RESEARCH ARTICLE

Fluoxetine Inhibits NLRP3 Inflammasome Activation: Implication in Depression

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Abstract

Background: Emerging evidence indicates that NLRP3 inflammasome-induced inflammation plays a crucial role in the pathogenesis of depression. Thus, inhibition of NLRP3 inflammasome activation may offer a therapeutic benefit in the treatment of depression. Fluoxetine, a widely used antidepressant, has been shown to have potential antiinflammatory activity, but the underlying mechanisms remain obscure.
Methods: We used a chronic mild stress model and cultured primary macrophage/microglia to investigate the effects of fluoxetine on NLRP3 inflammasome and its underlying mechanisms.
Results: We demonstrated that fluoxetine significantly suppressed NLRP3 inflammasome activation, subsequent caspase-1 cleavage, and interleukin-1β secretion in both peripheral macrophages and central microglia. We further found that fluoxetine reduced reactive oxygen species production, attenuated the phosphorylation of double-stranded RNA-dependent protein kinase, and inhibited the association of protein kinase with NLRP3. These data indicate that fluoxetine inhibits the activation of NLRP3 inflammasome via downregulating reactive oxygen species-protein kinase-NLRP3 signaling pathway. Correspondingly, in vivo data showed that fluoxetine also suppressed NLRP3 inflammasome activation in hippocampus and macrophages of chronic mild stress mice and alleviated chronic mild stress-induced depression-like behavior.
Conclusions: Our findings reveal that fluoxetine confers an antidepressant effect partly through inhibition of peripheral and central NLRP3 inflammasome activation and suggest the potential clinical use of fluoxetine in NLRP3 inflammasome-driven inflammatory diseases such as depression.

Keywords: fluoxetine, reactive oxygen species, NLRP3 inflammasome, depression, double-stranded RNA-dependent protein kinase

Introduction

Depression is a severe psychiatric disorder with a lifetime prevalence in excess of 15% and is the fourth leading cause of disability worldwide (DelMastro et al.,2015). Several lines of evidence have demonstrated that NLRP3 inflammasome plays a pivotal role in the pathophysiology of depression (Alcocer-Gomez and Cordero, 2014; Alcocer-Gomez et al., 2014; Choi and Ryter, 2014; Zhang et al., 2015). NLRP3 inflammasome has been found to be activated in patients with major depressive disorder (Alcocer-Gomez et al., 2015) and in rodent models of depression (Zhang et al., 2014), whereas antidepressant treatment could suppress NLRP3 inflammasome activation (Lu et al., 2014; Liu et al., 2015; Xue et al., 2015; Li et al., 2016). Stress-induced depressive behaviors require a

functional NLRP3 inflammasome (Pan et al., 2014). NLRP3 deletion inhibits these stress-like effects (Zhang et al., 2015). These findings indicate that NLRP3 inflammasome could be a target for new therapeutic interventions to treat depression in patients.

NLRP3 inflammasome is an intracellular multiprotein complex that consists of nod-like receptor protein 3, adaptor protein ASC, and procaspase-1 precursor (Schroder and Tschopp, 2014). This complex is noted for its broad array of activating stimuli, which include bacterial, fungal, and viral components; endogenous danger signals such as extracellular ATP; and environmental microparticles such as silica crystals (Martinon et al., 2006; Rathinam et al., 2012). NLRP3 inflammasome activation leads to the maturation of caspase-1 and the processing of its substrates, interleukin (IL)-1β and IL-18 (Shao et al., 2015). Normal activation of the NLRP3 inflammasome contributes to host defense (Jo et al., 2015). However, excessive activation of the NLRP3 inflammasome participates in the pathogenesis of a spectrum of diseases, including type 2 diabetes (Kim et al., 2016), rheumatoid arthritis (Choulaki et al., 2015), Alzheimer's disease (Lamkanfi and Dixit, 2014), and depression (Pan et al., 2014). Thus, the functional manipulation of the NLRP3 inflammasome has been demonstrated to be a promising therapeutic strategy for these diseases (Di Virgilio, 2014; Xue et al., 2015).

Fluoxetine, a selective serotonin reuptake inhibitor antidepressant, is commonly prescribed for treating depression due to its tolerability and safety (Montgomery, 1998; Garrison and Levin, 2000). Increasing evidence indicates that fluoxetine inhibits the production of proinflammatory cytokines including IL-1β, but the underlying mechanisms are still poorly understood (Liu et al., 2013; Branco-de-Almeida et al., 2014). Since the IL-1β secretion is tightly controlled by NLRP3 inflammasome, we thus hypothesize that fluoxetine might regulate NLRP3 inflammasome activation. To test this possibility, we prepared a chronic mild stress (CMS) model and cultured primary macrophage/microglia to investigate the effects of fluoxetine on NLRP3 inflammasome and its underlying mechanisms in vitro and in vivo. It was found that fluoxetine suppressed NLRP3 inflammasome activation via inhibiting the reactive oxygen species (ROS)-double-stranded RNA-dependent protein kinase (PKR)-NLRP3 signaling pathway in peripheral macrophages and central microglia. Furthermore, fluoxetine also inhibited NLRP3 inflammasome activation in hippocampus and macrophages of CMS mice. Our findings suggest fluoxetine may have promising clinical effects in NLRP3 inflammasome-driven inflammatory diseases such as depression.

