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ORIGINAL ARTICLE

Histamine Induces Microglia Activation and the Release of Proinflammatory Mediators in Rat Brain Via H_1R or H_4R

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Abstract

Histamine is a major peripheral inflammatory mediator and a neurotransmitter in the central nervous system. We have reported that histamine induces microglia activation and releases proinflammatory factors in primary cultured microglia. Whether histamine has similar effects in vivo is unknown. In the present study, we aimed to investigate the role of histamine and its receptors in the release of inflammatory mediators and activation of microglia in rat brain. We site-directed injected histamine, histamine receptor agonists or histamine receptor antagonists in the rat lateral ventricle using stereotaxic techniques. Flow cytometry was employed to determine histamine receptor expression in rat microglia. Microglia activation was assessed by Iba1 immunohistochemistry. The levels of tumour necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-10 (IL-10) were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits, TNF-α, IL-1β and IL-10 mRNA expressions were determined with Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). We found that all four types of histamine receptors were expressed in rat brain microglia. Histamine was able to induce microglia activation and subsequent production of the inflammatory factors TNF-α, IL-1β and IL-10, and these effects were partially abolished by H_1R and H_4R antagonists. However, H_2R and H_3R antagonists significantly increased production of TNF-α and IL-1β, and decreased IL-10 levels. The H_1R or H_4R agonists stimulated the production of TNF-α and IL-1β, while the H_2R or H_3R agonists increased IL-10 release. Our results demonstrate that histamine induces microglia activation and the release of both proinflammatory and anti-inflammatory factors in rat brain, thus contributing to the development of inflammation in the brain.

Keywords Microglia · Histamine · Histamine receptors · Agonist · Antagonist · Inflammatory mediators

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Introduction

The resident immune cells of CNS, microglia, play a pivotal role in the immune surveillance by avidly probing the brain in search of pathological sources such as infection, injury, and so on (Casano and Peri 2015; Graeber and Streit 2010; Kierdorf and Prinz 2017; Mrdjen et al. 2018). Increasing evidence has demonstrated that the microglia-mediated neuroinflammatory process is critically involved in the initiation and development of neurodegenerative disorders such as Parkinson's disease (PD) (Joers et al. 2017), Alzheimer's disease (AD) (Block et al. 2007; Katoh et al. 2001) and multiple sclerosis (Zrzavy et al. 2017). Neurotoxins, neuronal debris, injury, or other abnormal stimulation induces microglia activation and produces a host of inflammatory cytokines. Accumulation of these inflammatory cytokines is deleterious directly to neurons and subsequently induces further activation of microglia, which leads to a vicious cycle (Lenz and Nelson 2018; Ma et al. 2017). Therefore, inhibition of microglia M1 phenotype activation and the subsequent release of pro- inflammatory cytokines may eliminate deleterious effects of microglia and may potentially provide novel therapeutic strategies. However, the regulators and related mechanisms involved in microglia activation are not completely known.

As an endogenous biogenic amine, histamine is synthesized from L-histidine through the catalytic action of histidine decarboxylase. Mast cells and basophils are considered to be the main sources of histamine in peripheral tissues (Rocha et al. 2014). In the brain, histamine is produced mainly by histaminergic neurons, mast cells, and microglia (Chikahisa et al. 2013; Hu and Chen 2017; Rocha et al. 2016). Histamine is a ubiquitous mediator involved in a variety of physiological processes, such as regulation of pituitary hormones secretion, regulation of gastrointestinal and circulatory functions (Parsons and Ganellin 2006). Histamine also plays an important role in the CNS. Histamine can affect neurotransmission release and brain function (Shan et al. 2013, 2015). Histamine was dysregulated in neurodegenerative diseases such AD (Shan et al. 2012a, b, c) and PD (Shan et al. 2012a, b, c). In addition, histamine is a potent mediator of inflammation and a regulator of innate and adaptive immune responses. Four histamine receptors have been identified: H_1R, H_2R, H_3R and H_4R (Haas and Panula 2016). Histamine receptors are prominent in many brain regions. It has been discovered that brain histamine regulates satiety signalling, nociception, the sleep–wake cycle, motor circuits, and neuroimmune functions mainly through the four subtypes of receptors (Panula and Nuutinen 2013; Provensi et al. 2014). Increasing studies have shown that histamine played an important role in microglia activation in neurological diseases. For example, histamine could regulate activation of microglia by H_1R or H_4R in PD (Rocha et al. 2016; Zhou et al. 2019), resulting in the release of proinflammatory factors. Histamine released from mast cells (MCs) further stimulating microglia activation in AD (Theoharides et al. 2015). Histamine H1 receptor blockade prevents early microglia function, resulting in subsequent reduction in immune cell accumulation in MS (Barkauskas et al. 2015).

We have reported that histamine induce microglia activation and subsequently the release of proinflammatory factors in primary cultured microglia (Dong et al. 2014). The results suggested that histamine might participate in microglia activation and play an important role in neuroinflammation-related diseases. In the present study, we further investigated the effects of histamine and its receptors on microglia activation in vivo, and we also explored the effect of histamine on the release of inflammatory mediators in rat brain.

Materials and Methods

Reagents

Histamine was purchased from Sigma–Aldrich (St. Louis, MO, USA). H_1R agonist 2-pyridylethlamine dihydrochloride (2-pyridylethlamine), H_1R antagonist cetirizine dihydrochloride (cetirizine), H_2R agonist amthamine dihydrobromide (amthamine), H_2R antagonist ranitidine hydrochloride (ranitidine), H_3R agonist (*R*)-(−)-α-methylhistamine dihydrobromide ((*R*)-(−)-α-methylhistamine), H_3R antagonist carcinine ditrifluoroacetate (carcinine), H_4R agonist 4-methylhistamine dihydrochloride (4-methylhistamine) and H_4R antagonist A943931 dihydrochloride (A943931) were purchased from Tocris Bioscience (Bristol, UK). All the compounds were dissolved in sterile 0.9% sodium chloride. Rat TNF-α, IL-1β and IL-10 Immunoassay Kit were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Rabbit anti-Iba1 antibody was purchased from Wako Chemicals USA, Inc. Rabbit anti-H_1R and anti-H_2R antibodies were purchased from Alomone Labs (Jerusalem, Israel). Rabbit anti-H_3R antibody was purchased from Abcam (Hong Kong, China). And rabbit anti-H_4R was purchased from Santa Cruz (CA, USA). Fluorescein isothiocyanate (FITC) - conjugated mouse anti-OX-42 antibody and isotype control, phycoerythrin (PE)-conjugated goat anti-rabbit secondary antibody were purchased from BD (BD Biosciences, USA).

