of <25 MΩ was considered acceptable and was monitored periodically throughout the experiment. The experiment was terminated if the access resistance changed more than 15%. Whole-cell voltage clamp recordings were acquired with a MultiClamp 700 B amplifier and pClamp 10 software (Molecular Devices, USA), with a 3-kHz low-pass Bessel filter, and digitized at 10 kHz using a Digidata 1440A data acquisition system (Molecular Device, USA). For all experiments, 20 μM bicuculline (Sigma-Aldrich, USA) was added to eliminate $GABA_A$ receptor-mediated inhibitory postsynaptic currents.

Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded in voltage clamp at a holding potential of −70 mV. The sEPSC of LC neurons appeared as a rapidly rising and slowly decaying inward current. Each event of sEPSCs was detected and its amplitude and frequency were measured in 120 s recordings. All detected sEPSCs were monitored and checked visually so that all analyzed events had a typical sEPSC waveform.

In order to perform eEPSC, a glass monopolar stimulating electrode filled with aCSF was placed 100–300 μm caudal to the recording electrode and the afferents were stimulated at 0.1 Hz. Neurons were voltage clamped at −70 mV to record AMPAR-mediated evoked excitatory postsynaptic currents (AMPAR-eEPSCs). The eEPSC traces were constructed by averaging 12 eEPSCs (120s). Paired-pulse ratio was elicited by evoking two eEPSCs at a holding potential of −70 mV with an interstimulus interval of 50–250 ms. Paired-pulse traces were constructed by averaging 10 sweeps of paired pulses (100 s).

2.4. Chronic constriction injury (CCI) model

In order to achieve a neuropathic pain model, CCI surgery was performed on 10–12 days young rats. Animals were deeply anaesthetized, then sciatic nerve was exposed and four loose ligations were placed on the sciatic nerve by 6/0 chromic gut strings. Seven days after the CCI microsurgery rats were decapitated and the brain stem was cut in 300 μm horizontal slices, for patch clamp recording.

2.5. Data analysis

Data were analyzed off-line using Clampfit software. All values are expressed as mean ± SEM. The results were compared between neurons of neuropathic rats vs. neurons of control ones, using unpaired Student's *T*-test, $p < 0.05$ were considered as statistically significant.

3. Results

3.1. Passive membrane proprieties of LC neurons

Passive membrane proprieties were examined in LC neurons of both groups. Resting membrane potential was −50 ± 0.83 mV and −51 ± 1.8 mV in control and neuropathic brain slices, respectively. Membrane resistance varied from 208.6 ± 22.2 mΩ in control to 210.8 ± 17.3 mΩ in neuropathic rats. None of the measured passive membrane properties showed a significant difference among the CCI and control groups.