MATERIALS AND METHODS

Animals and Reagents

Male C57BL/6 mice (8–12 weeks) were maintained in specific pathogen-free facilities at the Animal Care Facility of Nanjing Medical University. All experimental procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all animals were treated according to protocols approved by Institutional Animal Care and Use Committee of Nanjing Medical University.

The following antibodies were used: goat anti-NLRP3 (sc-34410, Santa Cruz Biotechnology), rabbit anti-Caspase-1 (sc-514, Santa Cruz Biotechnology), goat anti-IL-1β (AF-401-NA, R&D), rabbit anti-phospho-PKR (Thr446) (11280, Signalway antibody), and rabbit anti-PKR (21272, Signalway antibody). Lipopolysaccharide (LPS) (*Escherichia coli*, 0111:B4), ATP, and fluoxetine were from Sigma. ROS-specific fluorescent probe CM-H2DCFDA was from Invitrogen. IL-1β ELISA kits was obtained from R&D Systems.

Cell Culture and Stimulation

Bone marrow-derived macrophages (BMDMs) were derived from tibia and femoral bone marrow cells and cultured for 7 days in Dulbecco's modified essential media complemented with 10% fetal bovine serum, 1% penicillin/streptomycin (vol/vol), and 50 nM GMSF. Cortical mixed glial cultures were generated from male C57BL/6 mice postnatal day 0 to 3 pups. Microglia were isolated by collecting the floating fraction of 10-day-old mixed glial cultures following 1 hour on a rotary shaker at 37°C at 250 rpm and plated in Dulbecco's modified essential media containing 4.5 g/L glucose, 2 mM L-glutamine, 1 mM sodium pyruvate, 10% fetal bovine serum, and 1% penicillin/streptomycin (vol/vol) on poly-d-lysine-coated 24-well culture plates. The purity of BMDMs and microglial cultures was >95% as determined with immunocytochemistry. Macrophages, microglia, RAW264.7 cells were stimulated with LPS (100 ng/mL) for 6 hours and then pulsed with 5 mM ATP for 30 minutes. In the experiments, cells were pretreated with the indicated concentrations of fluoxetine for 1 hour before LPS stimulation. Cell extracts and precipitated supernatants were analyzed by immunoblot and ELISA.

Cell Viability Assay

Cell viability was determined by the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) assay. BMDMs were plated into 96-well culture plates at a density of 5×10^4 cells/mL with 200 mL culture medium per well. Following treatment with different concentrations of fluoxetine, 20 mL MTT solution (5 mg/mL) was added to each well and incubated at 37°C for 4 hours. The medium was aspirated and 200 mL dimethyl sulfoxide was added. The absorbance value was measured using a multi-well spectrophotometer (Bio-Rad) at 490 nm.

CMS Procedure

Individually housed male mice were allowed to acclimate for 1 week and then were subjected to 5 weeks of stressors, which were mild and unpredictable in nature, duration, and frequency. Stressors included inversion of day/night light cycle, soiled cage bedding, 45° tilted cage, restraint, overnight food and water deprivation, and pairing with another stressed animal. All of

Table 1. The Stressors of a CMS Procedure

Stressor	Description	Duration (h)
Tilted caging	Each cage tilted to a 45-degree angle	18
Wet caging	200 mL water in 100 g sawdust bedding	12
Paired caging	Put mouse into cage of strange rat	12
Water/food deprivation	Deprivation water and food	12
Restraint stress	The mice are kept in closed and ventilated tubes	4
Intermittent illumination	2-h/2-h light/dark cycle	12
Continuous illumination	Continuous illumination in dark cycle	12

the stressors were shown in Table 1. Sucrose preference and the body weight of each animal were evaluated weekly until the end of the CMS. All the mice treated with CMS for 4 weeks when depressive behaviors occurred began receiving fluoxetine (10 mg/kg/d i.p) administration for 4 weeks. The CMS procedure was continued during the entire fluoxetine treatment period. The concentration of fluoxetine was consistent with previous research (Yang et al., 2013).

Sucrose Preference Test

Mice were given the choice to drink from 2 bottles for 10 hours; one contained a sucrose solution (1%) and the other contained only tap water. To prevent possible effects of side preference in drinking behavior, the positions of the bottles in the cage were switched after 5 hours. The animals were deprived of water for 12 hours before the test. The consumption of tap water, sucrose solution, and total intake of liquids was estimated simultaneously in the control and experimental groups by weighing the bottles. The preference for sucrose was measured as a percentage of the consumed sucrose solution relative to the total amount of liquid intake.

Tail Suspension Test

Mice tails were wrapped with tape from the base to the tip, covering about four-fifths of its length and fixed upside down on the hook. The immobility time of each mouse was recorded over a 6-minute period. Mice were considered immobile only when they hung passively and completely motionless. The time of immobility of the tail suspended mice during the last 4 minutes was measured with TailSuspScan (Clever Sys).

