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Original Article

Upregulation of the P2X7 receptor promotes Ca^{2+} accumulation and inflammatory response in post-stroke depression

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Abstract: Neuroinflammation is a critical process in post-stroke depression (PSD). The ionotropic P2X7 receptor (P2X7R) functions as an ATP-gated nonselective ion channel permeable to Ca^{2+}. We evaluated the role of P2X7R in the initiation of neuroinflammation induced by PSD by focusing on its interaction with Ca^{2+} channels. PSD symptoms were induced using a middle cerebral artery occlusion (MCAO) model and the administration of chronic mild stress (CMS). We used the sucrose preference and Morris Water Maze as depression screening tests. The expression level of P2X7R, accumulation of Ca^{2+} in brain tissues, and levels of proinflammatory markers were detected by the relevant biological experiments. The administration of MCAO+CMS induced anhedonia and memory deficit in model rats, which was indicative of the development of PSD. The progression of the PSD symptoms was associated with increased levels of P2X7R, Ca^{2+} accumulation in rat brain tissues, and proinflammatory markers. Moreover, the inhibition of P2X7R activity inhibited Ca^{2+} accumulation and suppressed proinflammatory markers, whereas the upregulation of P2X7R activity had the opposite effect. Inhibition of the Ca^{2+} channel further strengthened the effect of P2X7R inhibition on Ca^{2+} accumulation and proinflammatory markers. The upregulation of P2X7R would initiate Ca^{2+} accumulation and inflammatory response in brain tissues, which suggests a new therapeutic method for neuroinflammation related with PSD.

Keywords: P2X7 receptor, neuroinflammation, Ca^{2+}, post-stroke depression

Introduction

Post-stroke depression (PSD) is the most frequently observed neuropsychiatric sequela of an acute stroke, which affects approximately 1/3 of patients with stroke [1, 2]. PSD is defined as depression that occurs following a stroke in a patient who was previously not diagnosed with depression. PSD is clinically significant because it is detrimental to post-stroke rehabilitation and imposes a severe burden on caregivers. Currently, the management of PSD is essentially drug therapy, although the individual therapeutic effect is unclear [3, 4]. However, other treatment approaches, such as repetitive transcranial magnetic stimulation (rTMS), have shown a potential beneficial effect on patients with PSD although their clinical efficacy needs to be validated in large clinical cohorts [5]. Nonetheless, reports from recent meta-analyses indicate that the majority of PSD patients remain untreated or partially treated even after treatment with antidepressants [6, 7]. Husseini et al. reported that more than two-thirds of the 1450 patients with PSD in their study cannot be completely cured through conventional antidepressant treatments [8]. Thus, novel and effective therapeutic strategies are a necessary solution to promote recovery from PSD.

It is believed that PSD has a multifactorial and complicated etiology. Multiple biological factors have been reported to be relevant to PSD progression, such as genetic susceptibility, activation of the hypothalamo-pituitary-adrenal (HPA) axis, neurogenesis and inflammation [9]. Of these factors, inflammation plays a key role

in the pathogenesis of depression for a subset of depressed individuals [10]. Stroke is known to induce a systemic inflammatory response that triggers sickness behavior, wherein fatigue and PSD are predominant symptoms [11]. Increased cytokines (interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and IL-1β) are observed in patients with dysthymia or major depressive disorders [12-14]. A number of pathways contribute to PSD progression through promoting inflammation, among them, the activation of microglia is one of the proposed mechanisms that has recently elicited much interest [15].

Microglia, which approximately comprises 10% of all brain cells, is crucial for synapses development and behavioral regulation [16, 17]. Cell subsets are activated under pathological conditions, which are the primary coordinator and executor of many processes that affect neurons and other brain cells [15]. With regard to neuronal inflammation, activated microglia transform into a more motile state and migrate to the sites of damage [18], to promote inflammation and cause subsequent neurodegeneration [19]. Given the central role of microglia in the initiation of neuroinflammation and the pathogenesis of depression, researchers previously attempted to attenuate neuroinflammation by interfering with proinflammatory signaling in microglia. Huang et al. reported the use of nicardipine to inhibit neuroinflammation by blocking the Ca^{2+} channel in microglia [20]. In a study conducted by Liu et al., another calcium-channel blocker, verapamil, was found to protect against neuronal damage by inhibiting the activation of microglia [21]. These studies proved that the dysregulation of the Ca^{2+} channel in microglial cells contributed substantially to neuroinflammation, which is connected with PSD progression. Thus, research into the factors that modulate the Ca^{2+} channel in microglia will be helpful to the development of anti-PSD strategies.

Ionotropic P2X receptors (P2XR) are widely expressed in macrophages, dendritic cells, and microglial cells [22]. P2X7R, a typical subunit of the P2XR family, is assumed to have a standalone function in that agents which target P2X7R have been proposed as useful treatment strategies in various autoinflammatory diseases or for the amelioration of inflammatory pain [22]. Moreover, P2X7R has been reported to be a key player in microglial activation and proliferation [23]. Furthermore, it has been inferred that P2X7R drives the deleterious cycle of neuroinflammation by activating microglia [23].

Given that the upregulation of P2X7R causes a sustained increase in intracellular Ca^{2+}, we proposed a hypothesis that the neuroinflammation associated with PSD was induced by the accumulation of Ca^{2+} in microglia via upregulation of P2X7R. We aimed to verify this hypothesis in a PSD rat model and monitor the levels of P2X7R, proinflammatory cytokines, and Ca^{2+} with the progression of PSD. Subsequently, we intended to modulate the activities of P2X7R and the Ca^{2+} channel in PSD rats to confirm the central role of P2X7R in the Ca^{2+} channel and the subsequent neuroinflammation.

