

Research Article

Cannabinoid CB2 Receptor Mediates Nicotine-Induced Anti-Inflammation in N9 Microglial Cells Exposed to β Amyloid via Protein Kinase C

Ji Jia,¹ Jie Peng,¹ Zhaoju Li,¹ Youping Wu,¹ Qunlin Wu,¹ Weifeng Tu,¹ and Mingchun Wu²

¹Department of Anesthesiology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou 510010, China

²Department of Anesthesiology, Wuhan General Hospital of Guangzhou Military Command, Wuhan 430070, China

Correspondence should be addressed to Weifeng Tu; wftuyx02@163.com and Mingchun Wu; wumingchun007@126.com

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Background. Reducing β amyloid- ($A\beta$ -) induced microglial activation is considered to be effective in treating Alzheimer's disease (AD). Nicotine attenuates $A\beta$ -induced microglial activation; the mechanism, however, is still elusive. Microglia could be activated into classic activated state (M1 state) or alternative activated state (M2 state); the former is cytotoxic and the latter is neurotrophic. In this investigation, we hypothesized that nicotine attenuates $A\beta$ -induced microglial activation by shifting microglial M1 to M2 state, and cannabinoid CB2 receptor and protein kinase C mediate the process. **Methods.** We used $A\beta$ 1–42 to activate N9 microglial cells and observed nicotine-induced effects on microglial M1 and M2 biomarkers by using western blot, immunocytochemistry, and enzyme-linked immunosorbent assay (ELISA). **Results.** We found that nicotine reduced the levels of M1 state markers, including inducible nitric oxide synthase (iNOS) expression and tumor necrosis factor α (TNF- α) and interleukin- (IL-) 6 releases; meanwhile, it increased the levels of M2 state markers, including arginase-1 (Arg-1) expression and brain-derived neurotrophic factor (BDNF) release, in the $A\beta$ -stimulated microglia. Coadministration of cannabinoid CB2 receptor antagonist or protein kinase C (PKC) inhibitor partially abolished the nicotine-induced effects. **Conclusion.** These findings indicated that cannabinoid CB2 receptor mediates nicotine-induced anti-inflammation in microglia exposed to $A\beta$ via PKC.

1. Introduction

β amyloid- ($A\beta$ -) induced microglial activation plays a vital role in the pathogenesis of Alzheimer's disease (AD) [1, 2]. $A\beta$ binds to microglia and activates them to secrete inflammatory cytokines, including tumor necrosis factor α (TNF- α), interleukin- (IL-) 1 β and IL-6 [3]. Long-term inflammation in brain induces neuronal cell injury and even cell death [4]. Some latest investigations suggested that microglia can be activated into classic activated state (M1 state) or selective activated state (M2 state) [5–7]. In the M1 state, activated microglial cells produce proinflammatory cytokines, which are considered to be detrimental; in contrast, activated microglial cells of M2 state secrete anti-inflammatory cytokines and neurotrophins, including IL-10, brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF), which are regarded to

be beneficial. Therefore, shifting microglial M1 to M2 state is considered to be an effective therapy for AD [8].

Nicotine is a main constituent of tobacco, which induces neuroprotection against $A\beta$ both in vivo and in vitro [9, 10]. In addition, some studies showed that nicotine attenuates $A\beta$ -induced microglial activation [11, 12]. However, the mechanism of nicotine-induced neuroprotective effect against AD is still not clear. Traditionally, nicotinic acetylcholine receptor (nAChR) is believed to be involved in the nicotine-induced protection against $A\beta$ in neurons and microglia [12, 13]. Yet emerging evidences support that there might be a cross-talk between nicotine administration and the endocannabinoid system. It has been found that cannabinoid CB2 receptor antagonist is used to treat nicotine addiction [14], and CB2 receptor plays a relevant role in the rewarding, reinforcing [15], and motivational effects of nicotine [16]. Moreover, cannabis tetrahydrocannabinol (THC) reduces the incidence

of precipitated nicotine withdrawal signs in mice [17]. In addition, we have ever reported that cannabinoid CB1 receptor (CB1) is involved in nicotine-induced neuroprotection against $A\beta$ in neurons, and protein kinase C (PKC) mediates the protection [18]. It is proved that cannabinoid CB2 receptor (CB2) is expressed in microglial cells, and we have ever reported that upregulation of CB2 receptor shifts microglial M1 to M2 state, leading to neuroprotective effects [19]. However, whether CB2 receptor is involved in nicotine-induced anti-inflammation in microglial cells has not been reported.

In this study, we used microglial cells exposed to $A\beta$ to mimic the neuroinflammation of AD and hypothesized that CB2 receptor mediates nicotine-induced anti-inflammation in $A\beta$ -treated microglia by shifting microglial M1 to M2 state, and PKC may play a role in the anti-inflammation.

2. Materials and Methods

2.1. Materials. The N9 microglial cell-line was a gift from the Chinese Academy of Sciences. The nicotine, β amyloid 1–42 ($A\beta$), Iscove's modified Dulbecco's medium (IMDM), fetal bovine serum (FBS), and chelerythrine (protein kinase C inhibitor) were purchased from Sigma-Aldrich (St. Louis, MO, USA). CB2 receptor antagonist AM630 was purchased from Alexis Biochemicals (CH). The primary anti-CB2 and anti-PKC antibodies were purchased from Abcam Ltd. (Cambridge, UK). The anti-iNOS and anti-Arg-1 antibodies were purchased from Chemicon (USA). Bovine serum albumin (BSA), Cy3-labeled secondary antibody, FITC-labeled secondary antibody, anti-GAPDH antibody, and secondary horseradish peroxidase-conjugated goat anti-rabbit antibody were purchased from Beijing Cowin Bioscience Co., Ltd. (China). The DAPI staining solution was purchased from Beyotime (China). The enzyme-linked immunosorbent assay (ELISA) kits were purchased from PeproTech Inc. (USA).

