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The role of hippocampal brain-derived neurotrophic factor in age-related differences in neuropathic pain behavior in rats

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ABSTRACT

Aims: This study was aimed to explore the contribution of central brain-derived neurotrophic factor (BDNF) in the neuropathic pain pathogenesis using an aged rodent model.

Main methods: Adult and aged rats were randomly assigned to either a sciatic nerve ligation (SNL) group or a control skin sham surgery group. Sensory behavioral testing were performed on the day before surgery and on the 3rd, 7th, 14th, and 21st days after surgery, followed by measurement of BDNF protein levels in different brain regions. In another experiment, the hippocampal BDNF gene expression after SNL surgery was assessed at different time-points. Furthermore, the analgesic effects of intranasal BDNF administration were tested in SNL animals.

Key findings: Our behavioral results demonstrated that the hyperalgesia-like behavior after painful nerve injury has a higher incidence in aged rats compared with in adult animals. In particular, the hippocampal BDNF levels were inversely correlated with the probability of hyperalgesia-type behavior, in both brain-region specific and age-dependent manner. Time-course analysis showed that the hippocampal levels of BDNF mRNA in aged and adult rats started to decrease 7 and 14 days after surgery, respectively. However, the decrease was more pronounced in aged animals. Moreover, the repeated intranasal BDNF treatment could restore the central BDNF signaling, counteracting the age-related exacerbation of hyperalgesic behavior.

Significance: Our findings imply that hippocampal BDNF may be related with the pathogenesis of elderly neuropathic pain. Pharmacological data further suggest that brain BDNF may be modifiable in aged neuropathic animals, and therefore, represent a promising target for intervention.

1. Introduction

Neuropathic pain is pathological pain caused by a primary lesion or dysfunction in the somatosensory nervous system [1,2]. In the elderly population in particular, chronic neuropathic pain is a major individual and public-health burden because of its high prevalence and debilitating nature [3–5]. As such, the experts strongly agree in the importance of appropriate management for neuropathic pain in elderly people [6,7]. Currently, however, several types of chronic neuropathic pain are still difficult to treat due to resistance to conventional pain medications or adverse drug reactions [6–8]. Taking multiple comorbidities and geriatric syndromes (e.g., frailty, dementia, depression, falls, malnutrition, polypharmacy) into consideration, the management of geriatric neuropathic pain may be one of the most challenging problems facing an aging society.

To understand and treat the neuropathic pain in elderly individuals, it is necessary to unravel its underlying mechanisms. However, to date, little is known regarding age-specific changes in the neuropathic pain pathology. Conversely, the available evidence suggests that normal aging affects the physiological nociceptive system [9]. For example, there is some evidence of a minor age-related increase in experimental pain thresholds [9,10]. In contrast, a decrease in tolerance to high-intensity noxious stimuli was demonstrated with advancing age [9,11,12]. Furthermore, although central sensitization, a key pathogenesis of neuropathic pain, has been extensively studied in the spinal cord and brainstem [1,2,13,14], recent imaging studies in humans reported the pivotal roles of higher brain centers [14–16]. These brain regions also may be structurally and functionally affected in the aging process, resulting in increasing susceptibility to age-related pathology such as cognitive decline or more severe neurodegenerative disorders

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[17,18]. Taken together, we hypothesized that central pain processing mechanisms may contribute to the relative neuropathic pain risk in vulnerable older patients.

Neurotrophic factors, especially brain-derived neurotrophic factor (BDNF), are abundantly expressed in the nervous system, and BDNF is thought to be involved in neuronal survival, regeneration, and synaptic function through its high affinity receptor TrkB [19]. There is preclinical evidence demonstrating that BDNF may be associated with the development of spinal sensitization after nerve injury [20,21]. However, the possible involvement of BDNF at higher brain centers in pain-processing remains unknown [22]. In general, the decline in BDNF levels may be related with advanced brain aging and the resultant age-related neurological syndromes [23–25]. Therefore, we further hypothesized that age-related pain hypersensitivity may be related with changes in BDNF signaling within the brain.

In order to address these hypotheses, we compared the pain sensitivity and the brain BDNF levels after painful nerve injury between adult and aged rats. In addition, we examined the therapeutic potential of exogenous BDNF as a brain-targeted analgesic in aged neuropathic animals.

2. Methods

2.1. Animals and experimental designs

All experimental procedures on animals were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the International Association for the Study of Pain [26]. Adult (2–3 months old; 160–230 g body weight) and aged (19–24 months old; 540–680 g) male Wistar rats were purchased from Alfresa Shinohara Chemicals Corporation (Kochi, Japan). All animals were housed two *per* cage with standard 12-h cycle lighting, and were provided access to food and water ad libitum prior to and throughout the experimental protocol. Adult and aged rats were randomly assigned to either a sciatic nerve ligation (SNL) group or a control group that received skin sham surgery. The animals were further divided into three sets of experiments. Experiment 1 was designed to assess the pain-related behaviors and BDNF levels in several brain regions using a 2 × 2 experimental design: adult versus aged rats, by SNL versus skin sham control group (n = 12 in each group). Experiment 2 was conducted to observe the time course of hippocampal BDNF levels after SNL surgery (n = 6 in each group). In Experiment 3, to address the therapeutic potential of hippocampal BDNF for neuropathic pain, the analgesic activity of intranasal administered BDNF was evaluated (n = 12 in each group).