Forced Swim Test

Mice were individually forced to swim in an open cylindrical container (diameter, 15 cm; height, 25 cm) containing 14 cm of water at room temperature (about $22 \pm 1°C$) for 6 minutes. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. The duration of immobility was recorded during the last 4 minutes of the 6-minute testing period by TailSuspScan (Clever Sys). The immobility observed in this test is considered to reflect a state of despair.
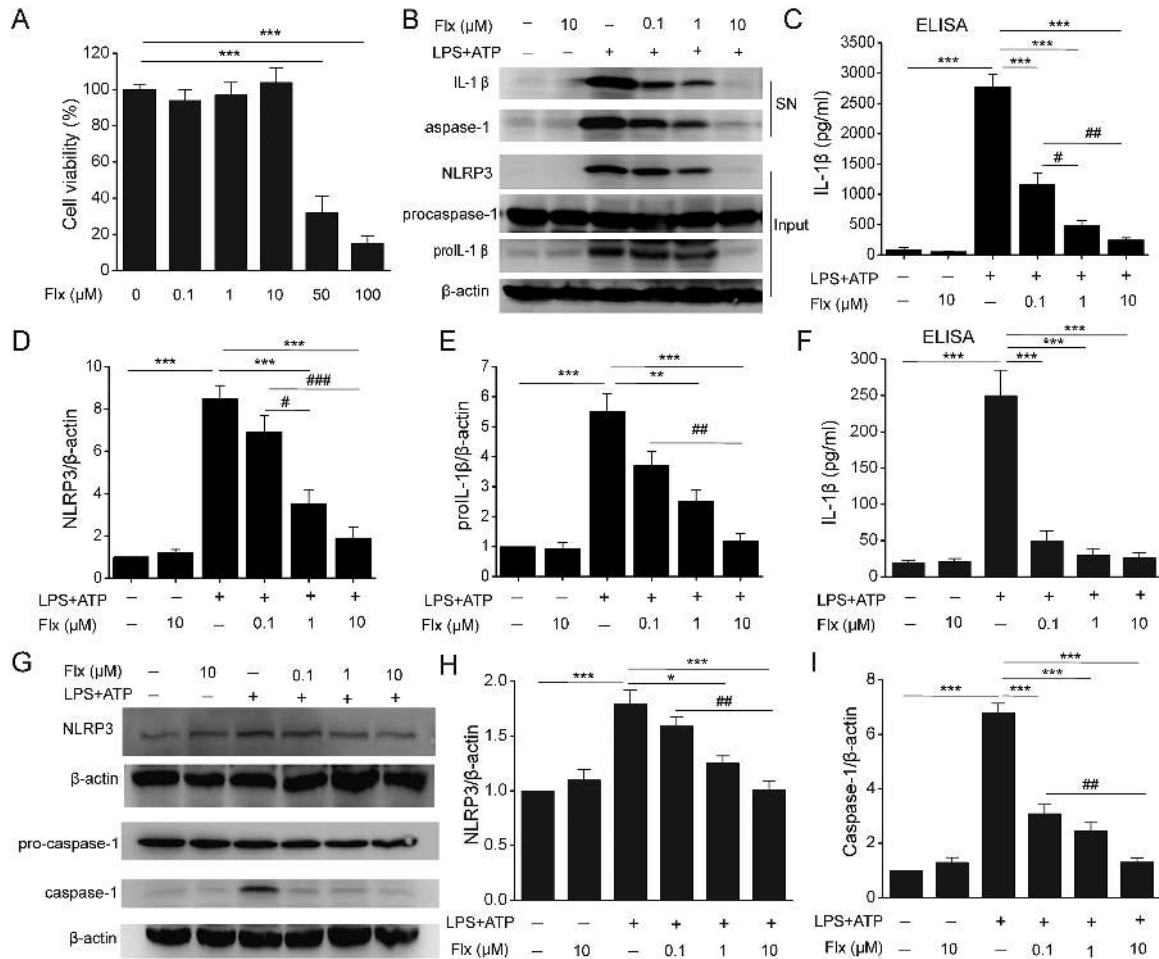
Figure 1. Fluoxetine inhibited NLRP3 inflammasome activation in bone marrow-derived macrophages (BMDMs) and RAW264.7 macrophages. BMDMs and RAW264.7 macrophages were incubated with indicated concentrations of fluoxetine (Flx, 0.1 μM, 1 μM, 10 μM) for 1 hour, then stimulated with LPS (100 ng/mL) followed by 30 minutes of ATP (5 mM) treatment. (A) The cytotoxicity of fluoxetine (Flx, 0.1 μM, 1 μM, 10 μM, 50 μM, 100 μM) was measured by 3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide (MTT) assay (***$P < .001$ vs Flx 0 μM). There were no significant differences among Flx 0 μM, Flx 0.1 μM, Flx 1 μM, and Flx 10 μM groups. (B) Immunoblot analysis of interleukin (IL)-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of BMDMs and immunoblot analysis of the NLRP3, proIL-1β, and procaspase-1 in lysates of those cells (input). (C) ELISA of IL-1β in supernatants of BMDMs. (D-E) Quantitative analysis of NLRP3 (D) and proIL-1β (E) in BMDMs. (F) ELISA of IL-1β secretion in supernatants of RAW264.7 macrophages. (G-I) Representative immunoblots (G) and quantitative analysis of NLRP3 (H) and caspase-1 (I) in RAW264.7 macrophages. Data are represented as means ± SEM from 2 replicates in 3 independent experiments. *$P < .05$, **$P < .01$, ***$P < .001$ vs LPS+ATP; #$P < .05$, ##$P < .01$, ###$P < .001$ vs LPS+ATP+Flx 0.1 μM. There were no significant differences between -LPS-ATP and -LPS-ATP + Flx 10 μM.

ELISA

Cell culture supernatants and serum were assayed for IL-1β with ELISA kits from R&D Systems according to the manufacturer's instructions.