Materials and methods

Middle cerebral artery occlusion (MCAO) model and administration of chronic mild stress (CMS)

As described in previous studies [24, 25], we induced PSD symptoms in SD rats using MCAO model in combination with the administration of CMS. After anesthesia, the neck skin was exposed and sterilized with iodine tincture. The left common carotid artery (LCCA) was exposed and a 4-0 silicon coating silk filament was introduced through a small incision in the LCCA into the distal left internal carotid artery for occlusion. After 30 min, Cerebral infarction occurred in the perfusion area. After two days, rats were administered with CMS for 17 d by referring to previous research [26]. Indeed, a cycle (136 h) included 112 h before swimming and 24 h (5 min swimming plus 2 h behavior restriction). During these 17 days, total 408 h equals 3 cycles. The final 5 min swimming and 2 h behavior restriction were considered as one day. All surgical protocols in experimental animals were approved by the Institutional Animal Care and Use Committee of the Third Affiliated Hospital of Chongqing Medical University (General Hospital), Chongqing, China. The standard for successful modeling was evaluated through neurological deficit score based on previous reports [24, 25].

Experimental design

The timeline of experimental procedures as follow: Before surgical produce, SD rats were cultured in standard conditions for 7 days to adapt environment, then underwent MCAO procedure for 2 day, CUMS for 17 days, followed by behavior evaluation and serum and tissue isolation.

To determine the effect of PSD induction on P2X7R expression and the role of P2X7R in PSD progression, we established three protocols in the present study. Group 1: sham group (MCAO administration without artery ligation), MCAO group (the standard MCAO operation), MCAO+CMS group (the standard MCAO operation and later to CMS administration, as described earlier). Group 2: sham group, MCAO group, MCAO+CMS group, MCAO+CMS+P2XR7 agonist group (MCAO+CMS+BzATP 10 μg/5 μL i.c.v.), MCAO+CMS+P2XR7 inhibitor group (MCAO+CMS+BBG 10 μg/5 μL i.c.v.). Group 3: sham group, MCAO group, MCAO+CMS group, MCAO+CMS+P2XR7 inhibitor group (MCAO+CMS+BBG 10 μg/5 μL i.c.v.), MCAO+CMS+PLM (phospholemman) inhibitor group (gavage of the PLM inhibitor Nimotop (20 mg/kg)), MCAO+CMS+P2XR7 inhibitor+PLM inhibitor group (coadministration of BBG and Nimotop).

After anesthesia, BzATP or BBG was injected into the lateral cerebral ventricle by a microinjector. Then, these agents were administered into the lateral cerebral ventricle over 10 minutes, with a total volume of 5 μL injected at 0.5 μL/min, prior to global cerebral ischemia. Similarly, the sham groups were anesthetized and treated with the same volume of saline by intracerebroventricular injection. All the experimental rats were sacrificed by a CO_2 euthanasia device (PVC, Wonderful Oasis Biotechnology, China) prior to the isolation of hippocampal tissues.

Sucrose preference test

In accordance with the previous researches [27, 28], the rats in each group were housed for 24 h in a home cage with two bottles containing 1% sucrose solution to adapt them to the ingestion of sugary water before the sucrose preference test. Then these two bottles were replaced with 1% sucrose solution and standard drinking water, respectively. After 24 h deprivation of water and food, each rat was provided two bottles, containing 1% sucrose solution and standard drinking water, respectively, in the home cage for 60 min. After 1 h, the two bottles were weighed, and the data was calculated with the formula: Sucrose preference test (%) = Sucrose solution intake/total intake × 100%. The results of the sucrose preference test were used to assess PSD-induced anhedonia.

Morris water maze

All rats were trained for 7 days before testing. A day before the test, the swimming abilities of rats were observed and rats that could not swim were excluded from the study. For the place navigation test, the mice were placed in the quadrant farthest from the platform among the 4 quadrants. Then, the rats were put into the water by selecting random entry points and allowed to swim freely for 60 s. Rats unable to find the platform within 60 s were directed to the platform for rest 10 s. We recorded the arrival time of the rats to the platform. For the spatial probe test, after removing the platform, the rats were given a probe trial, wherein they had to find the platform for 120 s, and the effective number of the original platform crossings was recorded by a camera. Both the place navigation and spatial probe tests were conducted three times for two consecutive days at the same time and in the same location.

Reverse transcription real-time PCR (RT-PCR)

We collected and grind the hippocampal tissues in CA1 region, and extracted total RNAs by TRIzol method (9109, Takara, USA). cDNA templates were generated using the Bestar™ qPCR RT kit (DBI Bioscience, China). PCR amplification was conducted using the final reaction mixture (20 μL) with 10 μL Bestar® SybrGreen qPCRmasterMix (2043, DBI Bioscience, China), 0.5 μL primers, 1 μL of the cDNA template, and 8 μL RNase-free H_2O; meanwhile, the melting curve was also analyzed after treatment on the Stratagene Real time PCR system (Mx3000P, Agilent, USA). The data was calculated according to $2^{-\Delta\Delta ct}$. Primer sequences were displayed as follows: P2X7R, forward: 5'-TCCAGAGCACGAATTATGGC-3', reverse: 5'-CAGAGGCAGGGAGGGAAA-3'; β-actin, forward: 5'-CATTGCTGACAGGATGCAGA-3', reverse: 5'-CTGCTGGAAGGTGGACAGTGA-3'.

