千葉大学学位申請論文

Effects of Antidepressants on Cytokine Alterations in Serum and Depressive-like Behavior After Lipopolysaccharide Administration in Mice

（リポポリサッカライド投与マウスにおける血清中サイトカイン変化と
うつ様症状に対する抗うつ薬の作用に関する研究）

千葉大学大学院医学薬学府

環境健康科学専攻 神経科学

（主任　橋本 謙二　教授）

大木 雄太

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Abstract

Accumulating evidence suggests that inflammation may play a role in the pathophysiology of major depressive disorder (MDD). Antidepressants, including selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), possess anti-inflammatory effects *in vitro*. Here, we examined the effects of SSRIs and SNRIs on lipopolysaccharide (LPS)-induced inflammation and depressive-like behavior in male mice. A single administration of LPS (0.5 mg/kg, i.p.) increased serum levels of the pro-inflammatory cytokine, tumor necrosis factor alpha (TNFα) and the anti-inflammatory cytokine, interleukin-10 (IL-10) in mice. Pretreatment with SSRIs (fluoxetine and paroxetine), SNRIs (venlafaxine and duloxetine), or 5-hydroxytryptophan (5-HTP), a precursor of serotonin, attenuated LPS-induced increases in TNFα, whereas it increased serum levels of IL-10, in mice treated with LPS. In the tail suspension test (TST), LPS increased the immobility time without affecting spontaneous locomotor activity, suggesting that LPS induced depressive-like behavior in mice. Treatment with fluoxetine (30 mg/kg) or paroxetine (10 mg/kg) significantly shortened LPS-induced increases of immobility time. These results suggested that antidepressants exert anti-inflammatory effects *in vivo,* and that the serotonergic system may partially mediate these effects. In addition, the anti-inflammatory effects of antidepressants may help alleviate the symptoms of LPS-induced depression in mice.

Abbreviation list

MDD, Major depressive disorder; TNFα, Tumor necrosis factor alpha; IL, Interleukin; NSAIDs, Non-steroidal anti-inflammatory drugs; MADRS, Montgomery-Asberg Depression Rating Scale; RA, Rheumatoid arthritis; LPS, Lipopolysaccharide; FST, Forced swimming test; TST, Tail suspension test; SSRI, Selective serotonin reuptake inhibitor; SNRI, Serotonin and norepinephrine uptake inhibitor; IFN, Interferon; 5-HTP, 5-Hydroxy-tryptophan; ELISA, Enzyme Immunosorbent Assay; i.p., Intraperitoneally; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; ANOVA, Analysis of variance; SERT, Serotonin transporter; IDO, Indoleamine-2, 3-dioxigenase.

1. Introduction

Major depressive disorder (MDD) is a common and recurrent mood disorder. The estimated lifetime prevalence is approximately 16.6% (Kessler et al., 2003). However, little is known about the pathophysiology of MDD (Hashimoto, 2009; Hashimoto et al., 2004). Accumulating evidence suggests that inflammation may play a role in the pathophysiology of MDD. Meta-analysis shows higher blood levels of pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), in drug-free depressive patients compared with healthy controls (Dowlati et al., 2010). A postmortem brain study showed elevated gene expression of pro-inflammatory cytokines in frontal cortex of people with a history of MDD (Shelton et al., 2011). A non-steroidal anti-inflammatory drug (NSAID) celecoxib was capable of augmenting the therapeutic efficacy of the selective serotonin reuptake inhibitor (SSRI) sertraline in MDD (Abbasi et al., 2012). Therapeutic administration of interferon-α (IFNα) in patients with hepatitis-C, increases pro-inflammatory cytokine levels in serum, leading to depressive symptoms. This increase of pro-inflammatory cytokines correlates positively with the severity of depression as indicated by the Montgomery-Asberg Depression Rating Scale (MADRS) (Bonaccorso et al., 2001; Wichers et al., 2007). Moreover, rheumatoid arthritis (RA) and the accompanying systemic inflammation shows high comorbidity with depression, and therapeutic administration of anti-TNFα antibodies reduces this high prevalence of depression (Uguz et al., 2009). These findings suggest that both peripheral and central inflammations are associated with depressive symptoms, and that inhibition of the inflammatory process could ameliorate these symptoms.

In animal studies, peripheral administration of bacterial endotoxin

lipopolysaccharide (LPS) (Kang et al., 2011; O'Connor et al., 2009) or TNFα (Kaster et al., 2012) induces depressive-like behavior in the forced swimming test (FST) and the tail suspension test (TST). LPS-induced depressive-like behavior is blocked by NSAIDs, while TNFα-induced depressive-like behavior is blocked by anti-TNFα antibodies (de Paiva et al., 2010; Kaster et al., 2012), suggesting a link between inflammation and depressive symptoms. SSRIs and serotonin and norepinephrine reuptake inhibitors (SNRIs) have been widely used as antidepressant drugs. *In vitro* assays show some antidepressants also possess anti-inflammatory properties, in central- and peripheral-derived cells (Diamond et al., 2006; Horikawa et al., 2010; Liu et al., 2011). For example, SSRIs, such as paroxetine and sertraline, inhibit interferon-γ (IFNγ)-induced TNFα and nitric oxide (NO) elevation in a murine microglial cell line (Horikawa et al., 2010). Venlafaxine shows anti-inflammatory effects through the suppression of IFNγ/IL-10 production ratio (Kubera et al., 2001). A meta-analysis shows that SSRI treatment decreases TNFα levels in human serum (Hannestad et al., 2011a), and alleviates depressive symptoms (Tuglu et al., 2003). Therefore, it is likely that the anti-inflammatory effects of antidepressants might contribute to their therapeutic efficacy in MDD patients.

