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JNJ10181457, a histamine H3 receptor inverse agonist, regulates *in vivo* microglial functions and improves depression-like behaviours in mice

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ABSTRACT

Brain histamine acts as a neurotransmitter and regulates various physiological functions, such as learning and memory, sleep-wake cycles, and appetite regulation. We have recently shown that histamine H3 receptor (H3R) is expressed in primary mouse microglia and has a strong influence on critical functions in microglia, including chemotaxis, phagocytosis, and cytokine secretion *in vitro*. However, the importance of H3R in microglial activity *in vivo* remains unknown. Here, we examined the effects of JNJ10181457 (JNJ), a selective and potent H3R inverse agonist, on microglial functions *ex vivo* and *in vivo*. First, we injected ATP, which is a typical chemoattractant, into hippocampal slices to investigate the effect of JNJ on chemotaxis. ATP-induced microglial migration toward the injected site was significantly suppressed by JNJ treatment. Next, we examined whether JNJ affected microglial phagocytosis in hippocampal slices and in the prefrontal cortex. Microglial engulfment of dead neurons induced by *N*-methyl-$_D$-aspartate was inhibited in the presence of JNJ. The increase in zymosan particle uptake by activated microglia in the prefrontal cortex was prevented by JNJ administration. Finally, we determined the importance of JNJ in a lipopolysaccharide (LPS)-induced depression model. JNJ reduced the LPS-induced upregulation of microglial pro-inflammatory cytokines and improved depression-like behaviour in the tail-suspension test. These results demonstrate the inhibitory effects of JNJ on chemotaxis, phagocytosis, and cytokine production in microglia inside the brain, and highlight the importance of microglial H3R for brain homeostasis.

1. Introduction

Histamine, which is a bioactive amine, regulates various physiological events, such as allergic reactions, gastric acid secretion, and itch sensation. In the central nervous system (CNS), histamine acts as a neurotransmitter. Neuronal histamine is enzymatically synthesized in histaminergic neurons [1], which are located in the tuberomammilary nucleus in the posterior hypothalamus and project their axons to the entire brain [2]. Among the four G protein-coupled histamine receptors (H1R–H4R), H1R, H2R, and H3R are widely distributed in the CNS and regulate various physiological functions. H1R and H2R are expressed as postsynaptic receptors in various areas of the CNS and control sleep-wake cycles and aggression [3,4]. In contrast, H3R, which is an inhibitory G protein-coupled receptor, is an autoreceptor in presynaptic membranes of histaminergic neurons and regulates histamine release [5]. H3R also acts as a heteroreceptor in non-histaminergic neurons and modulates the release of various neurotransmitters, including GABA and acetylcholine [6]. In addition, H3R is the most abundant histamine receptor in the CNS. Consistent with its abundance and strong impact on neurotransmitter release, H3R is involved in diverse brain functions, such as memory, cognition, appetite, and arousal [7]. These findings have attracted attention toward H3R in the CNS from a broad range of disciplines. Preclinical and clinical trials have studied the therapeutic effects of H3R inverse agonists on various neurological disorders, such as epilepsy and attention-deficit hyperactivity disorder [8–10]. Indeed, pitolisant, an H3R

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inverse agonist, was approved for the treatment of narcolepsy by the European Medicine Agency in 2016 [11].

Recent studies have revealed that H3R is also expressed in glial cells. Astrocytes, which are the most abundant cells in the CNS, express H3R, and activation of H3R enhances the expression and synthesis of neurotrophin-3 [12]. However, H1R, and not H3R, has a predominant role in astrocytic functions [13,14]. Our recent findings indicate that microglia, which are brain-resident macrophages, express H2R and H3R. Although we were not able to confirm the importance of H2R in primary mouse microglia, H3R was shown to regulate the levels of second messengers, such as intracellular Ca^{2+} and cyclic adenosine monophosphate, which in turn affect critical microglial functions including chemotaxis, phagocytosis, and cytokine secretion [15]. Thus, we hypothesize that H3R in microglia also plays a role in the CNS, and that H3R inverse agonists might exert therapeutic effects by regulating microglial H3R as well as neuronal H3R. Here we investigated the effects of a selective and potent H3R inverse agonist, JNJ10181457 (JNJ) [16], on microglial chemotaxis, phagocytosis, and cytokine production in *ex vivo* and *in vivo* assays, including in mouse models of human depression.

2. Materials and methods

2.1. Mice

CX3C chemokine receptor 1 (CX3CR1)-green fluorescent protein (GFP) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) [17]. All experiments were performed according to the Principles for Care and Use of Research Animals of Tohoku University, Sendai, Japan. All experiments involving animals are reported in accordance with Animal Research: Reporting of *in vivo* Experiments guidelines [18,19].

2.2. Organotypic hippocampal slice cultures

Organotypic hippocampal slices were prepared as described previously [20]. The hippocampus was cut into 120- (for chemotaxis assay) or 350 μm-thick (for *ex vivo* phagocytosis assay) sections using a MacIlwain tissue chopper (Mickle Laboratory Engineering; Gomshall Lane, UK).