Western blotting assay

The CA1 region of the hippocampal tissues was lysed with lysis buffer containing 1% PMSF, cocktail, and phosphatase inhibitors. The protein concentration was determined with the relevant Kit (Thermo, USA, #23227). We subjected 20 μg protein to a 10% SDS-PAGE at 100 V for 1.5 h, and then transferred it onto PVDF sheets. After washing and blocking, the membranes were processed with primary antibodies (P2X7R (1:500; CST, #13809), PLM (1:1000; Abcam), Cav1.2 (1:400; Alomone), and GAPDH (1:10,000; Abcam)) at 4°C overnight and secondary HRP IgG antibody (1:20,000, Boster, China, BA1051) for 1 h. The results were revealed using Beyo ECL Plus reagent (7seaBiotech, Shanghai, China, E002-5).

Enzyme-linked immunosorbent (ELISA) assay

Before euthanasia, we obtained 0.5 mL tail vein blood samples from the rats to measure inflammatory cytokines (TNF-α and IL-1β) [29, 30], at 12 h, 3 d, and 7 d in each group. We transferred 0.5 mL blood to sterile tubes and these samples were centrifuged, and the serum was stored at -70°C in microtubes. Serum levels of TNF-α and IL-1β were detected with the corresponding ELISA kits (Elabscience Biotechnology, China, E-EL-M0049 and E-EL-M0037). The levels of the cytokines were represented using a microplate reader (MULTISKAN MK3, Thermo, USA).

Detection of Ca^{2+} level

We used flow cytometry to determine the Ca^{2+} levels in CA1 regions of hippocampal tissues on the third day after the successful model establishment in line with previous research [31]. First, the microglia were isolated from the CA1 region as previously described [32, 33]. In brief: the hippocampal tissues were cut into small pieces and treated with 5 mL digestion buffer for 60 min at 37°C. After multiple blowing procedures, the sediments were suspended in 37% Percoll cellular separation buffer. Microglia in the supernatants and astrocytes at the bottom were enriched for 24 h, respectively. CD11b is a marker of microglia cells. We first isolated microglia cells and then detected Ca^{+}. Our current approach is based on previous research [34], in which CD11b-positive cells were eluted by removing the magnetic field, resulting in the isolation of approximately 93% viable CD11b-positive cells from sham and TBI mice (date show in Figure S1). The level of Ca^{2+} was further determined by flow cytometric detection with a fluorescent probe (S1052, Beyotime Technology, China).

Statistical analysis

All measurement data in the current study were represented as mean ± SD. The data was calculated using one-way analysis of variance with Duncan on SPSS version 19.0 (IBM, Armonk, NY, USA). And the significance was set at 0.05 (*P*-value).

Results

Behavioral analysis for depression

We induced symptoms of PSD through MCAO surgery combined with CMS. The model was assessed with the sucrose preference test. As shown in **Figure 1A**, rats in both MCAO and MCAO+CMS groups showed a significant decrease in sucrose intake versus those in the sham group ($P < 0.05$). Moreover, with respect to the MCAO group, rats in the MCAO+CMS group exhibited anhedonia in the sucrose preference test to a larger extent. In addition, the Morris Water Maze test (**Figure 1B**, **1C**) was carried out to further evaluate behavioral changes in the PSD model. In the place navigation test, MCAO rats took longer on average to find the platform than Sham rats, whereas MCAO+CMS rats attained a significantly higher time when compared with MCAO rats. In the spatial probe test, the number of platform crossings among the rats in the Sham group was 4.3 ± 0.6, whereas the number of platform crossings in the MCAO and MCAO+CMS groups were 2.3 ± 0.6 and 0.3 ± 0.6, respectively. The differences between Sham and MCAO, as well as MCAO and MCAO+CMS groups were significant. These data suggest that the PSD model was established in rats.

Induction of PSD symptoms induced inflammatory response, increased P2XR7 level, and caused Ca^{2+} accumulation in model rats

Serum samples were collected at 12 h, 3 d, and 7 d after different administrations from the rats that underwent Protocol I. The produc-