In this study, we examined the effects of two types of antidepressants (SSRI: fluoxetine, paroxetine, SNRI: venlafaxine, duloxetine) on LPS-induced inflammation, as measured by changes in the pro-inflammatory cytokine TNFα and anti-inflammatory cytokine interleukin-10 (IL-10) levels in mice. Furthermore, we evaluated the effects of fluoxetine and paroxetine on LPS-induced depressive-behavior in the TST.

2. Materials and methods

2.1. Animals

Male BALB/c mice were purchased from Japan SLC., Shizuoka, Japan and were used for all experiments at 8 weeks of age. Mice were housed in a room maintained at 23 ± 2 °C with a 12/12 hour light/dark cycle (lights on at 7:00 AM), and with free access to food and water. All animal care and use were in accordance with the Institutional Guidelines for Animal Care and Use (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan).

2.2. Drugs

Fluoxetine hydrochloride was synthesized at Otsuka Pharmaceutical Co., Ltd. Paroxetine hydrochloride (GlaxoSmithKlein plc., Brentford, UK), venlafaxine hydrochloride (Pfizer, Inc., New York, NY, USA), and duloxetine hydrochloride (Eli Lilly and Co., Indianapolis, USA) were purchased from the companies as stated. 5-Hydroxytryptophan (5-HTP) and LPS from Escherichia Coli (0127:B8) were obtained from Sigma-Aldrich Co. LLC. (St Louis, MO, USA).

2.3. Drug treatment and Enzyme-Linked Immunosorbent Assay (ELISA)

Vehicle (10 ml/kg, physiological saline injected intraperitoneally [i.p.]), fluoxetine, paroxetine, venlafaxine, or duloxetine (3, 10, or 30 mg/kg) were administered to mice. Vehicle (20 ml/kg, physiological saline injected i.p.) or 5-HTP (30, 100, or 300 mg/kg) were administered to mice. The stated doses of antidepressants were referred to the effective dose in behavioral tests (FST or TST) (Berrocoso et al., 2004; El Yacoubi et al., 2003; Redrobe et al., 1998a; Redrobe et al., 1998b; Wang et al., 2008; Wong et al., 2000). The dose range for 5-HTP was enough to induce a behavioral change (head-twitch) in a previous study, and was thus used here (Krisch and

Bole-Vunduk, 1994). Thirty minutes after drug administration, LPS (0.5 mg/kg, i.p.) was injected into mice. Blood samples were taken via a cardiac puncture, under isoflurane anesthesia (Mylan Inc., Tokyo, Japan), 90 minutes after LPS injection. Blood was coagulated and centrifuged at 2,000 g, for 20 minutes to generate serum samples. This experimental condition was referred to the report (Sugino et al., 2009). Serum samples were diluted with ELISA diluent solution (eBioscience Inc., San Diego, CA, USA) 20-fold to measure TNFα and 10-fold for IL-10. TNFα and IL-10 serum concentrations were measured using a Ready-SET-Go ELISA kit (eBioscience), according to manufacturer's instructions.

2.4. Spontaneous locomotor activity measurement

Thirty minutes after vehicle (10 ml/kg, physiological saline, i.p.), fluoxetine (30 mg/kg) or paroxetine (10 mg/kg) administration, mice were injected with vehicle (10 ml/kg, physiological saline, i.p.) or LPS (0.5 mg/kg). Twenty-four hours later, mice were placed in a novel cage, the same size as their home cage (14.3 cm × 35.3 cm), without bedding, and allowed to explore freely. Spontaneous locomotion was measured for 6 minutes using a passive infrared ray sensor in the Supermex system (Muromachi Kikai Co., Ltd., Tokyo, Japan). After measurements, blood samples were collected under isoflurane anesthesia for ELISA. After cervical dislocation, whole brain was taken and cut coronally at optic chiasm. Prefrontal cortex was obtained by carefully removing olfactory bulb, septum and striatum from anterior brain region and then used for real-time PCR analysis.

2.5. Tail suspension test

Thirty minutes after vehicle (10 ml/kg, physiological saline, i.p.), fluoxetine (30 mg/kg) or paroxetine (10 mg/kg) administration, mice were injected with vehicle (10

ml/kg, physiological saline, i.p.) or LPS (0.5 mg/kg). Twenty-four hours later, mice were suspended upside down by their tails. The immobility time was automatically measured for 6 minutes, using an analyzing system (Yamashita Giken, Tokushima, Japan). The experiment was carried out in a soundproof room.

2.6. Quantitative real-time RT-PCR

Total RNA from prefrontal cortex was extracted using the RNeasy Lipid Tissue Mini Kit (QIAGEN Inc., Valencia, CA, USA). RNA was reverse transcribed to cDNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen co., Carlsbad, CA, USA). Real-time reverse transcription PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System. TaqMan Gene Expression Assays (Applied Biosystems Co., Foster City, USA) for TNFα (Mm00443258_m1) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Mm99999915_g1), and TaqMan Fast Universal PCR Master Mix (Applied Biosystems Co.) were used for reaction mixtures, with 50 ng of cDNA template. GAPDH was used as the endogenous house keeping control gene.

