Original Article

Innate Immunity

Innate Immunity
18(3) 447–458

DOI: 10.1177/1753425911421512
ini.sagepub.com
SAGE

Effects of lithium on lipopolysaccharide-induced inflammation in rat primary glia cells

Sigalit Nahman[1,2], RH Belmaker[2] and Abed N Azab[1,3]

Abstract

Lithium is the gold-standard treatment for bipolar disorder, a severe mental illness. A large body of evidence suggests that inflammation plays a role in the pathogenesis of bipolar disorder and that mood stabilizers exhibit anti-inflammatory properties. However, contradicting findings have also been reported. In this study, we examined the effects of lithium on LPS-induced inflammation in rat primary glia cells. Cells were pre-treated with lithium (1 or 10 mM) for 6 or 24 h, after which, inflammation was induced by the addition of LPS (for another 18 h) to the culture medium. Thereafter, medium was collected and cells were harvested for further analyses. Levels of TNF-α, IL1-β and PGE_2 were determined by ELISA and NO levels by the Griess reaction assay. Expression levels of cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) were examined by Western blot analysis. We found that pre-treatment with lithium 10 mM (but not 1 mM) significantly reduced LPS-induced secretion of TNF-α, IL1-β, PGE_2 and NO. In addition, lithium significantly reduced the expression of COX-2 and iNOS. These findings indicate that lithium exhibits a potent anti-inflammatory effect. However, it's important to emphasize that this effect was obtained mainly under treatment with an extra-therapeutic concentration of the drug.

Keywords

Bipolar disorder, cytokines, inflammation, lithium, prostaglandins

Date received: 12 June 2011; revised: 31 July 2011; accepted: 3 August 2011

Introduction

Lithium is the classic pharmacotherapy for bipolar affective disorder.[1,2] Bipolar disorder (manic-depressive illness) is a severe and chronic mental illness, affecting approximately 1%–1.5% of the general population.[1,3] It is characterized by alternating shifts of mania and depression, both of which severely affect the mental, physical, social and functional status of affected patients.[1,4,5] Lithium is the most useful mood stabilizer for maintenance treatment of bipolar disorder.[1,2,6,7] The use of lithium is expected to further increase in coming years as it has recently been suggested as a treatment for other illnesses, including schizoaffective disorder,[8] neurodegenerative diseases, such as Alzheimer's disease[9–11] and amyotrophic lateral sclerosis.[12] Nevertheless, lithium therapy is complicated by two significant factors: firstly, it has a narrow therapeutic index, necessitating close monitoring of drug plasma concentration; and, secondly, it has several side effects, some of which are severe and occasionally irreversible.[1,13–15]

The mechanism of action of lithium in the treatment of bipolar disorder is not fully understood. Several hypotheses have been suggested to explain its therapeutic mechanisms;[16–18] however, a conclusive mechanism is still lacking.

Accumulating evidence suggests that lithium, and other mood stabilizers, exhibit anti-inflammatory properties[19–21] and that inflammation contributes to the pathological processes underlying bipolar disorder.[19–29] Moreover, inflammation has been extensively linked

[1]Department of Clinical Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-sheva, Israel
[2]Psychiatry Research Unit and Mental Health Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-sheva, Israel
[3]School for Community Health Professions, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-sheva, Israel

Corresponding author:
Abed Azab, Department of Nursing, School for Community Health Professions, Ben-Gurion University of the Negev, P.O.B 653, Beer-Sheva 84105, Israel.
Email: azab@bgu.ac.il

to the pathophysiology of depression.[23,30–41] Other neurological disorders, including schizophrenia,[42–44] Alzheimer's disease,[22,45,46] Parkinson's disease[47] and epilepsy[48] have also been associated with inflammation. The mechanism of the inflammatory responses involved in these illnesses is poorly understood. It is suggested that pro-inflammatory mediators, including prostaglandins (PGs), cytokines (e.g. IL1-β and TNF-α) and C-reactive protein play an important role. For example, bipolar patients had increased levels of pro-inflammatory mediators such as IL-6,[20,28,29] TNF-α[20,28,29] and C-reactive protein.[20,24,25,28] In addition, monocytes of bipolar patients were found to have a gene expression signature characterized by overexpression of multiple inflammatory genes.[27] Bipolar disorder has also been associated with altered regulation and synthesis of PGs.[19,21,26,27] The results regarding anti-inflammatory cytokines, such as IL-4 and IL-10 have been less consistent.[20,29] The connection between mood disorders and inflammation is further strengthened by a large body of evidence suggesting that patients with depression have increased levels of pro-inflammatory mediators,[22,30–33,35,36,41] while levels of anti-inflammatory cytokines are unchanged,[20,22,23] or either reduced.[41] Similarly, depression is characterized by alterations in the production and regulation of PGs.[19,21,36] Taken together, these, and other observations, laid the foundation for the rapidly developing 'inflammation hypothesis' of mood disorders.[20,21,31,33,49]

One of the cellular pathways that has been extensively linked with brain inflammation and the pathophysiology of mood disorders is that of the enzyme cyclooxygenase (COX),[19,21] which produces PGs. PGs (such as PGE_2) are pivotal mediators of tissue homeostasis and their aberrant regulation is known to cause deleterious pathophysiological effects.[50,51] The biosynthesis of PGs involves phospholipase A_2-mediated release of arachidonic acid (AA) from membrane phospholipids and its conversion to PGs by COX.[50,51] There are three COX enzymes, referred to as COX-1, COX-2 and COX-3. COX-1 produces PGs that are important for homeostasis and physiological functions and is expressed constitutively in most tissues.[52] COX-2 is an inducible enzyme, whose induction is believed to be a response to inflammatory or mitogenic stimuli.[52] Nevertheless, it's known that COX-1 and COX-2 have some over-lapping functions and that, in certain conditions, COX-1 can be up-regulated and contribute to inflammatory responses,[53,54] while COX-2 is constitutively expressed in some tissues (e.g. endothelium, brain and kidney) where it accounts for important physiological functions.[53,55] The regulation and function of COX-3 are still unknown.

Another pathway that is associated with brain inflammation is that of NO.[56–58] In the brain, NO is synthesized constitutively in post-synaptic neurons by the enzyme neuronal NO synthase (nNOS).[57] Alternatively, after inflammatory stimuli, NO is produced in glia cells by the enzyme inducible NOS (iNOS).[56] NO is a very active signalling molecule of the brain, associated with multiple cellular processes and signal transduction pathways.[58] Although it has been suggested that NO plays a role in the pathophysiology and treatment of bipolar disorder,[59,60] the results show an inconsistent pattern.

In line with the 'inflammation hypothesis' of mood disorders, mood-stabilizing drugs were found to exhibit potent anti-inflammatory properties.[19–21] Rapoport et al. have shown that mood stabilizers decrease brain concentration of PGE_2 and attenuate the turnover of AA.[19,21] For example, chronic lithium administration decreased AA turnover, COX-2 activity and PGE_2 concentration in the rat brain.[61] Similarly, chronic treatment with valproate[62] and Carbamazepine[63] reduced AA turnover, COX-2 activity and PGE_2 levels in rat brain, and chronic Lamotrigine administration decreased COX-2 expression in rat frontal cortex.[64] Consistent with a role for AA metabolites in the pathogenesis of bipolar disorder and the therapeutic mechanisms of mood-stabilizing drugs, treatment with celecoxib, a selective COX-2 inhibitor was found to be beneficial in a double-blind, placebo-controlled trial of bipolar patients.[49] Similarly, in double-blind trials of patients with major depression[33] and schizophrenia,[42,43] treatment with selective COX-2 inhibitors led to favourable effects. Furthermore, it was shown that chronic treatment with celecoxib alleviated depressive-like symptoms in rats and reversed the elevation in COX-2 expression and PGE_2 levels in the brain.[65] It has also been suggested that antipsychotic[43] and antidepressant drugs[66,67] confer anti-inflammatory properties. Alternatively, other studies, mostly conducted in non-brain cells, have given inconsistent results with regard to the effect of mood stabilizers on components of the inflammatory response. For example, in contrast to previous reports[61,62] that found lithium and valproate to decrease COX-2 protein levels in rat brain, lithium was found to *enhance* expression of COX-2 in renal and mammary cells.[68,69] Another study showed that the effect of lithium on COX-2 expression is tissue-specific, as it reduced expression in kidney inner medulla but increased expression in the renal cortex.[70] Thus, although many reports demonstrated that lithium attenuates the inflammatory response, contradictory findings have also been reported.