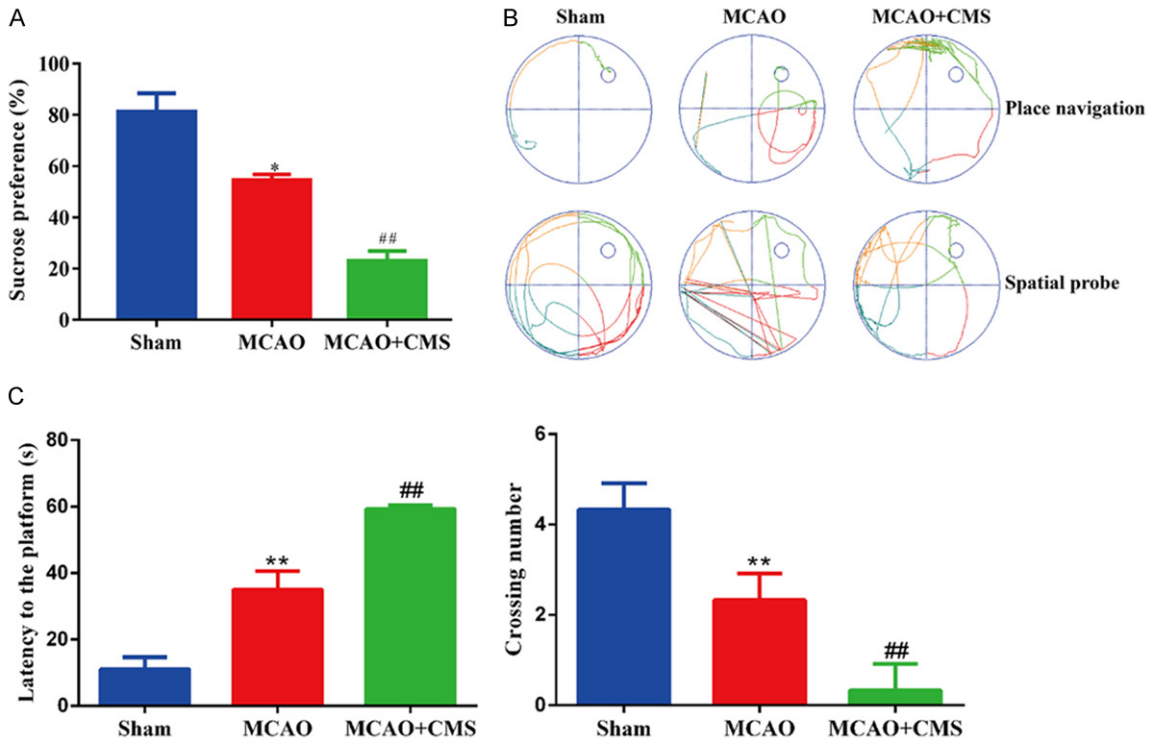

Figure 1. Behavioral test of depressive disorders. (A) The sucrose preference test and (B, C) Morris Water Maze test were conducted in all three models. The MCAO+CMS rats showed a significant anxiety-like anhedonia and despair-like behavior with cognitional impairment. $**P < 0.01$ vs Sham group; $##P < 0.01$ vs MCAO group. n = 5 for each group.

tion of cytokines, including TNF-α and IL-1β, was detected with ELISA. In association with the formation of PSD symptoms, the production of both cytokines was induced by the administration of MACO in combination with CMS (**Figure 2A** and **2B**). In addition, with the progression of time, increases in cytokine levels were gradually augmented (**Figure 2A** and **2B**). With regard to the target molecule that we focused upon in this study, the P2X7R expression was increased at both the mRNA and protein levels in a time-dependent manner (**Figure 2C** and **2D**). Besides, the data testified that the level of Ca^{2+} was dramatically higher in the MACO group relative to that in the sham group, while this elevation also could be prominently enhanced by CMS in the CA1 region of hippocampal tissues of the MACO rats (**Figure 2E**). As being previously reported, an increased level of P2X7R will activate Ca^{2+} accumulation [35]. Therefore, we speculated that the increase of Ca^{2+} level induced by CMS might be related to the upregulation of P2X7R in the CA1 region of hippocampal tissues collected from the MACO rats.

Modulation of P2X7R activity influenced anhedonia, inflammatory response, and Ca^{2+} channel activity in hippocampal tissues of model rats

To confirm the role of P2X7R in the progression of PSD, the activity of P2X7R was modulated with a specific agonist or inhibitor. The modulation of P2X7R activity influenced anhedonia, as detected by the sucrose preference test: the administration of P2X7R agonist (BzATP) further suppressed the rat's interest in sucrose (**Figure 3**), whereas the administration of a P2X7R inhibitor (BBG) restored their interest in sucrose, thereby confirming the key role of P2X7R activity in determining the emotional state of rat (**Figure 3**).

Furthermore, the administration of BzATP increased the activity of P2X7R in hippocampal tissues whereas the administration of BBG inhibited this activity (**Figure 4A** and **4B**). The upregulation of P2X7R increased the expressions of PLM and Cav2.1 (**Figure 4C**), which resulted in a further increase in the production

