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Original Article

Effect of Lipopolysaccharide on the Duration of Zolpidem-Induced Loss of Righting Reflex in Mice

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Zolpidem, a non-benzodiazepine hypnotic, is primarily used to treat insomnia. In a previous study, pior treatment with non-benzodiazepine receptor agonists was associated with inflammation. The present study aimed to clarify the association between the effects of zolpidem and inflammation in mice treated with lipopolysaccharide (LPS), a known model of inflammation. We assessed the zolpidem-induced loss of righting reflex (LORR) duration 24 h after LPS treatment in mice. Additionally, the expressions of γ-aminobutyric acid $(GABA)_A$ receptor subunit and K^+-Cl^- cotransporter isoform 2 (KCC2) mRNA in the hippocampus and frontal cortex were examined in LPS-treated mice. Pretreatment with LPS was associated with significantly prolonged duration of zolpidem-induced LORR compared to control mice. This effect was significantly attenuated by administering bicuculline, a $GABA_A$ receptor antagonist, or flumazenil, a benzodiazepine receptor antagonist, in LPS-treated mice. Compared to controls, LPS-treated mice showed no significant change in the expression of $GABA_A$ receptor subunits in the hippocampus or frontal cortex. Bumetanide, an Na^+-K^+-$2Cl^-$ cotransporter isoform 1 blocker, attenuated the extended duration of zolpidem-induced LORR observed in LPS-treated mice. LPS significantly decreased *Kcc2* mRNA expression in the hippocampus and the frontal cortex. These findings suggest that inflammation increases zolpidem-induced LORR, possibly through a reduction in KCC2 expression.

Key words: lipopolysaccharide, zolpidem, $GABA_A$ receptor, K^+-Cl^- cotransporters

Benzodiazepine receptor agonists are used to treat various conditions, including insomnia, and they exert hypnotic, anxiolytic, and anticonvulsant effects. Their frequent use – can be attributed to their fast-acting, effective action [1]. However, they are linked to a notably elevated risk of postoperative delirium, characterized by abnormal behavior, often accompanied by neuroinflammation [2-7]. Thus, the pharmacological mechanism of benzodiazepine receptor agonists may be associated with systemic inflammation.

Lipopolysaccharide (LPS) is a well-known bacterial toxin that can induce neuroinflammation [8-10]. We previously reported that LPS pretreatment 24 h in advance significantly increased the duration of pentobarbital-induced loss of righting reflex (LORR) in mice treated with benzodiazepine receptor agonists (diazepam and brotizolam) and a γ-aminobutyric acid–A ($GABA_A$) receptor agonist (muscimol) compared to that of mice treated with vehicle [11]. Thus, LPS pretreat-

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ment may induce an excessive increase in GABA activity [11-13]. Furthermore, LPS administration may play a specific role in the overactivation of $GABA_A$ receptors in the central nervous system. $GABA_A$ receptors mediate inhibitory synaptic action in mature neurons that maintain low intracellular chloride concentrations, the underlying mechanisms of which are related to chloride cation cotransporters (CCCs), including Na^+-K^+-$2Cl^-$ cotransporter isoform 1 (NKCC1) and K^+-Cl^- cotransporter isoform 2 (KCC2). In short, LPS administration alters $GABA_A$ receptor function through CCCs [14]. Thus, LPS-induced inflammation is speculated to result in the dysfunction of $GABA_A$ receptors associated with chloride concentration and mediated by NKCC1, thereby contributing to the effects of benzodiazepines.

Insomnia medications such as zolpidem, classified as a non-benzodiazepine hypnotic [15], are nonetheless known to act on $GABA_A$ receptors, as do benzodiazepines. This study aimed to examine how LPS-induced inflammation affects the duration of LORR by the non-benzodiazepine receptor agonist zolpidem. We also investigated how the expression of NKCC1 and KCC2 and the function of the $GABA_A$ receptor were affected by LPS-induced inflammation in mice.

Materials and Methods

Experimental animals. Animal experiments were conducted in accordance with the guidelines outlined in the Guide for Animal Experiments of Okayama University Advanced Science Research Center. The experimental protocol was approved by the Animal Care and Use Committee of Okayama University (OKU-2018746, OKU-2020216). A total of 132 ICR male mice with an initial weight of 28-35 g were acquired commercially from Jackson Laboratory (Yokohama, Japan). We used randomization to assign the mice to groups. The mice were housed in a temperature-controlled room with a constant 12-h light and dark cycle (lights on at 08 : 00), with six mice in each cage. Animals had unrestricted access to standard laboratory food and water. The room was maintained at 23 ± 1°C and a humidity level of approximately 60%.