2.2. Cell Culture. The N9 microglial cells were cultured in the IMDM medium containing 5% FBS, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 2 mM glutamine. The humidified atmosphere of the cell culture incubator consisted of 95% air and 5% CO_2 . The medium was changed every 3 days. The cells were passaged 2–3 times per week and used within 8 weeks.

2.3. Subcellular Fractionation of Proteins and Western Blot Analysis. N9 microglial cells were seeded into a 6-well plate at a density of 2×10^5 cells/well. After the treatments, the cells were homogenized on ice with the lysis buffer (0.3 M sucrose, 0.15 M NaCl, 20 mM Tris-HCl, 2 mM EDTA, 0.3 mM PMSF, and 10 μ g/mL leupeptin). Homogenates were centrifuged at $1000 \times g$ for 10 min at 4°C, and supernatant fractions were collected for ultracentrifugation. Cytosol and membrane fractions were separated by ultracentrifugation at $100,000 \times g$ for 90 min at 4°C. The supernatant constituted the cytosol fraction, and the pellet was resuspended and homogenized in the above lysis buffer with 0.2% Triton X-100 added. This resuspended fraction represented the membrane fraction.

The Bradford reagent was used to compare PKC concentration in each fraction, and 20 μ g of total lysate from each fraction was subjected to gel electrophoresis (12% bisacrylamide gel) and transferred to nitrocellulose membrane. Blots were blocked in 3% milk Tris-buffered saline Tween and probed with anti-PKC antibody (1:500) in 2% milk Tris-buffered saline Tween and probed with an anti-rabbit secondary antibody [20].

For Arg-1 and iNOS expressions, after the treatments, the total protein concentrations were quantified by Bradford reagent. Total protein lysates were subjected to 12% sodium dodecyl SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Membranes were incubated with rabbit anti-mouse primary antibody (anti-iNOS 1:1000; anti-Arg-1, 1:1000; anti-GAPDH, 1:1000) in phosphate buffered saline (PBS) with 0.1% Tween-20 overnight at 4°C and then incubated for 1 h at room temperature with anti-rabbit IgG. GAPDH served as the control. Expression was visualized by enhanced chemiluminescence. Image was assessed by using computerized analysis software (Bio-Rad Laboratories, Hercules, USA).

2.4. Immunocytochemistry. The microglial cells were seeded into a confocal microscopy special dish at a density of 2×10^4 cells. Then the microglial cells were divided into five groups: control, $A\beta$, nicotine (Nico) + $A\beta$, AM630 + Nico + $A\beta$ and AM630 + $A\beta$ groups. As CB2 receptor antagonist AM630 is lipid soluble, DMSO was used to dissolve AM630. After the dissolution, the DMSO containing AM630 was added into cell culture medium to treat cells, and the final concentration of DMSO was 0.005% (1/20000 in v/v). After the treatments, the cells were fixed with 4% paraformaldehyde solution for 1 h. Then, the cells were blocked with 5% BSA solution after being washed three times with PBS. The cells were incubated at 4°C overnight with the corresponding primary antibody (CB2, 1:50; iNOS, 1:200; Arg-1, 1:200). Then, the cells were incubated in Cy3-labeled (red) or FITC-labeled (green) secondary antibody solution (1:200) for 1 h at room temperature. At the end of the incubation, 200 μ L of DAPI staining solution was added into the dish. After 5-minute incubation, the dish was washed three times with PBS. Then the dish was observed by using a confocal microscope (FV10i, Olympus, Japan).

2.5. Enzyme-Linked Immunosorbent Assay. The supernatants of the cell culture were collected and measured for TNF- α , IL-6, IL-10, and BDNF concentrations by using the corresponding quantification ELISA kits according to the manufacturer's instructions. All the experiments were repeated three times. Results are expressed as picograms per litre.

2.6. Statistical Analysis. SPSS 13.0 for Windows was used to conduct statistical analysis. All data were expressed as means \pm standard deviation (SD). The results were compared by one-way ANOVA, followed by Tukey's Multiple Comparison Test. $P < 0.05$ was considered as statistical significance.