Animals

Male Sprague-Dawley (200–250 g) rats were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). All animals were housed in groups of five animals per cage under standard laboratory conditions with free access to food and water, a constant room temperature of 22 °C, 50–60% humidity, and a 12:12 day-night cycle. All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals

(publication no. 85–23, revised 1985) and the Guidelines for the Care and Use of Animals in Neuroscience Research by the Society for Neuroscience and approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University, NO: 14030126).

Flow Cytometry Analysis

Flow cytometry was employed to determine histamine receptor expression in rat microglia. As previously described (Dong et al. 2019), the dissociated cells from cerebral cortex tissues were incubated with rabbit anti-H_1R, anti-H_2R, anti-H_3R, anti-H_4R primary antibody or normal rabbit IgG overnight at 4 °C, then incubated with 1 μg/ml of FITC-conjugated goat anti-rabbit secondary antibody with PE-conjugated mouse anti-OX-42 antibody or isotype control (1:200) for 1 h at 37 °C. FACS Calibur flow cytometer (BD Biosciences, USA) was used to analyze the cells.

qRT-PCR

Unfixed cerebral cortex tissue was homogenized in 1 ml of Trizol reagent (Invitrogen), and RNA was isolated following the manufacturer's instructions. Reverse transcription was performed from 1 μg of total RNA for each sample using the Transcription First Strand cDNA Synthesis Kits (Roche) according to the manufacturer's instructions. Real-time PCR amplification was performed using the STEP ONE Real-time PCR Detection System (Foster City, CA) with the SYBR Green master mix (Applied Biosystems, Foster City, CA) in a final volume of 10 μl that contained 1 μl cDNA template from each sample. Primers are listed as the following: rat TNF-α forward TGCCTCAGCCTCTTCTCATT, reverse CCCATTTG GGAACTTCTCCT; rat IL-1β forward ACTATGGC AACTGTCCCTGAAC, reverse GTGCTTGGGTCCTC ATCCTG; rat IL-10 forward ACTGCTATGTTGCCTGCTCT T, reverse TCATTCTTCACCTGCTCCACT; rat GAPGH forward GGGTGTGAACCACGAGAAAT, reverse CCACAGTCTTCTGAGTGGCA. The cycling conditions were 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. The relative mRNA values were normalized to the GAPDH gene control values and calculated using the comparative cycle threshold (ΔΔCt) method.

Surgery and Compounds Administration

Following anaesthesia, rats were placed in the stereotaxic apparatus (Stoelting Instruments, USA). Guide cannulas (Plastic One) were inserted into the right lateral ventricle at the following coordinates: 1.5 mm lateral to the midline, 1.0 mm posterior to the bregma, and 3.8 mm deep (Zhou et al. 2019). The animals were allowed to recover in the animal facility for 14 days before use. Animals were handled daily to check the guide cannula and to familiarize the rats with the investigators.

To determine whether histamine can activate microglia, we administered histamine centrally by a unilateral injection in the lateral ventricle. The rats were randomly allocated to 4 groups (i.e., groups A-D) with 32 rats in each group, and investigators were blinded to the experimental treatment. Rats in groups B-D were intracerebroventricularly (i.c.v.) injected with 1 μl of histamine 20 μg/μl, 40 μg/μl and 80 μg/μl, respectively, and rats in group A (control rats) were injected with 1 μl 0.9% NaCl. Eight rats were randomly selected from each group and were sacrificed 0.5, 8, 24 and 72 h after compounds administration. To determine which histamine receptor mediated the effect of histamine on microglia activation, we intracerebroventricularly injected different H1-H4 antagonists 30 min prior to histamine. The rats were randomly allocated to six groups (group A-F) with 8 rats in each group. The rats in groups C-F were intracerebroventricularly injected with the H_1R antagonist cetirizine (10 μM), the H_2R antagonist ranitidine (10 μM), the H_3R antagonist carcinine ditrifluoroacetate (10 μM) and the H_4R antagonist A943931 (10 μM), respectively, and the rats of group A and B were injected with 0.9% NaCl. Thirty min later, the rats in groups B-F were intracerebroventricularly injected with 1 μl histamine (80 μg), and the rats in group A (control rats) were injected with 1 μl 0.9% NaCl. To determine the effect of H1-H4 agonists or antagonists on inflammatory mediator release, the H_1R agonist 2-pyridylethlamine, the H_2R agonist amthamine (100 μM), the H_3R agonist (R)-(−)-α-methylhistamine (100 μM) and the H_4R agonist 4-methylhistamine (100 μM) or the H1-H4 antagonists (10 μM) were intracerebroventricularly injected. Compounds were infused in a total volume of 1 μl using an UltraMicroPump III (World Precision Instruments Inc.) at a rate of 100 nl/min. After administration, the rat brain were collected for morphological or biochemical analyses.

Immunochemistry

Rats were anaesthetized by chloral hydrate and perfused first with 0.9% saline and then with cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and maintained in 4% paraformaldehyde overnight. The brains were cryopreserved in 30% sucrose in phosphate-buffered saline (PBS) and then stored at −70 °C until used. Free-floating sections (30 μm) encompassing the cerebral cortex were prepared using a cryostat. Tissue sections were incubated for one hour in 10% bovine serum albumin (BSA) with 0.3% Triton X-100 in 0.01 M phosphate-buffered saline, then overnight with rabbit anti-Iba1 primary antibody (1:200) at 4 °C. Tissue sections were washed and incubated in the following day with goat anti-rabbit secondary antibodies for one hour at room temperature. Immunostaining was visualized with 3, 3′-

diaminobenzidine, after which sections were counterstained with hematoxylin. The slides were scanned using a Leika 2500 (Leica Microsystems, Wetzlar, Germany) at 200 × magnification.