Drugs. The following drugs were used in this study: LPS (from *Escherichia coli* O127: B8; Sigma-Aldrich, St. Louis, MO, USA), zolpidem (ZOLPIDEM TARTRATE OD; Sawai Seiyaku Co., Tokyo, Japan), (+)-bicuculline (Sigma-Aldrich), flumazenil (FUJIFILM Wako Chemicals, Osaka, Japan), and bumetanide (Sigma-Aldrich). (+)-Bicuculline and flumazenil were suspended in 0.5% methylcellulose solution, and bumetanide was dissolved in 0.1N NaOH. Mice were intraperitoneally (i.p.) injected with LPS, (+)-bicuculline, and flumazenil at 10 mL/kg body weight and with zolpidem and bumetanide at 20 mL/kg body weight. Control mice were administered vehicle.

Behavioral study design for the duration of zolpidem-induced LORR. Behavioral experiments were conducted exclusively at 10 : 00-14 : 00. LORR was defined as the state in which the mice were lying on their back and unable to regain an upright position for at least 30 sec. The duration of LORR was defined as the time from the initial LORR until restoration. In the behavioral study, mice were divided into four groups: zolpidem, zolpidem + LPS, zolpidem + drug (bicuculline, flumazenil, zolpidem), and zolpidem + LPS + drug (bicuculline, flumazenil, zolpidem). First, the dosage of zolpidem required to achieve LORR was determined. Mice were injected with LPS one day before testing for zolpidem-induced LORR; bumetanide was administered 30 min before LPS administration, and bicuculline or flumazenil were administered 30 min before zolpidem administration. The doses of bumetanide, bicuculline, and flumazenil were selected based on previous reports. [11, 14] The duration of LORR was calculated by subtracting the time of LORR onset from that of LORR recovery. [11, 16, 17] The schedule used for the behavioral studies is shown in Fig. 1.

Serum cytokine measurements. As shown in Fig. 2, mice were divided into four groups, namely the

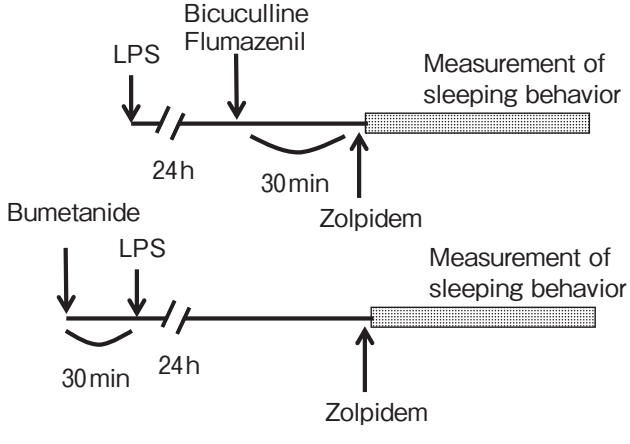

Fig. 1 Experimental design for the behavioral studies.

vehicle group, and 2-h, 5-h, and 24-h post-LPS-treatment groups. Animals were decapitated 2 h, 5 h, or 24 h after LPS administration, and blood samples were obtained from the decapitated mice at each of these time points. The serum levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were measured using commercially available enzyme-linked immunosorbent assay kits (Thermo Fisher Scientific Inc., Cleveland, OH, USA) following the manufacturer's instructions.

Measurement of mRNA expressions of Il6, Tnfa, $GABA_A$ receptor subunits (Gabra1, Gbarb2, and Gabrg2), Nkcc1, and Kcc2 by real-time quantitative polymerase chain reaction. The hippocampus and frontal cortex of individual animals were used to isolate the total RNA using Maxwell RSC® simply RNA Tissue Kit from Promega (Madison, WI, USA). ReverTra Ace® quantitative polymerase chain reaction (qPCR) RT