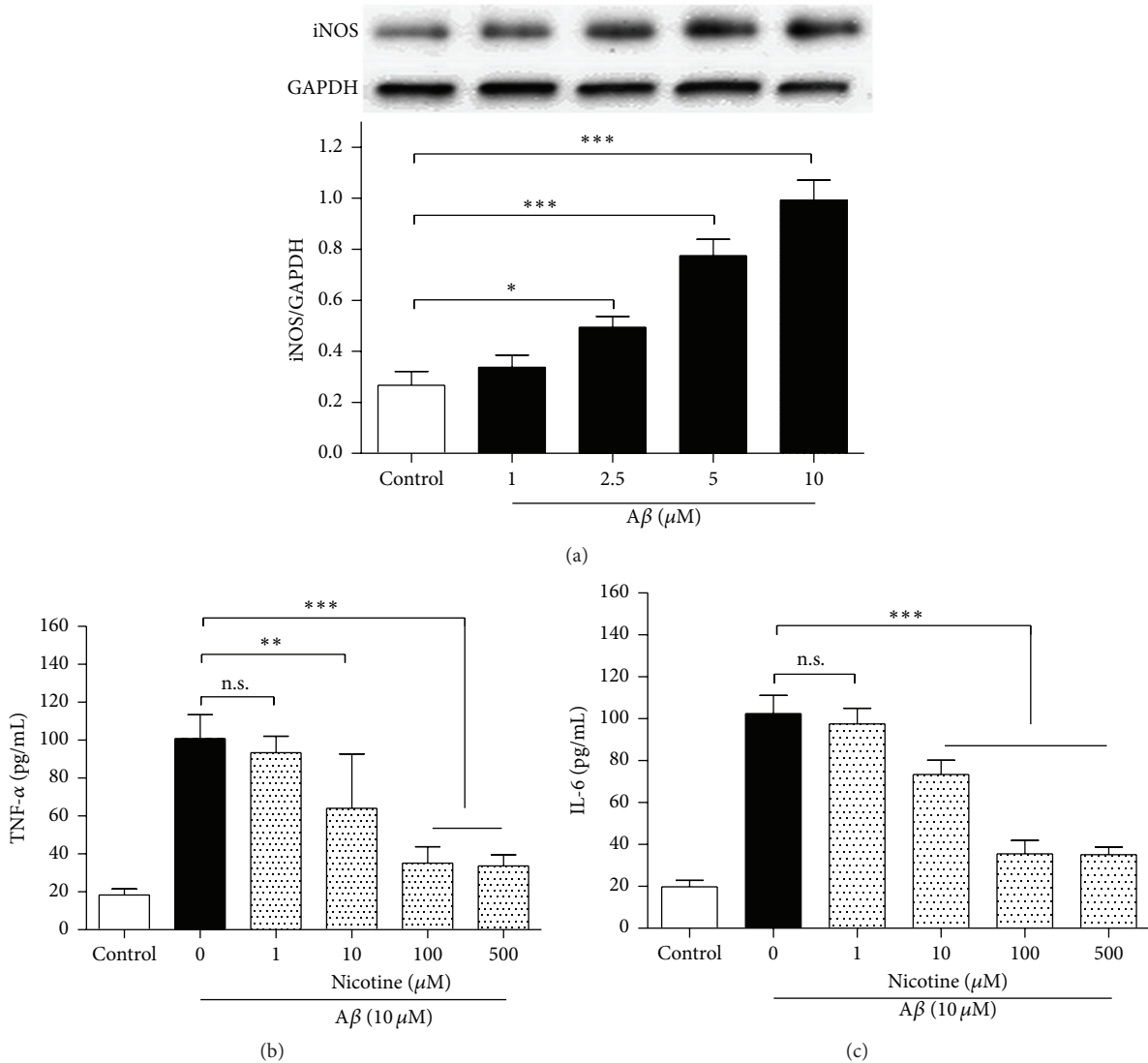


FIGURE 1: Nicotine decreased TNF-α and IL-6 releases from Aβ-stimulated microglial cells in a dose-dependent manner. (a) Aβ exposure increased iNOS expression. The N9 microglial cells were exposed to different concentrations of Aβ for 24 h (n = 4). (b) Nicotine decreased TNF-α release (n = 6). (c) Nicotine decreased IL-6 release (n = 6). Results are expressed as means ± SD, *P < 0.05, **P < 0.01, ***P < 0.001; n.s.: no significance.

3. Results

3.1. Nicotine Decreased TNF-α and IL-6 Releases in the Aβ-Stimulated N9 Microglia. To determine a suitable Aβ concentration, the N9 microglial cells were exposed to the medium containing different concentrations of Aβ for 24 h (Figure 1(a)); then western blot was used to evaluate the expression of inducible nitric oxide synthase (iNOS), a biomarker of microglial activation. The iNOS expression increased dose-dependently in the presence of Aβ, and 5 μM Aβ was used in the subsequent experiments.

Then, the N9 microglial cells were incubated in the medium containing different concentrations of nicotine plus 5 μM Aβ. Aβ exposure increased TNF-α and IL-6 concentrations in the medium significantly (Figures 1(b) and 1(c)); treatments of the cells with 10, 100, and 500 μM nicotine,

however, reduced the two proinflammatory factors releases obviously (P < 0.05), and 100 μM nicotine was taken in the following experiments.

3.2. CB2 Receptor Antagonist Reversed Nicotine-Induced Effects on iNOS and Arg-1 Expressions. To investigate nicotine-induced effects on microglial CB2 receptor expression, we used immunocytochemistry (Figure 2). The exposure of 100 μM nicotine for 24 h increased the CB2 protein expression obviously; however, the coadministration of 10 μM AM630, a selective CB2 receptor antagonist, markedly reversed the nicotine-induced upregulation of CB2 expression.

The microglial M1 state biomarker iNOS and M2 biomarker Arg-1 expressions were assessed by western blot

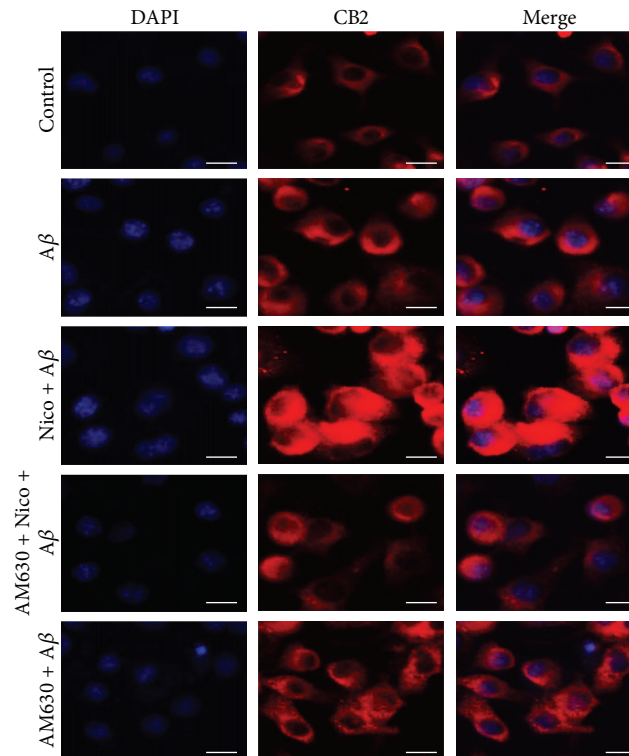


FIGURE 2: Nicotine increased CB2 receptor expression in microglial cells exposed to A β . The microglial cells were divided into five groups: control: cells cultured in drug-free medium; A β : cells cultured in the medium containing 5 μ M A β ; nicotine (Nico) + A β : cells cultured in the medium containing 10 μ M nicotine and 5 μ M A β ; AM630 + Nico + A β : cells cultured in the medium containing 10 μ M CB2 antagonist AM630, 10 μ M nicotine, and 5 μ M A β ; AM630 + A β : cells cultured in the medium containing 10 μ M AM630 and 5 μ M A β . After an incubation of 24 h, immunocytochemistry was used in investigating CB2 receptor expression (red), and the nuclei (blue) were counter-stained with DAPI staining solution. Bar = 20 μ m.