TNF-α, IL-1β and IL-10 Assay

The content of TNF-α, IL-1βand IL-10 in the rat brain tissue extracts was measured with a commercial ELISA kit from R&D Systems.

Statistical Analysis

All values are the means ± SEM. The significance of the difference between control and samples treated with various compounds was determined by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference test. Differences were considered significant at $P < 0.05$.

Results

Rat Brain Microglia Express Histamine H_1R, H_2R, H_3R, and H_4R

Ferreira R et al. have reported that the N9 microglia cell line expressed low levels of H_1R, H_2R, and H_3R at the mRNA level but a high level of H_4R (Ferreira et al. 2012). We previously reported that primary cultured microglia express all these histamine receptors, including H_1R, H_2R, H_3R and H_4R (Dong et al. 2014). In the present study, flow cytometry analysis showed that approximately 60, 22, 46 and 58% of microglia in cerebral cortex expressed H_1R, H_2R, H_3R, and H_4R, respectively (Fig. 1). The results indicate that H_1R, H_2R, H_3R, and H_4R are expressed in rat brain microglia.

Histamine Induces Rat Brain Microglia Activation and Inflammatory Cytokine Production

Activated microglia was detected with Iba1 immunohistochemistry. Immunohistochemical analysis at 0.5 h, 8 h, 24 h and 72 h showed that 20 μg histamine induced approximately 23, 27, 45 and 28% microglia activation, respectively; 40 μg histamine induced approximately 47, 61, 83 and 53% microglia activation, respectively; and 80 μg histamine induced 67, 82, 100 and 66% microglia activation, respectively. The study indicated that histamine at 40 and 80 μg induced activation of rat brain microglia at 0.5 h, peaked at 24 h, and decreased by 72 h (Fig. 2a). As neuroinflammation is mainly due to inflammatory factors from brain and their downstream signalling cascades, the levels of inflammatory factors were determined by ELISA, mRNA expressions were detected by qRT-PCR. As shown in Fig. 2b, histamine at 40 and 80 μg for 0.5 h increased TNF-α up to 404% and 529% that of the control, respectively, which peaked at 24 h and decreased at 72 h. IL-1β was increased by up to approximately 205% and 271% after administration of 40 and 80 μg histamine for 8 h. Likewise, the IL-10 level was significantly increased by up to approximately 589% and 845% after exposure to 40 and 80 μg histamine for 72 h, respectively. The mRNA expression levels are consistent with ELISA results (Fig. 2c). These results suggest that histamine could induce microglia activation and the release of the inflammatory factors TNF-α, IL-1β and IL-10 from rat brain.

Inhibition of Histamine-Induced TNF-α and IL-1β Production by H_1R or H_4R Antagonists

Then, antagonists for each of the four histamine receptor subtypes were used to determine which receptor subtype had an effect on histamine-induced microglia activation and proinflammatory factor release in the brain. As shown in Fig. 3, the H_1R antagonist cetirizine (10 μM) and the H_4R antagonist A943931 (10 μM) partially abolished histamine (80 μg, 24 h)-induced microglia activation (Fig. 3a) and release of the proinflammatory factors TNF-α and IL-1β (Fig. 3b). However, the H_2R antagonist ranitidine (10 μM) and the H_3R antagonist Carcinine ditrifluoroacetate (10 μM) increased histamine-induced microglia activation (differences are not statistically significant) but failed to effect histamine-induced TNF-α and IL-1β production. These results showed that the H_1R or H_4R antagonists inhibited histamine-induced microglia activation and TNF-α and IL-1β release in rat brain, suggesting that histamine may induce microglia activation and TNF-α and IL-1β release via H_1R and H_4R.

Effect of Histamine Receptor Agonists on Production of Inflammatory Cytokines in Rat Brain

To ascertain which histamine receptor subtype caused the release of inflammatory cytokines, agonists for the histamine receptor subtypes were used. The H_1R agonist 2-pyridylethlamine used at 100 μM for 8 h caused a significant increase in TNF-α and IL-1β production in the brain (Fig. 4a). The H_1R antagonist cetirizine (10 μM) partly abolished 2-pyridylethlamine (100 μM)-induced TNF-α and IL-1β release. However, 2-pyridylethlamine did not stimulate IL-10 release. In contrast, the H_2R agonist amthamine (100 μM) and the H_3R agonist (R)-(−)-α-methylhistamine (100 μM) used for 8 h failed to affect the production of TNF-α and IL-1β in rat brain (Fig. 4b,c), but after 24 h, they increased IL-10 by up to 609% and 751%, respectively. The corresponding antagonists blocked the effects. 4-methylhistamine, which actives the H_4R, was also used at 100 μM for 8 h. It increased the production of TNF-α and IL-1β. In addition, the H_4R