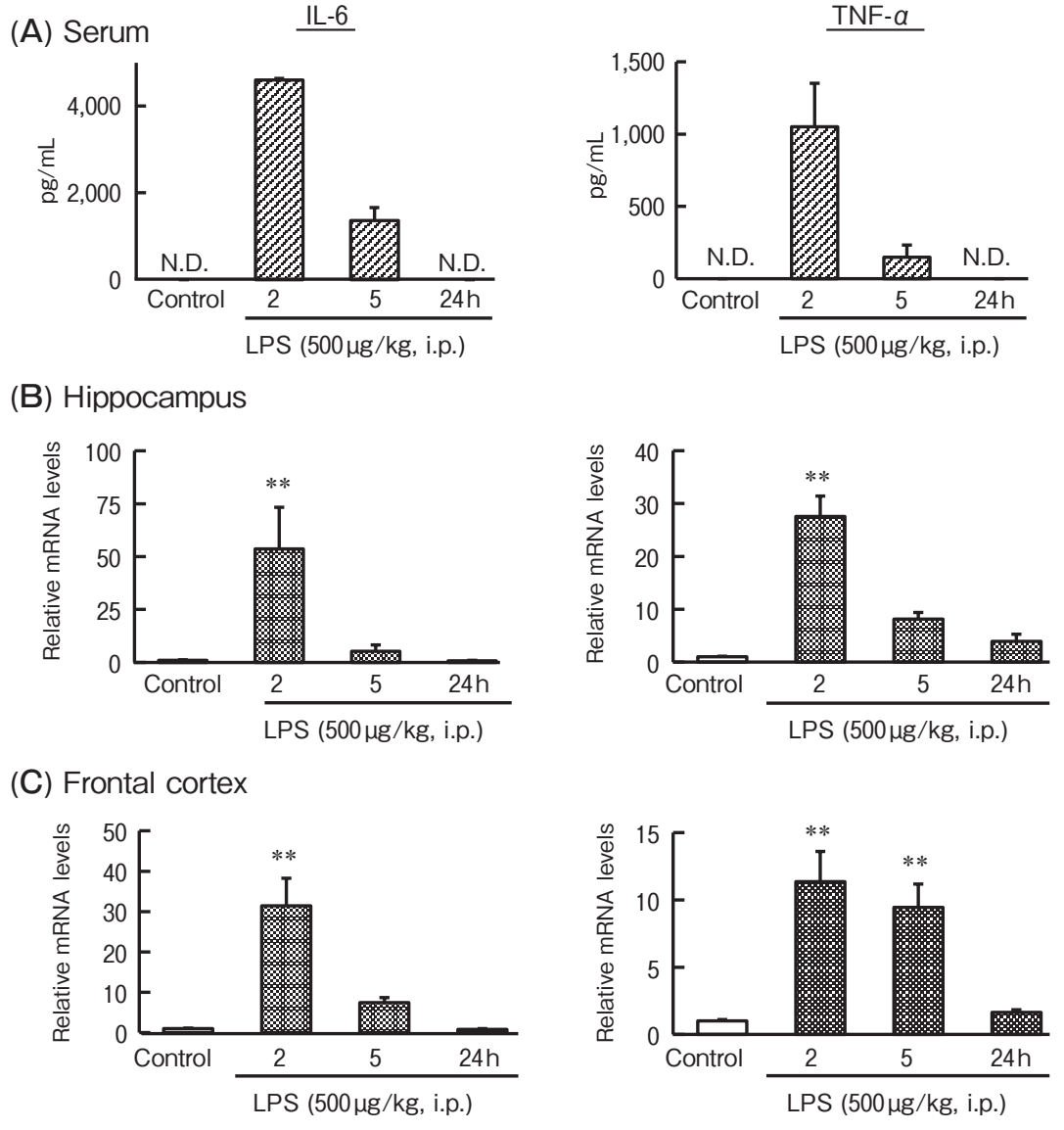

Fig. 2 Effects of LPS on serum IL-6 and TNF-α concentrations (A), hippocampal (B) and frontal cortex (C) *Il6* and *Tnfa* mRNA expression in mice. IL-6 and TNF-α concentrations and mRNA expression were observed at 2 h, 5 h, and 24 h after LPS administration (500 μg/kg, i.p.). Values are expressed as means ± SEMs (n = 4-6 for each group). Data were analyzed using a one-way analysis of variance, and group means were compared using Dunnett's test. $^{**}p < 0.01$ significantly different from the control group. N.D., not detected.

Master Mix from TOYOBO (Osaka, Japan) was used to perform reverse transcription on 1 μg of total RNA from each sample. Real-time PCR amplification was performed using the StepOnePlus™ Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences, target genes, and other related information are provided in Table 1. The PCR protocol consisted of an initial denaturation step at 95°C for 20 sec, followed by 40 cycles of denaturation at 95°C for 1 sec and annealing/extension at 60°C for 20 sec. Upon completion of the reaction, specificity was confirmed using melting curve analysis. To quantify the total expressions of the $GABA_A$ receptor α1 (*Gabra1*), β2 (*Gabrb2*), and γ2 (*Gabrg2*) subunits and those of *Il6*, *Tnfa*, *Nkcc1*, and *Kcc2*, forward and reverse primers and probes were designed (Integrated DNA Technologies, Coralville, IA, USA). The relative mRNA expression was normalized to that of *Gapdh*. The comparative cycle threshold (ΔΔCt) method was used for calculating each target gene [18].

