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TRANSFORMING GROWTH FACTOR-BETA IN THE RED NUCLEUS PLAYS ANTINOCICEPTIVE EFFECT UNDER PHYSIOLOGICAL AND PATHOLOGICAL PAIN CONDITIONS

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Abstract—Previous studies have demonstrated that the red nucleus (RN) participates in the modulation of neuropathic pain and plays both a facilitated role by pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β), and an inhibitory role through the anti-inflammatory cytokine IL-10. In this study, we sought to investigate the expressions and roles of transforming growth factor-beta (TGF-β), a potent anti-inflammatory cytokine, as well as its type 1 receptor (TGF-β-R1) in the RN in normal and neuropathic pain rats. Immunohistochemistry showed that TGF-β and TGF-β-R1 were constitutively expressed in the RN of normal rats, and co-localized with neurons and all three glial cell types, astrocytes, microglia and oligodendrocytes. Following spared nerve injury (SNI), the expression levels of TGF-β and TGF-β-R1 were significantly down-regulated in the RN contralateral (but not ipsilateral) to the nerve injury side of rats at one week and reached the lowest level at two weeks after SNI, and both of them were co-localized with neurons and oligodendrocytes but not with astrocytes and microglia. Microinjection of different doses of anti-TGF-β antibody (250, 125, 50 ng) into the unilateral RN of normal rats dose-dependently decreased the mechanical withdrawal threshold of contralateral (but not ipsilateral) hind paw and induced significant mechanical hypersensitivity, which was similar to mechanical allodynia induced by peripheral nerve injury. In contrast, microinjection of different doses of recombinant rat TGF-β1 (500, 250, 100 ng) into the RN contralateral to the nerve injury side of SNI rats dose-dependently increased the paw withdrawal threshold and significantly alleviated mechanical allodynia induced by SNI. These results suggest that TGF-β in the RN participates in nociceptive processing and plays antinociceptive effects under normal physiological condition and in the development of neuropathic pain induced by SNI.

Key words: red nucleus, transforming growth factor-beta, neuropathic pain, spared nerve injury.

INTRODUCTION

The red nucleus (RN) is an important nucleus of extracorticospinal tract, which comprises a critical subcortical relay station for a massive descending motor tract (the rubrospinal tract). Accumulating evidence has shown that the RN is involved in modulating muscle tension, motor learning, triggering conditioned motor responses, postural corrections, and the recovery of movement after spinal injury (Muir and Whishaw, 2000; Basso et al., 2002; Küchler et al., 2002; Lavoie and Drew, 2002; Zelenin et al., 2010). The RN neurons in the intact and decerebrate cat exhibit phasic discharge preferentially in the swing phase of locomotion, during which they influence the activity of flexor muscles (Lavoie and Drew, 2002). Unilateral lesions of the RN in rats give rise to a characteristic asymmetry in which abnormal braking and propulsive forces are produced during locomotion (Muir and Whishaw, 2000). In addition, electrical or chemical stimulation of the RN facilitates the low-threshold afferent-evoked jaw-opening reflex (JOR) and suppresses the high-threshold afferent-evoked JOR, suggesting that the RN is also involved in the control of jaw movements (Satoh et al., 2013).

Apart from the well-established roles in the motor system, the RN is also involved in pain processing and plays a descending regulatory role (Liu et al., 1991). The spontaneous discharges of neurons in the RN of rats have been recorded with microelectrode, and the discharge frequency of most RN neurons is changed by nociceptive stimulation of the peripheral nerve or limbs (Huang et al., 1992; Steffens et al., 2000). Microinjection of glutamic acid into the RN of normal rats could increase the pain threshold of tail flick reflex and this effect is blocked by an injection of lidocaine into the nucleus raphe magnus (NRM) (Liu et al., 1991). Our previous studies have shown that the expressions of tumor necrosis

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Abbreviations: ANOVA, analysis of variance; CCI, chronic constriction injury; IgG, immunoglobulin G; IL, Interleukin; IR, immunoreactivity; JOR, jaw-opening reflex; MOD, mean optical density; PWT, paw withdrawal threshold; RN, red nucleus; SNI, spared nerve injury; TGF-β, transforming growth factor-beta; TGF-β-R1, transforming growth factor-beta type 1 receptor; TNF-α, tumor necrosis factor-alpha.