and immunocytochemistry. Exposure of 100 μ M nicotine for 24 h significantly attenuated iNOS expression (Figures 3(a) and 3(b)) and upregulated Arg-1 expression (Figures 3(c) and 3(d)) in the A β -treated microglia; 10 μ M AM630, nevertheless, partially abolished the nicotine-induced effects on iNOS and Arg-1 expressions ($P < 0.05$). These findings indicated that cannabinoid CB2 receptor may mediate the nicotine-induced modulation of microglial M1/M2 states in the microglial cells exposed to A β .

3.3. CB2 Receptor Antagonist Reversed Nicotine-Induced Effects on TNF- α , IL-6, and BDNF Releases. Proinflammatory factors, including TNF- α and IL-6, are biomarkers of microglial M1 state; in contrast, anti-inflammatory factor IL-10 and neurotrophic factor BDNF are the biomarkers of microglial M2 state. In this study, compared with the cells treated with A β alone, exposure of 100 μ M nicotine for 24 h significantly decreased TNF- α (Figure 4(a)) and IL-6 (Figure 4(b)) concentrations ($P < 0.05$) and increased BDNF concentration (Figure 4(d)) in the medium ($P < 0.05$); coadministration of 10 μ M CB2 antagonist AM630, however, partially reversed the nicotine-induced effects on TNF- α , IL-6, and BDNF releases ($P < 0.05$). Interestingly, the concentration of anti-inflammatory IL-10 (Figure 4(c)) remained unchanged ($P > 0.05$). These findings indicated that CB2 receptor may mediate the nicotine-induced effects

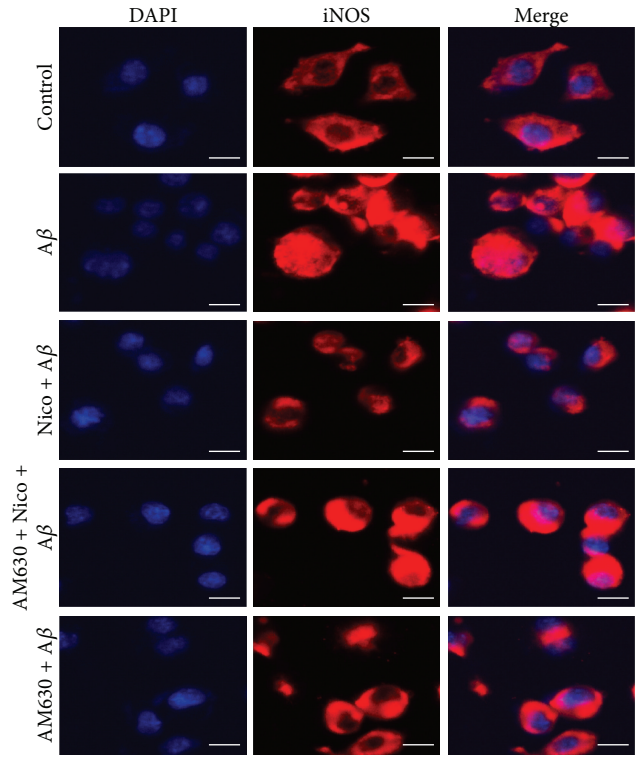
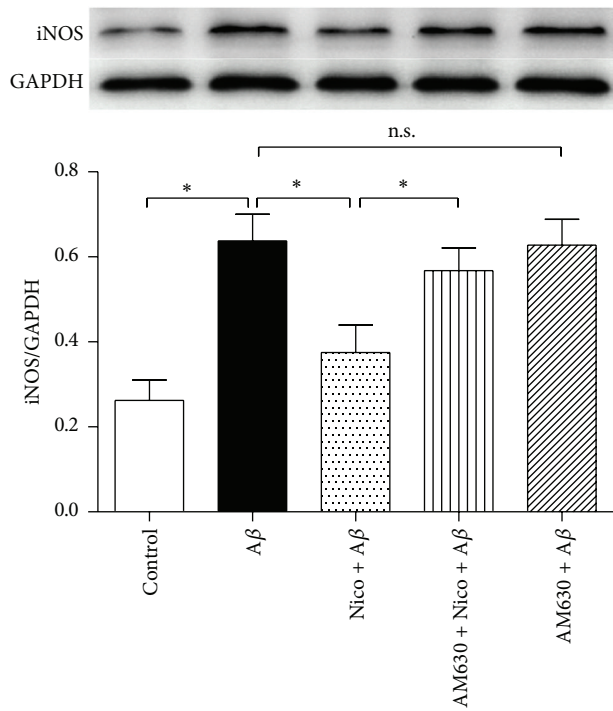
on the releases of proinflammatory and neurotrophic factors in the N9 microglia exposed to A β .

3.4. Protein Kinase C Inhibitor Abolished the Nicotine-Induced Anti-Inflammatory Effects. We also investigated the role of PKC in nicotine-induced anti-inflammations in A β -treated microglia. Compared with the cells exposed to 5 μ M A β alone (Figure 5), 100 μ M nicotine increased the expression of PKC in cell membrane significantly ($P < 0.05$), and CB2 receptor antagonist AM630 markedly reversed the nicotine-induced effect on PKC expression ($P < 0.05$).

Then, we assessed the role of PKC in nicotine-induced effects on cytokines. We found that 100 μ M nicotine exposure decreased TNF- α and IL-6 releases (Figures 6(a) and 6(b)) and increased BDNF release (Figure 6(d)) from the cells. Coadministration of 10 μ M PKC inhibitor chelerythrine (Che) abolished the nicotine-induced benefits significantly ($P < 0.05$). Meanwhile, the IL-10 concentration was still unchanged (Figure 6(c)). These results indicated that PKC may mediate the nicotine-induced anti-inflammations in the A β -treated N9 microglia.