The present study was undertaken to test the effects of lithium on LPS-induced inflammation in rat primary glia cells. Glia cells (microglia, astrocytes and oligodendrocytes) play a pivotal role in immune and inflammatory processes of the brain.[71,73] Microglia are the CNS equivalent of macrophages. They inspect the brain for tissue damage and infection and help in maintaining brain homeostasis by removing toxic debris and dead cells. In response to an inflammatory stimulus such as LPS, microglia release multiple inflammatory mediators

(such as NO, TNF-α and PGE_2).[71–74] Astrocytes are supportive to neurons and allow them to function properly.[71,72] Moreover, by producing growth factors and removing toxic cell debris, astrocytes enhance the viability and survival of neurons. Similarly to microglia, the induction of astrocytes by inflammatory stimuli induces morphological changes, followed by secretion of inflammatory mediators. Oligodendrocytes are pivotal for normal electrical communication between neurons.[72] They produce myelin which covers axons and facilitate the conduction of electrical impulses.[72] Therefore, primary glia cells are a model system that is highly relevant to the study of neurological disorders, including manic-depressive illness.

Materials and methods

Primary rat glia culture

Primary cultures of mixed glia cells were extracted from *Wistar* newborn (0–24 h) rat brains. The procedures were used in accordance with the Guidelines of the Faculty of Health Sciences Committee on the Use and Care of Laboratory Animals (Authorization # IL-04-01-2009). Glia cells extraction was performed as previously described.[75] Briefly, brains were removed under sterile conditions and washed four times with sterile 0.9% NaCl solution. Then, meninges and blood vessels were removed and the brains were minced, passed through a steel mesh (with 280 μm diameter clefts) and then through nylon sieves (120 μm pore size) to obtain single cells. The dissociated cells were pelleted twice by centrifugation at a low speed (1200 *g*) and washed with saline. The final cell suspension was in a high glucose (4.5%) DMEM containing 10% inactivated FCS, antibiotics (penicillin 100 unit/ml and streptomycin 100 μg/ml), 2 mM L-glutamine and 0.2 unit/ml insulin (regular). After cell count with a coulter, 10^6 cells/ml were seeded in 24-well plates that were coated with 0.01 mg/ml poly-L-lysine. The plates were incubated at 37°C in an atmosphere of 5% CO_2 in air. The medium was replaced after 1–3 d and once a wk thereafter. After 2–3 wk, when the cultures were almost free of neurons and contained mainly glia cells, experiments were conducted. For instance, at this stage, cultures contain >95% glia cells (~80% astrocytes, ~15% microglia) and less than 5% neurons, as described previously.[75]

Experimental procedure

Escherichia coli LPS (Sigma) was used to induce inflammation in glia cultures. Lithium was used at the following concentrations: 1 mM and 10 mM. We examined the effects of lithium on LPS-induced secretion of inflammatory mediators (including TNF-α, IL-1β, PGE_2 and NO) to the culture medium. Moreover, we determined the effect of lithium on expression level of COX-2 and iNOS—enzymes that are induced during inflammatory processes in the brain. Cells were treated with lithium for 6 h or 24 h *before* the addition of LPS to the culture medium, after which, LPS was added for *another* 18 h. Subsequently, samples were collected and stored at −20°C for further determination.

Determination of TNF-α, IL-1β and PGE_2 levels

Samples were assayed for TNF-α, IL-1β and PGE_2 protein content using ELISA kits, according to the manufacturer's protocols (R&D Systems). The optical density of standard and unknown samples was determined by using a spectrophotometer set to 450 nm. The detection limit of TNF-α and IL-1β assays was 62.5 pg/ml. The detection limit of the PGE_2 assay was 39 pg/ml. In the samples where the level of the examined inflammatory mediator was below the detection limit, results were expressed as 'undetectable'.

Determination of NO levels

Levels of NO in culture medium were determined by measuring the concentrations of nitrite (NO_2^-), a stable metabolite of NO, using the Griess reaction assay.[76] One hundred μl of samples and standard solution of known concentrations of $NaNO_2$ (0.39–12.5 μM) were transferred into a 96-well plate. These were mixed with an equal volume of Griess reagent (Sigma, St Louis, MO, USA), which contains 1% sulfanilamide and 0.1% *N*-1-naphthylethylendiamine dihydrochloride. The plate was incubated in the dark at 20–24°C for 15 min. The absorbance of the reaction products was measured at 540 nm using a spectrophotometer.

Western blot analysis

Glia cells were grown in small, poly-L-lysine-coated flasks, with $6x10^6$ cells seeded on each flask. Cells were harvested and lysed in 200 μl ice-cold lysis buffer [50 mM Tris HCl pH 7.5, 1 mM EDTA, 1 mM EGTA, Triton X-100 (0.1% w/v), 50 mM NaF, 1 mM sodium vanadate, 1 mM PMSF, 1 μM leupeptin, 20 u/ml aprotinin]. Lysates were transferred to Eppendorf tubes and incubated for 8 min on ice. Extracts were then centrifuged at 12,000 *g*, for 15 min at 4°C. Protein concentration in samples (supernatants) was determined by the method of Bradford.[77] Aliquots of samples, reconstituted with appropriate amounts of Laemmli's loading buffer and boiled for 5 min, were resolved (40 μg protein/well) on 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes (BioRad, Hercules, CA). Membranes were briefly exposed to Ponceau buffer to verify the transfer of protein to the membranes. Membranes were incubated 18–20 h (at 4°C) with primary antibodies against COX-2 and iNOS (Cayman, Ann Arbor, MI), followed by

incubation for 1 h with a HRP-conjugated secondary Ab (Jackson Immuno-Research Laboratories, Baltimore, PA). Actin was used as an internal control to normalize for loading variations and was detected using an anti-actin Ab (MP Biomedicals, Solon, OH). Immunocomplexes were visualized by enhanced chemiluminescence, using exposure to Fuji medical x-ray film for 5 sec. Bands were quantified by densitometric scanning using a 202D Bio Imaging System (Pharmacia, Bridgewater, NJ) with a Fuji-Film Thermal Imaging System FTI-500 (Fuji, Tokyo, Japan) and TINA software (Raytest, Straubenhardt, Germany).

Presentation of the data and statistical analysis

Each figure represents one out of several independent experiments (numbers are indicated in legends) in which similar results were obtained. In TNF-α, IL-1β, PGE_2 and NO experiments, each treatment group (e.g. Control, LPS) included 6–12 samples (wells). In each blot of a COX-2 or iNOS experiment, each treatment group represents one sample (flask). Figures of iNOS and COX-2 expression represent six independent experiments (blots). Results are expressed as mean ± SEM. Statistical evaluation of the results was carried out using a two-tailed student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results

Dose-dependent effect of LPS on secretion of NO, TNF-α, IL-1β and PGE_2 levels

Increasing concentrations of LPS (5–10,000 ng/ml) were added to the culture medium, which was collected 18 h after LPS administration. As seen in Figure 1, at a concentration of 100 ng/ml, LPS caused a prominent increase in NO (A), TNF-α (B) and IL-1β (C) secretion, which was similar to that reached under treatment with higher concentrations. Therefore, we decided to use 100 ng/ml LPS as the working concentration for NO, TNF-α and IL-1β experiments. On the other hand, LPS at 10–1000 ng/ml did not lead to prominent PGE_2 secretion (Figure 1D), as PGE_2 levels were low and significantly lower than those obtained under treatment with 5000 ng/ml LPS. Hence, we decided to use 5000 ng/ml LPS as the working concentration for PGE_2 induction.

Time-dependent effect of LPS on secretion of NO, TNF-α and PGE_2 levels

These experiments aimed to determine the time-response curves of inflammatory mediator secretion after induction with LPS. As seen in Figure 2, NO (A) levels peaked at 18 h, while TNF-α (B) levels peaked at 12 h