2.7. Statistical analysis

ELISA data were expressed as a percentage of the LPS-treated group. Data were presented as the mean ± standard error of the mean (S.E.M.). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed *post hoc* Dunnett test for ELISA or two-way ANOVA, followed *post hoc* Bonferroni test for behavioral evaluations and real-time PCR analysis, on Prism 5 (GraphPad Software Inc., La Jolla, USA). P values less than 0.05 was considered statistically significant.

3. Result

3.1. Effects of antidepressants on serum cytokine levels

In order to examine inflammation *in vivo*, we injected LPS (0.5 mg/kg), a component of the outer membrane of gram-negative bacteria, into mice. Ninety minutes after injection, blood was collected and serum levels of the pro-inflammatory cytokine TNFα and the anti-inflammatory cytokine IL-10 were determined. In saline-treated mice, TNFα and IL-10 levels were under our detection limits. Serum levels of TNFα and IL-10 increased after a single administration of LPS (**Table 1**).

Table.1. Effects of LPS on serum cytokine levels of mice

	TNFα (pg/mL)	**IL-10 (pg/mL)**
Saline	**N.D.**	**N.D.**
LPS	**4142 ± 650**	**481 ± 63**

Data are expressed as the mean ± SEM. (n = 7), N.D.: Not Detected.

We examined the effects of SSRIs (fluoxetine and paroxetine) and SNRIs (venlafaxine and duloxetine) on serum levels of TNFα and IL-10, in mice treated with LPS (0.5 mg/kg). Mice were pretreated with the drug 30 minutes prior to LPS injection, and blood was collected 90 minutes after LPS injection. Pretreatment with fluoxetine (3, 10, or 30 mg/kg) or paroxetine (3, 10, or 30 mg/kg) significantly attenuated LPS-induced TNFα increases, in a dose-dependent manner (fluoxetine; $F(3, 22) = 15.34$, $P < 0.01$, paroxetine; $F(3, 23) = 20.31$, $P < 0.01$) (**Figure 1A and 1C**). Both fluoxetine (3, 10, or 30 mg/kg) and paroxetine (10, or 30 mg/kg) significantly increased

serum levels of IL-10 in mice treated with LPS also in a dose dependent manner (fluoxetine; $F(3, 23) = 37.68$, $P < 0.01$, paroxetine; $F(3, 24) = 41.66$, $P < 0.01$) (**Figure 1B and 1D**).