Statistical analysis. All data are expressed as means ± standard errors of the mean (SEMs). Statistical analysis was performed using Excel-Tokei ver. 7.0 (Esumi Co. Ltd., Tokyo, Japan). The data were analyzed using a two-tailed Student's *t*-test, while one-or two-way analysis of variance was used to evaluate the other data. Multiple comparison tests (Dunnett's and Tukey's tests) were then conducted to determine statistical significance, which was set at $p < 0.05$.

Results

Effects of LPS on IL-6 and TNF-α serum concentrations. LPS administration increased serum levels of IL-6 and TNF-α at 2 h and 5 h after the treatment, respectively. However, serum concentrations of IL-6 and TNF-α were normalized after 24 h (Fig. 2A).

Effects of LPS administration on the mRNA expression of Il6 and Tnfa in the hippocampus and frontal cortex. Il6 mRNA expression significantly increased in the hippocampus and frontal cortex of mice at 2 h after LPS administration (hippocampus: $F[3,19] = 6.32$, $p < 0.01$; frontal cortex: $F[3,17] = 14.26$, $p < 0.01$; Fig. 2B, C), whereas that of *Tnfa* significantly increased at 2 h or 5 h after LPS administration (hippocampus: $F[3,17] = 32.15$, $p < 0.01$; frontal cortex: $F[3,15] = 12.25$, $p < 0.01$; Fig. 2B, C).

Effects of zolpidem on LORR duration. To determine the optimal dose of zolpidem for administration, the duration of LORR was measured at three doses (50, 75, and 100 mg/kg i.p.). The incidence of LORR following zolpidem treatment was 75% at 50 mg/kg and 100% at ≧75 mg/kg (Table 2). Therefore, a 75 mg/kg dose was used in further experiments.

Effects of bicuculline and flumazenil on the duration of zolpidem-induced LORR mediated by LPS. We measured the duration of zolpidem-induced LORR 24 h after LPS administration. This phenomenon was significantly extended upon LPS administration (Fig. 3). We studied whether the extension of zolpidem-induced LORR by LPS was related to the $GABA_A$ receptor (bicuculline: $GABA_A$ receptor antagonist) and benzodiazepine receptor (flumazenil: benzodiazepine receptor antagonist). Bicuculline injection significantly decreased the duration of LORR in LPS-treated mice but not in control mice (LPS: $F[1,11] = 23.14$, $p < 0.01$; bicuculline: $F[1,11] = 2.81$, $p = 0.12$; LPS × bicuculline: $F[1,11] = 1.19$, $p = 0.30$; Fig. 3A). Flumazenil significantly decreased the prolongation of LORR in LPS-treated mice but not in control mice (LPS: $F[1,11] = 32.74$, $p < 0.01$; flumazenil: $F[1,11] = 5.83$, $p < 0.05$; LPS × flumazenil: $F[1,11] = 0.58$, $p = 0.46$; Fig. 3B).

Effects of LPS on $GABA_A$ receptor subunits. We focused on the site of the $GABA_A$ receptor that could bind benzodiazepines. LPS did not affect the expression of the $GABA_A$ receptor subunits (*Gabra1*, *Gabrb2*, and *Gabrg2*) in the hippocampus or frontal cortex at 24 h after the treatment (Fig. 4).

Effects of LPS and bumetanide on the duration of zolpidem-induced LORR. We evaluated whether bumetanide, an NKCC1 blocker, could prevent the duration of zolpidem-induced LORR. Bumetanide (30 mg/kg, i.p.) attenuated zolpidem-induced LORR in normal mice. Administration of LPS significantly increased the duration of zolpidem-induced LORR. Bumetanide blocked the increased duration of zolpidem-induced LORR (LPS: $F[1,11] = 38.44$, $p < 0.01$; bumetanide: $F[1,11] = 16.07$, $p < 0.01$; LPS × bumetanide: $F[1,11] = 6.40$, $p < 0.05$; Fig. 5).

Effects of LPS on Nkcc1 and Kcc2 mRNA expressions in the hippocampus and frontal cortex. To clarify whether *Nkcc1* and *Kcc2* mRNA expression changed during inflammation, we examined their expression at three time points (2 h, 5 h, and 24 h after LPS injection). *Nkcc1* mRNA expression remained unchanged in LPS-induced inflammation (hippocam-

Table 1 Oligonucleotide sequences of the primer sets used for reverse transcriptase–polymerase chain reaction

