Original Paper

Activation of Microglia by Histamine and Substance P

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Key Words
Microglial activation • Histamine • Substance P • CNS immune inflammation

Abstract
Background: Activated microglia perform many of the immune effector functions typically associated with macrophages. However, the regulators involved in microglial activation are not well defined. Because microglia play a pivotal role in immune surveillance of the CNS, we studied the effect of the neuromediators histamine and substance P on microglia. ***Methods:*** The induction of microglial activation by histamine and substance P was examined using primary cultured microglia. Fluorescent images were acquired with a confocal microscope. The levels of TNF-α and IL-6 were measured with a commercial ELISA kit. Intracellular reactive oxygen species (ROS) levels were determined by dichlorodihydrofluorescein oxidation. The mitochondrial membrane potential was assessed with the MitoProbe™ JC-1 assay kit. ***Results:*** We found that the neuromediators histamine and substance P were able to stimulate microglial activation and the subsequent production of ROS and proinflammatory factors TNF-α and IL-6. These effects were partially abolished by antagonists of the histamine receptors H1 and H4 and of the substance P receptors NK-1, NK-2 and NK-3. Histamine induced mitochondrial membrane depolarization in microglia. ***Conclusions:*** These results indicate that the neuromediators histamine and SP can trigger microglial activation and release of pro-inflammatory factors from microglia, thus contributing to the development of microglia-mediated inflammation in the brain.

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769

Zhu et al.: Microglia Activation by Histamine and SP

Introduction

Microglia, the resident immune cells in the brain, play a pivotal role in immune surveillance of the central nervous system (CNS). Consequently, these cells are likely to play an important role in determining either the development of protective immune responses or the progression of damaging inflammation during CNS diseases [1-4]. Microglia respond to traumatic injury and the presence of infectious organisms by migrating to the site of injury, where they proliferate. When subjected to abnormal stimulation, such as neurotoxins, neuronal debris, or injury, microglia become gradually activated and produce a host of factors, including tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), interleukin-6 (IL-6), nitric oxide (NO), and reactive oxygen species (ROS). Accumulation of these proinflammatory and cytotoxic factors is directly deleterious to neurons and induces further activation of microglia, resulting in a vicious cycle [5, 6]. Thus, the inhibition of microglial activation and subsequent inflammatory processes may identify novel therapeutic strategies for eliminating the deleterious effects of microglia [3]. However, the regulators and mechanisms involved in microglial activation are not fully known.

Histamine is a potent mediator of inflammation and a regulator of the innate and adaptive immune responses. Histamine induces local inflammation reactions either directly by acting on target cells or indirectly by activating other humoral and/or cellular effector systems. Histamine released from mast cells can promote Th1 and Th2 cell activation [7]. Four histamine receptors have been identified: H_1, H_2, H_3 and H_4 [8-11]. Substance P (SP) is widely distributed in the central and peripheral nervous systems of vertebrates [12]. SP enhances lymphocyte proliferation, immunoglobulin production, and cytokine secretion from lymphocytes, monocytes, macrophages, and mast cells. The SP-induced release of inflammatory mediators, such as cytokines, oxygen radicals, arachidonic acid derivatives, and histamine, potentiates tissue injury and stimulates further leukocyte recruitment, thereby amplifying the inflammatory response [13]. SP can also increase vascular permeability, thus facilitating the development of an inflammatory response [14]. SP is an important proinflammatory neuropeptide that functions as an immunoneuromodulator in the brain [15, 16]. This was demonstrated by inhibiting the SP-SP receptor interactions *in vivo*, which diminished the inflammatory responses [17], and by using mice genetically devoid of SP-SP receptor interactions [18]. Central to the ability of SP to augment inflammation is its role in modulating the function of myeloid cells, such as macrophages and dendritic cells, via SP-specific neurokinin (NK) receptors [19, 20]. SP is a ligand for the NK-1 receptor and also displays some affinity for the NK-2 receptor [21]. As such, substance P has the potential to interact with a number of CNS cell types that bear substance P receptors, including neurons, astrocytes and oligodendrocytes. It has also been reported that microglia expresses the SP gene and NK-1 receptor [22]. However, little is known about the role of the neuromediators histamine and SP in brain microglia activation.

We have previously reported that the mast cell tryptase induces microglial activation and release of inflammatory factors [23]. In this study, we investigate whether the neuromediators histamine and SP can affect microglial activation and the subsequent production of proinflammatory factors.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were purchased from Gibco-BRL (Grand Island, NY, USA). Histamine, SP, and 3-(4, 5-dimethylthiazol-2-yl)-2-,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The H_1R antagonist cetirizine dihydrochloride (cetirizine), H_2R antagonist ranitidine hydrochloride (ranitidine), H_3R antagonist carcinine ditrifluoroacetate (carcinine), H_4R antagonist A943931 dihydrochloride (A943931), RP 67580 (a potent and selective tachykinin NK_1 receptor antagonist), GR 159897 (a potent and selective non-peptide neurokinin

NK$_2$ receptor antagonist) and SB 218795 (a potent, selective and competitive non-peptide NK$_3$ receptor antagonist) were purchased from Tocris Bioscience (Bristol, UK). The mouse anti-rat ED8 (anti-CD11b/CD18) monoclonal antibody (a marker for complement receptor 3 of activated microglia) was purchased from AbD Serotec (Raleigh, NC, USA). Fluoroshield mounting medium with 4,6-diamidino-2-phenylindole (DAPI) was purchased from Abcam (HK, China). The Rat IL-6 Immunoassay Kit and Rat TNF-α Immunoassay Kit were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). The LIVE green reactive oxygen species detection kit and MitoProbe™ JC-1 assay kit were purchased from Molecular Probes, Invitrogen (Carlsbad, CA, USA).