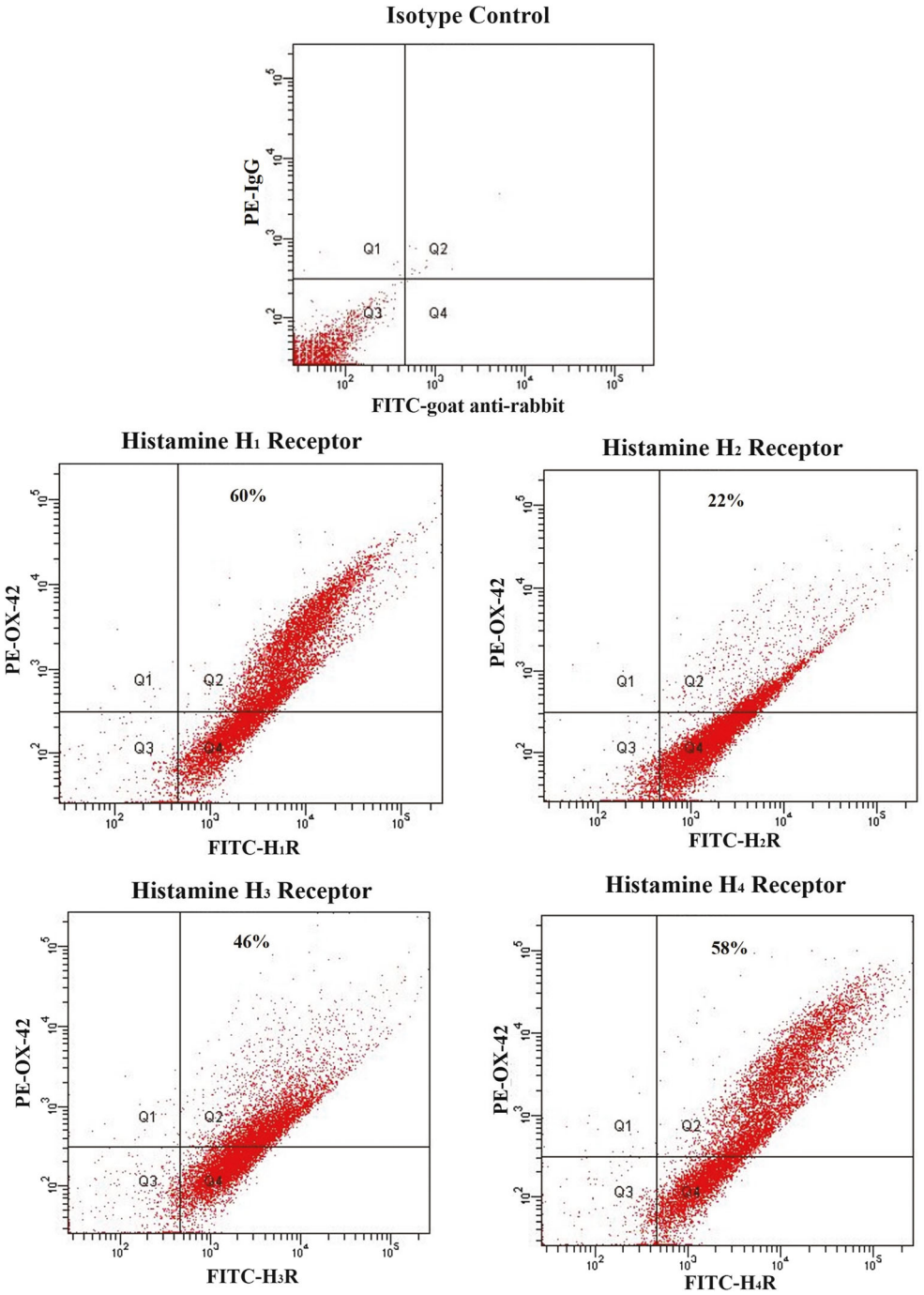

Fig. 1 Rat brain microglia express histamine H_1R, H_2R, H_3R, and H_4R. Flow cytometry analysis was used to determine the receptor expression of H_1R, H_2R, H_3R and H_4R in the rat cerebral motor cortex microglia

antagonist A943931 (10 μM) abolished 4-methylhistamine (100 μM) induced TNF-α and IL-1β release (Fig. 4d). Similarly, it had no effect on IL-10 production. qRT-PCR confirmed the ELISA results (Fig. 4e–h). These results showed that the agonists of H_1R or H_4R stimulated TNF-α and IL-1β release, while the agonist of H_2R or H_3R affected IL-10 release, suggesting that all histamine receptors are involved in the production of inflammatory factors in rat brain.

Effect of Histamine Receptor Antagonists on Production of Inflammatory Cytokines in Rat Brain

The antagonists of H_1R or H_4R inhibited histamine-induced microglia activation and TNF-α and IL-1β release from rat brain, and the agonist of H_1R or H_4R stimulated the production of TNF-α and IL-1β. Thus, we used the histamine receptor antagonists to investigate whether antagonists alone influenced