2.2. Experimental model of neuropathic pain

The SNL surgery was performed according to the method originally described previously [27], but we did not excise the adjacent paraspinous muscles or the articular processes. Under anesthesia with isoflurane (1.5%–2%) in oxygen, the right paravertebral region was exposed via lumbar incision, and the L6 transverse process was removed. The L5 and L6 spinal nerves were then tightly ligated with a 6–0 silk suture and transected distal to the ligature. The fascia was closed with 4–0 resorbable Vicryl polyglactin sutures, and the skin was closed with 3 surgical staples. Skin sham control animals received anesthesia, lumbar skin incision, and skin closure with 3 staples.

2.3. Behavioral testing for confirmation of hyperalgesia

All behavioral experiments were carried out with the investigators blinded to treatment conditions. In Experiment 1, sensory testing was performed on the day before surgery (baseline) and on the 3rd, 7th, 14th, and 21st days after surgery. For pharmacological experiments, additional testing was performed before and after administration as indicated in the results section. Animals were initially familiarized with the testing environment for 4 h or more, and then placed in clear plastic enclosures upon a 1/4–in wire grid. Mechanical withdrawal threshold and hyperalgesia-like behavior were evaluated using von Frey fibers and needle stimulation, respectively, according to previous studies [28–30].

2.3.1. Paw withdrawal threshold (PWT)

Mechanical allodynia at the hind-paw was assessed by von Frey fibers. (Smith and Nephew Inc., Germantown, WI). Fibers with forces of 0.57, 0.84, 1.39, 2.27, 4.03, 5.13, 6.92, 11.0, 14.2, and 24.6 g were applied in increasing order. The filament was perpendicularly applied from underneath the mesh openings to stimulate the plantar surface at the medial aspect adjacent to the wound, and the force of each application was made optimal and consistent by having the filament bent to the same extent. A method of constant stimuli was used in which all animals were tested with the full range of fibers regardless of their responses. The withdrawal response was scored either as none or positive if the paw was removed or licked. If there was no response, the value of 25 g was assigned as a threshold.

2.3.2. Probability of hyperalgesia-like behavior (PHB)

A Quincke 22–gauge spinal needle was used for detecting elevated sensory responsiveness after surgery, and this procedure was performed using a previously described highly specific technique [28]. Briefly, the plantar surface of each hind paw was touched with the tip of the needle, which was applied with pressure adequate to indent but not penetrate the skin. Five needle applications were delivered in random order to each paw and repeated 5 min later for a total of 10 applications per session. These mechanical stimuli produced either a normal brief reflexive withdrawal or a hyperalgesia-type response that included sustained ($>$ 1 s) paw lifting, shaking, and grooming. As the latter response occurs only after true SNL but not sham exposure of the nerve alone, and only on the side ipsilateral to the injury, this may be accepted as an indication of a neuropathic pain state [28]. The degree of hyperalgesia was expressed as the difference between the probabilities of hyperalgesia response.

2.4. Tissue collection and enzyme-linked immunosorbent assay

Recent clinical and preclinical evidence suggests that multiple cortical and subcortical brain areas, specifically including the anterior cingulate cortex (ACC), prefrontal cortex (PFC), thalamus, cerebellum, hippocampus, and amygdala, are involved in pain processing [15,16,31–33]. Furthermore, it has been reported that BDNF protein is highly expressed in some of these regions [34]. Therefore, we selected and investigated these six brain regions.

Experimental tissues and serum were collected after completion of behavioral testing on the 21st day after skin sham or SNL surgery. Animals were sacrificed by decapitation under deep isoflurane anesthesia, and whole brains were rapidly harvested. The 6 different brain regions were dissected according to the dissection method described by Glowinski and Iversen [35]. Each sample was homogenized with a polytron homogenizer in ice-cold lysis buffer (10 mM NaCl, 1.5 mM $MgCl_2$, 20 mM HEPES, 20% glycerol, 0.1% Triton X-100, 1 mM dithiothreitol, pH = 7.4) containing protease inhibitors cocktail (P8340, Sigma-Aldrich, St Louis, MO, USA). The homogenates were centrifuged (11,000 × g, 20 min, 4 °C), and the supernatants were aliquoted and frozen at −80 °C until required for Enzyme-Linked Immunosorbent Assay (ELISA). The protein concentrations of BDNF in the supernatant were analyzed in duplicate using the ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The data were normalized and expressed as pg BDNF per mg tissue (pg/mg). Following the sample collections, all SNL animals were confirmed for accuracy of the initial surgery by laminectomy.