Microglia-enriched cultures

Rat primary microglial cells were prepared according to a previously described protocol with slight modifications [24]. Briefly, tissues from whole brains of postnatal (P1–P2) Sprague–Dawley rats were triturated, and the cells were then plated on poly-D-lysine precoated cell culture flasks in DMEM containing 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO$_2$/95% air. After reaching a confluent monolayer of glial cells (10–14 days), microglia were separated from astrocytes by shaking off for 5 h at 100 r.p.m. and replated on 24-well culture plates at a density of 10^5 cells/cm^2. After plating the microglia-enriched population for 24 h, the cells were treated with lipopolysaccharide (LPS), histamine or substance P (SP) alone. The enriched microglia culture was >98% pure, as determined by OX-42-IR.

Cell viability assay

Cell viability was measured by the Thiazolyl blue (MTT) method. Briefly, cells were collected and seeded in 96-well plates at a density of 10^5 cells/cm^2. After incubation for 48 h, the cells were exposed to fresh medium containing LPS (0.01 μg/ml) or various concentrations (0.001, 0.01, 0.1 and 1 μg/ml) of histamine or SP at 37°C. LPS was used as a positive control for microglial activation. After incubation for up to 24 h, 20 μL MTT tetrazolium salt (dissolved in Hank's balanced salt solution at a final concentration of 5 mg/mL) was added to each well, and the cells were placed in a CO$_2$ incubator for 4 h. Finally, the medium was aspirated from each well, and 150 μL of DMSO was added to dissolve the formazan crystals. The absorbance of each well was measured using a Dynatech MR5000 plate counter at test and reference wavelengths of 570 and 630 nm, respectively.

Immunofluorescence

To determine the effect of histamine and SP on microglial activation, cells were fixed with 4% paraformaldehyde for 30 min. Nonspecific binding was then blocked by incubating the cells in a solution of 5% BSA and 0.1% Triton X-100 for 1 h at room temperature. The microglia were incubated with the ED8 monoclonal antibody (1:300), which recognizes complement receptor 3 (CD11b/CD18), overnight at 4°C. After three washes with PBS, the microglia were incubated with a PE-conjugated secondary antibody (1:200), and nuclei were stained with DAPI. After three washes in PBS, the cells were smeared on glass slides, and coverslips were placed and sealed with nail polish. Fluorescent images were acquired by using a confocal microscope.

TNF-α and IL-6 assays

The amounts of TNF-α and IL-6 in the culture medium were measured with commercial ELISA kits from R&D Systems.

Intracellular reactive oxygen species assay

The production of intracellular reactive oxygen species (ROS) was measured by DCFH oxidation. The DCFH-DA reagent passively enters cells, where it is de-acetylated by an esterase to produce the nonfluorescent DCFH. Inside the cell, DCFH reacts with ROS to form DCF, the fluorescent product. For this assay, 10 mM DCFH-DA was dissolved in methanol and diluted 500-fold in HBSS, to give a 20 μM concentration of DCFH-DA. Enriched-microglia cultures, seeded (5×10^4) in 96-well plates, were then exposed to DCFH-DA for 1 h, followed by a 2-h treatment with HBSS containing several concentrations of LPS, histamine or substance P. After incubation, the fluorescent signal from dichlorofluorescein (DCF; excitation 495 nm, emission 529 nm) was registered every 2 min, for up to 20 min of incubation, in a Perkin-Elmer LS-5B Luminescence

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771

Zhu et al.: Microglia Activation by Histamine and SP

Spectrometer (Perkin-Elmer, Oak Brook, IL, USA) [25, 26]. To calculate the amount of intracellular ROS produced, the mean signal of the control group was subtracted from that of each treatment group. Four independent sets of samples were analyzed.

Measurement of mitochondrial membrane potential in microglia

The mitochondrial membrane potential ($\Delta\Psi_m$) of microglia was assessed with the MitoProbe™ JC-1 Assay Kit (Molecular Probes, Invitrogen). JC-1 exhibits potential-dependent accumulation in mitochondria, resulting in a fluorescence emission shift from green (~529 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. The potential-sensitive color shift is due to the concentration-dependent formation of red fluorescent J-aggregates. Microglia, suspended in 1 ml PBS at approximately 1×10^6 cells/ml, were incubated with 2 μM of JC-1 for 15 minutes at 37°C. The cells were washed and resuspended in 500 μl PBS and then analyzed on a flow cytometer with 488 nm excitation using emission filters appropriate for Alexa Fluor 488 dye and R-phycoerythrin. A decrease in the red/green fluorescence intensity ratio was interpreted as a loss of $\Delta\Psi_m$, whereas an increase in the ratio was interpreted as a gain in $\Delta\Psi_m$.

Statistical Analysis

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

Results

Histamine and SP induced microglial activation

Cell survival, measured by 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide (MTT) analysis, revealed that a 24-h incubation with LPS (0.01 μg/ml) or various concentrations (0.001, 0.01, 0.1 and 1 μg/ml) of histamine or SP had no effect on microglia viability (date not shown).