2.3. Chemotaxis assay

Hippocampal slices were pre-incubated with or without 10 μM JNJ (Tocris Bioscience; Bristol, UK) containing dissection buffer with the following composition: Modified Eagle's Medium (MEM)/Hank's Balanced Salt Solution (HBSS) (Life Technologies; Carlsbad, CA, USA), 10 mM Tris-HCl (pH 7.2), 25 mM HEPES (Life Technologies) supplemented with 100 IU/mL penicillin G potassium (Wako Pure Chemical Industry; Osaka, Japan) and 100 μg/ml streptomycin sulphate (Wako). Injection of 3 mM ATP (Wako) was performed using a microinjector (BJ-110; BEX; Tokyo, Japan) with the following specifications: diameter of pipette tip, 8 μm; pressure, 0.04 MPa; duration, 100 m sec. Images were captured every 30 s using a fluorescent microscope (IX-71; Olympus; Tokyo, Japan). Microglial chemotaxis was analysed as described previously [21]. Briefly, the fluorescent intensity in region Rx (100 μm in radius from the injected site) at each time point Rx(t) was subtracted from the first image Rx(0). Microglial chemotaxis at any time point is calculated using the equation $Rx(t) - Rx(0)$.

2.4. Ex vivo phagocytosis assay

The hippocampal slices were pre-incubated for 1 h at 37 °C with slice culture medium with the following composition: 50% MEM/HBSS, 25% HBSS (Life Technologies), 25% horse serum (HyClone Laboratories; Logan, UT, USA), and 25 mM HEPES supplemented with 100 IU/mL penicillin G potassium and 100 μg/ml streptomycin sulphate. After stimulation with 50 μM *N*-methyl-$_D$-aspartate (NMDA) (Tokyo Chemical Industry; Tokyo, Japan) in the presence or absence of 1 μM JNJ for 3 h, the slices were incubated with 0.5 μg/ml of propidium iodide (PI) (Tokyo Chemical Industry), which is a membrane-impermeable fluorescent dye for DNA, with or without 1 μM JNJ for 21 h. Fluorescent images were captured using a Nikon C2si microscope (Nikon; Tokyo, Japan). Microglial phagocytosis was evaluated using Bz-9000 (Keyence; Osaka, Japan), and the images were analysed using Hybrid Cell Count image analysis software (Keyence).

2.5. Immunohistochemistry for CD68

Fixed slices were incubated with a primary antibody for CD68 (Abcam; Cambridge, UK) in 20% bovine serum albumin (Sigma-Aldrich; St. Louis, MO, USA) in phosphate-buffered saline (Wako) overnight at 4 °C. The sections were then treated with a secondary antibody.

2.6. Quantitative real-time PCR

RNA isolation, reverse transcription, and PCR were performed as described previously [15]. The sequences of the primers are listed in Table 1.

2.7. In vivo phagocytosis assay

In vivo phagocytosis was assayed as described previously [22]. Yeast-derived zymosan particles conjugated with Alexa 568 (Thermo Fisher Scientific Inc.; Waltham, MA, USA) was stereotaxically injected into the prefrontal cortex at the following coordinates: anterior, 0.3 mm; lateral, ± 1.2 mm; and ventral, 1.6 mm from bregma. We intracranially co-administered 10 μg/uL of lipopolysaccharide (LPS) (Sigma-Aldrich) and/or 10 μM JNJ with the zymosan particles. We injected the mice with 10 mg/kg JNJ or saline intraperitoneally for the next 3 consecutive days. Four days after the zymosan injection, brain coronal sections (50 μm-thick) were prepared using a vibratome (Leica; Wetzlar, Germany) after perfusion with 4% paraformaldehyde (Wako). Phagocytosis was quantified by counting the numbers of engulfed beads in microglia using a fluorescent microscope and appropriate software (Keyence).

Table 1
Primer sequences for PCR.

Gene	Primer sequence	Product size (bp)
GAPDH		
Sense	5′-AGAACATCATCCCTGCATCC-3′	91
Antisense	5′-CACATTGGGGGTAGGAACAC-3′	
CD68		
Sense	5′-ACTGGTGTAGCCTAGCTGGT-3′	85
Antisense	5′-CCTTGGGCTATAAGCGGTCC-3′	
IL-1β		
Sense	5′-TGCCACCTTTTGACAGTGATG-3′	138
Antisense	5′-TGATGTGCTGCTGCGAGATT3′	
IL-6		
Sense	5′-CCGGAGAGGAGACTTCACAG-3′	134
Antisense	5′-CAGAATTGCCATTGCACAAC-3′	
TNF-α		
Sense	5′-CCCTCACACTCACAAACCAC-3′	133
Antisense	5′-ACAAGGTACAACCCATCGGC-3′	

GAPDH, glyceraldehyde-6-phosphate dehydrogenase; IL-1β, interleukin-1β; TNF-α, tumour necrosis factor α.

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