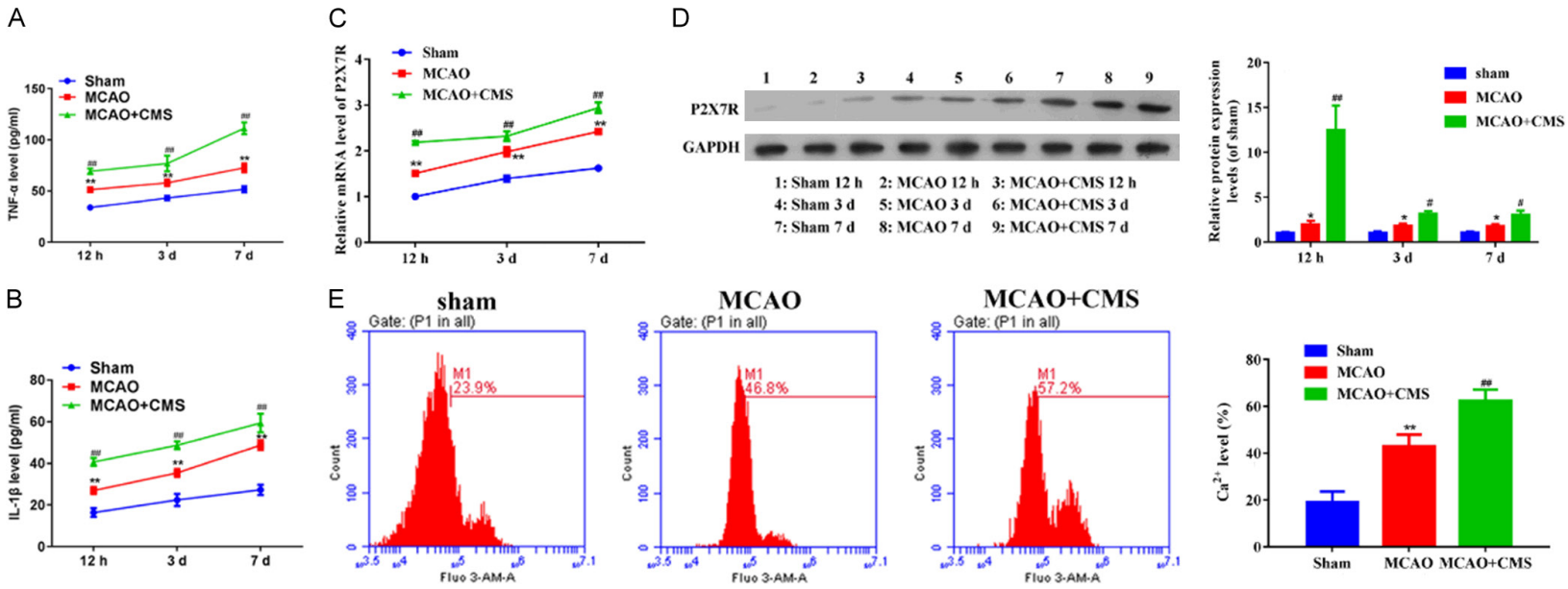

Figure 2. Induction of PSD symptoms induced inflammatory response, increased P2XR7 level, and caused Ca^{2+} accumulation in model rats. Serum samples were collected from rats at 12 h, 3 d, and 7 d after the model induction and subjected to ELISA for the detection of TNF-α and IL-1β levels. A. Quantitative analysis results of TNF-α level. B. Quantitative analysis results of IL-1β level. The CA1 region samples of hippocampus tissues were collected from rats at 12 h, 3 d, and 7 d after model induction and subjected to Western blotting to detect P2X7R expression at the mRNA and protein levels. Samples collected 3 d after model induction were subjected to flow cytometry to detect Ca^{2+} accumulation. C. Results of quantitative analysis of RT^2-PCR detection of P2X7R mRNA level. D. Representative images and results of quantitative analysis of Western blotting for the detection of P2X7R protein level. E. Representative images and results of quantitative analysis of flow cytometric detection of Ca^{2+} accumulation. $^{**}P < 0.01$ vs Sham group; $^{\#\#}P < 0.01$ vs 12 h. n = 5 for each group.

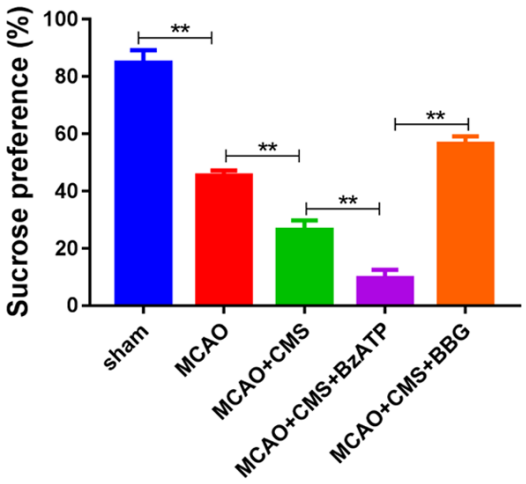

Figure 3. Modulation of P2X7R activity influenced rat's interest in sucrose. **P < 0.01. n = 5 for each group.

of TNF-α and IL-1β that were originally induced by PSD at 12 h, 3 d, and 7 d after the various administrations in rats in Protocol II (**Figure 4D** and **4E**). In contrast, the inhibition of P2X7R resulted in the suppressed expressions of PLM and Cav2.1 (**Figure 4C**) as well as the decreased production of TNF-α and IL-1β (**Figure 4D** and **4E**) that was mediated in a time-dependent manner. Furthermore, the up-regulation of P2X7R increased the Ca^{2+} level in hippocampal tissues collected from rats that were sacrificed 3 d after the different experimental administrations (**Figure 4F**). When P2X7R expression was inhibited by a specific inhibitor, the level of Ca^{2+} was reduced (**Figure 4F**), confirming the regulatory function of P2X7R on Ca^{2+} channel activity.

Interaction between P2X7R and Ca^{2+} channel determined the function of P2X7R

The interaction between P2X7R and the Ca^{2+} channel was further explored by subjecting MACO+CMS rats to the coadministration of BBG and a PLM inhibitor (Nimotop). The administration of Nimotop had a similar effect on the rat's interest in sucrose as that of BBG, and the coadministration of both inhibitors restored this interest (**Figure 5**).

As shown in **Figure 6A**, the administration of Nimotop further suppressed the expression of PLM and Cav1.2, which was already inhibited by the BBG. Moreover, the inhibitory effect of BBG on the levels of TNF-α (**Figure 6B**), IL-1β (**Figure 6C**), and Ca^{2+} (**Figure 6D**) was strengthened by Nimotop, indicating the presence of an interaction between the P2X7R and Ca^{2+} channel in rats with PSD.