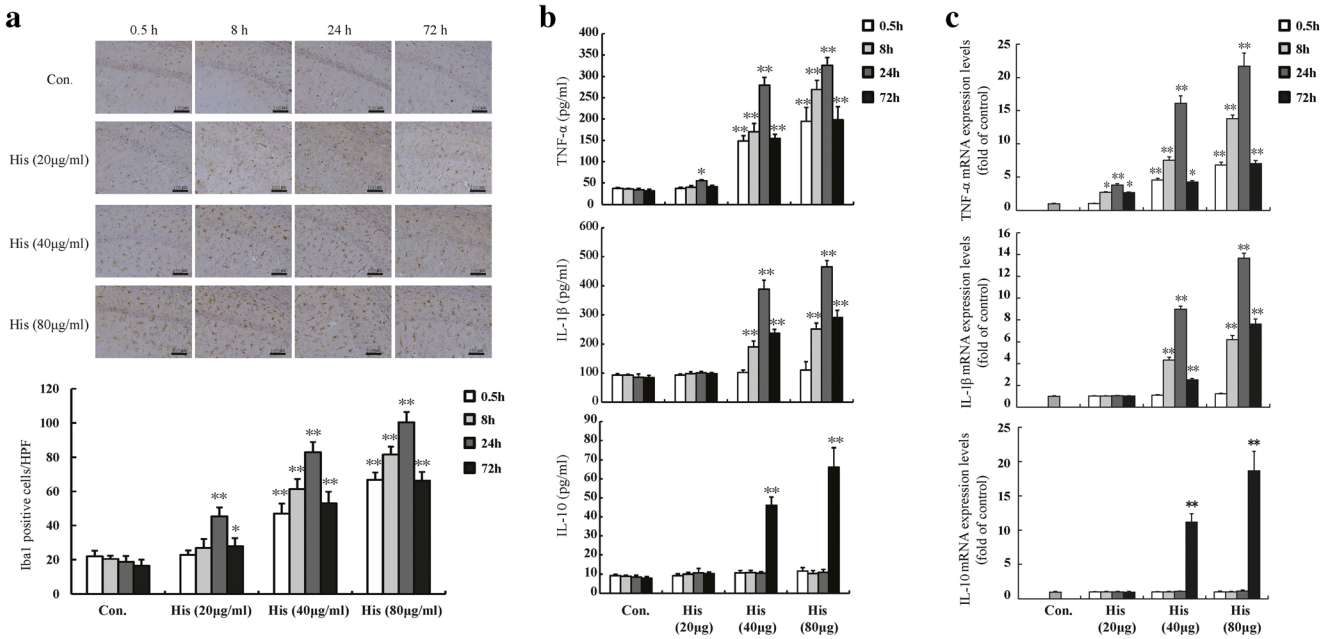

Fig. 2 Activation of rat brain microglia and TNF-α, IL-1β and IL-10 production by histamine. Rats were intracerebroventricularly injected with 0.9% NaCl or 20 μg, 40 μg or 80 μg histamine and were sacrificed 0.5, 8, 24 and 72 h after compounds administration. **a** Immunohistochemistry analysis of the activation of microglia. **b** Release of TNF-α, IL-1β and IL-10 induced by histamine. **c** TNF-α, IL-1β and IL-10 mRNA expression induced by histamine. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group. Data are presented as the mean ± SEM

production of inflammatory cytokines in the brain. As shown in Fig. 5, the H_1R antagonist cetirizine (10 μM) and the H_4R antagonist A943931 (10 μM) alone had no effect on inflammatory cytokine (i.e., TNF-α, IL-1β and IL-10) release and mRNA expressions. However, the H_2R antagonist ranitidine (10 μM) and the H_3R antagonist carcinine ditrifluoroacetate (10 μM) used alone for 8 h significantly increased protein and mRNA expressions of TNF-α and IL-1β but decreased IL-10 protein and mRNA level after 24 h. The H_1R and H_4R antagonists have a greater effect on TNF-α and IL-1β release and mRNA expressions than the H_2R and H_3R antagonists. These results further indicated that histamine was able to induce TNF-α and IL-1β release in rat brains mainly via H_1R and H_4R and induce IL-10 release via H_2R and H_3R. Activation of H1R and H_4R has proinflammatory effects and activation of H_2R and H_3R has anti-inflammatory effects.

Discussion

Histamine is now commonly acknowledged not only as an inflammatory mediator in peripheral tissues but also as a neurotransmitter in CNS. It has become an important factor in the pathogenesis of allergic and autoimmune diseases because of its proinflammatory features. Histamine exerts various effects on different cells by reason of the differential expression of histamine receptors. The discovery of new histamine sources in the brain and new histamine receptors has made it clear that histamine has an increasingly defined role in the CNS. Our in vitro study showed for the first time that all four histamine receptors are expressed in primary cultured microglia, histamine can upregulate expression of H_1R and H_4R, and histamine induces microglia activation and the subsequent release of TNF-α and IL-6 from microglia (Dong et al. 2014). Frick et al. reported that histamine can regulate microglia in vivo via the H4 receptor (Frick et al. 2016). Microglia are the residential macrophages of the CNS, which associates them with the immune system. From the early stages of development to the homeostasis of the CNS, they are involved in the biology and pathology of the CNS (Lannes et al. 2017). It has been shown that activation of microglia is an early sign that usually precedes and causes neuronal death in chronic neurodegenerative diseases (Gao et al. 2003; Minghetti 2005; Block and Hong 2005). Therefore, inhibition of microglia M1 phenotype activation and subsequent neuroinflammation may provide a prospective clinical therapy for neuroinflammation-related neurodegenerative disorders. Histamine was thought to have a dual role in neurological diseases, evidences have shown its crucial involvement in the modulation of microglia-mediated neuroinflammation (Barata-Antunes et al. 2017). Histamine modulated microglial motility and cytokine release, and it was an important modulator of microglial phagocytosis and ROS production, both components involved in the vulnerability and cell death of DA neurons in the SN in vivo (Rocha et al. 2016). A decrease in NF-κB and NADPH oxidase 2 with an increase in arginase 1 and P2Y12 receptor was induced by histamine only in the ALS in-ammatory environment, but not in the healthy microglia, together with an increase in IL-