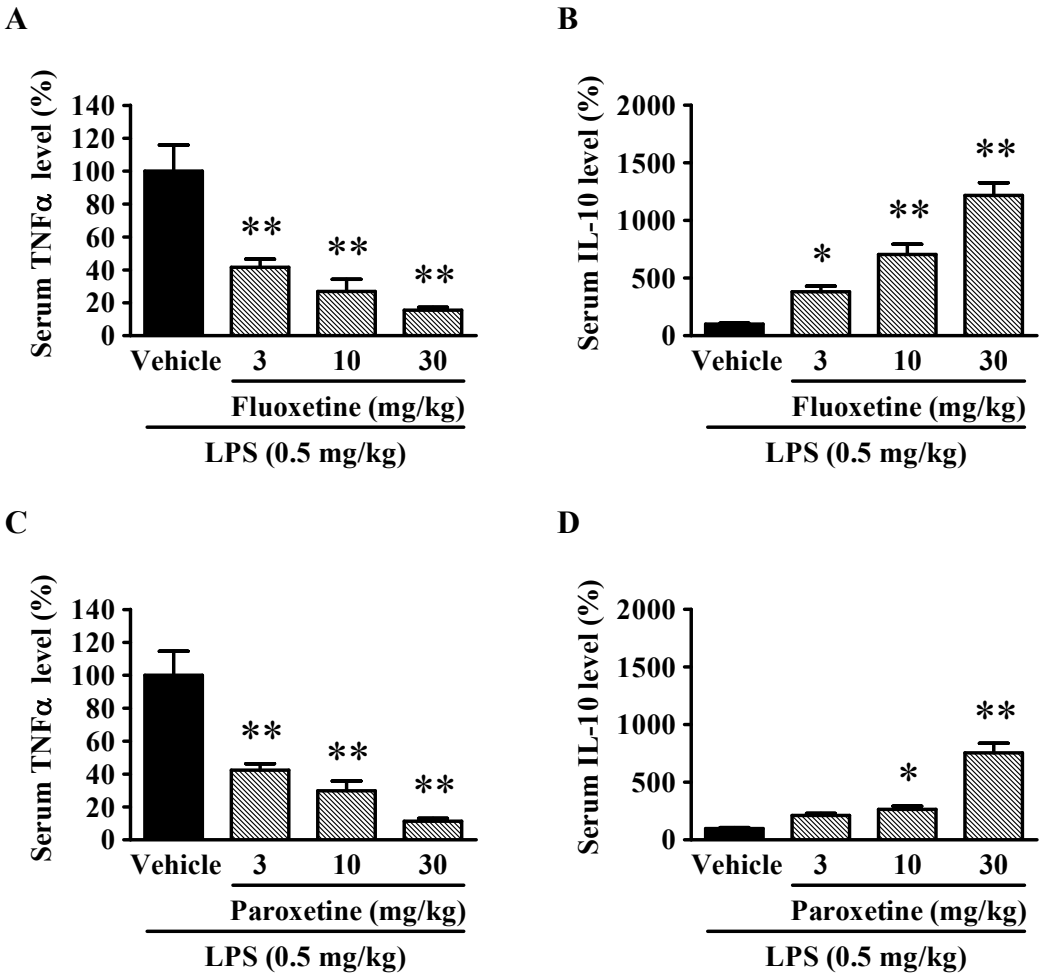

Figure.1. Effects of fluoxetine and paroxetine on LPS-induced cytokine increase in serum. Thirty minutes after a single i.p. administration of vehicle (10 ml/kg), fluoxetine (3, 10, or 30 mg/kg) or paroxetine (3, 10, or 30 mg/kg), LPS (0.5 mg/kg, i.p.) was injected. Blood sample was collected 90 minutes after LPS injection. Serum concentration of TNFα and IL-10 was measured with ELISA. (A) Fluoxetine, TNFα. (B) Fluoxetine, IL-10. (C) Paroxetine, TNFα. (D) Paroxetine, IL-10. Data are expressed as percentage of vehicle + LPS treated group, and data were shown the mean ± SEM (n = 6-7). *$P < 0.05$, **$P < 0.01$ compared with vehicle + LPS treated group.

Similarly, venlafaxine (10, or 30 mg/kg) and duloxetine (3, 10, or 30 mg/kg) significantly attenuated LPS-induced TNFα increases (venlafaxine; $F(3, 22) = 3.811$, $P < 0.05$, duloxetine; $F(3, 23) = 9.445$, $P < 0.01$) (**Figure 2A and 2C**). Venlafaxine (3, 10, or 30 mg/kg) and duloxetine (10, or 30 mg/kg) significantly increased serum levels of IL-10 in mice treated with LPS, in a dose-dependent manner (venlafaxine; $F(3, 23) = 15.93$, $P < 0.01$, duloxetine; $F(3, 24) = 50.45$, $P < 0.01$) (**Figure 2B and 2D**).