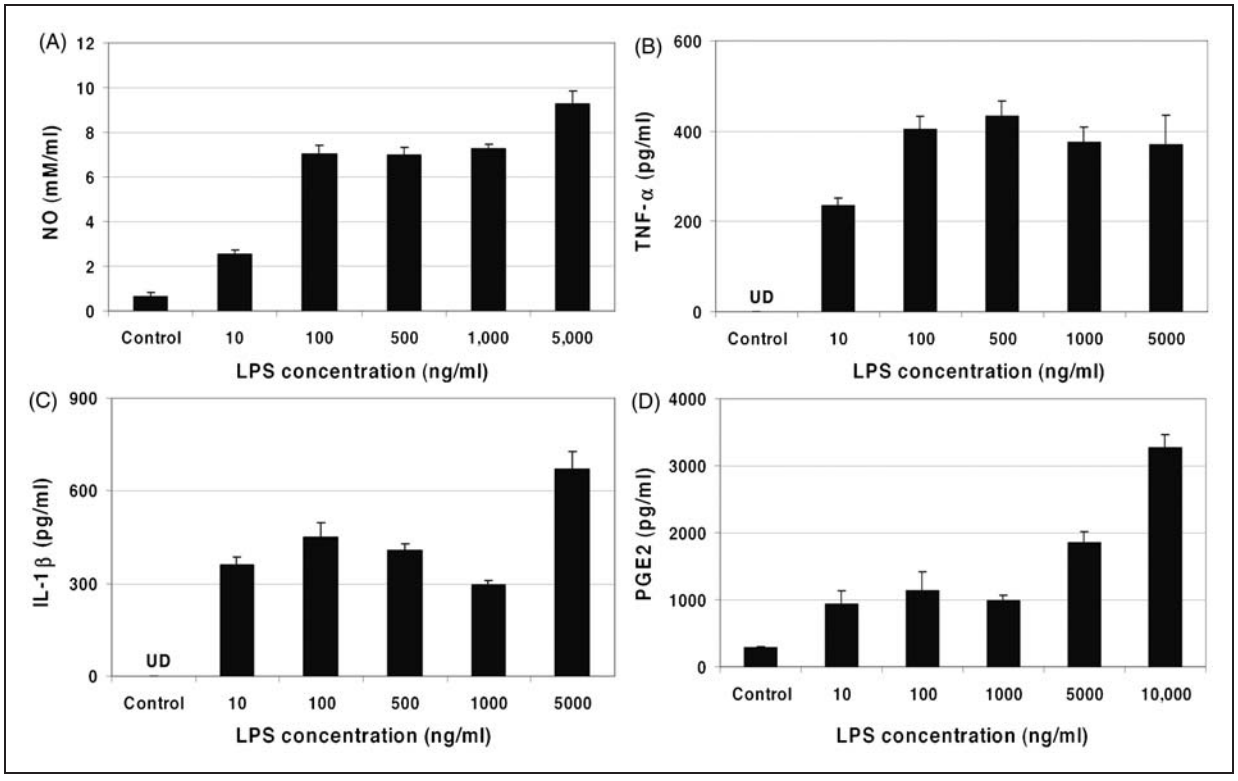

Figure 1. Dose-dependent effects of LPS on NO, TNF-α, IL-1β and PGE_2 secretion. Primary rat glia cells were treated with 10–10,000 ng/ml LPS for 18 h. NO (A) levels were determined by the Griess reaction assay and TNF-α (B), IL-1β (C) and PGE_2 (D) levels by ELISA. The data express the results of one out of three independent experiments with similar results. Values are mean ± SEM of 6–12 samples. UD, undetectable.

after LPS 100 ng/ml exposure. Moreover, PGE_2 secretion (Figure 2C) gradually increased reaching its peak at 30 h of LPS exposure. Based on these results, we decided to examine the effects of lithium on secretion of inflammatory mediators at 18 h post LPS induction. This is because at 18 h post-LPS induction, the levels of all the examined inflammatory mediators were prominently elevated.

Effects of lithium on LPS-induced secretion of NO, TNF-α, IL-1β and PGE_2

To test the effect of lithium on LPS (100 ng/ml)-induced secretion of NO, TNF-α and IL-1β, cells were treated with lithium for 6 h or 24 h before the addition of LPS to the culture medium. The rationale behind the use of this experimental protocol was to resemble an acute (6 h) and 'chronic' (24 h) lithium treatment. As seen in Figure 3, pretreatment with 1 mM lithium for 6 h (A) and 24 h (B) before LPS did not significantly alter LPS-induced secretion of NO. On the other hand, pretreatment with 10 mM lithium for 6 h (C) and 24 h (D) significantly reduced LPS-induced secretion of NO. Similarly, as seen in Figure 4, pretreatment with 1 mM lithium for 6 h (A) and 24 h (B) did not reduce LPS-induced secretion of TNF-α. On the other hand, pretreatment with 10 mM lithium for 6 h (C) and 24 h (D) significantly reduced LPS-induced secretion of TNF-α. Moreover, as seen in Figure 5, pretreatment with 1 mM lithium for 6 h (A) significantly reduced LPS-induced secretion of IL-1β. Likewise, pretreatment with 10 mM lithium for 6 h (C) and 24 h (D) also significantly reduced LPS-induced secretion of IL-1β. Contrastingly, 1 mM lithium did not alter IL-1β levels when the drug was given 24 h before LPS (B). Furthermore, similar results were obtained in regard to the effect of lithium on LPS (5000 ng/ml)-induced secretion of PGE_2. In these experiments, we used ibuprofen (a non-selective COX inhibitor) as a positive control. As seen in Figure 6, pretreatment with 1 mM lithium for 6 h (A) and 24 h (B) did not significantly alter LPS-induced secretion of PGE_2. Alternatively, pretreatment with 10 mM lithium for 6 h (C) and 24 h (D) significantly reduced LPS-induced secretion of PGE_2. In all PGE_2 experiments, ibuprofen totally abolished LPS-induced secretion of PGE_2.

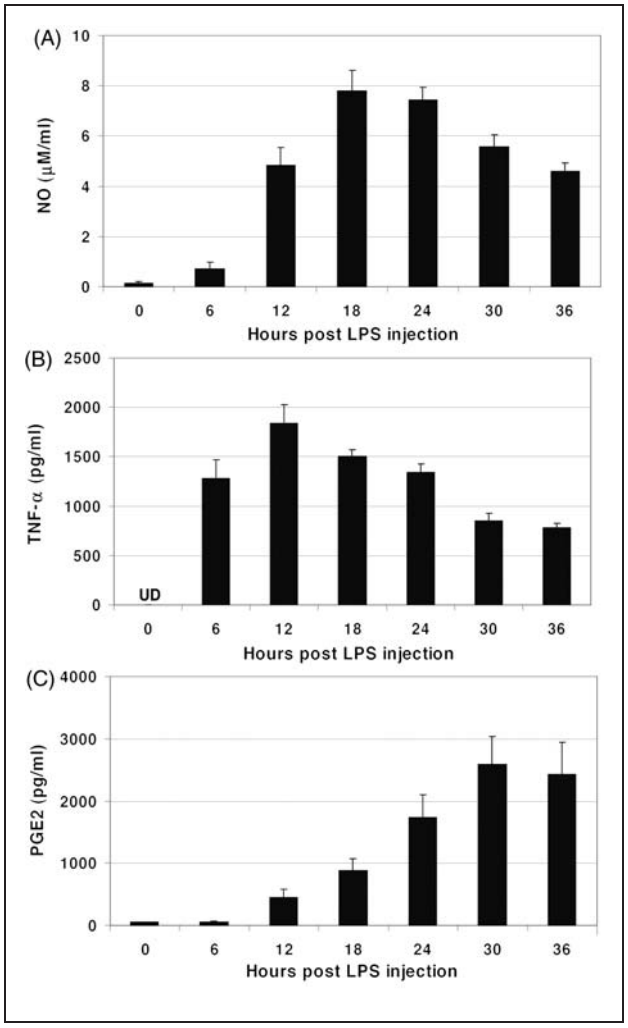

Figure 2. Time-dependent effects of LPS on NO, TNF-α and PGE_2 secretion. Primary rat glia cells were treated with 100 ng/ml LPS (A, B) or 5 μg/ml LPS (C) for the indicated times, at which media was collected for NO (A), TNF-α (B) and PGE_2 (C) determination. The NO levels were determined by the Griess reaction assay and TNF-α and PGE_2 levels by ELISA. The data express the results of one out of three independent experiments with similar results. Values are mean ± SEM of 6–12 samples. UD, undetectable.

Taken together, these results generally indicate that, while a therapeutically relevant concentration of lithium (1 mM) did not reduce LPS-induced secretion of the tested inflammatory mediators, a higher concentration (10 mM) significantly reduced the levels of all of them (NO, TNF-α, IL-1β and PGE_2).

Effects of lithium on LPS-induced expression of iNOS and COX-2

These experiments were conducted in order to examine whether the effects of lithium on LPS-induced secretion of NO and PGE_2 are caused by the suppression of iNOS and COX-2 expression. Lithium was added to the culture medium at 6 h before treatment with LPS (5000 ng/ml), and LPS was added for another 18 h. As seen in Figure 7, lithium alone did not alter iNOS expression and was similar to control. Pretreatment with lithium (1 or 10 mM) significantly reduced LPS-induced expression of iNOS. Moreover, the effects of lithium on COX-2 expression were similar to its effect on iNOS. As seen in Figure 8, lithium alone did not significantly alter COX-2 expression and was similar to control. Pretreatment with lithium 10 mM (but not 1 mM) significantly reduced LPS-induced expression of COX-2.