Activated microglia were detected with the monoclonal antibody ED8, which recognizes complement receptor 3 (CR3). LPS (0.01 μg/ml) was used as a positive control for microglial activation. Upon incubation with LPS (0.01 μg/ml), histamine (0.1 μg/ml) or SP (0.1 μg/ml) for 24 hours, the ED8-positive expression (in red) was remarkably upregulated (Fig. 1). These results suggest that the neuromediators histamine and SP could activate microglia.

Histamine and SP induced TNF-α release from microglia

As microglia-mediated neuroinflammation is mainly due to an excess of proinflammatory and cytotoxic factors from activated microglia and theses factors' downstream signaling cascades, the levels of proinflammatory factors were determined. As shown in Fig. 2, incubation with LPS (0.01 μg/ml) for 24 h significantly increased the production of TNF-α (P<0.01 vs. control). Incubation with histamine at 0.001, 0.01, 0.1 and 1 μg/ml for 24 h produced a concentration-dependent increase in TNF-α levels to 572%, 965%, 1637% and 3413% of the control, respectively. Similarly, SP enhanced TNF-α production in microglia to 765%, 1374% and 2155% of the control at concentrations of 0.01, 0.1 and 1 μg/ml, respectively. Exposure to LPS (0.01 μg/ml) or histamine (0.1 μg/ml) for 30 min, 2 h, 6 h and 24 h enhanced the production of TNF-α. SP (0.1 μg/ml) exerted similar effects on TNF-α production (Fig. 3). These results indicate that histamine and SP could induce TNF-α release from microglia.

Histamine and SP induced IL-6 release from microglia

Incubation with LPS (0.01 μg/ml) for 24 h significantly enhanced the microglial production of IL-6 to 727% of the control (P<0.01). The IL-6 level was also significantly elevated, to 466% and 548% of the control, after exposure to histamine at 0.1 and 1 μg/ml, respectively. However, lower concentrations of histamine (0.001 and 0.01 μg/ml) increased the IL-6 level but not in a statistically significant way compared with the control. SP also

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772

Zhu et al.: Microglia Activation by Histamine and SP

Fig. 1. Histamine and SP induced microglial activation. Primary microglia were treated with LPS (0.01 µg/ml), histamine (0.1 µg/ml) or SP (0.1 µg/ml) for 24 hours. LPS was used as a positive control for microglial activation. The cells were stained with the ED8 antibody and observed by confocal microscopy, revealing an upregulation of ED8-positive expression (red) in activated microglia. The blue staining represents DAPI. Scale bar: 50 µm.
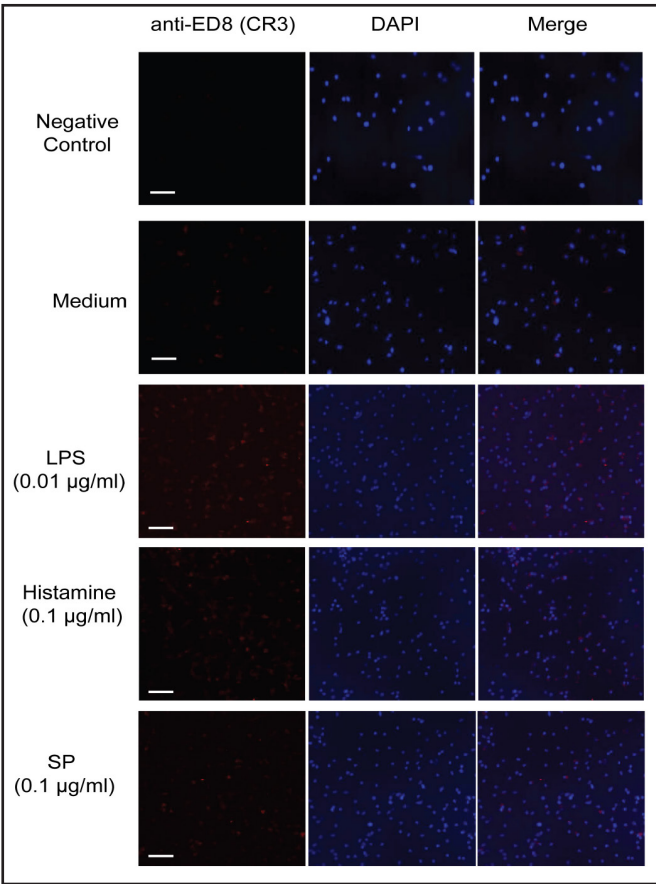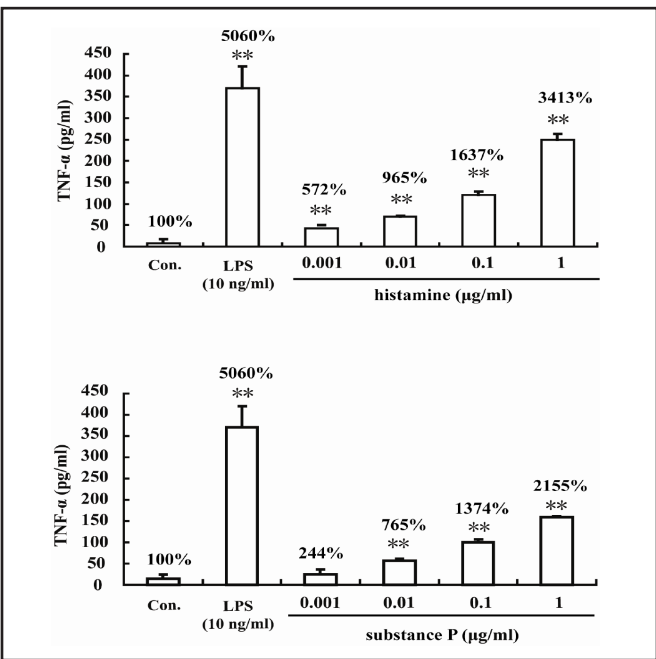