ROS Detection

Intracellular ROS was measured with the ROS-specific fluorescent probe CM-H2DCFDA. BMDMs were incubated with 25 μM H_2DCF-DA for 30 minutes and washed twice with PBS. Fluorescence was measured at $OD_{485-530}$ using a multi-well spectrophotometer. The ROS production intensity of untreated control cells was arbitrarily set to 100%.

Coimmunoprecipitation

The cell lysates from BMDMs were immunoprecipitated with anti-NLRP3 antibody followed by protein A/G plus agarose (Santa Cruz Biotechnology). After washing, bound proteins were eluted from the beads and analyzed by immunoblot for PKR.

Western-Blotting Analysis

Cell lysates were homogenized in lyses buffer (Beyotime, China) and protein concentration was determined by the Bradford assay (Bio-Rad, Hercules, CA). The analysis of protein was performed according to standard SDS-PAGE. Proteins were separated on 10% to 15% Tris-HCl polyacrylamide gels (Bio-Rad) and transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking, the blots were incubated with specific antibodies as described above in Tris-buffered saline with Tween 20 overnight at 4°C, and then with horseradish peroxidase conjugated secondary antibodies. Immunoreactive bands were detected by enhanced chemiluminescence plus detection reagent (Pierce, Rockford, IL) and analyzed using an Omega 16ic Chemiluminescence Imaging System (Ultra-Lum, CA).

Statistical Analysis

Data are presented as mean ± SEM. Comparisons between multiple groups were made using a 2-tailed, unequal-variance Student's t test, or ANOVA followed by Tukey's posthoc analysis. Differences were considered significant at $P < .05$.

RESULTS

Fluoxetine Inhibits NLRP3 Inflammasome Activation in Macrophages

MTT assay was performed to determine its cytotoxicity to BMDMs and found fluoxetine (0.1 μM, 1 μM, and 10 μM) treatment had no effect on cell viability (Figure 1A) (F = 0.462, P = .717). To determine the effects of fluoxetine on NLRP3
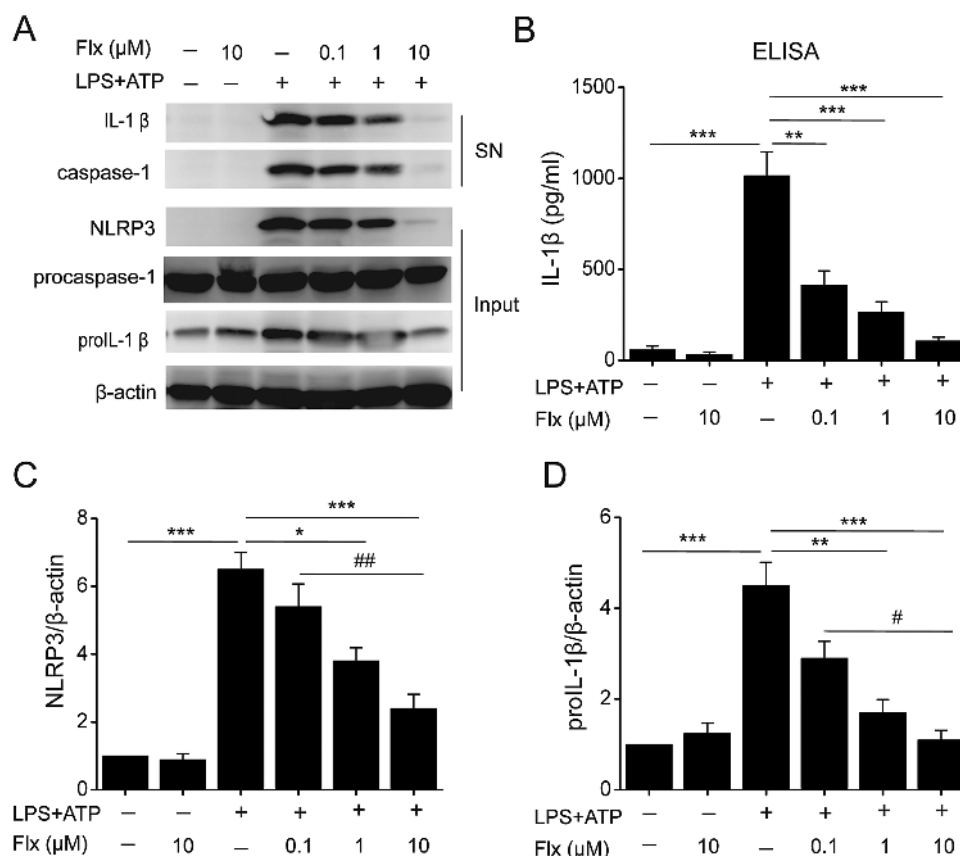
Figure 2. Fluoxetine suppressed NLRP3 inflammasome activation in microglia. Microglia were incubated with indicated concentrations of fluoxetine (Flx, 0.1 μM, 1 μM, 10 μM) for 1 hour, then stimulated with LPS (100 ng/mL) followed by 30 minutes of ATP (5 mM) treatment. (A) Immunoblot analysis of interleukin (IL)-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of microglia and immunoblot analysis of the NLRP3, proIL-1β, and procaspase-1 in lysates of those cells (input). (B) ELISA of IL-1β in supernatants of microglia. (C-D) Quantitative analysis of NLRP3 (C) and proIL-1β (D) in microglia. Data are represented as means ± SEM from 2 replicates in 3 independent experiments. *P < .05, **P < .01, ***P < .001 vs LPS+ATP; #P < .05, ##P < .01 vs LPS+ATP+Flx 0.1 μM. There were no significant differences between -LPS-ATP and -LPS-ATP + Flx 10 μM.