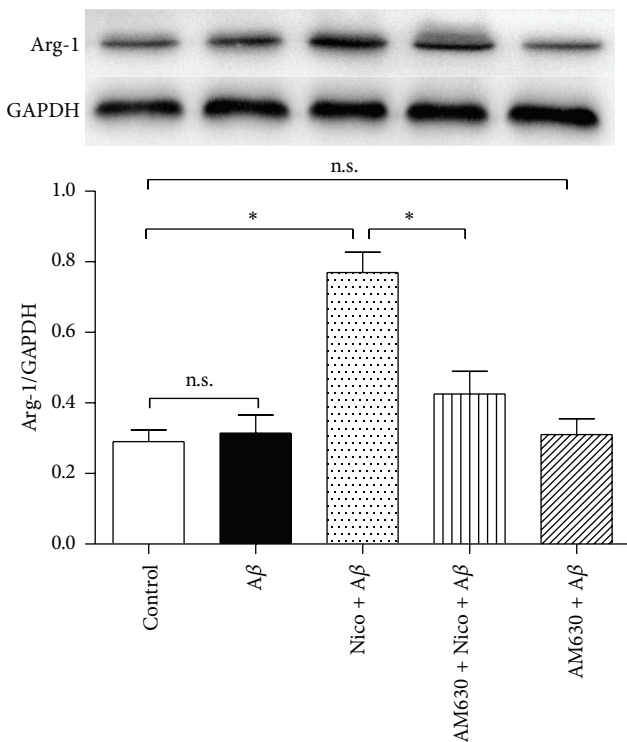
4. Discussion

In this study, we found that nicotine attenuated A β -induced microglial activation, decreased the markers' level

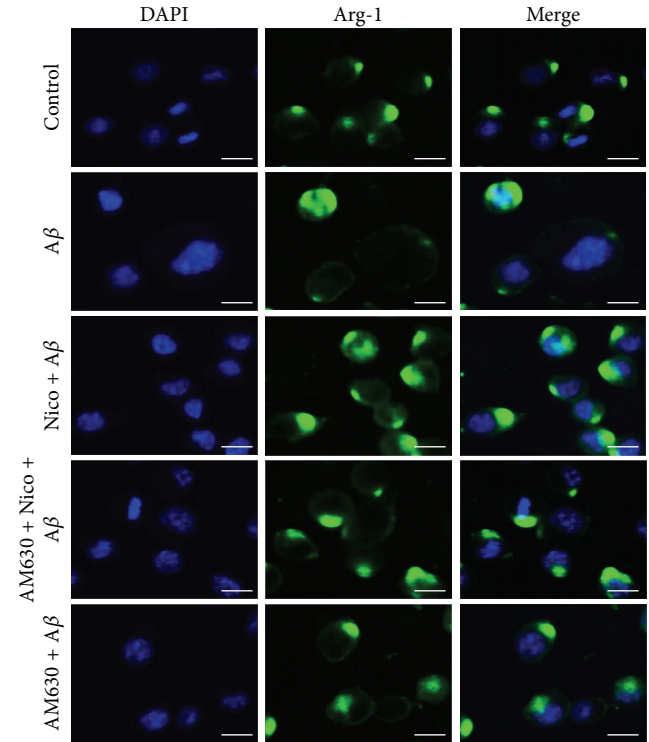


(a)

(b)



(c)



(d)

FIGURE 3: CB2 receptor antagonist reversed nicotine-induced effects on iNOS and Arg-1 protein expressions. The microglial cells were divided into five groups: control: cells cultured in drug-free medium; A β : cells cultured in the medium containing 5 μ M A β ; nicotine (Nico) + A β : cells cultured in the medium containing 10 μ M nicotine and 5 μ M A β ; AM630 + Nico + A β : cells cultured in the medium containing 10 μ M CB2 antagonist AM630, 10 μ M nicotine, and 5 μ M A β ; AM630 + A β : cells cultured in the medium containing 10 μ M AM630 and 5 μ M A β . After an incubation of 24 h, western blot ($n = 4$) and immunocytochemistry were used to evaluate iNOS (a-b) and Arg-1 (c-d) expressions. Results are expressed as means \pm SD, * $P < 0.05$; n.s.: no significance. Bar = 10 μ m.