Discussion

Depression is closely intertwined and mutually reinforced with neuro-inflammatory [10]. The promotion of depression by an inflammatory response as well as the promotion of an inflammatory response by depression has clear health consequences. A neuroinflammatory process can be initiated by multiple important mechanisms, including the psychological effects of circulating mediators and a direct effect of activation of microglia [36]. However, the mechanisms causing neuroinflammation are not fully understood. In our study, based on the behavioral analysis for depression, we first successfully established PSD model through MCAO surgery and CMS administration.

Neuroinflammation caused by microglia activation has both beneficial and detrimental functions in the nervous system [37, 38]. The activation of microglial cells during neuroinflammation can lead to the clearance of debris or invading pathogens or to the production of neurotrophic factors that maintain the hemostasis of the nerve microenvironment [19]. From the reports of previous studies, we infer that the activation of microglial cells is closely related to the function of Ca^{2+} channels, and the application of a Ca^{2+} channel blocker could prevent neuroinflammation by blocking calcium dysregulation. For example, study used nicardipine to inhibit neuroinflammation by blocking the Ca^{2+} channel in microglia [20] and Liu et al. employed another Ca^{2+} channel blocker, verapamil, to protect against neuronal damage by inhibiting calcium dysregulation [21]. Therefore, the modulation of Ca^{2+} channel activity might be a promising strategy for anti-neuroinflammatory strategies, which can also be used for the management of PSD in the clinic. In our study, we further confirmed that both inflammatory response and Ca^{2+} accumulation were significantly enhanced in PSD model rats, suggesting that the modulation of Ca^{2+} is critical in PSD during neuroinflammation.

P2X7R is an ATP-gated nonselective ion channel permeable to Ca^{2+} [39, 40]. Most P2X7Rs

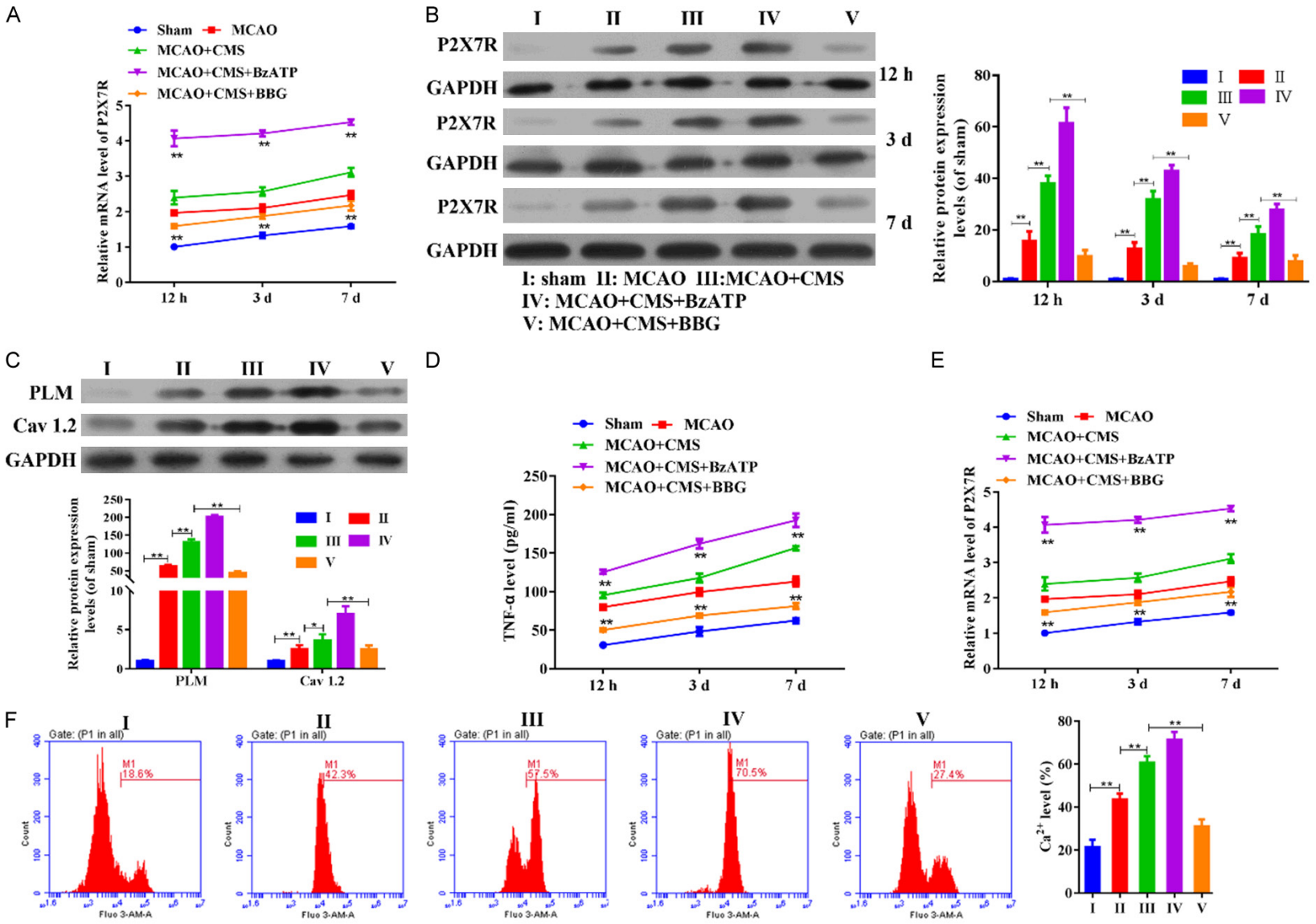

Figure 4. Modulation of P2X7R activity influenced the inflammatory response and Ca^{2+} channel activity in model rats. PSD rats were further subjected to the administration of a P2X7R agonist (BzATP) or P2X7R inhibitor (BBG). A. Results of a quantitative analysis of RT^2-PCR detection of P2X7R mRNA level in brain tissues collected 12 h, 3 d, and 7 d after model induction. B. Representative images of Western blotting for the detection of P2X7R protein level in brain tissues collected