inflammasome activation, LPS-primed BMDMs were pretreated with fluoxetine before ATP challenge. Fluoxetine indeed reduced NLRP3-dependent caspase-1 activation and IL-1β maturation in a concentration-dependent manner (Figure 1B-C) (F = 82.249, P < .01). It has been reported that fluoxetine can repress LPS-induced NF-κB activation (Du et al., 2014), so we then examined whether fluoxetine had an impact on priming for inflammasome activation. As shown in Figure 1D-E, fluoxetine significantly decreased LPS-induced NLRP3 (F = 34.599, P < .01) and pro-IL-1β expression (F = 24.202, P < .01). The observed inhibitory effects of fluoxetine on NLRP3 inflammasome activation were also confirmed in RAW264.7 macrophages (Figures 1F-I, F = 32.306, P < .01). These results demonstrate that fluoxetine inhibits NLRP3 inflammasome activation in macrophages.

Fluoxetine Suppresses NLRP3 Inflammasome Activation in Microglia

Microglia, the resident macrophages of the CNS, are the major sites contributing to the inflammatory responses in the brain (Kim et al., 2015) and play a crucial role in the pathogenesis of depression (Dheen et al., 2007; Irwin et al., 2012; Brites and Fernandes, 2015; Yirmiya et al., 2015). Therefore, we then sought to study the regulation of NLRP3 inflammasome by fluoxetine in microglia. Similar to the results obtained in the macrophages, fluoxetine inhibited ATP-induced caspase-1 activation and

IL-1β production (Figure 2A-B) (F = 29.815, P < .01) in microglia. Moreover, fluoxetine also reduced the expression of NLRP3 (F = 30.162, P < .01) and pro-IL-1β (Figure 2C-D, F = 19.732, P < .01). These data indicate that fluoxetine indeed can block NLRP3 inflammasome activation in microglia.

The ROS-PKR-NLRP3 Signaling Pathway Mediates the Inhibitory Effect of Fluoxetine on NLRP3 Inflammasome in Macrophages

Our preceding data have strongly demonstrated that fluoxetine inhibited NLRP3 inflammasome. To dissect the underlying molecular mechanisms, we first measured the ROS production, which is believed to be a common NLRP3 activator (Martinon, 2015). Fluoxetine significantly decreased ATP-induced ROS production as detected by the fluorescence of CM-H$_2$DCFDA in BMDMs (Figure 3A-B) (F = 26.807, P < .01). It has been demonstrated that ROS induces double-stranded PKR phosphorylation and activates NLRP3 (Lu et al., 2013). We then determined if fluoxetine could alter PKR activity. As shown in Figure 3C, PKR was effectively phosphorylated in BMDMs following ATP stimulation at 30 minutes (F = 16.5, P < .01). Pretreatment with fluoxetine significantly reduced the phosphorylation of PKR (Figure 3D) (F = 18.146, P = .001) and the interaction between PKR and NLRP3 (Figure 3E) (F = 73.049, P < .01). These data indicate that fluoxetine blocks NLRP3 inflammasome by inhibiting ROS-PKR-NLRP3 signaling pathway.
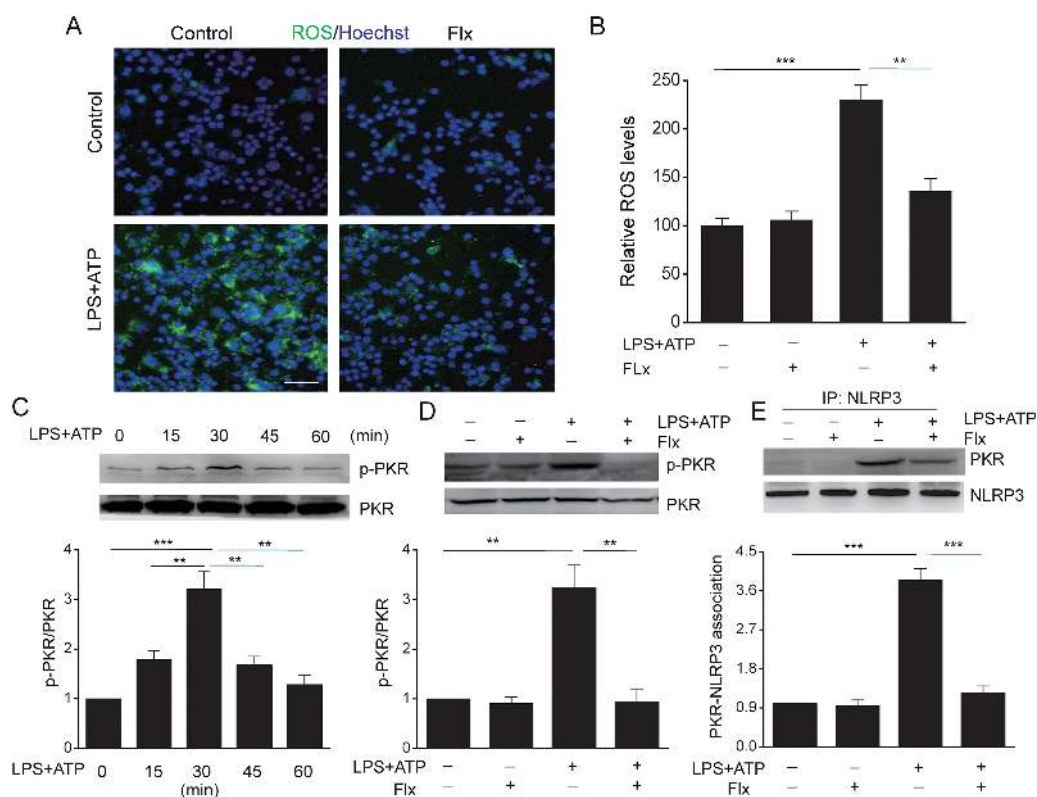
Figure 3. Fluoxetine reduced ATP-induced ROS production, RNA-dependent protein kinase (PKR) phosphorylation and the association of PKR with NLRP3 in macrophages. Bone marrow-derived macrophages (BMDMs) were pretreated with fluoxetine (Flx, 10 μM) for 1 hour, then stimulation with LPS+ATP. (A) Cells were stained with H$_2$DCF-DA labeling and images captured with a fluorescence microscope. (B) The quantitative analysis of ROS levels by a Titertek Fluoroskan II microtiter fluorescence plate reader. (C) LPS-primed BMDMs were stimulated with ATP at indicated time points and phosphorylated and total PKR were determined by Western-blot analysis (**P < .01, ***P < .001 vs LPS+ATP 30 min). (D-E) BMDMs were pretreated with fluoxetine (Flx, 10 μM) for 1h, followed by stimulation with LPS+ATP for 30 minutes. (D) Representative immunoblots and quantitative analysis of phosphorylated and total PKR. (E) Immunoprecipitation and immunoblot analysis of the PKR-NLRP3 interaction. Data are represented as means ± SEM from 2 replicates in 3 independent experiments. **P < .01, ***P < .001 vs LPS+ATP. There were no significant differences among -LPS-ATP, -LPS-ATP+Flx, and +LPS+ATP+Flx.