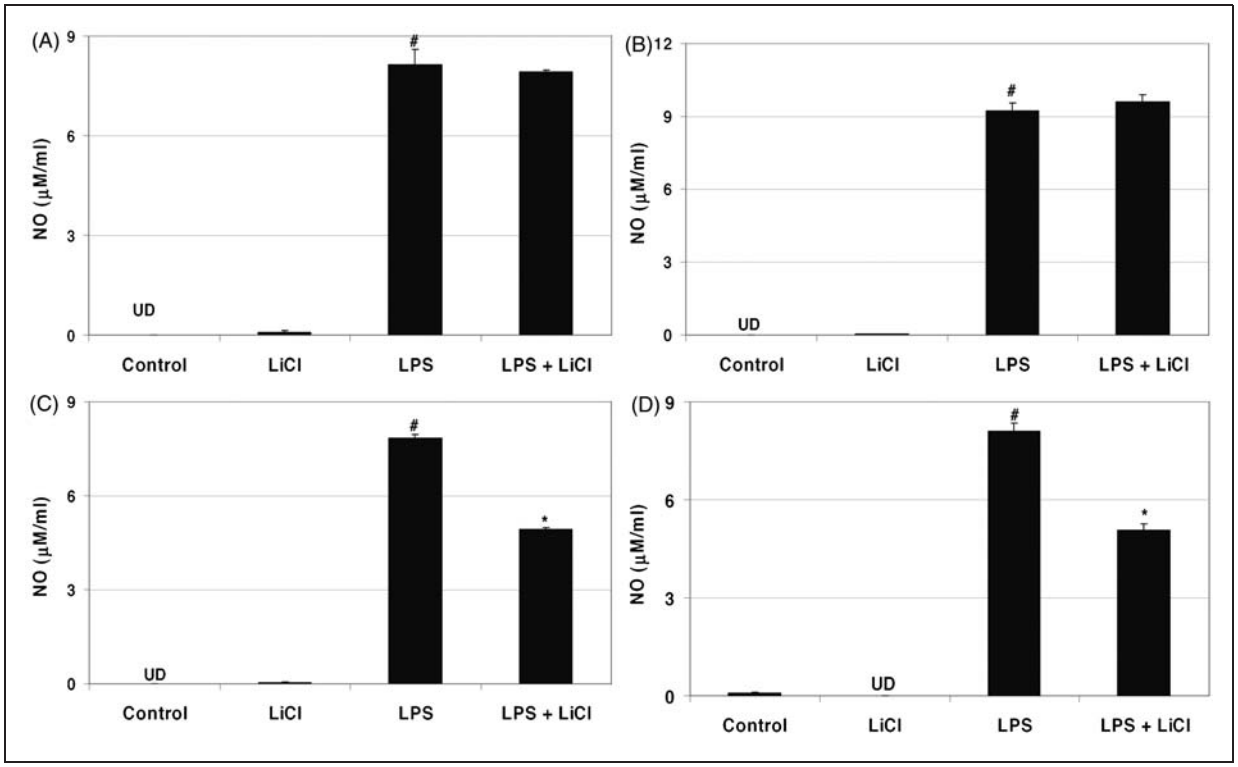

Figure 3. Effect of lithium on LPS-induced secretion of NO. Primary rat glia cells were pretreated with lithium 1 mM (A, B) or 10 mM (C, D) for 6 h (A, C) or 24 h (B, D) before the addition of LPS (100 ng/ml) for another 18 h. The NO levels were determined by the Griess reaction assay. The data express the results of one out of five independent experiments with similar results. Values are mean ± SEM of 6–12 samples. UD, undetectable. $^{\#}P < 0.05$ vs. Control, $^{*}P < 0.05$ vs. LPS.

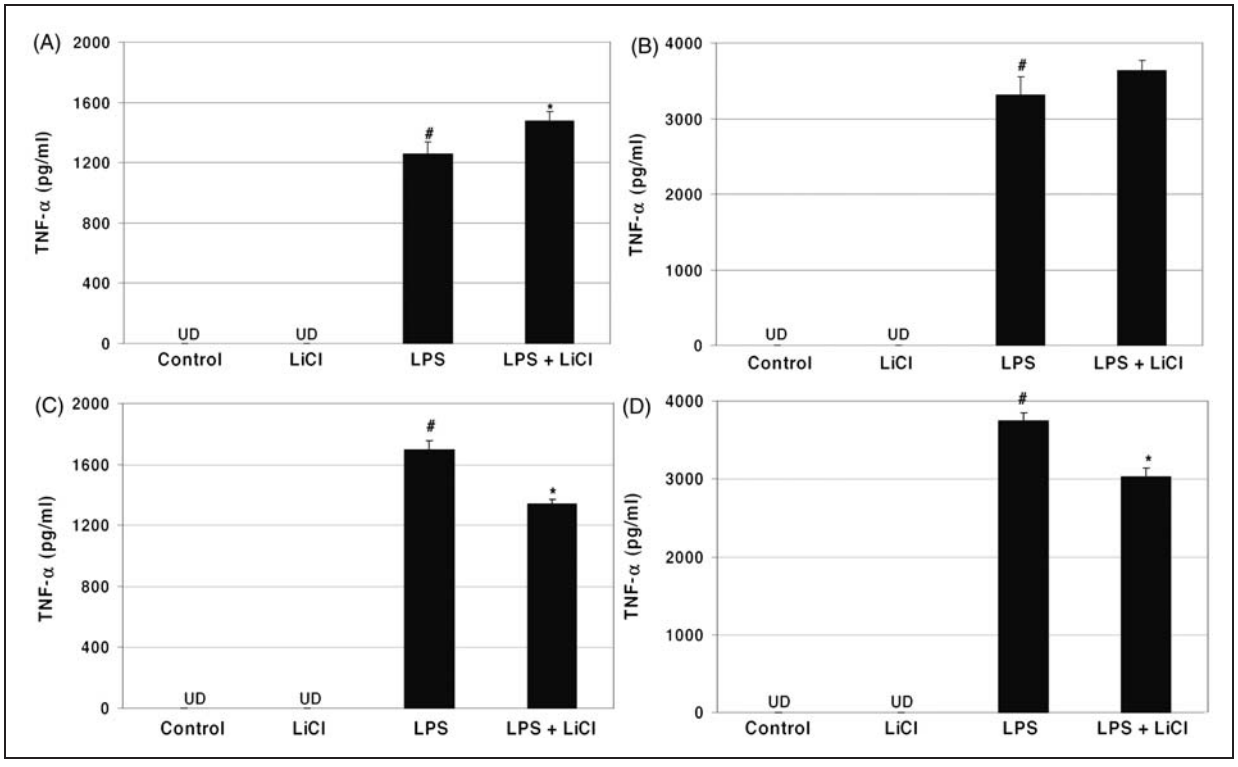

Figure 4. Effect of lithium on LPS-induced secretion of TNF-α. Primary rat glia cells were pretreated with lithium 1 mM (A, B) or 10 mM (C, D) for 6 h (A, C) or 24 h (B, D) before the addition of LPS (100 ng/ml) for another 18 h. The TNF-α levels were determined by ELISA. The data express the results of one out of three independent experiments with similar results. Values are mean ± SEM of 6–12 samples. UD, undetectable. $^{\#}P < 0.05$ vs. Control, $^{*}P < 0.05$ vs. LPS.