Fig. 2. Effects of histamine (0.001, 0.01, 0.1 and 1 µg/ml) and SP (0.001, 0.01, 0.1 and 1 µg/ml) on TNF-α release from microglia. LPS (0.01 µg/ml) was used as a positive control for microglial activation. $^{**}P < 0.01$ *vs.* the control group. The data are presented as the mean ± SEM of three independent experiments.

enhanced IL-6 production, with a minimum effective dose of 0.1 µg/ml (Fig. 4). Exposure to LPS (0.01 µg/ml) and histamine (0.1 µg/ml) for 30 min, 2 h, 6 h and 24 h increased the production of IL-6, and SP (0.1 µg/ml) had similar effects on IL-6 production (Fig. 5). These results indicate that histamine and SP could induce the production of IL-6 in microglia.
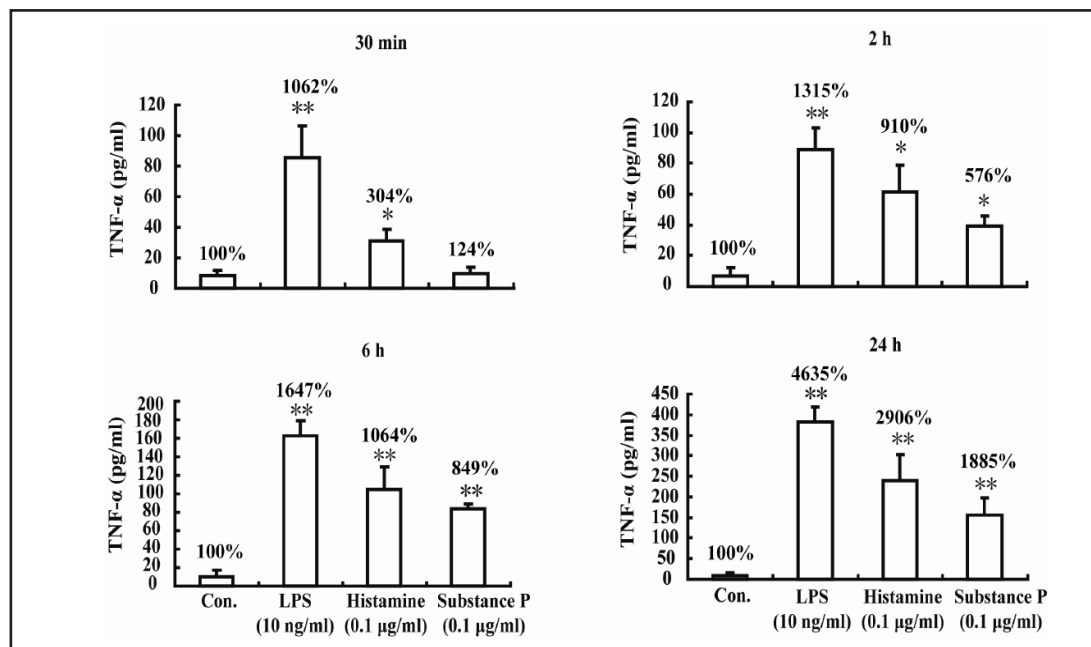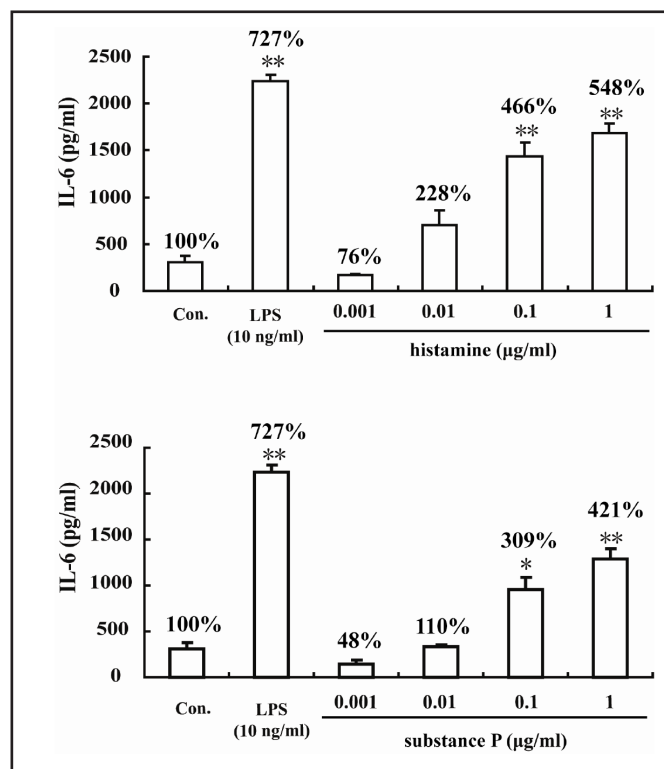
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773

Zhu et al.: Microglia Activation by Histamine and SP

Fig. 3. Effects of exposure to histamine (0.1 µg/ml) and SP (0.1 µg/ml) for 30 min, 2 h, 6 h and 24 h on TNF-α release from microglia. LPS (0.01 µg/ml) was used as a positive control for microglial activation. $^*P < 0.05$, $^{**}P < 0.01$ *vs.* the control group. The data are presented as the mean ± SEM of three independent experiments.