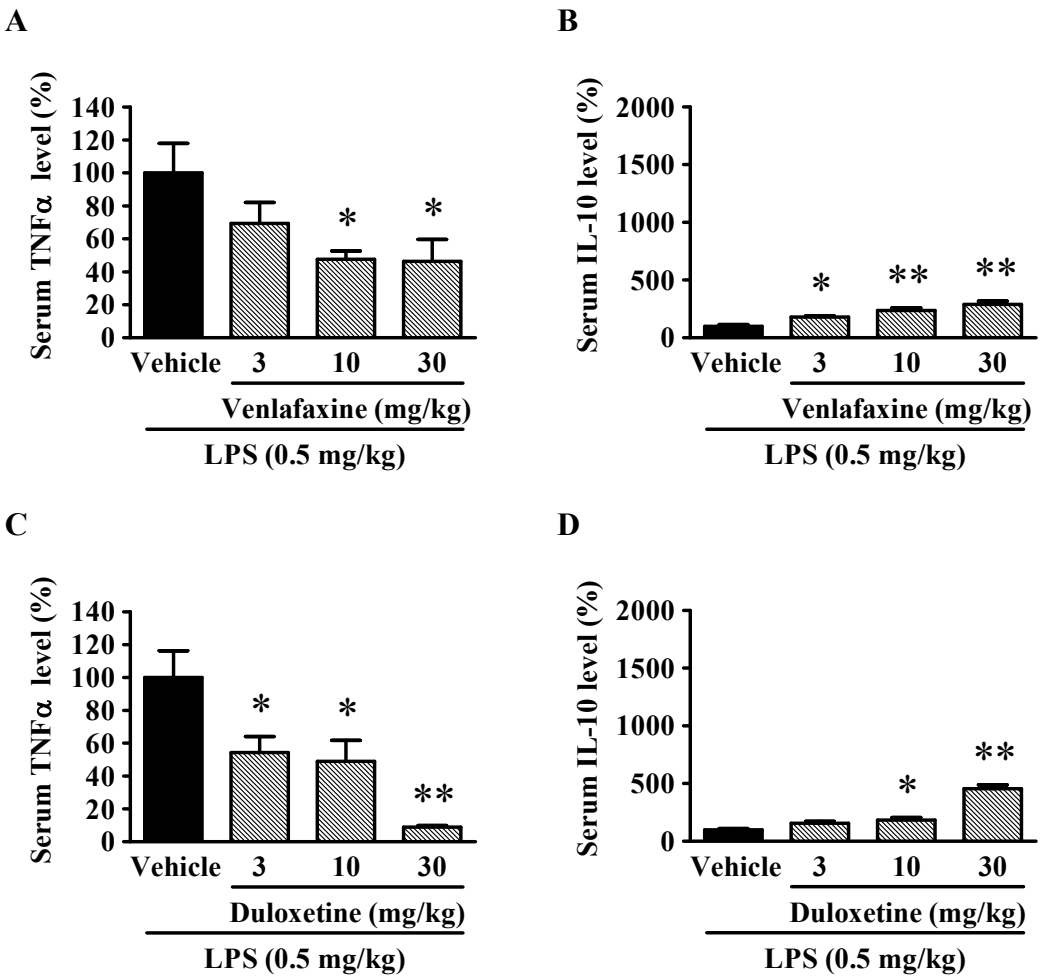

Figure.2. Effects of venlafaxine and duloxetine on LPS-induced cytokine increase in serum. Thirty minutes after a single i.p. administration of vehicle (10 ml/kg), venlafaxine (3, 10, or 30 mg/kg) or duloxetine (3, 10, or 30 mg/kg), LPS (0.5 mg/kg, i.p.) was injected. Blood sample was collected 90 minutes after LPS injection. Concentration of TNFα and IL-10 was measured with ELISA. (A) Venlafaxine, TNFα. (B) Venlafaxine, IL-10. (C) Duloxetine, TNFα. (D) Duloxetine, IL-10. Data are expressed as percentage of vehicle + LPS treated group, and data were shown the mean ± SEM (n = 6-7). *P < 0.05, **P < 0.01 compared with vehicle + LPS treated group.

To determine whether the serotonergic system makes an essential contribution to the anti-inflammatory effects of antidepressants, we pretreated LPS-challenged mice with 5-HTP, a precursor of serotonin. Pretreatment with 5-HTP (30, 100, or 300 mg/kg) significantly attenuated LPS-induced increases in TNFα, in a dose dependent manner ($F(3, 24) = 62.05$, $P < 0.01$) (**Figure 3A**). Furthermore, pretreatment with 5-HTP also increased serum levels of IL-10 in mice treated with LPS, in a dose-dependent manner ($F(3, 24) = 40.17$, $P < 0.01$). Two doses (100 or 300 mg/kg) of 5-HTP were statistically significant (**Figure 3B**).

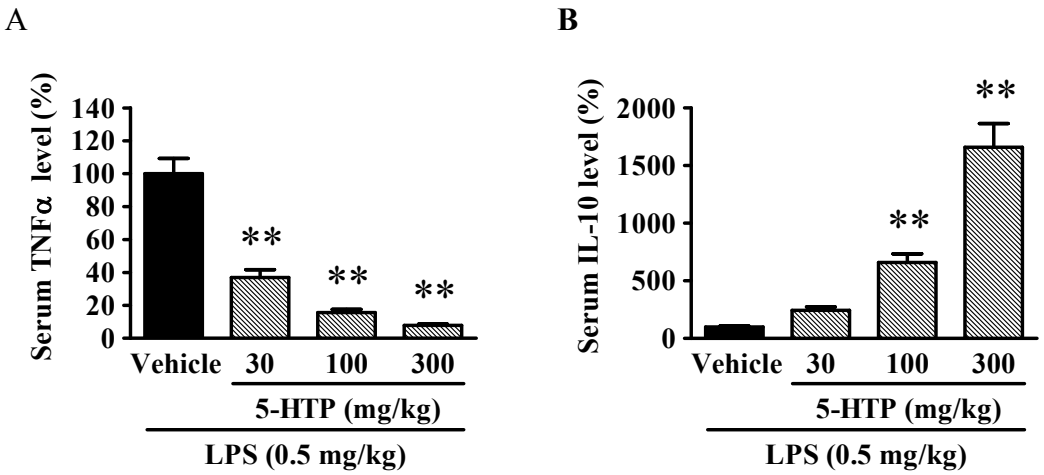

Figure.3. Effects of 5-HTP on LPS-induced cytokine increase in serum. Thirty minutes after a single i.p. administration of vehicle (20 ml/kg) or 5-HTP (30, 100, or 300 mg/kg), LPS (0.5 mg/kg, i.p.) was injected. Blood sample was collected 90 minutes after LPS injection. Concentration of TNFα and IL-10 was measured with ELISA. (A) 5-HTP, TNFα. (B) 5-HTP, IL-10. Data are expressed as percentage of vehicle + LPS treated group, and data were shown the mean ± SEM (n = 5-7). **P < 0.01 compared with vehicle + LPS treated group.

3.2. Behavioral evaluations

We performed behavioral evaluations using the spontaneous locomotor activity tests and TST 24 hours after LPS (0.5 mg/kg) injection. This time course was selected because LPS could induce depressive-like behavior in mice 24 hours after LPS injection (O'Connor et al., 2009). Mice were pretreated with fluoxetine (30 mg/kg), 30 minutes prior to LPS injection. Spontaneous locomotor activity did not differ between saline- and LPS-treated mice, and fluoxetine (30 mg/kg) pretreatment had no effects on spontaneous locomotor activity in saline- and LPS-treated mice (LPS, $F(1, 26) = 2.296$, $P = 0.1418$; fluoxetine, $F(1, 26) = 0.8042$, $P = 0.3781$; interaction, $F(1, 26) = 3.873$, $P = 0.0598$) (**Figure 4A**). Next we examined anti-inflammatory effects of fluoxetine on LPS-induced behavioral changes in the TST. The immobility time of LPS-treated mice was significantly higher than that of saline-treated mice ($P < 0.05$). Fluoxetine (30 mg/kg) significantly shortened immobility time, both in saline- ($P < 0.01$) and LPS-treated mice ($P < 0.01$) (LPS, $F(1, 43) = 13.02$, $P < 0.01$; fluoxetine, $F(1, 43) = 29.08$, $P < 0.01$; interaction, $F(1, 43) = 0.5137$, $P = 0.477$) (**Figure 4B**).