12 h, 3 d, and 7 d after model induction. C. Representative images and results of quantitative analysis of Western blotting detection of PLM and Cav1.2 protein levels in brain tissues collected 3 d after model induction. D. Results from the quantitative analysis of ELISA detection of the TNF-α level in serum samples collected 12 h, 3 d, and 7 d after model induction. E. Results from the quantitative analysis of ELISA detection of the IL-1β level in serum samples collected 12 h, 3 d, and 7 d after model induction. F. Representative images and results from quantitative analysis of flow cytometric detection of Ca^{2+} accumulation in brain tissues collected 3 d after model induction. $^{**}P < 0.01$ vs MCAO+CMS group; $^{**}P < 0.01$. n = 5 for each group.

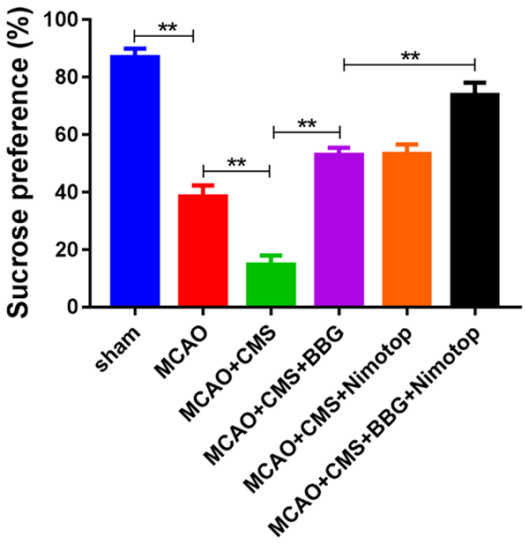

Figure 5. Inhibition of Ca^{2+} channel activity strengthened the effect of P2X7R inhibition on rat's interest in sucrose. $^{**}P < 0.01$. n = 5 for each group.

are expressed in excitable or epithelial/endothelial tissues and bind with effectors to render numerous functions [39, 41, 42]. Moreover, the ability of P2X7R to act as a direct conduit for Ca^{2+} influx underlies its multiple roles in Ca^{2+}-based signaling responses [22]. Thus, we hypothesized that the modulation of P2X7R might represent a novel strategy for handling PSD by suppressing central nervous system cell activation via the Ca^{2+} channel. And our results also revealed that the level of P2X7R was also prominently elevated in PSD model rats. Besides, we certified that the administration of a P2X7R inhibitor reversed the production of proinflammatory cytokines and activities of Ca^{2+} channels in rats of the PSD model, whereas the administration of a P2X7R agonist strengthened the impairments induced by PSD. Moreover, we uncovered for the first time that interaction between P2X7R and Ca^{2+} channel can determine the function of P2X7R in PSD. Given that inflammation plays a key role in the initiation of PSD [10, 36], the effect of P2X7R on the level of inflammatory responses represents the possibility that the inhibition of P2X7R would attenuate PSD progression.

Besides determining the role of P2X7R in the progression of PSD, we conducted subsequent assays to explain the interaction between P2X7R and Ca^{2+} channels in the activation of calcium dysregulation. PSD rats were co-administered P2X7R and Ca^{2+} inhibitors. Based on the inflammatory responses and molecular expressions, we inferred that the inhibition of the Ca^{2+} channel further promoted the effect induced by P2X7R inhibition. As discussed earlier, P2X7R is a well-characterized ion channel that is permeable to Ca^{2+} [39, 40]; thus, P2X7R inhibition should exert its effect on calcium dysregulation by influencing the level of Ca^{2+}. Our results partially support the theory that the inhibition of the Ca^{2+} channel further suppressed an inflammatory response that was firstly inhibited by P2X7R inhibition. From our inference of the role of P2X7R in the progression of PSD, we understand that this synergistic effect of Ca^{2+} channel inhibition on P2X7R inhibition would contribute to the attenuation of PSD.

Collectively, the findings of this study showed that the induction of PSD symptoms would induce the expression of P2X7R, which would result in the dysregulation of Ca^{2+} in the cells. Subsequently, abnormal levels of Ca^{2+} would initiate an inflammatory response in brain tissues and, finally, promote the progression of PSD. Fortunately, the inhibition of the P2X7R and Ca^{2+} channel both inhibited this inflammatory response and attenuated PSD. Based on our results, P2X7R is a promising target for future research into the development of anti-PSD strategies as well as other treatments for disorders associated with Ca^{2+} dysregulation. However, there are limitations to the current study. More function (such as apoptosis) and mechanism (such as the regulatory genes of P2X7R) also need to be further explored.