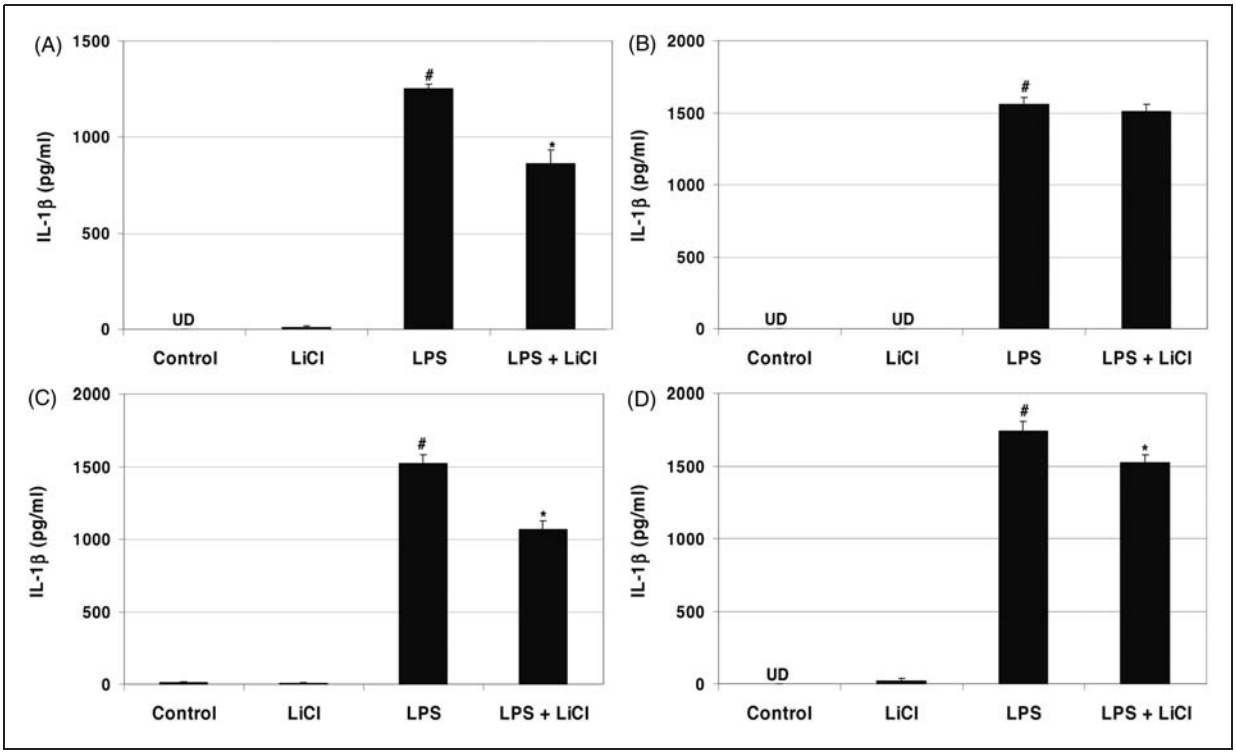

Figure 5. Effect of lithium on LPS-induced secretion of IL-1β. Primary rat glia cells were pretreated with lithium 1 mM (A, B) or 10 mM (C, D) for 6 h (A, C) or 24 h (B, D) before the addition of LPS (100 ng/ml) for another 18 h. The IL-1β levels were determined by ELISA. The data express the results of one out of three independent experiments with similar results. Values are mean ± SEM of 6–12 samples. UD, undetectable. $^{\#}P < 0.05$ vs. Control, $^{*}P < 0.05$ vs. LPS.

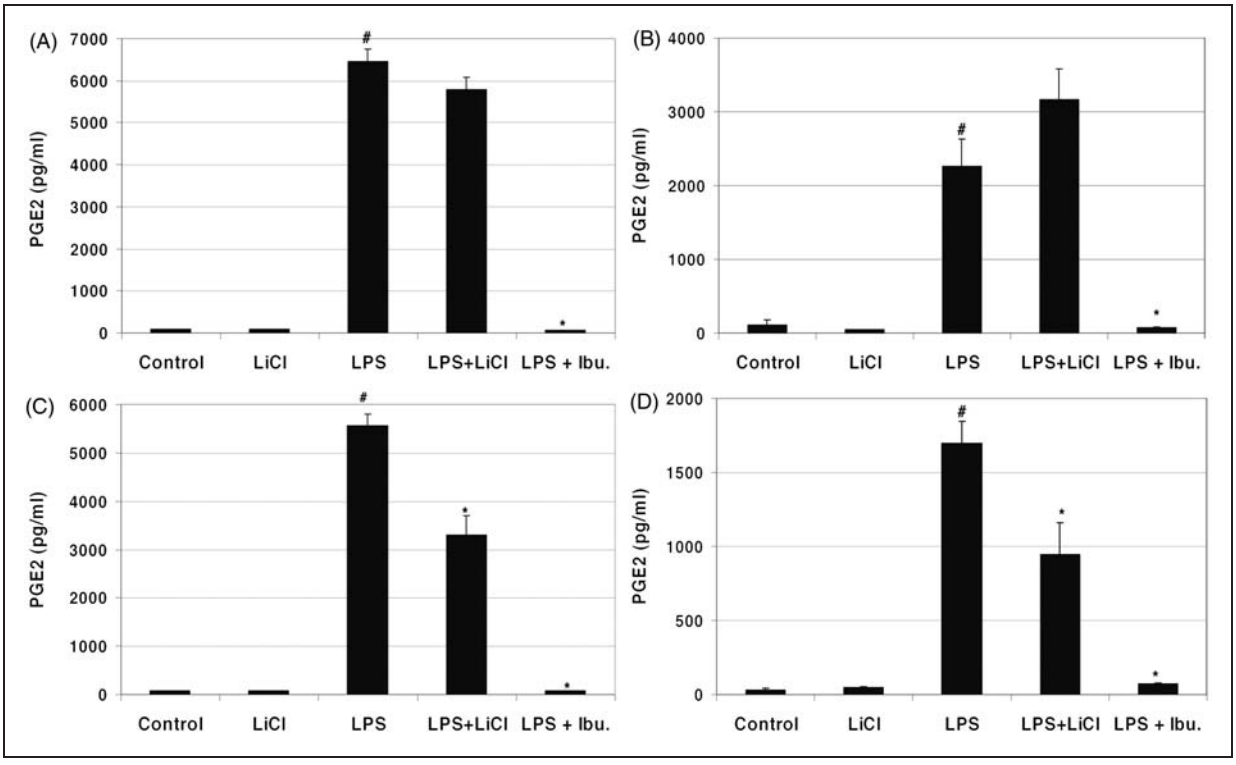

Figure 6. Effect of lithium on LPS-induced secretion of PGE_2. Primary rat glia cells were pretreated with lithium 1 mM (A, B) or 10 mM (C, D) for 6 h (A, C) or 24 h (B, D) before the addition of LPS (5 μg/ml) for another 18 h. Ibuprofen (Ibu.) 100 μM was administered at the same time as lithium and served as a positive control. PGE_2 levels were determined by ELISA. The data express the results of one out of three independent experiments with similar results. Values are mean ± SEM of 6–12 samples. $^{\#}P < 0.05$ vs. Control, $^{*}P < 0.05$ vs. LPS.

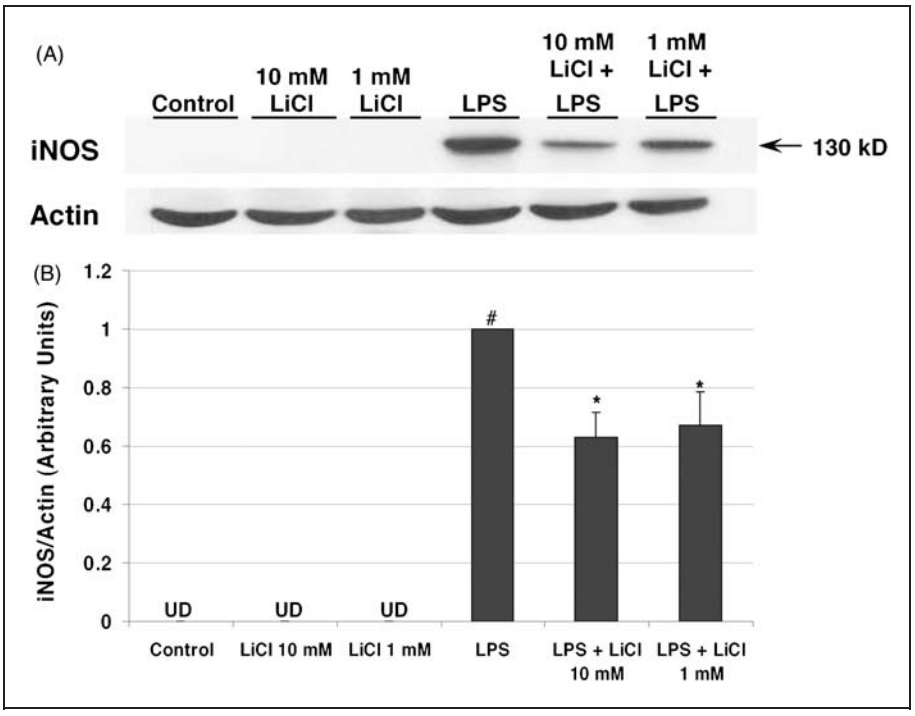

Figure 7. Effect of lithium on iNOS expression. Primary rat glia cells were treated with lithium 1 or 10 mM at 6 h before LPS (5 μg/ml) induction for 18 h. Thereafter, cells were lysed as described in 'Materials and methods' and aliquots of total cell protein (40 μg per lane) were separated on 10% SDS/polyacrylamide gel. The iNOS protein was identified with a specific anti-iNOS Ab. Actin was used as an internal control to normalize for loading variations. (A) An immunoblot of iNOS expression representing one experiment. (B) Quantitative analysis of six immunoblots showing that pretreatment with lithium (1 or 10 mM) significantly reduced LPS-induced expression of iNOS. Bars represent mean + SEM of relative iNOS expression, quantified densitometrically from 6 independent experiments. Owing to variations in LPS-induced expression of iNOS in different experiments, the level of expression induced by LPS in each experiment was arbitrarily expressed as 1. UD, undetectable. $^{\#}P < 0.05$ vs. Control, $^{*}P < 0.05$ vs. LPS.