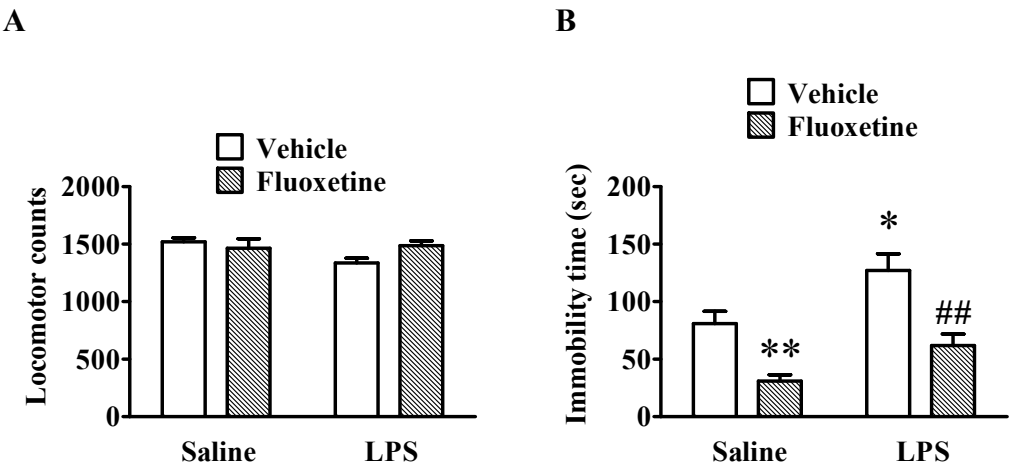

Figure.4. Antidepressant-like effect of fluoxetine on LPS-treated mice. Thirty minutes after a single i.p. administration of vehicle (10 ml/kg) or fluoxetine (30 mg/kg), saline (10 ml/kg) or LPS (0.5 mg/kg, i.p.) was injected. Behavioral tests were performed 24 hr following saline or LPS injection. (A) Spontaneous locomotor activity was measured for 6 minutes. (B) The duration of immobility time in TST was measured for 6 minutes. Data are shown the mean ± SEM (n = 6-12). *P < 0.05, **P < 0.01 compared with vehicle + saline treated group. $^{\#\#}$P < 0.01 compared with vehicle + LPS treated group.

Next, we examined effects of paroxetine in behavioral tests. LPS (0.5 mg/kg, 24 hrs) didn't affect spontaneous locomotor activity. Paroxetine (10 mg/kg) had no effect on spontaneous locomotor activity in saline- and LPS-treated mice (LPS, $F(1, 28) = 0.0466$, $P = 0.8306$; paroxetine, $F(1, 28) = 0.3442$, $P = 0.5621$; interaction, $F(1, 28) = 0.8267$, $P = 0.371$) (**Figure 5A**). In the TST, LPS induced depressive-like behavior ($P < 0.01$), and pretreatment with paroxetine (10 mg/kg) significantly attenuated the increase of immobility time in LPS-treated mice ($P < 0.01$), without affecting saline-treated mice (LPS, $F(1, 43) = 2.854$, $P = 0.0984$; paroxetine, $F(1, 43) = 8.24$, $P < 0.01$; interaction, $F(1,43) = 13.9$, $P < 0.01$) (**Figure 5B**).

Next, we collected blood and brain (prefrontal cortex) samples immediately after measuring spontaneous locomotor activity. At this time point, serum levels of TNFα and IL-10 were under detectable limits (**data not shown**). Levels of TNFα mRNA in the prefrontal cortex of LPS-treated mice were significantly higher than levels in saline-treated mice ($P < 0.01$). Pretreatment with paroxetine (10 mg/kg) significantly ($P < 0.01$) attenuated increases in TNFα mRNA induced by LPS (LPS, $F(1, 28) = 22.39$, $P < 0.01$; paroxetine, $F(1, 28) = 11.52$, $P < 0.01$; interaction, $F(1, 28) = 6.489$, $P < 0.05$) (**Figure 5C**). In contrast, IL-10 mRNA levels in the prefrontal cortex were undetectable (**data not shown**).

