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Adult hippocampal neurogenesis in neuropathic pain and alkyl glycerol ethers treatment

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ABSTRACT

Neuropathic pain manifested by a number of sensory symptoms is often accompanied by disorders of higher nervous activity, such as memory impairment, depression, anxiety, anhedonia, etc. This emphasizes the involvement of supraspinal structures including the hippocampus in neuropathic pain pathogenesis. In the present study, we focused on the impact of chronic neuropathic pain on hippocampal neurogenesis and microglial state. In addition, we test the effect of alkyl glycerol ethers on hippocampal neuronal and microglial plasticity as well as behavioral parameters. Neuropathic pain was induced using the model of sciatic nerve chronic constriction injury. We found an impairment of working memory and locomotor activity in animals with neuropathic pain, which was prevented by alkyl glycerol ethers treatment. Sciatic nerve ligation in mice contributed to the decrease in hippocampal neurogenesis intensity. Alkyl glycerol ethers administration significantly reduced this effect. Neuropathic pain-associated neurogenesis reduction was accompanied by an increased percentage of Iba1-labeled area in the CA1 hippocampal region on the 14th and 28th days after surgery. In addition, we observed a decrease in hippocampal pro-inflammatory microglia marker CD86 immunostaining on day 28 after surgery in alkyl glycerol ethers-treated mice with sciatic nerve ligation. These results are consistent with data on pro- and anti-inflammatory cytokines expression in the hippocampus. Alkyl glycerol ethers administration increased IL-10 and decreased IL-1β hippocampal expression in animals with neuropathic pain. Taken together, these data suggest that neuropathic pain-behavior in rodents is accompanied by changes in microglia polarization, thereby contributing to neurogenesis impairment and cognitive disturbances. Alkyl glycerol ethers prevented M1 microglial activation, contributing to the maintenance of normal neurogenesis levels within the hippocampus and normalizing working memory.

1. Introduction

Neuropathic pain is a condition that occurs when the somatosensory nervous system is affected due to central and peripheral nervous system disorders. Neuropathic pain manifests itself as a diverse combination of positive (allodynia, hyperalgesia) and negative (hypoesthesia) sensory symptoms. Patients with neuropathic pain are difficult to treat, and sometimes impossible to relieve the pain syndrome. This type of pain cannot be treated with traditional analgesics, such as non-narcotic analgesics and non-steroidal anti-inflammatory drugs. A number of studies demonstrate, that patients with chronic pain may exhibit additional complex symptoms, including chronic fatigue, anxiety, depression, insomnia and cognitive disturbance (Schnurr and MacDonald, 1995; Argoff, 2007). It has been shown that cognitive impairments, especially disruptions of short-term memory are observed in patients with neuropathic pain (Jamison et al., 1988; Eccleston, 1994). Numerous studies demonstrate that spinal microglia play a significant role in the neuropathic pain response (Ji and Suter, 2007; Coull et al., 2005). However, it is assumed that microglia in the supraspinal nerve centers play a significant role in the formation of both the neuropathic pain response and its cognitive consequences (Apkarian et al., 2009).

Numerous studies have demonstrated the relationship between hippocampal neurogenesis, microglial activation, and cognitive disorders in neurological and neurodegenerative diseases (Fan et al., 2007; Biscaro et al., 2012). Adult neurogenesis in the hippocampus is a

Abbreviations: CNS, central nervous system; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; NO, nitric oxide; ROS, reactive oxygen species; TGF-β, transforming growth factor-beta; AGE, alkyl glycerol ethers; CCI, chronic constriction injury; PBS, phosphate buffer saline; DG SGZ, dentate gyrus subgranular zone; DCX, doublecortin; PFA, paraformaldehyde; PCNA, proliferating cell nuclear antigen; SNI, spared nerve injury

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notable process due to its unique impact on cognition, memory, and behavior (Aimone et al., 2014). It is demonstrated that adult neurogenesis in mice is blocked by inflammation-associated microglia, but induced by alternatively activated microglia secreting anti-inflammatory cytokines (Butovsky et al., 2006). Microglia play a major role in the central nervous system (CNS) immune response. Microglial cells are resident macrophages that participate in the early detection and removal of pathogens from the CNS via the recruitment of adaptive immune system cells. The innate and adaptive immune responses caused by microglia include the release of proinflammatory and anti-inflammatory mediators. Depending on the produced mediators and their functions, the activated microglia is divided into 2 phenotypes: M1 "classical activation" and M2 "alternative activation". M1 phenotype is associated with the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), superoxide, nitric oxide (NO), and reactive oxygen species (ROS). M2 microglia predominantly produce anti-inflammatory cytokines IL4, IL-10, IL-13, IFN-α, and transforming growth factor- β (TGF-β) which are closely associated with tissue repair and extracellular matrix reconstruction. Several recent studies have focused on the role of spinal microglia in the development of neuropathic pain. However, little is known about the effect of neuropathic pain on the phenotype of brain microglia, cytokine production, influences on neurogenesis, and higher cerebral functions. Thus, determining the effects of neuropathic pain on cognitive function can be based on detecting the molecules expressed by microglia and understanding their influence on the neurogenesis process.