Discussion

The major finding of our study is that lithium reduced LPS-induced inflammation in rat primary glia cells. This confirms previous reports in which lithium reduced LPS-induced inflammation in mouse glia cells.[78,79] Lithium reduced production of the pro-inflammatory mediators NO, TNF-α, IL-1β and PGE_2 after stimulation with bacterial endotoxin. Moreover, lithium attenuated LPS-induced expression of iNOS and COX-2, which generate NO and PGE_2, respectively.

The accepted therapeutic level of lithium in bipolar patients is 0.6–1.2 mM. It is important to emphasize that, in the current study, the anti-inflammatory effects of lithium were obtained at a concentration of 10 mM, which is extra-therapeutic but widely used in lithium's pharmacology research. At a therapeutically-relevant concentration (1 mM), lithium did not significantly reduce LPS-induced inflammation. It is worth noting that other studies that reported a significant anti-inflammatory effect for lithium after LPS induction also used extra-therapeutic concentrations (10–20 mM) of the drug.[78–80] Moreover, the therapeutic efficacy of lithium is observed mainly when it is given as a prophylactic treatment against recurrent manic, or depressive, episodes.[1,2] The acute clinical effect of lithium alone on mania or depression is low. Thus, if (brain) inflammation is indeed associated with clinical exacerbations, a therapeutic effect of lithium may necessitate much higher doses than those given for prophylactic treatment. This may explain why high doses of lithium are necessary in order to observe a potent anti-inflammatory effect.

Interestingly, Bosetti et al. have shown that chronic treatment with lithium, which led to a therapeutically-relevant plasma concentration (0.74 + 0.03 mM), significantly reduced COX-2 activity and PGE_2 levels in rat brain.[61] The discrepancy between the results of our study and those of the previous report[61] may stem from methodological differences. Importantly, Bosetti et al. did not examine the effects of lithium on LPS (or other inflammatory stimuli)-induced inflammation, but rather determined its effects on basal COX-2 activity and PGE_2 levels.[61] In addition, in their study, lithium was administered for a total duration of 6 wk. Therefore, although in our study lithium 1 mM did not cause a significant reduction in secretion of inflammatory mediators and expression of inflammatory-associated enzymes (except for iNOS), a long-term exposure to lithium (as in bipolar patients) may still

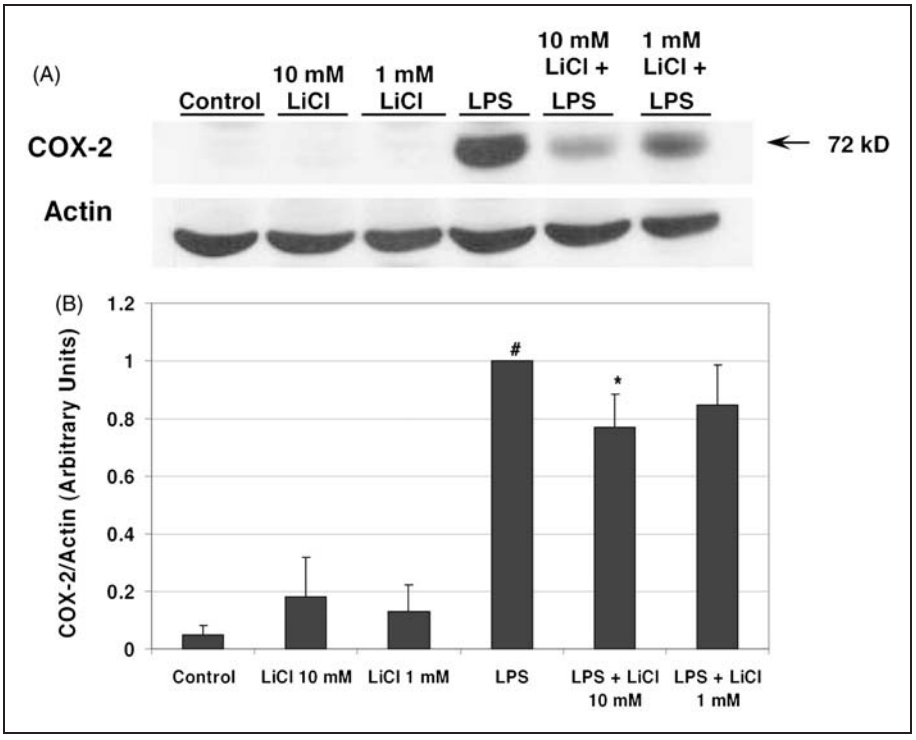

Figure 8. Effect of lithium on COX-2 expression. Primary rat glia cells were treated with lithium 1 or 10 mM at 6 h before LPS (5 μg/ml) induction for 18 h. Thereafter, cells were lysed as described in 'Materials and Methods' and aliquots of total cell protein (40 μg per lane) were separated on 10% SDS/polyacrylamide gel. COX-2 protein was identified with a specific anti-COX-2 Ab. Actin was used as an internal control to normalize for loading variations. (A) An immunoblot of COX-2 expression representing one experiment. (B) Quantitative analysis of six immunoblots showing that pretreatment with lithium 10 mM significantly reduced LPS-induced expression of COX-2. Bars represent mean + SEM of relative COX-2 expression, quantified densitometrically from six independent experiments. Owing to variations in LPS-induced expression of COX-2 in different experiments, the level of expression induced by LPS in each experiment was arbitrarily expressed as 1. $^{\#}P < 0.05$ vs. Control, $^{*}P < 0.05$ vs. LPS.

lead to significant anti-inflammatory effects. This may also be true as in our experimental protocol, LPS was used to induce an *acute* inflammatory response. The magnitude of a possible (chronic) inflammatory process in bipolar patients is not necessarily as high as the one induced by an acute LPS stimulation in vitro. A lower magnitude inflammatory response may be well 'contained' by chronic treatment with 1 mM lithium. In our study, lithium was administered at 6 h and 24 h before exposure to LPS. The rationale for using these two times was to mimic a short-term (acute) and a long-term (chronic) treatment protocols. We are aware that treatment with lithium for a total of 42 h is not a typical 'chronic' treatment protocol; however, we aimed to prolong, as much as possible, the exposure time to lithium in order to examine whether a time-dependent effect exists. Although the results indicate that there is no significant difference between the two treatment courses (pretreatment with lithium for 6 h versus 24 h), it cannot be ruled out that a long-term (4–6 wk) treatment protocol will lead to different results. On the other hand, it is possible that even a long-term treatment with lithium 1 mM will not lead to potent (significant) anti-inflammatory effects. We could not conduct a long-term treatment protocol because of a technical limitation—when we begin experiments with the primary glia cultures, cells are already 2–3 wk old (after extraction) and they remain viable for another 1–2 wk. This does not allow conduction of a long-term (4–6 wk) treatment protocol with lithium.

Nevertheless, the results of our study support the accumulating evidence indicating that lithium and other mood-stabilizing drugs[61–64,81–84] exert anti-inflammatory properties and that inflammation may contribute to the pathophysiological mechanisms underlying bipolar disorder.[49]

The precise mechanism by which lithium inhibits inflammation is not known. Several pharmacological properties of lithium may account for its anti-inflammatory effect. For example, lithium inhibits the multi-functional enzyme glycogen synthase kinase-3 (GSK-3).[17,85,86] Inhibition of GSK-3 has been associated with attenuation of inflammatory responses in different experimental models.[78–80,87–90] Consistently, lithium inhibition of GSK-3 led to a significant reduction in the secretion of inflammatory mediators and expression of inflammatory enzymes.[78–80,87] Relevant to the present study, Jope and co-workers have shown that lithium (20 mM) significantly reduced LPS-induced secretion of IL-6 in mouse primary astrocytes[78] and decreased

the levels of IL-6, NO, iNOS and COX-2 in mouse microglia BV-2 cells.[79] Moreover, inhibition of GSK-3 by lithium was found to decrease the transcriptional activity of NFκ-B,[80,87] which may explain the anti-inflammatory effect of lithium. Other pharmacological effects of lithium may also contribute to its anti-inflammatory activity.