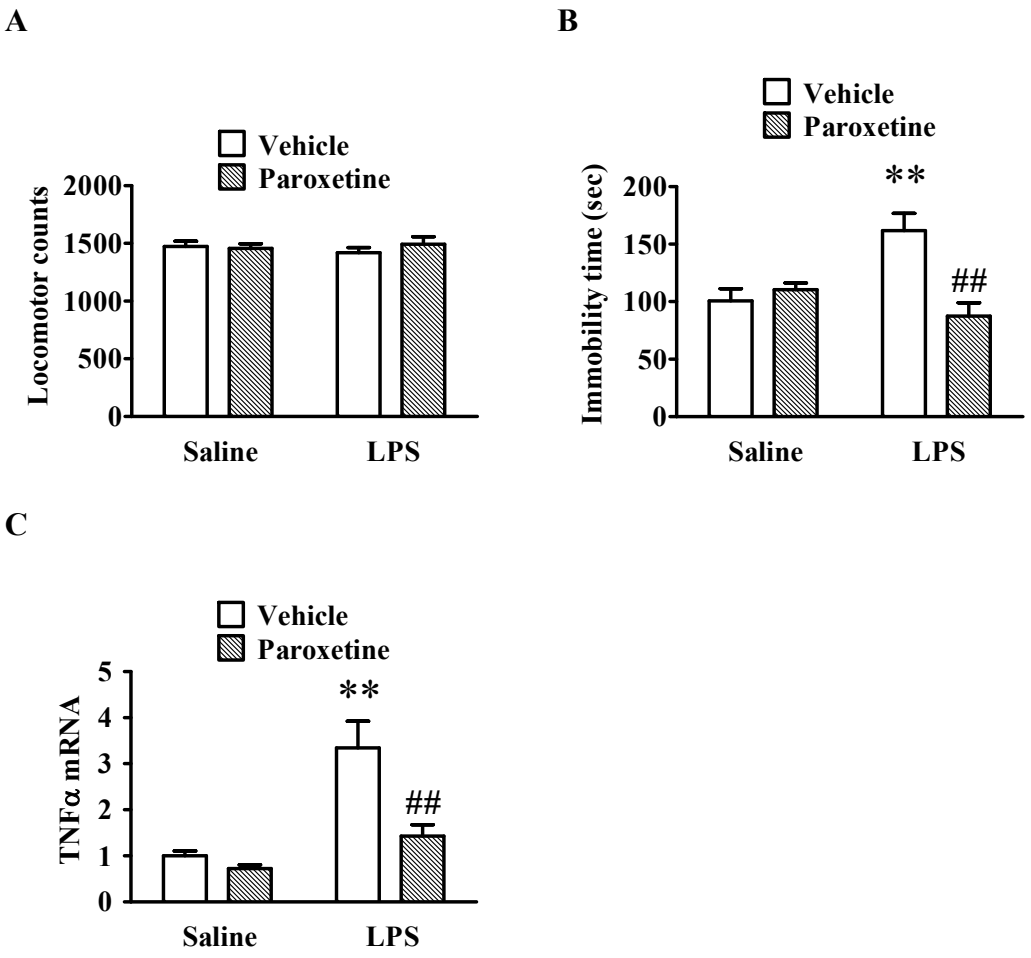

Figure.5. Antidepressant-like effect of paroxetine on LPS-treated mice. Thirty minutes after a single i.p. administration of vehicle (10 ml/kg) or paroxetine (10 mg/kg), saline (10 ml/kg) or LPS (0.5 mg/kg, i.p.) was injected. Behavioral tests were performed 24 hr following saline or LPS injection. (A) Spontaneous locomotor activity was measured for 6 minutes. (B) The duration of immobility time in TST was measured for 6 minutes. (C) Immediately after measurement of spontaneous locomotion, prefrontal cortex was collected and real-time PCR was performed for TNFα mRNA analysis. Data were shown the mean ± SEM (n = 8-12). **P < 0.01 compared with vehicle + saline treated group. $^{\#\#}$P < 0.01 compared with vehicle + LPS treated group.

4. Discussion

This study showed that antidepressants such as SSRIs and SNRIs possess anti-inflammatory action in the periphery, as measured by decreased TNFα levels and up-regulated IL-10 levels in LPS-treated mice. In the TST, we demonstrated that LPS-treated mice displayed depressive-like behavior 24 hours after LPS injection, and that fluoxetine and paroxetine showed antidepressant-like effects in this model.

It has been reported that SSRIs and SNRIs possess anti-inflammatory effects *in vitro* (Horikawa et al., 2010; Kubera et al., 2001). This study suggests that serotonin uptake inhibition may be an important process in the anti-inflammatory action of these drugs. Both SSRIs and SNRIs exert their therapeutic effect by inhibiting the serotonin transporter (SERT) in the brain, thereby increasing extracellular serotonin concentrations. In the periphery, SERT is expressed in platelets and regulates plasma serotonin levels (Mercado and Kilic, 2010). SSRIs inhibit peripheral SERT while sharply increasing plasma serotonin levels (Ortiz and Artigas, 1992). Serotonin receptor subtypes 4 and 7 are expressed in monocytes and their stimulation decreases LPS induced TNFα release (Durk et al., 2005). Furthermore, 5-HTP, which is converted to serotonin by the aromatic L-amino acid decarboxylase, also decreased TNFα levels in LPS-treated mice, suggesting that serotoninergic activation may be essential for anti-inflammatory action. Taken together, it is likely that the inhibitory effects of SSRIs and SNRIs on LPS-induced TNFα increases are the result of serotonin receptor stimulation. This activation is mediated through increases in plasma serotonin levels induced by SERT inhibition in the peripheral system.

IL-10 is one of the most essential anti-inflammatory cytokines. It acts by inhibiting the production of pro-inflammatory cytokines such as TNFα and IL-6 (de

Waal Malefyt et al., 1991). The inhibitory effects of TNFα production stimulated by acute administration of SSRIs and SNRIs would in part be due to the upregulation of IL-10, in addition to serotonin receptor stimulation, although the precise mechanisms for this up-regulation by these antidepressants remain unknown. In this study, we found that 5-HTP increased serum levels of IL-10 in mice. Therefore, it seems that serotonin plays an important role in IL-10 upregulation induced by SSRIs and SNRIs.