Fluoxetine Suppresses NLRP3 Inflammasome Activation via Inhibiting the Association of PKR with NLRP3 in Hippocampus of CMS Mice

Increasing evidence suggest that NLRP3 inflammasome is involved in the pathogenesis of depression (Alcocer-Gomez et al., 2015; Zhang et al., 2015). We then wanted to know whether fluoxetine can prevent NLRP3 inflammasome-driven inflammation in CMS-induced model of depression. To address this question, we prepared the CMS model and found that fluoxetine administration significantly improved depressive behavior, including increased sucrose preference (Figure 4A) (F=11.923, P<.01) and decreased duration of immobility time in TST (Figure 4B) (F=21.342, P<.01) and FST (Figure 4C) (F=9.106, P<.01). Meanwhile, CMS significantly increased NLRP3 expression, caspase-1 cleavage and IL-1β production in hippocampus, and this effect was reversed by fluoxetine treatment (Figures 4D-G, F=11.698, P<.01). In addition, we also found that fluoxetine significantly inhibited the CMS-induced PKR phosphorylation (Figure 4H) (F=15.799, P<.01) and an association between PKR-NLRP3 (Figure 4I) (F=18.402, P=.001) in hippocampus. These data suggest that fluoxetine indeed inhibits CMS-induced NLRP3 inflammasome activation via suppression of the interaction between PKR and NLRP3 in mice.
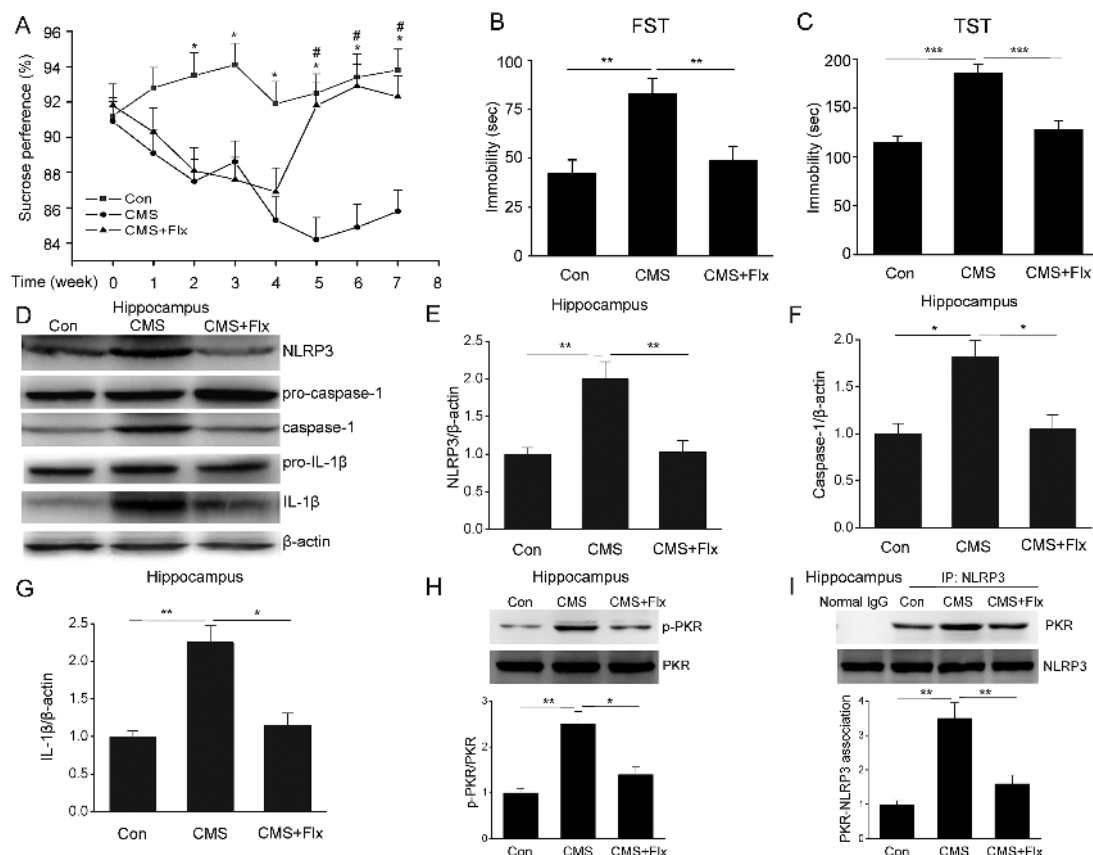
Fluoxetine Inhibits NLRP3 Inflammasome Activation in Macrophages of CMS Mice