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factor-alpha (TNF-α), interleukin-1β (IL-1β) and nerve growth factor (NGF) are up-regulated in the RN of rats with spared nerve injury (SNI), and microinjection of their corresponding antibodies reduces mechanical allodynia induced by SNI (Li et al., 2008b; Wang et al., 2008; Jing et al., 2009). On the contrary, repeated microinjection of recombinant rat TNF-α into the RN induces a significant mechanical allodynia in normal rats (Zhang et al., 2013). Furthermore, our recent study demonstrates that IL-10, an anti-inflammatory cytokine, is also up-regulated in the RN of rats with SNI, and microinjection of recombinant rat IL-10 into the RN suppresses mechanical allodynia induced by SNI (Wang et al., 2012). These results strongly suggest that the RN participates in nociceptive processing and plays both a facilitated role and an inhibitory role by different neurotransmitters and cytokines.

The transforming growth factor-beta (TGF-β) family, serving as anti-inflammatory cytokines, has been implicated in the regulation of development, disease and tissue repair in the nervous system (Böttner et al., 2000; Buckwalter et al., 2006). Several recent studies demonstrate that TGF-β also plays an important role in normal nociceptive processing and the regulation of pathological pain (Echeverry et al., 2009, 2013; Lantero et al., 2012, 2014; Mika et al., 2013; Chen et al., 2013, 2014). However, the expression and roles of the RN TGF-β under physiological and pathological pain conditions are still unknown. Thus, the purpose of this study was to explore the expression patterns of TGF-β and its type 1 receptor (TGF-β-R1) in the RN and also their biological roles under normal physiological condition and in the development of neuropathic pain induced by SNI.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats weighing 200–230 g were used for the study, all of which were purchased from the Experimental Animal Center of Shaanxi Province, China. All animals were housed with *ad libitum* access to food and water and maintained on a 12-h/12-h light/dark cycle. The experiment was carried out in accordance with the Institutional Animal Care Committee of Xi'an Jiaotong University and the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

SNI

The SNI was performed as reported previously (Bourquin et al., 2006). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and three terminal branches of the sciatic nerve were exposed by direct incision of the skin and a section of the biceps femoris muscle in the right thigh. The tibial and common peroneal branches were tight-ligated with 5-0 silk sutures and sectioned distal to the ligation, removing 2–4 mm of the distal nerve stump. Great care was taken to avoid contacting or stretching the intact sural nerve. Muscle and skin were closed in two layers. In the sham-operated group, rats were treated in the same way, but the nerves were not lesioned. Rats of SNI were used for further experiments only when the withdrawal threshold of right hind paw was less than 4.0 g in response to von Frey filament stimulation.

Immunohistochemistry

After nerve injury, rats in different groups (three rats for each group) were anesthetized and perfused transcardially with Bouin's fluid (300-ml saturate nitroxanthic acid solution, 100-ml 40% formaldehyde and 20-ml glacial acetic acid). The brain tissues containing the RN were removed, postfixed in Bouin's fluid for 1 day and then dehydrated by 30% sucrose. All brain tissues were embedded in OCT and sectioned coronally into 10-μm-thick sections using an ultramicrotome. One section from 100 μm was picked for the following experiment and three sections were used for analyzing one animal.

After routine treatments of ice acetone, 3% hydrogen peroxide and normal goat serum blocking solution, sections were incubated with rabbit anti-rat TGF-β (1:200; Abcam, Cambridge, MA, USA) or anti-rat TGF-β-R1 antibody (1:50; Abcam) overnight at 4 °C. Sections were then incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) for 30 min, and subsequently reacted with DAB for staining. As a control, the primary antibody was omitted or isotypic antibody (normal rabbit IgG) was used to confirm immunospecificity. To further confirm the specificity of TGF-β staining, excessive soluble TGF-β1 (1 μg/μl; Sino Biological Inc., China) was used to block the primary anti-TNF-β antibody.

Histological sections were viewed with Olympus BX-51 microscope and the images were captured with Olympus DP71 camera. TGF-β and TGF-β-R1 immunoreactivity (IR) were quantified using Image J software (National Institute of Health, Bethesda, MA, USA). Briefly, digital images from each rat were opened under Image J program and converted to 8-bit grayscale, allowing the computer to distinguish between areas of IR and background. Following a standardized elimination of background through adjusting the threshold, the RN area was selected and the mean optical density (MOD) and area ratio of TGF-β- and TGF-β-R1-positive cells (area of positive signal/area of region of interest) were measured (http://rsb.info.nih.gov/ij/).

Immunofluorescence staining

Two weeks after nerve injury, rats in different groups (three rats for each group) were anesthetized and sacrificed by cardiac bleeding. The brain tissues containing the RN were rapidly harvested and stored at −80 °C after embedded in OCT. All brain tissues were sectioned coronally into 10-μm-thick sections using an ultramicrotome.

After routine treatments with acetone and normal goat serum blocking solution, sections were incubated with the primary antibodies of rabbit anti-rat TGF-β (1:500; Abcam) or anti-rat TGF-β-R1 (1:50; Abcam) mixed with