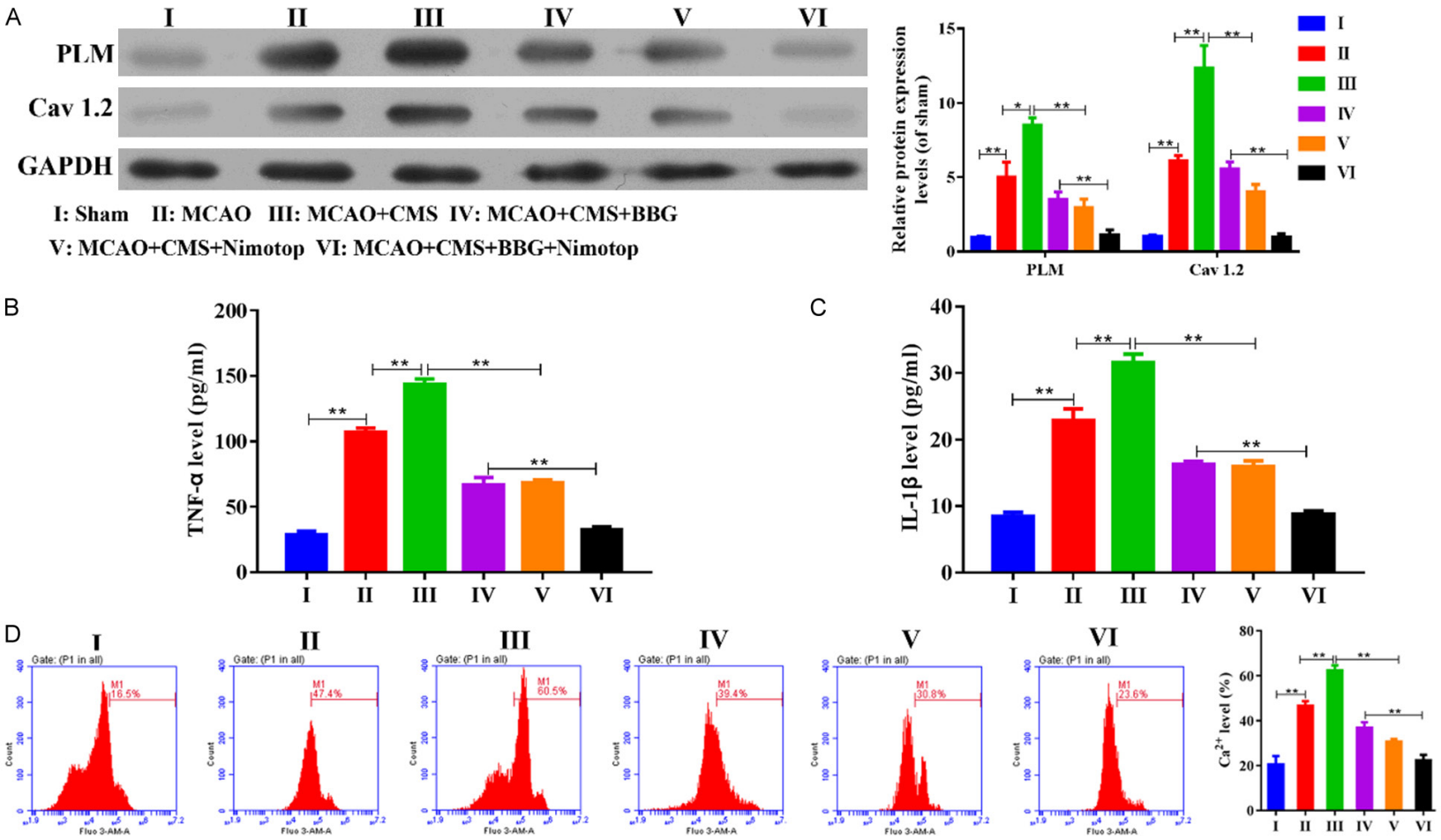

Figure 6. Interaction between P2X7R and Ca^{2+} channel determined the function of P2X7R in the initiation of inflammatory responses. PSD rats were further subjected to the coadministration of a P2X7R inhibitor (BBG) and a PLM inhibitor (Nimotop). A. Representative images and results from the quantitative analysis of Western blotting for the detection of PLM and Cav1.2 protein levels in brain tissues collected 3 d after model induction. B. Results from the quantitative analysis of ELISA detection of TNF-α level in serum samples collected 3 d after model induction. C. Results from the quantitative analysis of ELISA detection of IL-1β level in serum samples collected 3 d after model induction. D. Representative images and results from the quantitative analysis of flow cytometric detection of Ca^{2+} accumulation in brain tissues collected 3 d after model induction. $^{*}P < 0.05$, $^{**}P < 0.01$. n = 5 for each group.

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Disclosure of conflict of interest

None.

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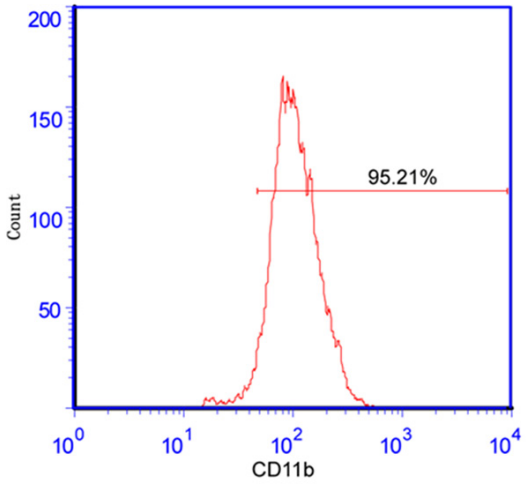

Figure S1. The expression of CD11b-positive cells from sham and TBI mice.