Cytokines such as IL-1β and TNF-α affect cognition, learning and memory. Furthermore, considerable data associated cytokines to CNS neurotransmission and behavior. For example, TNF-α has been reported to reduce serotonin availability in the synaptic cleft by increasing the activity of serotonin transporter.[91] Contrastingly, IL-1β was shown to stimulate the release of serotonin, norepinephrine and dopamine in rat hypothalamus.[92] These findings suggest that cytokines play a role in brain function and affect behavior. We examined the effects of lithium on LPS-induced secretion of IL-1β and TNF-α. Lithium 1 and 10 mM significantly reduced IL-1β levels. This result was consistent in multiple experiments, irrespective of the pretreatment time with lithium (6 h or 24 h). Therefore, the inhibitory effect of lithium on IL-1β secretion may exert a therapeutic effect. On the other hand, only at a concentration of 10 mM did lithium significantly reduce TNF-α levels; at a concentration of 1 mM, lithium significantly increased TNF-α levels. The mechanism underlying the difference in the effect of lithium on IL-1β and TNF-α is not understood. However, the increase in TNF-α levels under treatment with 1 mM lithium is consistent with previous reports in which therapeutic concentrations of lithium significantly increased plasma TNF-α levels in bipolar patients.[93,94] Furthermore, the mood stabilizer valproate has been shown to reduce the release of TNF-α, interferon-γ, NO and reactive oxygen species in primary rat neuron-glia cultures.[83] Another study showed that valproate reduces TNF-α and IL-6 production.[81] In our study, lithium 1 and 10 mM led to a significant reduction in LPS-induced iNOS expression. However, only lithium 10 mM significantly decreased LPS-induced secretion of NO. In this regard, it is worth noting that the effect of lithium 1 mM on expression of iNOS (and COX-2) was not consistent in all experiments that we conducted. In most of the experiments, lithium 1 mM did not reduce LPS-induced expression of iNOS and COX-2. However, in some experiments it did reduce the enzyme expression. This discrepancy in lithium's effect explains the relatively large standard errors seen in Figures 7 and 8 (iNOS and COX-2, respectively). We do not know the exact reason for this inconsistency, however, a different magnitude response-to-LPS may be a partial explanation. Namely, that the same LPS concentration led to a different extent expression of iNOS and COX-2 in various experiments.

In summary, the present study demonstrates that lithium (10 mM) reduced LPS-induced inflammation in primary rat glia cells. More research is needed to elucidate the exact association between brain inflammation and the pathogenesis of bipolar disorder and the mechanisms by which mood stabilizers inhibit inflammation.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Belmaker RH. Bipolar disorder. *N Engl J Med* 2004; 351: 476–486.
2. Gershon S, Chengappa KN and Malhi GS. Lithium specificity in bipolar illness: a classic agent for the classic disorder. *Bipolar Disord* 2009; 11: 34–44.
3. Narrow WE, Rae DS and Robins LN. Revised prevalence estimates of mental disorders in the United States: using clinical significance criterion to reconcile 2 surveys' estimates. *Arch Gen Psychiatry* 2002; 59: 115–123.
4. Simon GE, Bauer MS, Ludman EJ, et al. Mood symptoms, functional impairment, and disability in people with bipolar disorder: specific effects of mania and depression. *J Clin Psychiatry* 2007; 68: 1237–1245.
5. Soreca I, Fagiolini A, Frank E, et al. Relationship of general medical burden, duration of illness and age in patients with bipolar I disorder. *J Psychiatr Res* 2008; 42: 956–961.
6. Lenox RH, McNamara RK, Papke RL, et al. Neurobiology of lithium: an update. *J Clin Psychiatry* 1998; 59: 37–47.
7. Goodwin FK. Rationale for long-term treatment of bipolar disorder and evidence for long-term lithium treatment. *J Clin Psychiatry* 2002; 63: 5–12.
8. Baethge C, Gruschka P, Berghöfer A, et al. Prophylaxis of schizoaffective disorder with lithium or carbamazepine: outcome after long-term follow-up. *J Affect Disord* 2004; 79: 43–50.
9. Noble W, Planel E, Zehr C, et al. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc Natl Acad Sci USA* 2005; 102: 6990–6995.
10. Engel T, Goñi-Oliver P, Gómez de Barreda E, et al. Lithium, a potential protective drug in Alzheimer's disease. *Neurodegener Dis* 2008; 5: 247–249.
11. Jope RS, Yuskaitis CJ and Beurel E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. *Neurochem Res* 2007; 32: 577–595.
12. Fornai F, Longone P, Cafaro L, et al. Lithium delays progression of amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 2008; 105: 2052–2057.
13. Gitlin M. Treatment-resistant bipolar disorder. *Mol Psychiatry* 2006; 11: 227–240.
14. Bowden CL. Making optimal use of combination pharmacotherapy in bipolar disorder. *J Clin Psychiatry* 2004; 65: 21–24.
15. Gitlin M. Lithium and the kidney: an updated review. *Drug Saf* 1999; 20: 231–243.
16. Berridge MJ and Irvine RF. Inositol phosphates and cell signaling. *Nature* 1989; 341: 197–204.
17. Klein PS and Melton DA. A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci USA* 1996; 93: 8455–8459.
18. Manji HK, Moore GJ and Chen G. Lithium at 50: have the neuroprotective effects of this unique cation been overlooked? *Biol. Psychiatry* 1999; 46: 929–940.
19. Rao JS, Lee HJ, Rapoport SI, et al. Mode of action of mood stabilizers: is the arachidonic acid cascade a common target? *Mol Psychiatry* 2008; 13: 585–596.
20. Goldstein BI, Kemp DE, Soczynska JK, et al. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. *J Clin Psychiatry* 2009; 70: 1078–1090.

21. Rapoport SI, Basselin M, Kim HW, et al. Bipolar disorder and mechanisms of action of mood stabilizers. *Brain Res Rev* 2009; 61: 185–209.
22. O'Brien SM, Scully P, Scott LV, et al. Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. *J Affect Disord* 2006; 90: 263–267.
23. Liu JC, Yang YY, Chou YM, et al. Immunologic variables in acute mania of bipolar disorder. *J Neuroimmunol* 2004; 150: 116–122.
24. Huang TL and Lin FC. High-sensitivity C-reactive protein levels in patients with major depressive disorder and bipolar mania. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31: 370–372.
25. Dickerson F, Stallings C, Origoni A, et al. Elevated serum levels of C-reactive protein are associated with mania symptoms in outpatients with bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31: 952–955.
26. Begemann M, Sargin D, Rossner MJ, et al. Episode-specific differential gene expression of peripheral blood mononuclear cells in rapid cycling supports novel treatment approaches. *Mol Med* 2008; 14: 9–10.
27. Padmos RC, Hillegers MH, Knijff EM, et al. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. *Arch Gen Psychiatry* 2008; 65: 395–407.
28. Soczynska JK, Kennedy SH, Goldstein BI, et al. The effect of tumor necrosis factor antagonists on mood and mental health-associated quality of life: novel hypothesis-driven treatments for bipolar depression? *Neurotoxicology* 2009; 30: 497–521.
29. Kauer-Sant'Anna M, Kapczinski F, Andreazza AC, et al. Brain-derived neurotrophic factor and inflammatory markers in patients with early- vs. late-stage bipolar disorder. *Int J Neuropsychopharmacol* 2008; 12: 447–458.
30. Maes M, Bosmans E, Suy E, et al. Immune disturbances during major depression: upregulated expression of interleukin-2 receptors. *Neuropsychobiology* 1990–1991; 24: 115–120.
31. Maes M. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2011; 35: 664–675.
32. Licinio J and Wong ML. The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection. *Mol Psychiatry* 1999; 4: 317–327.
33. Müller N, Schwarz MJ, Dehning S, et al. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry* 2006; 11: 680–684.
34. Vaccarino V, Brennan ML, Miller AH, et al. Association of major depressive disorder with serum myeloperoxidase and other markers of inflammation: a twin study. *Biol Psychiatry* 2008; 64: 476–483.
35. Gimeno D, Kivimäki M, Brunner EJ, et al. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med* 2009; 39: 413–423.
36. Dimopoulos N, Piperi C, Psarra V, et al. Increased plasma levels of 8-iso-PGF2alpha and IL-6 in an elderly population with depression. *Psychiatry Res* 2008; 161: 59–66.
37. Manev H, Manev R and Vidovich MI. Depression, inflammation, and cardiovascular disease: is 5-lipoxygenase the missing link? *J Am Coll Cardiol* 2008; 51: 1990–1991.
38. Milaneschi Y, Corsi AM, Penninx BW, et al. Interleukin-1 receptor antagonist and incident depressive symptoms over 6 years in older persons: the InCHIANTI study. *Biol Psychiatry* 2009; 65: 973–978.
39. Howren MB, Lamkin DM and Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009; 71: 171–186.
40. Miller AH, Maletic V and Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009; 65: 732–741.
41. Dhabhar FS, Burke HM, Epel ES, et al. Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. *J Psychiatr Res* 2009; 43: 962–969.
42. Müller N, Riedel M, Scheppach C, et al. Beneficial antipsychotic effects of celecoxib add-on therapy compared to risperidone alone in schizophrenia. *Am J Psychiatry* 2002; 159: 1029–1034.
43. Müller N, Ulmschneider M, Scheppach C, et al. COX-2 inhibition as a treatment approach in schizophrenia: immunological considerations and clinical effects of celecoxib add-on therapy. *Eur Arch Psychiatry Clin Neurosci* 2004; 254: 14–22.
44. Haack M, Hinze-Selch D, Fenzel T, et al. Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. *J Psych Res* 1999; 33: 407–418.
45. McGeer PL. Cyclo-oxygenase-2 inhibitors: rationale and therapeutic potential for Alzheimer's disease. *Drugs Aging* 2000; 17: 1–11.
46. Firuzi O and Pratico D. Coxibs and Alzheimer's disease: should they stay or should they go? *Ann Neurol* 2006; 59: 219–228.
47. Whitton PS. Inflammation as a causative factor in the aetiology of Parkinson's disease. *Br J Pharmacol* 2007; 150: 963–976.
48. Turrin NP and Rivest S. Innate immune reaction in response to seizures: implications for the neuropathology associated with epilepsy. *Neurobiol Dis* 2004; 16: 321–334.
49. Nery FG, Monkul ES, Hatch JP, et al. Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. *Hum Psychopharmacol* 2008; 23: 87–94.
50. Goetzl EJ, An S and Smith WL. Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. *FASEB J* 1995; 9: 1051–1058.
51. Smith WL, Garavito RM and DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 1996; 271: 33157–33160.
52. Smith WL, DeWitt DL and Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 2000; 69: 145–182.
53. Wallace JL. Selective COX-2 inhibitors: is the water becoming muddy? *TiPS* 1999; 20: 4–6.
54. Coruzzi G, Menozzi A and Dobrilla G. Novel non-steroidal anti-inflammatory drugs: what we have learned from animal studies. *Curr Drugs Targets Inflamm* Allergy 2004; 3: 43–61.
55. McAdam BF, Catella-Lawson F, Mardini IA, et al. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci USA* 1999; 96: 272–277.
56. Merrill JE, Murphy SP, Mitrovic B, et al. Inducible nitric oxide synthase and nitric oxide production by oligodendrocytes. *J Neurosci Res* 1997; 48: 372–384.
57. Knowles RG and Moncada S. Nitric oxide synthases in mammals. *Biochem J* 1994; 298: 249–258.
58. Moncada S and Bolanos JP. Nitric oxide, cell bioenergetics and neurodegeneration. *J Neurochem* 2006; 97: 1676–1689.
59. Selek S, Savas HA, Gergerlioglu HS, et al. The course of nitric oxide and superoxide dismutase during treatment of bipolar depressive episode. *J Affect Disord* 2008; 107: 89–94.
60. Savas HA, Gergerlioglu HS, Armutcu F, et al. Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. *World J Biol Psychiatry* 2006; 1: 51–55.
61. Bosetti F, Rintala J, Seemann R, et al. Chronic lithium down-regulates cyclooxygenase-2 activity and prostaglandin E_2 concentration in rat brain. *Mol Psychiatry* 2002; 7: 845–850.