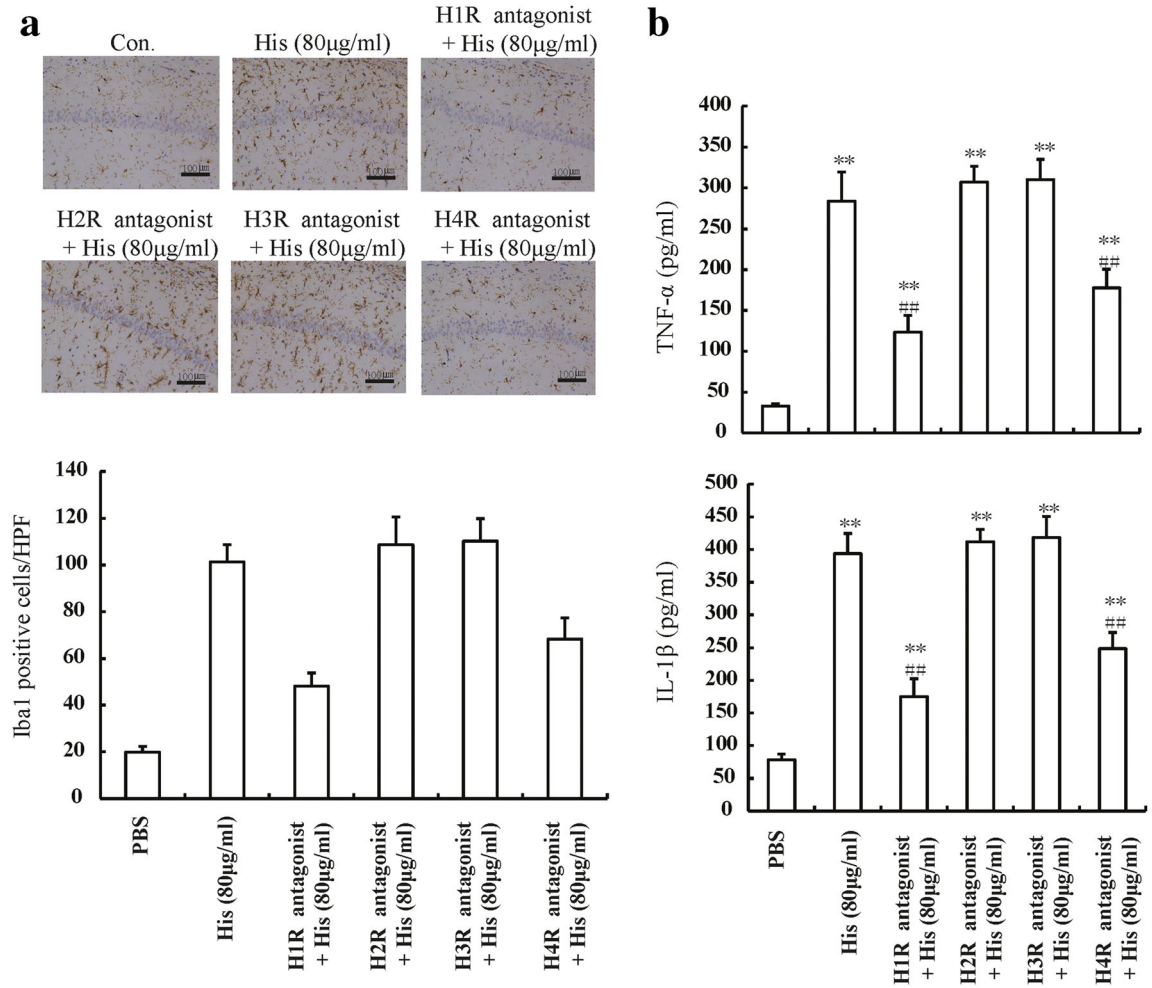

Fig. 3 Effects of HR antagonists on histamine-induced microglia activation and TNF-α and IL-1β release. **a** Immunohistochemistry analysis of the activation of microglia. H_1R antagonist cetirizine (10 μM), H_2R antagonist ranitidine (10 μM), H_3R antagonist Carcinine ditrifluoroacetate (10 μM), and H_4R antagonist A943931 (10 μM) were intracerebroventricularly injected into rats 30 min before addition of histamine (80 μg) for 24 h. **b** Release of TNF-α and IL-1β from rat brain. $^{**}P < 0.01$ vs. the control group. $^{\#\#}P < 0.01$ vs. histamine (80 μg) treatment group. Data are presented as the mean ± SEM

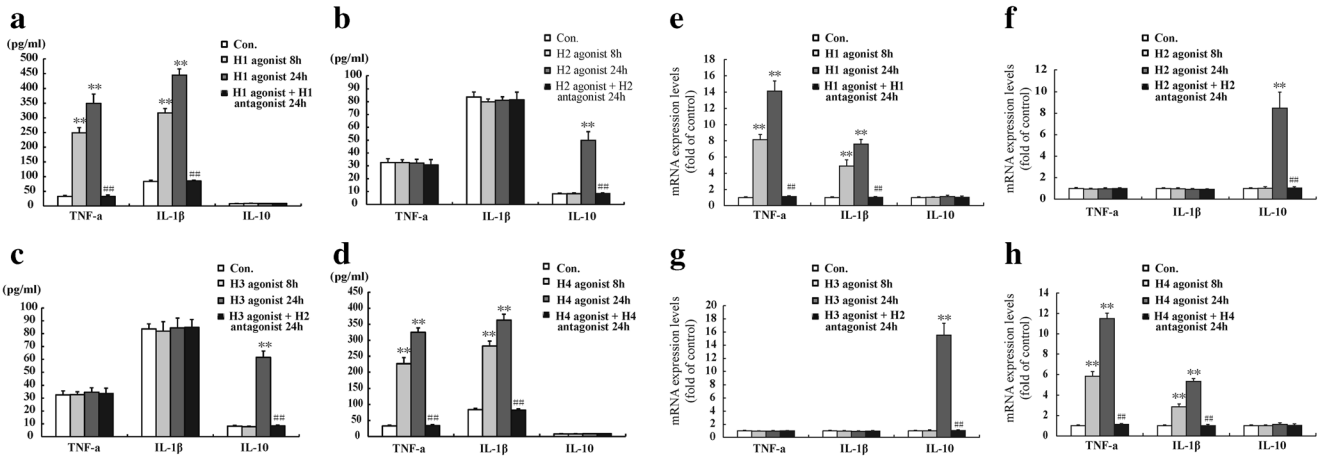

Fig. 4 Effect of agonists specific to histamine receptor subtypes (H_1R [**a**], H_2R [**b**], H_3R [**c**], and H_4R [**d**]) on TNF-α, IL-1β and IL-10 release and effect of agonists specific to histamine receptor subtypes (H_1R [**e**], H_2R [**f**], H_3R [**g**], and H_4R [**h**]) on TNF-α, IL-1β and IL-10 mRNA expression. Rats were intracerebroventricularly injected with 0.9% NaCl, H_1R agonist 2-pyridylethlamine (100 μM), H_2R agonist amthamine (100 μM), H_3R agonist(R)-(−)-α-methylhistamine (100 μM), and H_4R agonist 4-methylhistamine (100 μM). $^{**}P < 0.01$ vs. the control group. $^{\#\#}P < 0.01$ vs. the response to HR antagonist treatment groups. Data are presented as the mean ± SEM