(**A**) *Tnf* and *Il6*

Gene symbol	Accession number	Forward (5′ to 3′)	Reverse (5′ to 3′)	Amplicon (bp)	Position (5′ to 3′)
Tnf	NM_013693.3	aagcctgtagcccacgtcgta	ggcaccactagttggttgtctttg	122	435–556
Il6	NM_031168.2	ccacttcacaagtcggaggctta	gcaagtgcatcatcgttgttcatac	112	218–329
Gapdh	NM_001289726.1	aaggtcatcccagagctgaa	gcttcaccaccttcttgatg	136	741–876

(**B**) *Gabra1*, *Gabrb2*, and *Gabrg2*

Gene symbol	Accession number	Forward (5′ to 3′)	Reverse (5′ to 3′)	Probe (5′ to 3′)	Amplicon (bp)	Position (5′ to 3′)
Gabra1	NM_010250.5	tgagagctgaatgcccaatg	tctgctacaaccactgaacg	FAM/cctgcccac/ZEN/taaaattcggaagctatgc/IABkFQ	145	1,262–1,406
Gabrb2	NM_008070.5	gtgtgattctcaagggtctaat	tggattcccgagtctcttct	FAM/tgaaagtaa/ZEN/ggaggtgggacacagc/IABkFQ	98	2,413–2,510
Gabrg2	NM_177408.7	gctacttcaccatccagacttac	ggcaggaacagcatccttat	FAM/attccctgc/ZEN/acactcatcgtggtc/IABkFQ	92	1,342–1,433
Gapdh	NM_001289726.1	aaggtcatcccagagctgaa	gcttcaccaccttcttgatg	FAM/accaccaac/ZEN/tgcttagccccccctg/IABkFQ	136	741–876

(**C**) *Nkcc1* and *Kcc2*

Gene symbol	Accession number	Forward (5′ to 3′)	Reverse (5′ to 3′)	Probe (5′ to 3′)	Amplicon (bp)	Position (5′ to 3′)
Nkcc1	NM_009194.3	ctgccgagagtaaaggagttg	agagcatcacaccccaaatg	FAM/aacatgcag/ZEN/cggactaatacaccctt/IABkFQ	87	938–1,024
Kcc2	NM_001355480.1	atttcgctattaccactggactctct	cccggtactcgatgtacttataaatg	FAM/cctctgcct/ZEN/ggccctcatgttcat/IABkFQ	137	1,876–2,012
Gapdh	NM_001289726.1	aaggtcatcccagagctgaa	gcttcaccaccttcttgatg	FAM/accaccaac/ZEN/tgcttagccccccctg/IABkFQ	136	741–876

Tnf, tumor necrosis factor; *Il6*, interleukin 6; *Gabra1*, gamma-aminobutyric acid A receptor, subunit alpha 1; *Gabrb2*, gamma-aminobutyric acid A receptor, subunit beta 2; *Gabrg2*, gamma-aminobutyric acid A receptor, subunit gamma 2; *Nkcc1*, Na^+-K^+-$2Cl^-$ cotransporter isoform 1; *Kcc2*, K^+-Cl^- cotransporter isoform 2; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; bp, base pair.

Table 2 Incidence of zolpidem-induced loss of righting reflex in mice

Dose (mg/kg, i.p.)	Duration of righting reflex loss (minutes, mean ± SEM)	Number of animals with righting reflex loss (n = 8 per group)
50	31.8 ± 5.1	6
75	51.9 ± 2.6	8
100	88.6 ± 13.2	8

Values are expressed as the mean ± SEM.