IL-10 is a key regulator of depression symptoms (Roque et al., 2009). IL-10 knockout mice show depressive-like behavior compared with wild-type mice, and this behavior is reversed by IL-10 administration. Conversely, mice overexpressing IL-10 display antidepressant-like behavior compared with wild-type mice (Mesquita et al., 2008). Furthermore, IL-10 suppresses the HPA axis, thereby inhibiting the secretion of corticosterones by the adrenal gland (Roque et al., 2009). From these reports, it is likely that IL-10 up-regulation induced by SSRIs and SNRIs contributes to the therapeutic effect seen in MDD.

In the TST, LPS-treated mice showed prolonged immobility time, with no change in spontaneous locomotor activity, compared with saline-treated mice. This finding is in agreement with previous reports (Kang et al., 2011; O'Connor et al., 2009). The report states that locomotor activity in mice decreases transiently 6 hours after LPS injection and recovers at around 24 hours after injection. This short term decrease in locomotor activity after LPS injections is called sickness-behavior, and is due to acute inflammation. Sickness behavior is distinguishable from depressive behavior and can be monitored by recording spontaneous locomotor activity (Frenois et al., 2007). In this study, behavioral changes were observed 24 hours after LPS challenge and there were no differences in spontaneous locomotor activity between saline- and LPS-treated mice.

From this, we conclude that our LPS-treated mice showed depressive-like behavior in this study. We found that serum levels of TNFα and IL-10 were undetectable 24 hours after LPS challenge, suggesting that within the periphery, LPS-induced increases of these cytokines had tapered off within this timeframe. Interestingly, we found that, 24 hours after LPS injection, TNFα mRNA levels in the brain were significantly higher than those of saline-treated mice. It has been proposed that peripheral inflammation may relay signals to the central nervous system (Krishnadas and Cavanagh, 2012). Peripheral LPS injections induced depressive-like behaviors through the pro-inflammatory cytokine-induced, indoleamine-2, 3-dioxigenase (IDO) enzyme (O'Connor et al., 2009), and SERT activation in the brain (Zhu et al., 2010). Therefore, peripheral and central inflammation would be involved in depressive-like behavior in mice treated with LPS.

Fluoxetine and its active metabolite, norfluoxetine occupy mouse brain SERT, 24 hours after fluoxetine administration (Hirano et al., 2004). Taking this into consideration, it is not surprising that fluoxetine shortened immobility time at 24 hours, in saline-treated mice. Fluoxetine also shortened immobility time in LPS-treated mice, suggesting that fluoxetine was effective in treating LPS-induced depressive-like behavior. On the other hand, paroxetine disappeared from mouse brains 24 hours after administration (Hirano et al., 2004). At this time point, paroxetine showed no antidepressant-like effect in saline-treated mice (**Figure 5B**). However, paroxetine attenuated LPS-induced depressive-like behavior. Furthermore, paroxetine pretreatment inhibited LPS-induced TNFα mRNA increases in prefrontal cortex. It is likely that paroxetine attenuated LPS-induced increases in TNFα in the periphery and in doing so, inhibited inflammation in the brain and following depressive-like behavior. In addition, paroxetine inhibits IFN-γ induced microglial activation (Horikawa et al., 2010),

suggesting that paroxetine may inhibit LPS-induced TNFα mRNA increases in the brain, by inhibiting microglial activation. It has been reported that inhibiting inflammatory processes in the periphery ameliorated depressive-like behavior induced by peripheral LPS challenge (Kang et al., 2011). Therefore, inhibition of inflammatory processes in the periphery may be enough to prevent inflammation-induced depressive-like behavior. Recently, Arakawa et al. (2012) reported that minocycline, a potent inhibitor of microglial activation, produced antidepressant-like effects on the learned helplessness rats, an animal model of depression. These results suggest that the anti-inflammatory properties of antidepressants confer an antidepressant-like effect on inflammation-induced depressive-like behavior.

Clinical studies have shown that IFNα induces depressive symptoms in hepatitis C patients, and that this symptom onset can be prevented by pretreatment with SSRIs (McNutt et al., 2012; Schaefer et al., 2012). Citalopram reduces endotoxin-induced fatigue in human (Hannestad et al., 2011b). Our findings on fluoxetine and paroxetine in the treatment of LPS-induced depressive-like behavior lend support to these clinical studies (McNutt et al., 2012; Schaefer et al., 2012). MDD patients have higher levels of pro-inflammatory cytokines in their blood (Dowlati et al., 2010), and inflammation in frontal cortex was observed in people with a history of MDD (Shelton et al., 2011). SSRIs decrease TNFα levels in MDD patients (Hannestad et al., 2011a) and the decrease is associated with therapeutic effect (Tuglu et al., 2003). From these evidences, inflammation in the periphery and brain might be involved in the pathophysiology of depressive symptoms in human. Therefore, SSRIs and SNRIs may in part ameliorate depressive symptoms, by inhibiting peripheral and central inflammation through their known anti-inflammatory effects.

In conclusion, these findings suggest that antidepressants such as SSRIs and SNRIs possess anti-inflammatory effects. They are capable of decreasing LPS-induced TNFα increases and up-regulating IL-10 levels in serum. It is these anti-inflammatory effects which inhibit the depressive-like behavior seen in LPS-treated mice. Therefore, the anti-inflammatory effects of SSRIs and SNRIs may partially contribute to their therapeutic effect in MDD.

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