Several lines of evidence have demonstrated that peripheral inflammation plays an important role in the progression of depression (Reader et al., 2015). Since macrophages are the main cells responsible for peripheral inflammation, we presume that fluoxetine might regulate NLRP3 inflammasome activation in macrophages in a CMS-induced model of depression. To verify this hypothesis, we first measured the levels of IL-1β in serum and found that fluoxetine treatment significantly decreased serum IL-1β production in the CMS model (Figure 5A) (F=18.937, P<.01). As IL-1β mature is dependent on NLRP3 inflammasome, we then cultured the BMDMs from CMS mice to investigate the impact of fluoxetine on NLRP3 inflammasome. As shown in Figures 5B-E, the expression of NLRP3 (F=29.21, P<.01), caspase-1 activation (F=25.201, P<.01), and subsequent IL-1β mature (F=73.957, P<.01) in BMDMs is significantly increased in CMS mice, and this enhancement was abolished by fluoxetine treatment. These results indicate that fluoxetine could inhibit CMS-induced NLRP3 inflammasome activation in a macrophage in model of depression.

Discussion

The most important finding presented here is that fluoxetine inhibits the NLRP3 inflammasome activation in vitro and in vivo. Activation of the NLRP3 inflammasome requires 2 signals (Franchi et al., 2014; Elliott and Sutterwala, 2015). The first signal is induced by endogenous molecules or microbial that activates NF-κB to increase the expression of NLRP3 and proIL-1β, which is a prerequisite for inflammasome activation (Bauernfeind et al., 2009). The second signal directly activates the NLRP3

Figure 4. Fluoxetine inhibited chronic mild stress (CMS)-induced NLRP3 inflammasome activation via suppression of the association RNA-dependent protein kinase (PKR) with NLRP3 in hippocampus of mice. (A) Sucrose preference (n=18/groups; *P<.05, #P<.05 vs CMS). (B) Tail suspension test (n=18/groups). (C) Forced swim test (n=18/groups). Representative immunoblots (D) and quantitative analysis of NLRP3 (E), caspase-1 (F) and IL-1β (G) in hippocampus of CMS mice (n=4/groups). (H) Representative immunoblots and quantitative analysis of phosphorylated and total PKR (n=4/groups). (I) Immunoprecipitation and immunoblot analysis of the PKR-NLRP3 interaction (n=4/groups). Data are represented as means ± SEM. *P<.05, **P<.01 vs CMS.
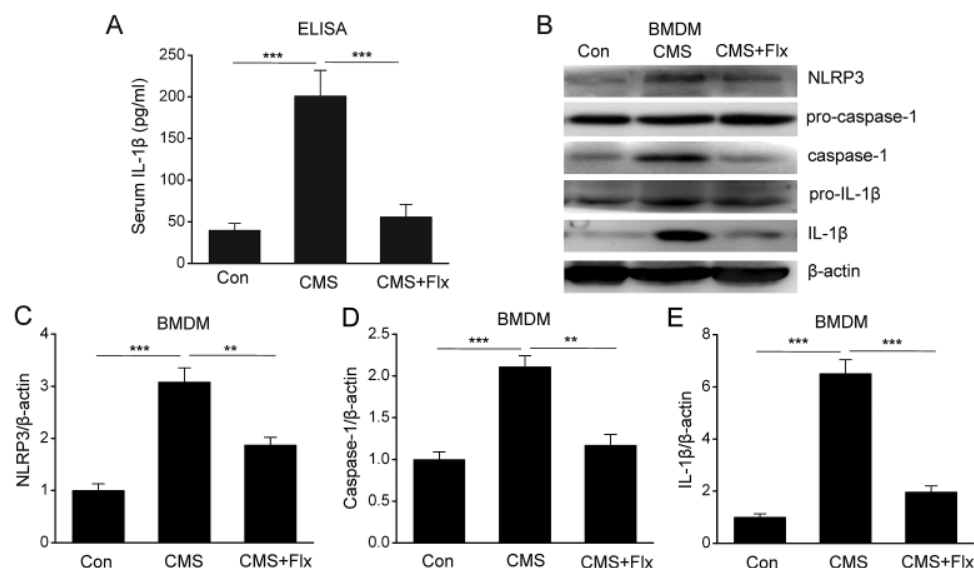
Figure 5. Fluoxetine inhibited NLRP3 inflammasome activation in bone marrow-derived macrophages (BMDMs) of chronic mild stress (CMS) mice. (A) Serum interleukin (IL)-1β levels by ELISA (n=18/groups). Representative immunoblots (B) and quantitative analysis of NLRP3 (C), caspase-1 (D), and IL-1β (E) in BMDMs of CMS mice (n=4/groups). Data are represented as means ± SEM. $^{**}P<.01$, $^{***}P<.001$ vs CMS.