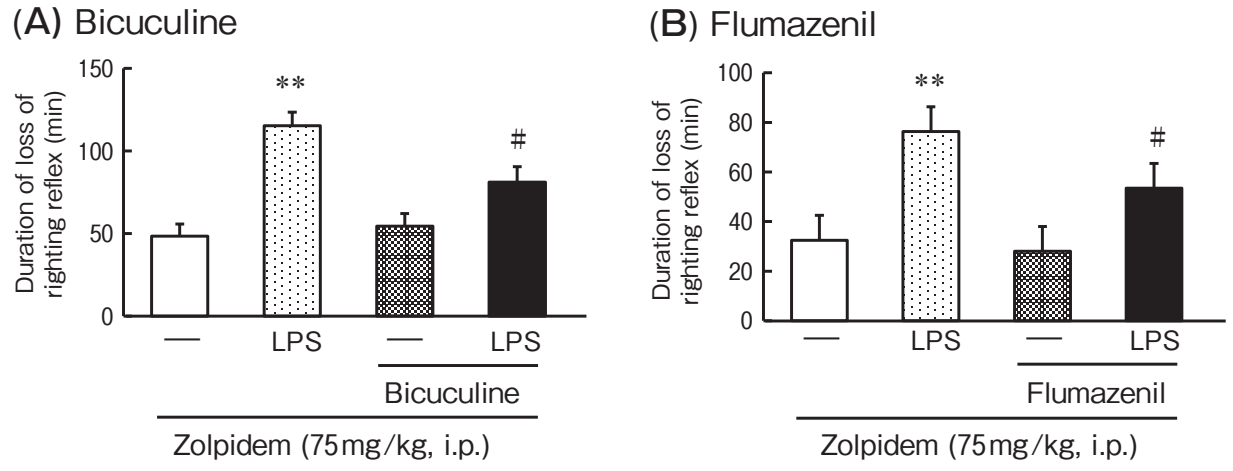

Fig. 3 Effects of bicuculline (**A**) and flumazenil (**B**) on the duration of the zolpidem-induced loss of righting reflex in LPS-treated mice. LPS (500 μg/kg, i.p.) was injected 1 day before the zolpidem-induced loss of righting reflex test. Bicuculline (3 mg/kg, i.p.) and flumazenil (10 mg/kg) were administered to LPS-treated mice 30 min before zolpidem administration (75 mg/kg, i.p.). Values are expressed as means ± SEMs (n = 6 for each group). Data were analyzed using a two-way analysis of variance, and group means were compared using Tukey's test for multiple comparisons. $^{**}p < 0.01$ significantly different from the control group, $^{\#}p < 0.05$ significantly different from the LPS group.

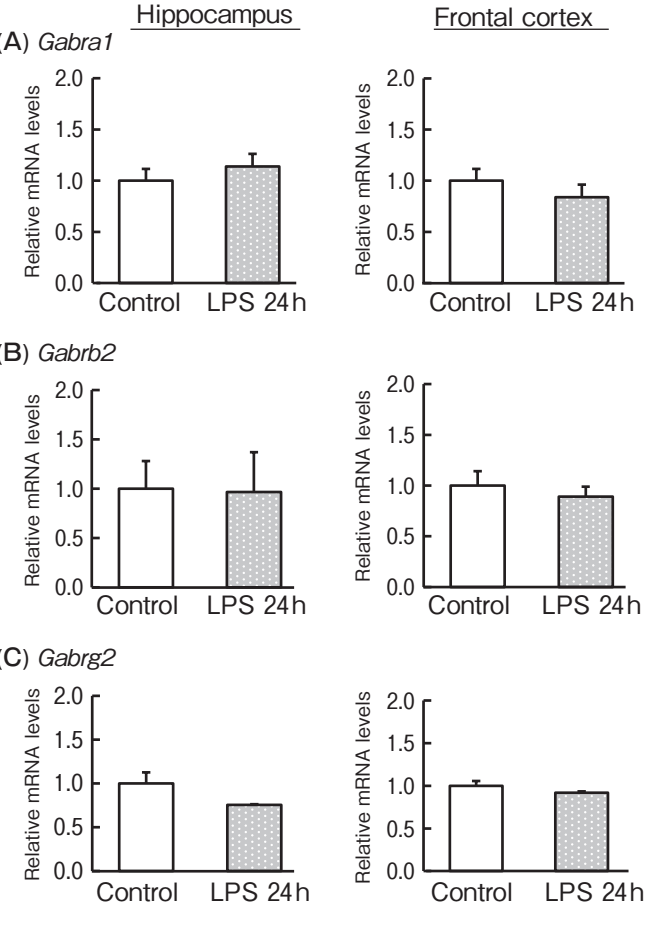

Fig. 4 Effect of LPS on the mRNA expression of *GABA$_A$ receptor subunits* (**A**, *Gabra1*; **B**, *Gabrb2*; **C**, *Gabrg2*) in the hippocampus and frontal cortex of mice. Mice were injected with LPS (500 μg/kg, i.p.) 1 day before decapitation. Values are expressed as means ± SEMs (n = 5-6 for each group). Data were analyzed using an unpaired Student's *t*-test.

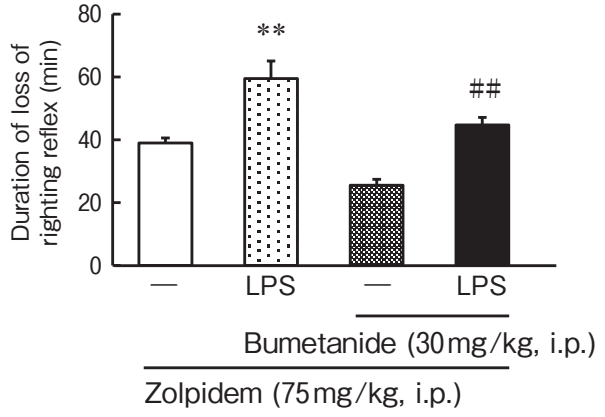

Fig. 5 Effects of bumetanide on the duration of zolpidem-induced loss of righting reflex in LPS-treated mice. LPS (500 μg/kg, i.p.) was injected 1 day before the zolpidem-induced loss of righting reflex test. Bumetanide (30 mg/kg, i.p.) was administered 30 min before LPS administration. Values are expressed as means ± SEMs (n = 6 for each group). Data were analyzed using a two-way analysis of variance, and group means were compared using Tukey's test for multiple comparisons. $^{**}p < 0.01$ significantly different from the control group, $^{\#\#}p < 0.01$ significantly different from the LPS group.