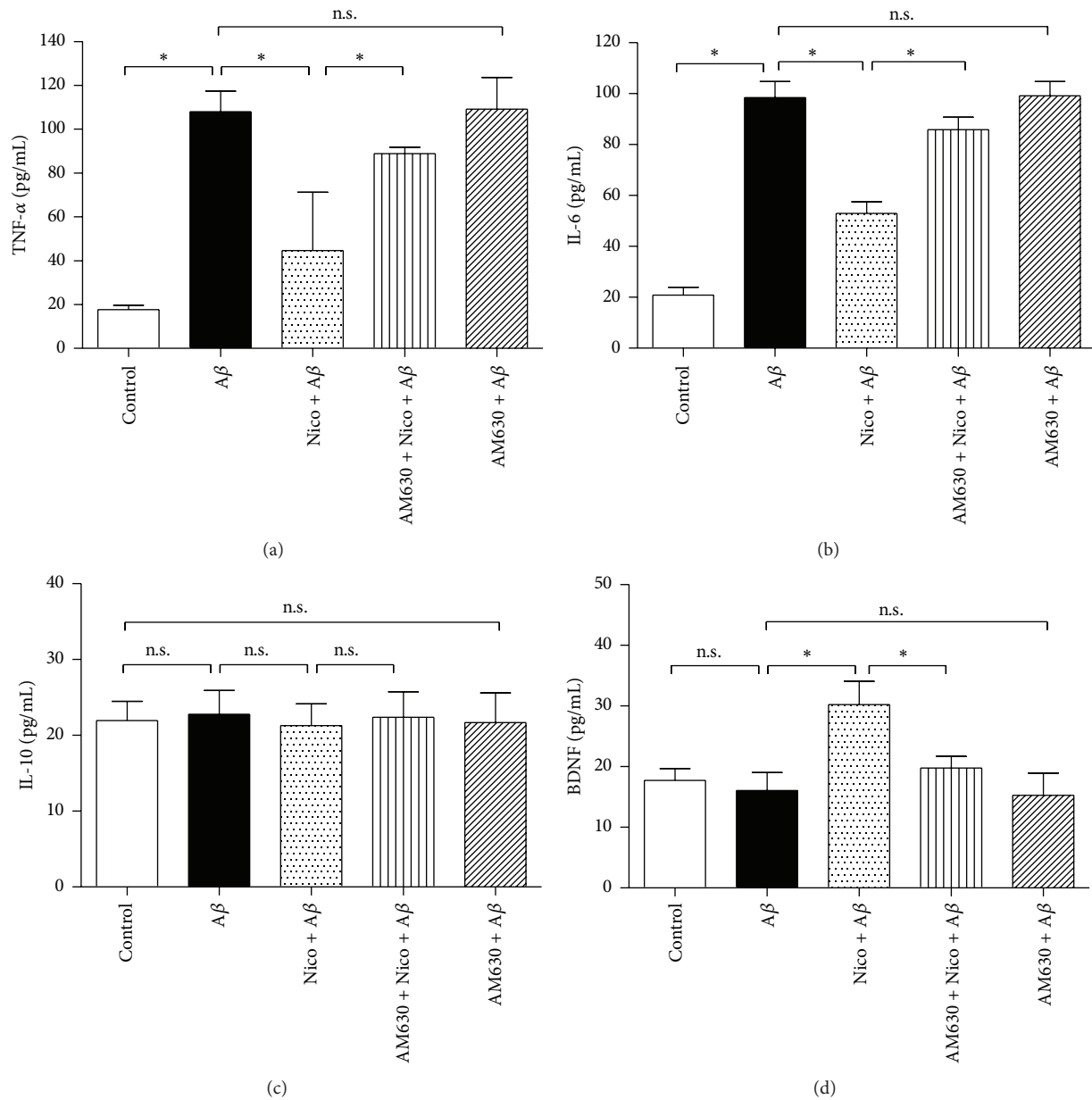


FIGURE 4: CB2 receptor antagonist partially abolished nicotine-induced effects on TNF- α , IL-6, and BDNF releases. The microglial cells were divided into five groups: control: cells cultured in drug-free medium; A β : cells cultured in the medium containing 5 μ M A β ; nicotine (Nico) + A β : cells cultured in the medium containing 10 μ M nicotine and 5 μ M A β ; AM630 + Nico + A β : cells cultured in the medium containing 10 μ M CB2 antagonist AM630, 10 μ M nicotine, and 5 μ M A β ; AM630 + A β : cells cultured in the medium containing 10 μ M AM630 and 5 μ M A β . After an incubation of 24 h, concentrations of TNF- α (a), IL-6 (b), IL-10 (c), and BDNF (d) in the supernatants were assessed by using the corresponding reagent kit ($n = 6$). Results are expressed as means \pm SD, * $P < 0.05$; n.s.: no significance.

of microglial M1 state, including iNOS, TNF- α , and IL-6, and increased the markers' level of M2 state, including Arg-1 and BDNF. Coadministration of cannabinoid CB2 antagonist AM630 or PKC inhibitor chelerythrine, however, significantly reversed the nicotine-induced effects on the markers of microglial M1 and M2 states. Our findings indicated that nicotine attenuates A β -induced microglial activation by shifting microglial M1 to M2 state via cannabinoid CB2 receptor, and PKC may mediate the process.

Microglial activation is involved in a variety of neurological conditions, including AD, Parkinson's disease (PD), and traumatic brain injury [21–23]. Therefore, inhibiting microglial activation is believed to be an effective therapy for these neurological disorders. Some current investigations indicated that microglia of resting state can be activated into classic activated state (M1 state) or alternative activated state (M2 state) [5–7]. Microglia of M1 state can secrete high levels of proinflammatory factors, which are considered to

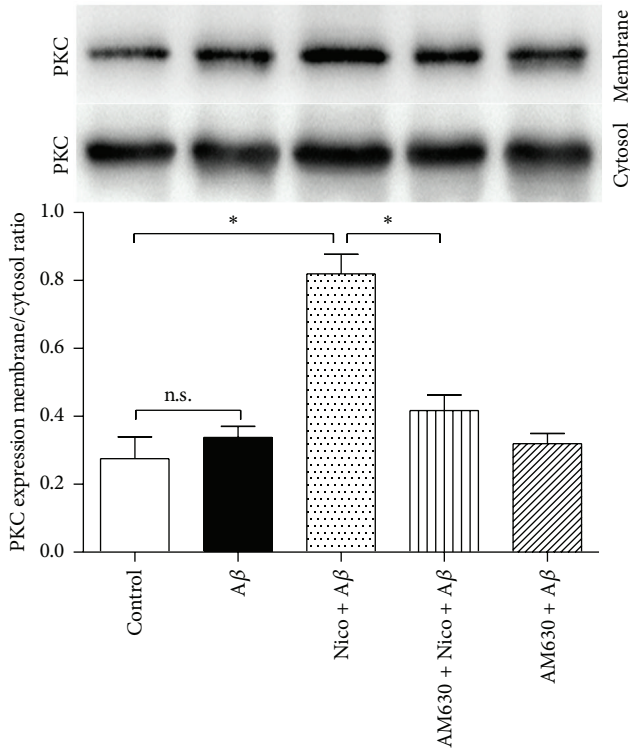


FIGURE 5: CB2 receptor antagonist abolished nicotine-induced effect on PKC expression. The microglial cells were divided into five groups: control: cells cultured in drug-free medium; A β : cells cultured in the medium containing 5 μ M A β ; nicotine (Nico) + A β : cells cultured in the medium containing 10 μ M nicotine and 5 μ M A β ; AM630 + Nico + A β : cells cultured in the medium containing 10 μ M CB2 antagonist AM630, 10 μ M nicotine, and 5 μ M A β ; AM630 + A β : cells cultured in the medium containing 10 μ M AM630 and 5 μ M A β . After an incubation of 24 h, western blot was used to detect PKC expression ($n = 4$). Results are expressed as means \pm SD, * $P < 0.05$; n.s.: no significance.