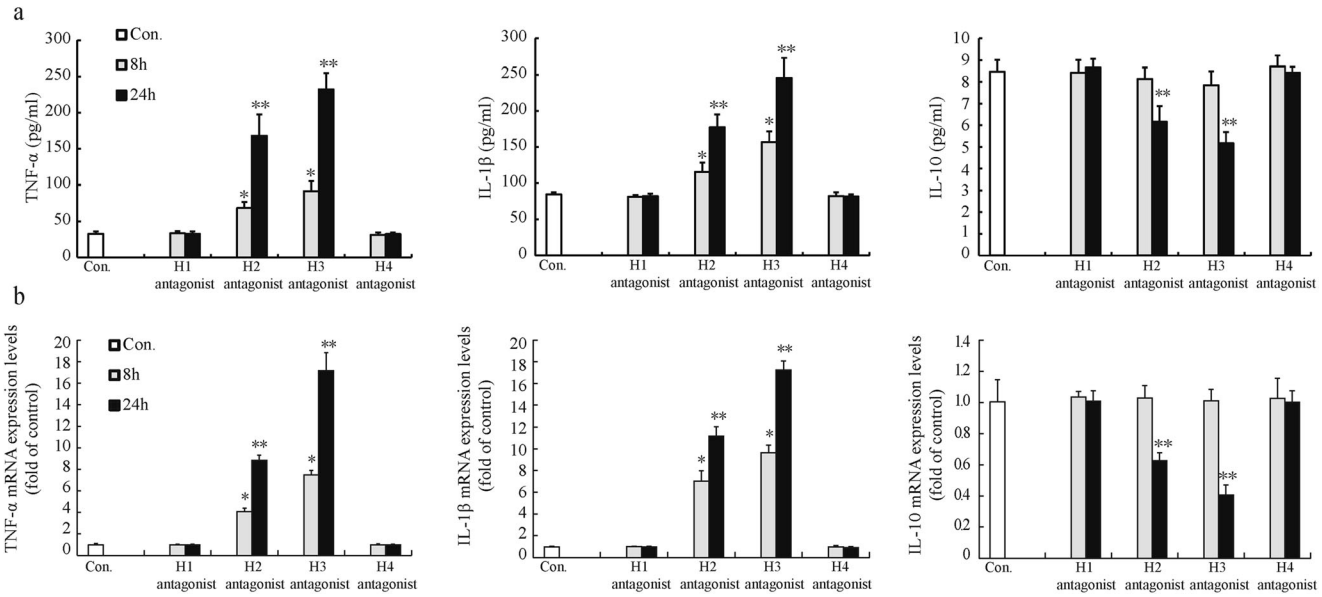

Fig. 5 Effects of HR antagonists on TNF-α, IL-1β and IL-10 release (**a**), and effects of HR antagonists on TNF-α, IL-1β and IL-10 mRNA expression (**b**). Rats were intracerebroventricularly injected with 0.9% NaCl, H_1R antagonist cetirizine (10 μM), H_2R antagonist ranitidine (10 μM), H_3R antagonist carcinine ditrifluoroacetate (10 μM), and H_4R antagonist A943931 (10 μM). $^*P < 0.05$, $^{**}P < 0.01$ vs. the control group. Data are presented as the mean ± SEM

6, IL-10, CD163, and CD206 phenotypic markers in SOD1-G93A cells (Apolloni et al. 2017). Here, we aimed to reveal the role of histamine and its receptors in microglia activation and the production of inflammatory cytokines in the rat brain. A previous study has reported that injection of 100 and 250 nmol of histamine into the substantia nigra could induce microglia activation (Vizuete et al. 2000), which may be consistent with the present findings. Proinflammatory cytokines, such as TNF-α and IL-1β, are detrimental and associated with brain diseases, especially when present at elevated concentrations (Pozzi et al. 2018). Upregulated TNF-α has been demonstrated in various neurodegenerative diseases, including acute (e.g., stroke and head trauma) and chronic (e.g., AD, Parkinson's disease, and amyotrophic lateral sclerosis) diseases (Tweedie et al. 2007). IL-1β, as an important mediator of neuronal injury, also plays diverse roles in the CNS. A large number of reports have indeed shown that IL-1β is involved in brain diseases (Girard et al. 2010; Griffin et al. 2006; Iori et al. 2016; Krakowiak et al. 2017; Lin and Edelson 2017; Murray et al. 2015), such as Alzheimer disease, multiple sclerosis, epilepsy, stroke, and even neurodevelopmental disorders, including schizophrenia and autism. Recent research has indicated that IL-1β could selectively affect cell-to-cell communication in the brain through targeting specific synaptic pathways (Han et al. 2017; Mishra et al. 2012). IL-10 plays a critical role in preventing inflammatory and autoimmune pathologies by limiting the immune response to pathogens and microbial flora (O'Garra and Vieira 2007). However, the regulation of IL-10 production by central nervous cells remains unknown. The concentration course study demonstrated as little as 40 μg of histamine could stimulate approximately half maximal microglia activation, suggesting that histamine is a potent stimulus for microglia activation.

Cytokines are a broad class of small proteins secreted by various cell types including microglia. They also have pro-inflammatory and anti-inflammatory properties. Microglia share comparative properties in reaction to acute or prolonged stimuli, and are classified into two extreme phenotypes: the classically activated M1 phenotype and the alternatively activated M2 phenotype (Yang et al. 2017). Differences between these two phenotypes range from morphological changes to alteration of representative cytokines (Hu et al. 2015). Classically activated M1 microglia release pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8, and TNF-α et al. (Orihuela et al. 2016). M2 microglia are characterized by producing anti-inflammatory cytokines such as IL-10. IL-10 played a key role in phagocytic microglia to engulf apoptotic cells (Cianciulli et al. 2015). M1 and M2 microglia are defined by their contradictory functions and distinctive biomarkers. And co-localization of representative biomarkers of both activation states in the same microglia indicated concurrence of M1 and M2 phenotype, which was found in traumatic brain injuries (TBI) (Morganti et al. 2016), amyotrophic lateral sclerosis (ALS) (Chiu et al. 2013) and spinal cord injury (SCI) (Shechter et al. 2013). In this study, we found that histamine-induced TNF-α release occurred first, followed by IL-1β release, and that IL-10 release occurred last, suggesting that TNF-α, IL-1β and

IL-10 release may by regulated by different signalling pathways in the brain.