Fig. 4. Effects of histamine (0.001, 0.01, 0.1 and 1 µg/ml) and SP (0.001, 0.01, 0.1 and 1 µg/ml) on IL-6 release from microglia. LPS (0.01 µg/ml) was used as a positive control for microglial activation. $^*P < 0.05$, $^{**}P < 0.01$ *vs.* the control group. The data are presented as the mean ± SEM of three independent experiments.

Histamine and SP induced ROS production in microglia

The production of ROS is another important indicator of microglial activation. Therefore, the effects of different treatments on the ROS levels were examined by fluorometry. As demonstrated in Fig. 6, the incubation with LPS (0.01 µg/ml) for 2 h induced the production

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774

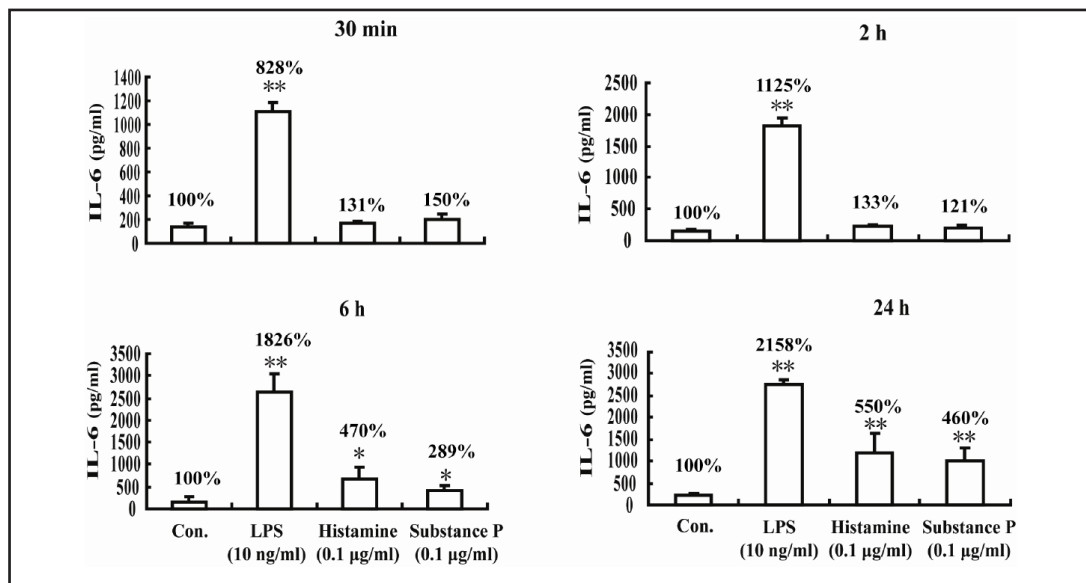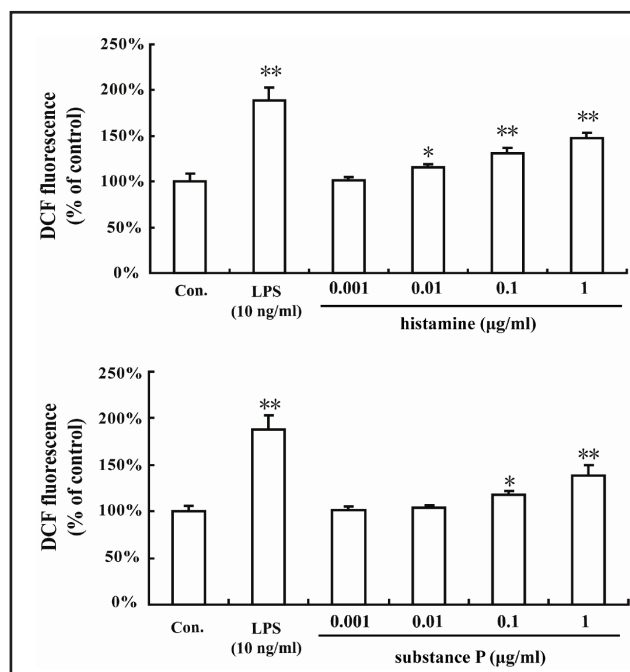Zhu et al.: Microglia Activation by Histamine and SP

Fig. 5. Effects of exposure to histamine (0.1 µg/ml) and SP (0.1 µg/ml) for 30 min, 2 h, 6 h and 24 h on IL-6 release from microglia. LPS (0.01 µg/ml) was used as a positive control for microglial activation. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the control group. The data are presented as the mean ± SEM of three independent experiments.

Fig. 6. Effects of histamine (0.001, 0.01, 0.1 and 1 µg/ml) and SP (0.001, 0.01, 0.1 and 1 µg/ml) on ROS production in microglia. The quantification of DCFH-DA fluorescence intensity is shown. LPS (0.01 µg/ml) was used as a positive control for microglial activation. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* the control group. The data are presented as the mean ± SEM of four independent experiments.

of ROS to 188% of the control level (P<0.01 vs. control). A 2-h incubation with histamine at 0.01, 0.1 and 1 µg/ml produced a concentration-dependent increase in the ROS level, to 115%, 131% and 148% of the control, respectively. Similarly, SP increased ROS production in microglia to 118% and 138% of the control at concentrations of 0.1 and 1 µg/ml, respectively.