be harmful; in contrast, microglia of M2 state may produce a variety of anti-inflammatory cytokines and neurotrophins, which are believed to be beneficial. For this reason, shifting microglial M1 to M2 state is regarded to be helpful in treating microglial activation-induced neurological conditions. In brains of AD patients, A β deposition is a typical pathological character, which can induce microglial activation and even neuron death [24, 25]. And some recent investigations showed that A β can activate microglia into M1 state to secrete inflammatory cytokines [26, 27]. Thus, in this study, we used A β -stimulated microglia to mimic the neuroinflammatory response of AD. The mouse N9 microglial cells used in this study, like primary cultured microglia, can be polarized into M1 or M2 state and secrete the markers of microglial M1 and M2 states, such as iNOS, TNF- α , IL-6, and Arg-1, in the presence of stimulus. So we used N9 microglia to study microglial M1 and M2 states in this investigation [28, 29]. Nicotine is neuroprotective, and at present, most investigations about nicotine's neuroprotective effects are associated with the activation of nAChR in neuronal cells [12, 13]. However, some studies of nicotine indicated that there

might be a cross-talk between nicotine administration and the activation of the endocannabinoid system. For instance, cannabinoid CB2 receptor antagonist is used in treating nicotine addiction [14]. In addition, cannabis THC reduces the incidence of precipitated nicotine withdrawal signs in mice [17], and Chen et al. reported that nicotine attenuates brain ischemia/reperfusion injury in rats via cannabinoid CB1 receptor [30]. Moreover, in a previous study, we found that nicotine protects neurons against A β neurotoxicity via CB1 receptor [18]. In this investigation, we found that nicotine inhibited A β -induced microglial activation by ameliorating M1/M2 states, leading to the result that less proinflammatory factors and more BDNF were released; CB2 receptor antagonist, nevertheless, partially abolished the nicotine-induced anti-inflammatory and neurotrophic effects. Meanwhile, the level of IL-10, an anti-inflammatory cytokine, did not change significantly; therefore these findings may show that nicotine-induced neuroprotective effects are mainly by decreasing the production of proinflammatory cytokines and increasing the release of neurotrophins, but not by enhancing the secretion of anti-inflammatory factors. Similarly, some studies indicated that nicotine treatment induces neuroprotection by increasing the protein expression level of BDNF in gestational and postnatal rat brain [31, 32]. How does nicotine stimulate CB2 receptor and increase its expression in microglia remains a question to be answered. At present, most studies about the relationship between nicotine administration and cannabinoid system are related to the addiction of nicotine [33–35]. Although nAChRs, including α 3-, α 4-, and α 7-AChRs, are believed to be involved in nicotine-induced bioactivities [36–38], and a study reported that α 3-nAChR and CB2 receptor were expressed in the same neural cells of brain tissue in mice [39], additional studies are needed to explore the mechanism that nicotine stimulates CB2 receptor and increases its expression.

PKC is a widely expressed family of serine/threonine kinases. It has been demonstrated that PKC plays a role in neuroprotections against brain ischemic injury [40]. And Xu et al. reported that PKC mediates isoflurane (an inhalable anesthetic) induced anti-inflammation against lipopolysaccharide plus interferon γ in microglial cells [41]. In addition, we have ever reported that PKC is involved in nicotine-induced protection against A β in neurons [18]. Thus, we investigated the role of PKC in this study. We found that CB2 antagonist AM630 significantly attenuated nicotine-induced upregulation of PKC expression in the cell membrane, and PKC inhibitor chelerythrine markedly abolished the nicotine-induced effects on the secretion of cytokines, suggesting that PKC may mediate nicotine-induced anti-inflammation in N9 microglial cells exposed to A β . Similarly, Chen et al. reported that PKC mediated nicotine-induced neuroprotection against A β in neurons [42]. PKC is expressed both in cytosol and in cell membrane. PKC can be activated, and after the activation, PKC can translocate from cytosol to cell membrane. Such translocation has been identified to be the hallmark of PKC activation [43]. Cannabinoid CB2 receptor is a G protein-coupled receptor, which is expressed mainly in cell membrane [44]. Therefore, we infer that nicotine activated microglial CB2 receptor and