pus: $F[3,28] = 0.96$, $p = 0.43$; frontal cortex: $F[3,20] = 0.35$, $p = 0.79$; Fig. 6A) whereas *Kcc2* mRNA expression in the hippocampus and frontal cortex significantly decreased 2 h and 5 h after LPS administration, respectively (hippocampus: $F[3,20] = 2.89$, $p = 0.06$; frontal cortex: $F[3,20] = 3.50$, $p < 0.05$; Fig. 6B).

Discussion

We previosely reported that at 24 h after administration, LPS significantly increased the duration of pentobarbital-induced LORR treated with diazepam, brotizolam and muscimol compared with vehicle treatment [11]. This result suggests that inflammation enhances benzodiazepine receptor agonist activity through the $GABA_A$ receptor activity. In a mouse model of inflammation, we examined the duration of LORR induced by zolpidem, a widely used hypnotic non-benzodiazepine agent. LPS administration strengthened the effects of zolpidem by extending the duration of zolpidem-induced LORR in the mice. Zolpidem markedly increased the duration of LORR in inflammatory conditions; this increment is intriguing from the viewpoint of the mechanism of action. Furthermore, the stimulatory action of zolpidem was

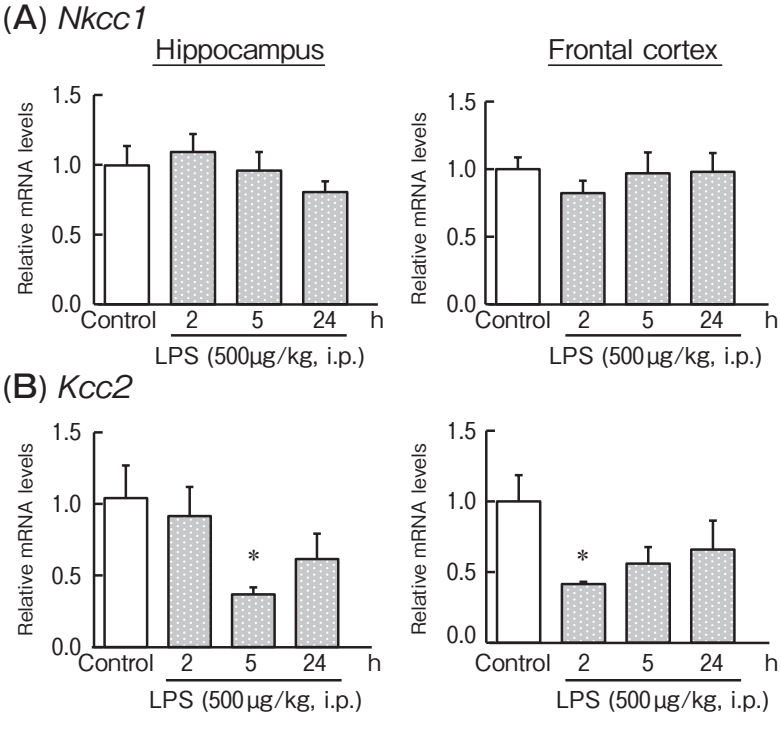

Fig. 6 Effects of LPS on *Nkcc1* (**A**) and *Kcc2* (**B**) mRNA expressions in the hippocampus and frontal cortex of mice. Hippocampal *Nkcc1* and *Kcc2* mRNA expressions were observed at 2 h, 5 h, and 24 h after LPS administration (500 μg/kg, i. p.). Values are expressed as the means ± SEMs (n = 6 for each group). Data were analyzed using a one-way analysis of variance, and group means were compared using Dunnett's test. $^{*}p < 0.05$, significantly different from the control group.

significantly decreased by bicuculline, a $GABA_A$ receptor antagonist, and flumazenil, a benzodiazepine receptor antagonist. Flumazenil, a benzodiazepine receptor antagonist, has been shown to reverse the hypnotic effects of zolpidem, a non-benzodiazepine agent by the competitive pharmacodynamic antagonism on the benzodiazepine site [19]. Based on such findings, it appears that the stimulatory effects of zolpidem are mediated by the benzodiazepine-binding site on the $GABA_A$ receptor. In contrast, LPS administration increased serum IL-6 and TNF-α concentrations and *Il6* and *Tnfa* mRNA expression in the hippocampus and frontal cortex at 2 h and 5 h after administration, respectively. In particular, inflammatory conditions, as evidenced by the presence of pro-inflammatory cytokines, may be altered by benzodiazepine activity through $GABA_A$ receptor function [20, 21]. The enhancing effect of $GABA_A$ receptors and benzodiazepine receptor function may be related to the initial increase in IL-6 and TNF-α levels following LPS administration in mice.