The effect of receptor antagonists on histamine- and SP-induced inflammatory factors and ROS release

We next examined the effects of antagonists of specific histamine receptors and the tachykinin receptor on inflammatory factors induced by histamine or SP and on ROS release from microglia. As shown in Fig. 7A, the H_1R antagonist Cetirizine (10 µM) and H_4R
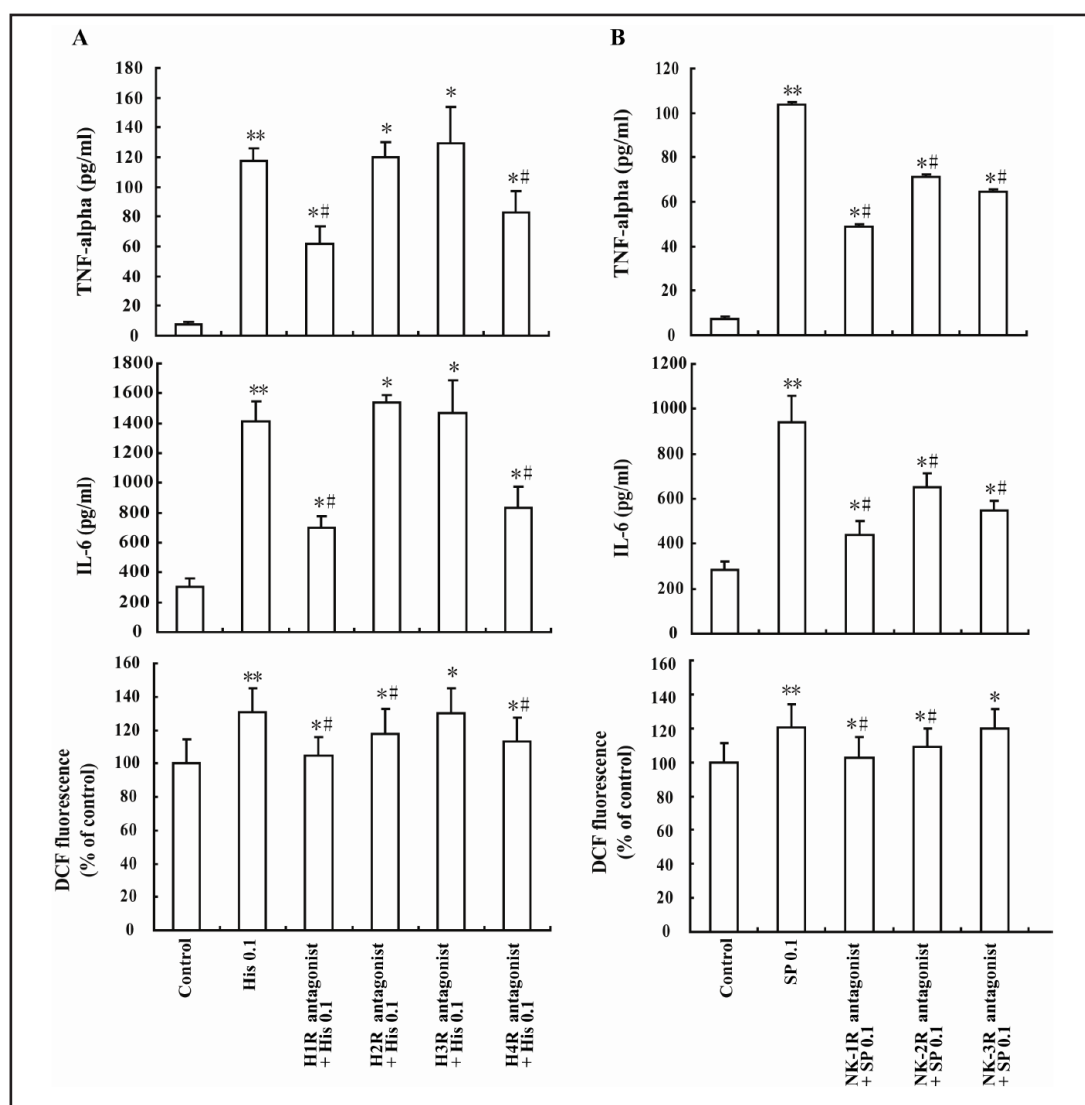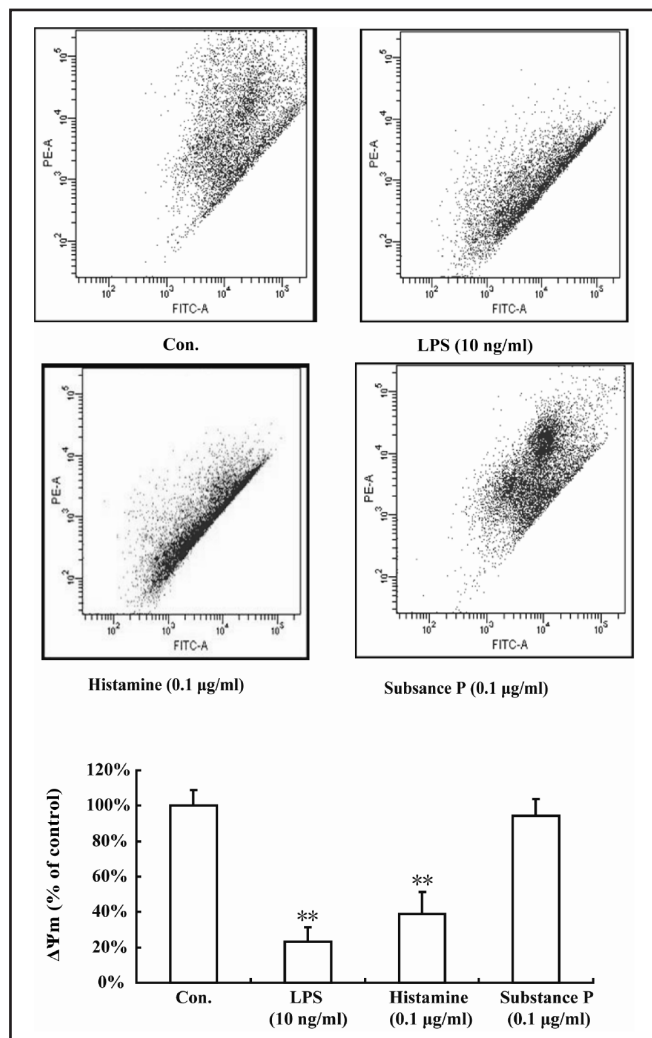
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775