Our additional experiments demonstrated that the antagonists of H_1R or H_4R alone failed to affect TNF-α, IL-1β and IL-10 production, and they partly blocked histamine-induced TNF-α and IL-1β release. However, H_2R or H_3R antagonists by themselves induced a significant increase in TNF-α and IL-1β release and inhibited IL-10 production but had no effect on histamine-induced TNF-α and IL-1β production. Meanwhile, the agonists of H_1R or H_4R stimulated the rat brain to produce TNF-α and IL-1β in vivo, while the H_2R or H_3R agonist affected IL-10 levels. Therefore, these data suggest that histamine appears to induce microglia activation and TNF-α and IL-1β release via a similar mechanism, which depends at least partially on activation of H_1R and H_4R, but IL-10 release is through activation of H_2R and H_3R. It is widely accepted that histamine exerts the stimulatory effects on the immune system through H_1R and exerts the inhibitory effects through H_2R (Medina et al. 1999). This concept has also been supported by a multiple sclerosis model, which showed that histamine's dual effects are due to the different pathophysiological features of the four histamine receptors. Therefore, H_1R and H_4R may be involved in exacerbating the disease, while H_2R and H_3R may be effective in ameliorating the disease (Saligrama et al. 2012). This finding may help explain the relationships between the production of inflammatory factors and histamine receptors. However, the exact mechanism requires further detailed study. The mitochondrial membrane potential and the downstream MAPKs have been demonstrated to regulate microglia activation and the release of proinflammatory factors (Akundi et al. 2005; Ciallella et al. 2005; Lund et al. 2005; Waetzig et al. 2005). Our previous results have also suggested that histamine could induce microglia activation by reducing the mitochondrial membrane potential and regulating the downstream MAPKs. Our present results suggest that effects of histamine on microglia are likely not only proinflammatory through H_1R and H_4R but also anti-inflammatory through H_2R and H_3R.

Shan (Shan et al. 2019) reported that H_4R agonist 4-methylhistamine inhibited LPS-induced secretion of IL-1β and TNF-α at concentrations of 0.1 μM and 1 μM. The H_4R antagonist JNJ77777120 significantly blocked the anti-inflammatory effects of 4-methylhistamine. All of these results come from microglia HAPI cells. We have reported that H_4R agonist 4-methylhistamine, used at 0.1 to 100 μM for 24 h, produced a concentration dependent increase in TNF-α and IL-6 release from microglia. However, H_4R antagonist A943931 (10 μM) alone failed to affect the productions of TNF-α and IL-6 in microglia (Dong et al. 2014). We also found that H_4R agonist 4- methylhistamine had no effect on LPS-induced inflammatory cytokines releases from microglia (data not shown). This discrepancy may be due to the fact that primary cultured microglia are more sensitive than cell lines. Activation of H_4 receptor alone can induce inflammatory cytokines releases from primary cultured microglia. However, it's known that LPS has a strong pro-inflammatory effect on microglia. Activation of H_4 receptor on primary cultured microglia is not enough to synergize the inflammatory effect caused by LPS on primary cultured microglia. In the present study, we found that H_4R agonist 4-methylhistamine increased the production of TNF-α and IL-1β release in rat cerebral cortex. The pro-inflammatory effect of H_4R agonists under normal condition in vivo study is consistent with our in vitro study. The role of H_4R in LPS-induced neuroinflammation in vivo need to be further invested. The Santa Cruz H_4R antibody sc M120 was used to evaluate H_4R expression on microglia in this study and our previous study (Dong et al. 2014). However, Silke reported that the H4R antibodies (Santa Cruz sc M120 and sc H110) have no specificity on H_4R protein in flow cytometric analysis and Western blot analysis. Commercially available H4R antibodies that have been validated are needed to further confirm H4R expression in microglia.

A large amount of brain histamine is contained not only in neurons but also in brain mast cells (Borriello et al. 2017; Dong et al. 2017). Mast cells, as a main source of histamine in the CNS, have been proved to play a role in the pathogenesis of the experimental autoimmune encephalomyelitis (EAE), experimental allergic neuritis, and experimental autoimmune demyelinating diseases (Elieh-Ali-Komi and Cao 2017; Yin et al. 2017). A previous study suggested that the "first responders" in brain injury are activated mast cells rather than microglia (Jin et al. 2009). Recent studies indicate that mast cells actively participated in the pathogenesis of inflammation through the release of proinflammatory mediators in vivo. We have previously reported that *corticotropin-releasing hormone* (CRH)-stimulated human mast cells (HMC-1) activate microglia and induce TNF-α and IL-6 release (Zhang et al. 2016), suggesting the importance of mast cells in microglia activation and the release of proinflammatory factors in the CNS. It has been reported that mast cell activation leads to accumulation of cerebral histamine (Biran et al. 2008), which suggests that local histamine concentration may be sufficient for microglia activation. We also reported that the released histamine from mast cells acts on microglia activation and that mast cell-microglia crosstalk contributes to neuroinflammatory disease (Dong et al. 2017). The functions of histamine receptors may be more interesting and important in neuroinflammatory-related diseases. It is technically challenging to use in vivo *experiments, such as those presented here, to certify the effect of microglia in histamine-induced inflammatory mediator release.* Therefore, a better understanding of the roles and the molecular mechanisms of histamine receptors, especially microglial histamine receptors, in activating microglia and regulating inflammatory processes in the CNS is required, and more research is needed for these areas of neuroimmunology.

Conclusion

We demonstrate that histamine induces activation of microglia in vivo, and prompts proinflammatory cytokine release via H_1R and H_4R but stimulates IL-10 release via H_2R and H_3R in the rat brain. These results suggest that histamine plays an important role in microglia activation and neuroinflammation-related diseases. Histamine H_1 and H_4 antagonists may be used as prospective clinical therapeutic tools to inhibit histamine-induced microglia activation and subsequent neuroinflammation, as well as neuroinflammation-related neurodegenerative disorders.

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Compliance with Ethical Standards

Disclosure Statement The authors declare that there are no conflicts of interest regarding the publication of this paper.

Statement on the Welfare of Animals This article does not contain any studies with human participants performed by any of the authors.

All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication no. 85–23, revised 1985) and the Guidelines for the Care and Use of Animals in Neuroscience Research by the Society for Neuroscience and approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University, NO: 14030126).

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