Despite the presence of a large number of anesthetics in the pharmaceutical market, limited success has been achieved in the treatment of neuropathic pain (Finnerup et al., 2010). In most cases, methods of anesthesia are ineffective. This fact suggests the need for other treatment options to target chronic neuropathic pain as well as the cognitive consequences (Scholz and Woolf, 2007). Considering the involvement of immune cells and glia in the pathogenesis of neuropathic pain, the development of therapeutic strategies aimed at immune responses or modulating glial functions may be an important step in the treatment of neuropathic pain. A promising group of compounds capable of influencing the microglial state is alkyl glycerol ethers (AGE) obtained from marine organisms. According to previously described studies, administration of AGE is able to cause peripheral macrophage activation (Yamamoto et al., 1988) and increased immune response (Sy et al., 1994). Nevertheless, all studies concerning the effect of alkyl glycerol ethers on the immune response are limited to only the peripheral immune system, and the effect on brain resident macrophages has not yet been considered.

In the present study, we focused on the impact of chronic neuropathic pain on hippocampal neurogenesis and the microglial state. In addition, we tested the effect of alkyl glycerol ethers on hippocampal neuronal and microglial plasticity as well as behavioral parameters.

2. Materials and methods

2.1. Animals and surgery

Experiments were performed using 3-month old male C57BL/6 mice. The animals were housed two to four per cage with a 12-h light–dark cycle and ad lib access to food and water. To reduce stress, the mice were handled for 5 min once a day during five consecutive days before the experiments. All procedures were approved by the Animal Ethics Committee at National Scientific Center of Marine Biology Far Eastern Branch, Russian Academy of Sciences, according to the Laboratory Animal Welfare guidelines. Neuropathic pain was produced using the model of chronic constriction injury (CCI) of the sciatic nerve (Bennett and Xie, 1988). In brief, animals were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.), then the right sciatic nerve was exposed and three ligatures (3 silk gut sutures; ETHICON, USA) were placed around the nerve proximal to the trifurcation with 1 mm between each ligature. The ligatures were tightened loosely until a slight twitching of the ipsilateral hindlimb. Animals in the sham group received surgery identical to those described but without nerve ligation.

Table 1
AGE composition in final preparation.

Component, %	Saturated AGE	Trivial name
14:0[a]	1.26 ± 0.16 [b]	
16:0	94.39 ± 0.03	chimyl alcohol
18:0	4.35 ± 0.37	batyl alcohol
Σsat	100 ± 0.00	

[a] Indicated by chain length and double bond of alkyl chain in alkyl glycerol ether.
[b] % to total components (mean value ± standard deviation (n = 5)).

2.2. Drugs

2.2.1. Preparation of the alkyl-glycerol ethers

The squid Berryteuthis magister liver was obtained from Nakhodka Active Marine Fishery Base (Russia) and stored at −200 °C. Extraction of total lipids was performed according to Bligh and Dyer (1959). Saponification of lipids was carried out by conventional technique (Christie, 2003). The lipid mixture after hydrolysis and acidification was dissolved in acetone in a ratio of 1:5 at room temperature and incubated for 24 h at −20 °C (Ermolrenko et al., 2016). Recrystallization of obtained sediment was performed for complete AGE separation from other lipids.

The AGE content in resulting product was over 99% where chimyl alcohol was the main component −94% (Table 1). The chemical structure of chimyl and batyl alcohol is shown in Fig. 1.

2.2.2. Animal treatment

Alkyl glycerol ethers preparation was administered to mice as a water emulsion at a dose of 250 mg/kg using the oral gavage technique. The period of administration was 2 weeks from the day of surgery. Animals were divided into 3 groups (n = 42): "Sham" group − sham-operated animals treated with vehicle (water) (n = 14); "CCI" group − animals with sciatic nerve constriction injury treated with vehicle (water) (n = 14); "CCI + AGE" group (n = 14) − AGE-treated mice with sciatic nerve constriction injury. Half of the animals (7 mice per group) were sacrificed 14 days after the surgery on the day of the last AGE administration. The second half of animals were sacrificed 28 days after surgery, i.e. 2 weeks after the discontinuation of AGE treatment.

2.3. Behavioral tests

All mice were exposed to behavioral tests 2 and 4 weeks after surgery. Determination of thermal hyperalgesia was carried out weekly. All tests were performed during the light cycle between 7:00 AM. and 7:00 P.M. To minimize olfactory cues from previous trials, each apparatus was thoroughly cleaned with 10% ethanol after each animal. To

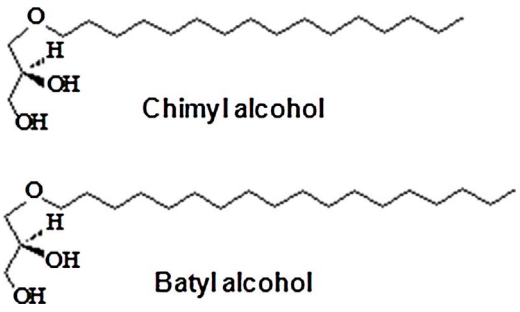

Fig. 1. The chemical structure of chimyl and batyl alcohols.