Zhu et al.: Microglia Activation by Histamine and SP

Fig. 7. Effects of receptor antagonists on histamine- and SP-induced inflammatory factors and ROS release. (A) The 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 added to microglia 30 min before treatment with histamine (0.1 μg/ml) for 24 h. (B) The NK-1R antagonist RP67580 (10 μM), NK-2R antagonist GR159897 (3 μM) and NK-3R antagonist SB 218795 (3 μM) were added to microglia 30 min before treatment with SP (0.1 μg/ml) for 24 h. $^*P < 0.05$, $^{**}P < 0.01$ *vs.* the control group. $^\#P < 0.05$ *vs.* the histamine (His) or substance P (SP) (0.1 μg/ml) treatment groups. The data are presented as the mean ±SEM of four independent experiments.

antagonist A943931 (10 μM) partially abolished the histamine (0.1 μg/ml)-induced release of TNF-α and IL-6 from microglia. In contrast, the H_2R antagonist Ranitidine (10 μM) and H_3R antagonist Carcinine ditrifluoroacetate (10 μM) had little effect on histamine-induced TNF-α and IL-6 production. However, the antagonists of H_1R, H_2R and H_4R could partially abolish histamine-induced ROS production. In this study, we also found that the selective NK-1 receptor antagonist RP67580 (10 μM), selective NK-2 receptor antagonist GR159897 (3 μM) and NK-3 receptor antagonist SB 218795 (3 μM) could partially abolished the SP (0.1 μg/ml)-induced release of TNF-α and IL-6 from microglia. The NK-1 and NK-2 receptor antagonists could also partially abolish SP-induced ROS production (Fig. 7B). These results indicate that histamine was able to induce the release of inflammatory factors from microglia

Fig. 8. Effects of histamine (0.1 µg/ml) and SP (0.1 µg/ml) on mitochondrial depolarization in microglia. The graph shows quantification of the mitochondrial membrane potential, expressed as the fluorescence intensity ratio of J-aggregates to JC-1 monomers (red:green). LPS (0.01 µg/ml) was used as a positive control for microglial activation. $^{**}P < 0.01$ *vs.* the control group. The data are presented as the mean ± SEM of three independent experiments.

primarily via H_1R and H_4R, whereas SP induced inflammatory factor release from microglia not only through the NK-1 receptor but also the NK-2 and NK-3 receptors.

Histamine induced the loss of mitochondrial membrane potential in microglia

As changes in the mitochondrial membrane potential ($\Delta\Psi_m$) have been shown to be involved in microglial activation and production of proinflammatory factors, the molecular probe JC-1 was used to detect the effect of histamine and SP on variations in the microglial mitochondrial membrane potential. As shown in Fig. 8, after exposure to LPS (0.01 µg/ml) for 30 min, most microglia displayed a loss or collapse of $\Delta\Psi_m$, indicated by a fluorescence shift of JC-1 from red-orange to greenish-yellow. Similarly, incubation with histamine (0.1 µg/ml) for 30 min reduced the $\Delta\Psi_m$ of microglia. However, exposure to SP (0.1 µg/ml) for 30 min to 24 h caused no change in the $\Delta\Psi_m$ of microglia. These data indicate that histamine could induce mitochondrial membrane depolarization in microglia, whereas SP had no effect on the microglial $\Delta\Psi_m$.