We also explored the potential correlation between alterations in *Gabra1*, *Gabrb2*, and *Gabrg2* expression and variations in LORR duration. The $GABA_A$ receptor comprises a five-subunit complex (two α subunits, two β subunits, and one γ subunit). $GABA_A$ receptors containing α1 subunits are associated with sleep, antiepileptic effects, and sedation. Benzodiazepines bind to the benzodiazepine receptor located on the $GABA_A$ receptor between the α and γ subunits [22]. We examined the effects of LPS on the $GABA_A$ receptor subunits (*Gabra1*, *Gabrb2*, and *Gabrg2*). The expression of *Gabra1*, *Gabrb2*, and *Gabrg2* did not change after LPS administration. Therefore, it is unlikely that the mechanism underlying the prolongation of LORR by LPS is related to changes in the expression of *Gabra1*, *Gabrb2*, and *Gabrg2*. Some agents directly activate the $GABA_A$ receptor, whereas others influence the chloride ion (Cl^-) channel opening directly. The role ofintracellular Cl^- concentration is regulated in postsynaptic neuron responses caused by the activation of $GABA_A$ receptors [23]. However, because an experiment for directly measuring Cl^- could not be performed, we focused on NKCC1 and KCC2 transporters. These transporters modify the inhibitory effect of GABAergic function by changing the intracellular chloride concentration [24, 25]. First, we focused on NKCC1, which moves Cl^- into cells. Bumetanide was used as an NKCC1 blocker [26, 27]. Bumetanide significantly inhibited the LPS-induced increase in the duration of zolpidem-induced LORR by LPS administration. This may be attributed to the increasing effect of zolpidem-induced LORR or an increase in intracellular Cl^- concentration through NKCC1 mediated by LPS administration.

Our study focused on the hippocampus and frontal cortex, which have many α1 (ω1) subunits and are affected by zolpidem, investigating the effects of LPS on the mRNA of *Nkcc1* and *Kcc2* in the hippocampus and frontal cortex of mice. *Nkcc1* mRNA expression was predicted to increase owing to LPS administration. However, LPS administration did not alter *Nkcc1* mRNA expression in the hippocampus or frontal cortex. In contrast, the mRNA levels of *Kcc2* decreased after LPS administration (at both 2 and 5 h). In a previous study, LPS reduced KCC2 expression in treated primary cultured rat cells, and IL-1β was important for maintaining KCC2 expression [28]. Thus, we hypothesized that KCC2 downregulation may be related to changes in LPS-induced pro-inflammatory cytokines.

Furthermore, the stimulatory effect of zolpidem-induced LORR may be attributed to the failure to regulate the function of the intracellular Cl^- efflux by LPS administration. In other words, inflammatory conditions increased the inhibitory effects of the $GABA_A$ receptor, resulting in a high intracellular Cl^- concentration through a reduction in the movement of Cl^- out of the cells owing to decreased KCC2 expression. Therefore, higher intracellular Cl^- concentrations may be at least in part responsible for the enhancement of zolpidem-induced LORR. However, this study has some limitations. First, we did not investigate the effects of LPS administration on intraneuronal Cl^- levels. Further studies are required to assess these effects.

Clinically, the inflammatory conditions investigated in this study may represent post-operative conditions in which benzodiazepine receptor agonists are sometimes used. It is well known that the incidence of post-operative delirium is significantly higher in patients treated with benzodiazepine receptor agonists [2-5]. We think that these effects may be closely related to the relationship between inflammation and GABA activity. This study indicated that the stimulatory effect of zolpidem may contribute to KCC2 dysfunction in LPS-treated mice. Consistent with previous findings, our results provide insights into the pharmacological effects of benzodiazepines and non-benzodiazepines, including

oversedation, decreased responsiveness, and drowsiness under inflammatory conditions. Notably, caution should be exercised when using benzodiazepines in cases of inflammation, because inflammation was found to disturb the equilibrium of Cl^- in $GABA_A$ receptors in this study. However, the detailed mechanisms of Cl^- transporters and $GABA_A$ receptor function during inflammation require further investigation.

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