inflammasome to induce caspase-1 cleavage, leading to the maturation of the proinflammatory cytokines IL-1β and IL-18 (Schroder and Tschopp, 2014). In the present study, we found that fluoxetine reduced LPS-induced expression of NLRP3 and proIL-1β. We also observed that fluoxetine suppressed activation of caspase-1 and the maturation of IL-1β in response to NLRP3 activators such as ATP. These data indicate that fluoxetine inhibits both the priming (signal 1) and the activation of the NLRP3 inflammasome (signal 2).

Our studies have further revealed the molecular mechanism underlying the regulation of NLRP3 inflammasome activation by fluoxetine. The production of ROS is believed to be a common activator of the NLRP3 inflammasome (Martinon, 2015). It has been demonstrated that ROS can enhance PKR phosphorylation (Pyo et al., 2008), which plays a crucial role in the activation of NLRP3 inflammasome (Lu et al., 2013). In this study, we found that fluoxetine not only decreased the ROS production but also reduced the activity of PKR and PKR-NLRP3 association in macrophages. These data demonstrate that fluoxetine inhibits the activation of NLRP3 inflammasome through controlling the production of ROS and the association of NLRP3 with PKR. Fluoxetine reduces the level of ROS, which attenuates the phosphorylation of PKR and the PKR-NLRP3 interaction and subsequently inhibits the NLRP3 inflammasome activation. Taken together, these findings indicate that the ROS-PKR-NLRP3 signal pathway contributes to the inhibition of the NLRP3 inflammasome activation by fluoxetine.

In addition, we provide evidence that fluoxetine ameliorates CMS-induced depressive behavior via inhibiting peripheral and central NLRP3 inflammasome activation. Several studies demonstrate that NLRP3 inflammasome plays a critical role in the pathogenesis of depression (Alcocer-Gomez et al., 2014; Deng et al., 2015). Thus, the NLRP3 inflammasome may represent a novel therapeutic target for depression. In this study, we used a CMS-induced anhedonia model of depression and found that CMS led to activation of the NLRP3 inflammasome in the hippocampus and BMDMs. Fluoxetine treatment abolished CMS-induced activation of NLRP3 inflammasome, accompanied by improved depressive behavior including a significant increase in the sucrose preference and decrease in the duration

of immobility time. These data suggest that suppression of peripheral and central NLRP3 inflammasome activation may contribute to the improved depressive behaviors observed in fluoxetine-treated mice.

In conclusion, our study demonstrates that fluoxetine suppresses CMS-induced NLRP3 inflammasome activation in the hippocampus and in BMDMs, accompanied by improvement of depressive behavior. Furthermore, fluoxetine inhibits the activation of NLRP3 inflammasome via downregulating ROS-PKR-NLRP3 signaling pathway in macrophages and microglia (Figure 6). Collectively, our findings reveal that fluoxetine confers an antidepressant effect partly through inhibition of peripheral
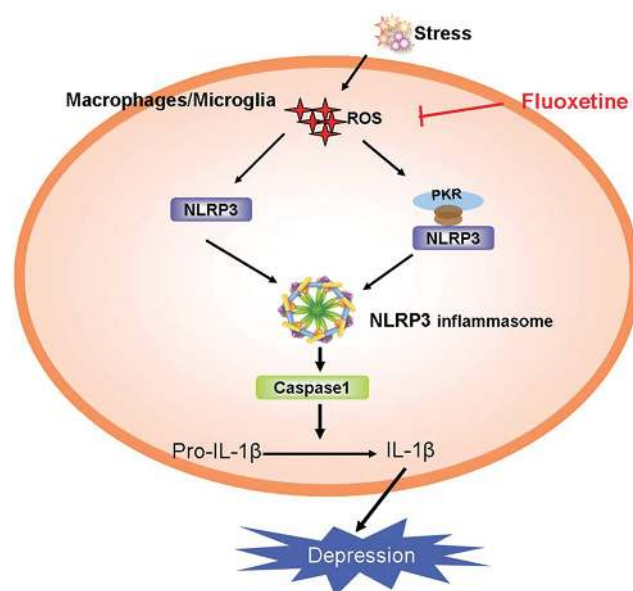
Figure 6. Schematic layout of showing fluoxetine inhibits NLRP3 inflammasome activation via ROS- RNA-dependent protein kinase-NLRP3 signaling pathway in depression. Fluoxetine decreases the level of ROS, which attenuates PKR phosphorylation and the interaction of PKR with NLRP3, and subsequently inhibits the NLRP3 inflammasome activation.

and central NLRP3 inflammasome activation and suggest the promising clinical use of fluoxetine in NLRP3 inflammasome-driven inflammatory diseases such as depression.

Acknowledgments

Statement of Interest

None.

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