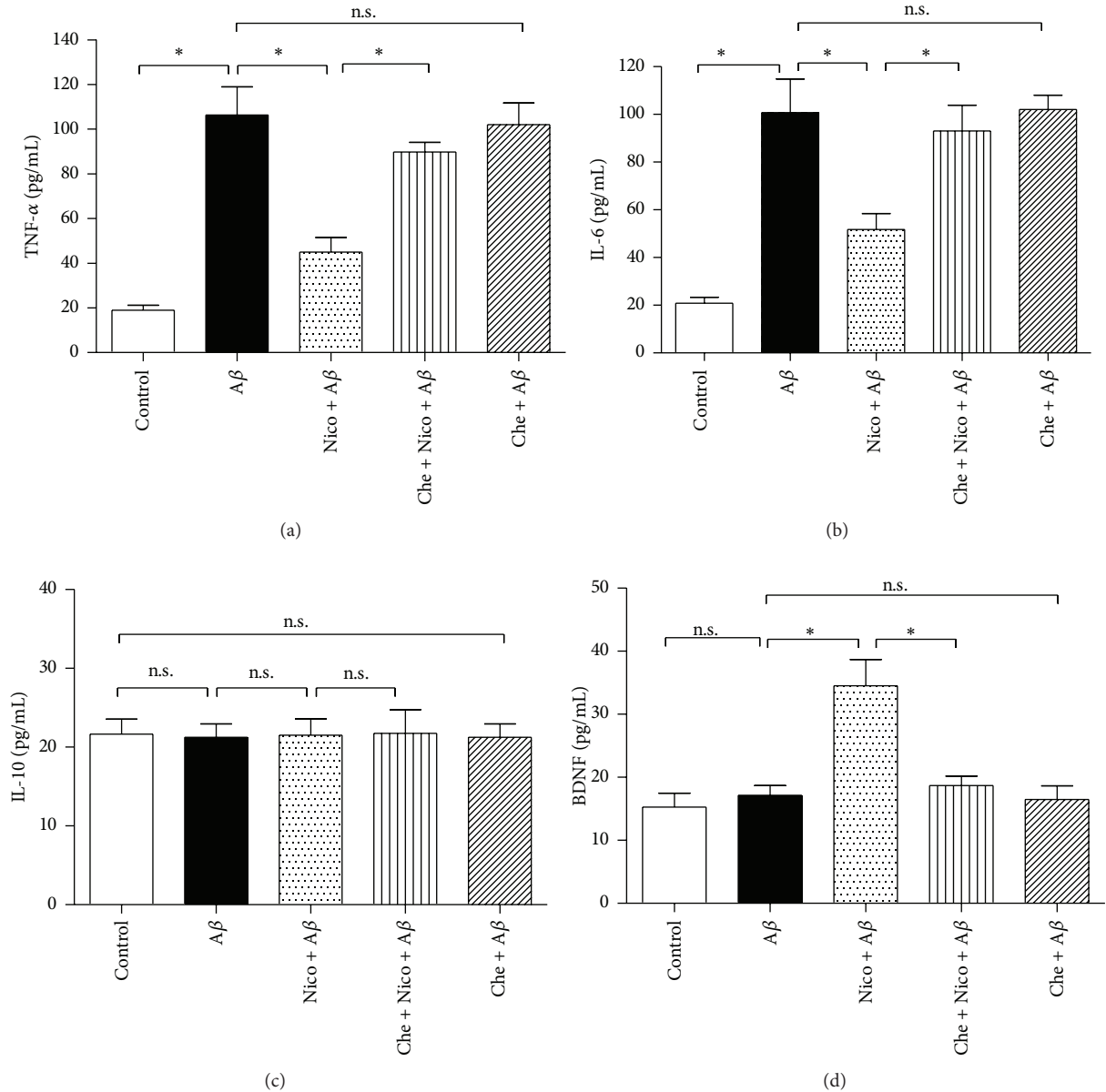


FIGURE 6: PKC inhibitor reversed nicotine-induced effects on TNF- α , IL-6, and BDNF releases. The microglial cells were divided into five groups: control: cells cultured in drug-free medium; A β : cells cultured in the medium containing 5 μ M A β ; nicotine (Nico) + A β : cells cultured in the medium containing 10 μ M nicotine and 5 μ M A β ; Che + Nico + A β : cells cultured in the medium containing 10 μ M PKC inhibitor chelerythrine (Che), 10 μ M nicotine, and 5 μ M A β ; Che + A β : cells cultured in the medium containing 10 μ M chelerythrine and 5 μ M A β . After an incubation of 24 h, concentrations of TNF- α (a), IL-6 (b), IL-10 (c), and BDNF (d) were assessed by using the corresponding reagent kit ($n = 6$). Results are expressed as means \pm SD, * $p < 0.05$; n.s.: no significance.

then induced an activation of PKC protein, followed by the decrease of microglial M1 markers, and the increase of microglial M2 markers, resulting in anti-inflammatory and neuroprotective effects (Figure 7).

However, there are still some limitations in our study. First, at present, at least 11 isoforms of PKC have been identified [45], including PKC α , PKC γ , and PKC ϵ , where isoform of PKC which mediates the nicotine-induced effects in this study has not been investigated. Second, this study is just in vitro; therefore the results of this study should

be verified in vivo. In this investigation, we found that cannabinoid CB2 receptor mediated nicotine-induced anti-inflammation against A β in microglia by shifting microglial M1 to M2 state via PKC. Our findings of this study offered a novel pharmaceutical target of nicotine and a new therapeutic target for the treatment of AD.

In summary, this study showed that nicotine attenuates A β -induced microglial activation by modulating microglial M1/M2 states, and the effects are mediated by cannabinoid CB2 receptor and PKC.

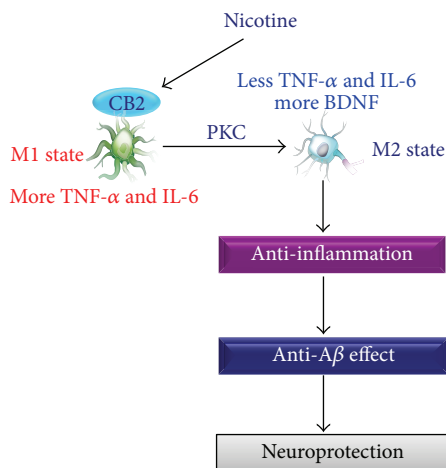


FIGURE 7: Hypothetical model of CB2 receptor mediates nicotine-induced neuroprotection against $A\beta$ toxicity in microglia. Nicotine exposure increases microglial CB2 receptor expression, which then shifts microglial M1 to M2 state, leading to the result that less TNF- α and IL-6 and more BDNF are released from the cells, and the process is mediated by PKC. Then the nicotine-induced anti-inflammation may happen, resulting in anti- $A\beta$ effect and neuroprotection.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Ji Jia, Jie Peng, and Zhaoju Li contributed equally to this work.

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