62. Chang MC, Contreras MA, Rosenberger TA, et al. Chronic valproate treatment decreases the in vivo turnover of arachidonic acid in brain phospholipids: a possible common effect of mood stabilizers. *J Neurochem* 2001; 77: 796–803.
63. Bazinet RP, Rao JS, Chang L, et al. Chronic carbamazepine decreases the incorporation rate and turnover of arachidonic acid but not docosahexaenoic acid in brain phospholipids of the unanesthetized rat: relevance to bipolar disorder. *Biol Psychiatry* 2006; 59: 401–407.
64. Lee HJ, Ertley RN, Rapoport SI, et al. Chronic administration of lamotrigine downregulates COX-2 mRNA and protein in rat frontal cortex. *Neurochem Res* 2008; 33: 861–866.
65. Guo JY, Li CY, Ruan YP, et al. Chronic treatment with celecoxib reverses chronic unpredictable stress-induced depressive-like behavior via reducing cyclooxygenase-2 expression in rat brain. *Eur J Pharmacol* 2009; 612: 54–60.
66. Castanon N, Leonard BE, Neveu PJ, et al. Effects of antidepressants on cytokine production and actions. *Brain Behav Immun* 2002; 16: 569–574.
67. Lim CM, Kim SW, Park JY, et al. Fluoxetine affords robust neuroprotection in the postischemic brain via its anti-inflammatory effect. *J Neurosci Res* 2009; 87: 1037–1045.
68. Rao R, Hao CM and Breyer MD. Hypertonic stress activates glycogen synthase kinase 3beta-mediated apoptosis of renal medullary interstitial cells, suppressing an NFkappaB-driven cyclooxygenase-2-dependent survival pathway. *J Biol Chem* 2004; 279: 3949–3955.
69. Haertel-Wiesmann M, Liang Y, Fantl WJ, et al. Regulation of cyclooxygenase-2 and periostin by Wnt-3 in mouse mammary epithelial cells. *J Biol Chem* 2000; 275: 32046–32051.
70. Kotnik P, Nielsen J, Kwon TH, et al. Altered expression of COX-1, COX-2, and mPGES in rats with nephrogenic and central diabetes insipidus. *Am J Physiol Renal Physiol* 2005; 288: F1053–F1068.
71. Aschner M. Immune and inflammatory responses in the CNS: modulation by astrocytes. *Toxicol Lett* 1998; 102-103: 283–287.
72. Allen NJ and Barres BA. Glia – more than just brain glue. *Nature* 2009; 457: 675–677.
73. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* 1996; 19: 312–318.
74. Shemi D, Azab AN and Kaplanski J. Time-dependent effect of LPS on PGE2 and TNF-α production by ray glial brain culture: influence of COX and cytokine inhibitors. *J Endotoxin Res* 2001; 6: 377–381.
75. Tenenbaum M, Azab AN and Kaplanski J. Effect of estrogen against LPS-induced inflammation and toxicity in primary rat glial and neuronal cultures. *J Endotoxin Res* 2007; 13: 158–166.
76. Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 1982; 126: 131–138.
77. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254.
78. Beurel E and Jope RS. Lipopolysaccharide-induced interleukin-6 production is controlled by glycogen synthase kinase-3 and STAT3 in the brain. *J Neuroinflammation* 2009; 6: 9.
79. Yuskaitis CJ and Jope RS. Glycogen synthase kinase-3 regulates microglial migration, inflammation, and inflammation-induced neurotoxicity. *Cell Signal* 2009; 21: 264–273.
80. Martin M, Rehani K, Jope RS, et al. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat Immunol* 2005; 6: 777–784.
81. Ichiyama T, Okada K, Lipton JM, et al. Sodium valproate inhibits production of TNF-alpha and IL-6 and activation of NF-kappaB. *Brain Res* 2000; 857: 246–251.
82. Rao JS, Bazinet RP, Rapoport SI, et al. Chronic treatment of rats with sodium valproate downregulates frontal cortex NF-kappaB DNA binding activity and COX-2 mRNA. *Bipolar Disord* 2007; 9: 513–520.
83. Peng GS, Li G, Tzeng NS, et al. Valproate pretreatment protects dopaminergic neurons from LPS-induced neurotoxicity in rat primary midbrain cultures: role of microglia. *Mol Brain Res* 2005; 134: 162–169.
84. Chen PS, Wang CC, Bortner CD, et al. Valproic acid and other histone deacetylase inhibitors induce microglial apoptosis and attenuate lipopolysaccharide-induced dopaminergic neurotoxicity. *Neuroscience* 2007; 149: 203–212.
85. Jope RS. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. *Trends Pharmacol Sci* 2003; 24: 441–443.
86. Stambolic V, Ruel L and Woodgett JR. Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr Biol* 1996; 6: 1664–1668.
87. Hoeflich KP, Luo J, Rubie EA, et al. Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature* 2000; 406: 26–27.
88. Whittle BJ, Varga C, Pósa A, et al. Reduction of experimental colitis in the rat by inhibitors of glycogen synthase kinase-3beta. *Br J Pharmacol* 2006; 147: 575–582.
89. Cuzzocrea S, Crisafulli C, Mazzon E, et al. Inhibition of glycogen synthase kinase-3beta attenuates the development of carrageenan-induced lung injury in mice. *Br J Pharmacol* 2006; 149: 687–702.
90. Cuzzocrea S, Mazzon E, Di Paola R, et al. Glycogen synthase kinase-3beta inhibition attenuates the degree of arthritis caused by type II collagen in the mouse. *Clin Immunol* 2006; 120: 57–67.
91. Irwin MR and Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. *Brain Behav Immun* 2007; 21: 374–383.
92. Shintani F, Kanba S, Nakaki T, et al. Interleukin-1 beta augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. *J Neurosci* 1993; 13: 3574–3581.
93. Guloksuz S, Cetin EA, Cetin T, et al. Cytokine levels in euthymic bipolar patients. *J Affect Disord* 2010; 126: 458–462.
94. Himmerich H, Koethe D, Schuld A, et al. Plasma levels of leptin and endogenous immune modulators during treatment with carbamazepine or lithium. *Psychopharmacology* 2005; 179: 447–451.