Discussion

Microglial activation has been shown to be an early sign that often precedes and triggers neuronal death in chronic neurodegenerative diseases [3, 5, 27]. A key proinflammatory protein synthesized and released by activated microglia is TNF-α [28]. The presence of

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777

Zhu et al.: Microglia Activation by Histamine and SP

elevated levels of TNF-α has been documented in many forms of neurodegenerative disease of both chronic (e.g., AD, Parkinson's disease, and amyotrophic lateral sclerosis) and acute (e.g., stroke and head trauma) types [29]. A recent study highlighted the crucial role of IL-6, one of the proinflammatory proteins released by activated microglia, within the CNS [30]. In the CNS, IL-6 can trigger inflammation-related cellular responses, including neurogenesis, gliogenesis, cell growth, cell survival, myelination and demyelination [31-33]. IL-6 is normally expressed at relatively low levels in the brain, but it is elevated in the cerebral spinal fluid and brain homogenates in the presence of brain injury or inflammation [34]. Therefore, the inhibition of microglial activation and subsequent neuroinflammation may offer a therapeutic benefit for neuroinflammation-related neurodegenerative disorders. The factors responsible for the overactivation of microglia are largely undefined. Here, we demonstrate that the neuromediators histamine and SP can induce microglial activation and release of inflammatory factors.

A significant amount of brain histamine is contained not only in neurons but also in brain mast cells [35, 36]. The results presented in this study demonstrate that the interaction between microglia and histamine may modulate the activation of microglia and the production of IL-6, TNF-α and ROS in the activated microglia. However, the current understanding of histamine's effect on TNF-α and IL-6 release from microglia is limited. We found that as little as 10 ng/ml of histamine was able to induce approximate increases of 5.7-fold in TNF-α release and 3.6-fold in IL-6 release from microglia, indicating that histamine is a potent stimulus of microglial activation. However, histamine did not induce the release of TNF-α in the N9 microglia cell line [37], possibly because the microglia cell line is less sensitive than primary cultured microglia. In addition, the N9 microglia cell line is mouse-derived, and there are differences in microglial activation between rat-derived and mouse-derived cells [38]. In the present study, we also found that histamine was able to induce enhanced ROS production. ROS are important molecules secreted by microglia [39]. A considerable amount of evidence suggests that oxidative stress induced by microglia-derived ROS is a major contributor to neurodegeneration [40, 41]; this process involves activation of the p38 and JNK MAPKs through the upstream MKK signaling. The mitochondrial membrane potential and the downstream MAPKs have been shown to regulate microglial activation and the production of proinflammatory factors from microglia [42-45]. In the present study, we demonstrated that histamine induces mitochondrial membrane depolarization in microglia. These results suggest that histamine can induce microglial activation by reducing the mitochondrial membrane potential and regulating the downstream MAPKs.

It is now widely accepted that the neuropeptide SP can play an important role in augmenting inflammatory responses at peripheral sites. In particular, it has been demonstrated that SP can promote the immune functions of peripheral macrophages [46]. Binding of this neuropeptide to SP receptors (NK-1R) augments the production of proinflammatory monokines such as IL-1, IL-6, and TNF-α [47, 48]. Furthermore, SP can induce a respiratory burst in macrophages, resulting in the production of reactive oxygen intermediates [49]. However, much less is known about the role of this neuropeptide in the initiation and/or maintenance of inflammatory responses at the site of its most ubiquitous distribution, the CNS. Luber-Narod reported that substance P enhances the secretion of tumor necrosis factor-alpha from neuroglial cells stimulated with lipopolysaccharide [50]. In the present study, we found that SP could induce the release of TNF-α and IL-6 from microglia. However, SP had no effect on the microglial mitochondrial membrane potential. Lai examined the expression of substance P and its receptor in human fetal brain microglia [51]. The ability of substance P to modulate immune cell function depends on its interaction with its receptor neurokinin-1, which is expressed in a variety of immune cells, including microglia [52]. We also found that SP could increase ROS generation in microglia; we therefore propose that SP induces the production of proinflammatory factors in microglia through elevated levels of mitochondria-derived ROS and phosphorylation by downstream MAPKs. Substance P was suggested to activate NK-1, but this has yet to be demonstrated, and a contribution of the tachykinin receptors NK-2 and NK-3 cannot be ruled out. In this study,

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778

Zhu et al.: Microglia Activation by Histamine and SP

we found that antagonists of NK-1R, NK-2R or NK-3R could partially abolish the release of SP-induced proinflammatory factors. These results suggest that not only the NK_1 receptor but the NK_2 and NK_3 receptors are also involved in SP-induced microglial activation. However, the induction of microglial ROS production by SP occurs primarily through the NK-1 and NK-2 receptors. Understanding the detailed mechanism of SP-induced microglial activation requires further study.

Our subsequent experiments showed that histamine appears to induce the release of TNF-α and IL-6 from microglia via a similar mechanism, which depends at least partially on the activation of H_1R and H_4R. It is generally thought that the stimulatory effects of histamine on the immune system are mediated by H_1R, whereas the inhibitory effects most frequently involve H_2R [53]. This concept has also been supported by a model of multiple sclerosis (MS), which showed that the dual effects of histamine are due to different pathophysiological features of four histamine receptors. Thus, H_1R and H_4R may participate in exacerbating the disease, and H_2R and H_3R may be effective in ameliorating the disease [54]. These results indicate that histamine most likely exerts a proinflammatory effect on microglia via H_1R and H_4R. However, we found that histamine induces ROS production mainly through H_1R, H_2R and H_4R; thus, this might involve a different mechanism from that of proinflammatory factor release.

In summary, we have shown here that the neuromediators histamine and SP can induce microglial activation and the subsequent production of proinflammatory factors. These results suggest that histamine and SP might play an important role in microglial activation and neuroinflammation-related diseases and further our understanding of the regulators and mechanisms involved in the activation of microglia.

Disclosure Statement

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

Acknowledgements

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780

Zhu et al.: Microglia Activation by Histamine and SP

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