Quinolinic Acid Decreases Mental Activity in Mice by Damaging Brain Neurons through the Activation of N-methyl-D-aspartate Receptors: Proposed Relationship to Human Mental Fatigue

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It is generally known that many psychological factors, such as stress or insomnia, cause mental fatigue. However, the detailed mechanisms underlying mental fatigue have not been clarified. We speculated that mental fatigue may be caused by neuronal brain damage through the activation of N-methyl-D-aspartate (NMDA) receptors by quinolinic acid (QUIN), one of the metabolites of tryptophan in the kynurenine pathway. In the present study, we tested this hypothesis in mice using a home cage equipped with a running wheel; voluntary wheel-running reflected mental activity. In normal mice, wheel-running activity was not affected by intraperitoneal administration of QUIN, but it was significantly decreased by intracerebroventricular administration of QUIN. In restraint stress-loaded mice, whose blood-brain barriers were weakened, wheel-running activity was significantly decreased by intraperitoneal administration of QUIN, and this effect was inhibited by memantine hydrochloride (MEM), an NMDA receptor antagonist. Intraperitoneal administration of lipopolysaccharide (LPS) induced a decrease in wheel-running activity and a concomitant increase in blood and brain QUIN levels. MEM inhibited the LPS-induced decrease in wheel-running activity, but it did not affect the increase in blood and brain QUIN levels. The number of hippocampal neurons was significantly decreased by LPS treatment, and this effect was inhibited by MEM. These results suggest that QUIN, which is produced via tryptophan metabolism, decreases mental activity in mice by damaging brain neurons through the activation of NMDA receptors. This finding supports, at least in part, our hypothesis that the same mechanism contributes to causing human mental fatigue.

Key words — quinolinic acid, mental fatigue, neuronal damage, N-methyl-D-aspartate receptor, tumor necrosis factor-α, memantine

INTRODUCTION

In recent years, the metabolites of the essential amino acid tryptophan (TRP) have become of interest because they may play a role in causing several diseases. TRP is metabolized in the serotonin pathway and in the kynurenine pathway. In humans, more than 90% of the TRP ingested during a meal is thought to be metabolized in the kynurenine pathway. In this pathway, TRP is finally metabolized into NAD via intermediates that include kynurenine and quinolinic acid (QUIN). Among these TRP metabolites, QUIN acts as an agonist of the N-methyl-D-aspartate (NMDA) receptor, an excitatory amino acid receptor in the brain. Therefore, the increase in QUIN activates NMDA receptors excessively and causes neuronal damage.2–4) Indeed, it has been suggested that increases in brain QUIN participate in causing several neurodegenerative diseases.5,6) In the kynurenine pathway, TRP is metabolized first by two enzymes, tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). TDO is a “housekeeping” enzyme that is constitutively active in the liver. Therefore, TRP is usually metabolized in the liver by TDO.7) However, under specific conditions such as infection, inflammation or immune activation, IDO activity is induced in extrahepatic organs such as the brain, lung, in-
Fatigue is classified roughly into physical and mental fatigue. It is generally accepted that physical fatigue mainly arises from a disorder of energy metabolism in muscle, which is not limited to lactic acid accumulation, caused by long-duration or intensive exercise. However, the detailed mechanisms underlying mental fatigue have not been clarified, although various psychological factors such as stress or insomnia participate in it. It is well known that fatigue often develops in patients with the above-mentioned diseases. IFNs, which are used for the treatment of chronic hepatitis C and some cancers, are known to cause fatigue as a side effect. In addition, LPS causes a so-called “sickness behavior” that is characterized by a fatigue-like decrease in locomotor activity in mice in which increases in IDO and TNF-α are implicated. Therefore, we speculated that fatigue in these diseases or treatments is caused by damage to brain neurons through the activation of NMDA receptors by QUIN, which is increased through the induction of IDO by TNF-α and/or IFNs. Moreover, it is known that TNF-α is also produced in the blood and the brain during many kinds of stress. Taken together, we hypothesized that the increase in brain QUIN and subsequent neuronal damage through the activation of NMDA receptors participates not only in the causes of fatigue during the above-mentioned diseases or treatments but also in those of mental fatigue.

Animal models of mental fatigue have been made so far by the methods assumed the condition that fatigue is caused in humans. For example, rats are housed in a cage filled with water to a height of about 1.5 cm for several days to induce fatigue by sleep-disturbance. Or, rats are given polyriboinosinic : polyribocytidyllic acid (poly I : C), a synthetic double-stranded RNA, to induce fatigue assumed that by viral infection. However, the relationship between fatigue and neuronal brain damage has not been examined in these fatigue models. Therefore, one aim of the present study is to clarify such relationship by suitable methods.

As for the method to evaluate the extent of fatigue, weight-loaded forced swimming test is used in the fatigue models mentioned above. However, this method is not thought to be suitable for the evaluation of mental activity, because the swimming time in this test is largely dependent on physical endurance. Wheel-running activity is also used to evaluate the extent of fatigue. It is generally accepted that wheel-running in mice is an index of their locomotor activity because mice naturally willingly rotate the running wheel when they are placed in it. However, if the running wheel is used alone, wheel-running will almost entirely reflect physical performance or endurance because mice have difficulty resting in the wheel. When the home cage is equipped with a running wheel, mice can freely move between the wheel and the adjacent home cage, where the mice can take a rest when they are tired. Moreover, the mice are permitted not only to run in the wheel at their pleasure but also to stay in the home cage when they do not feel like running, even if they are physically tough. Therefore, it is supposed that voluntary wheel-running reflects mental activity if the home cage equipped with a running wheel is used. In the present study, we used this apparatus to test the above-mentioned hypothesis by evaluating the mental activity of QUIN- or LPS-treated mice.

MATERIALS AND METHODS

Animals —— Male ddY mice were purchased at 5 weeks old from Japan SLC Inc. (Hamamatsu, Japan). The animals were used in the experiments after 1 week of acclimation and were housed in an air-conditioned room (23 ± 2°C, humidity: 55 ± 10%) under a 12 hr dark/12 hr light cycle (lights on from 8:00 until 20:00) with free access to water and food. This study was approved by the Animal Experiment Committee of Kampo Research Laboratories, Kracie Pharma, Ltd., (Takaoka, Japan) in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

Drugs —— QUIN (Wako Pure Chemicals, Osaka, Japan), memantine hydrochloride (MEM; Sigma-Aldrich, St. Louis, MO, U.S.A.) and LPS (Escherichia coli (E. coli) O26:B6; Difco Laboratories, Detroit, MI, U.S.A.) were dissolved in pyrogen-free saline (Otsuka Pharmaceutical Fac-
Fig. 1. The Effect of QUIN on Wheel-Running Activity in Normal Mice

QUIN was administered intraperitoneally (i.p.; 100 mg/kg) or intracerebroventricularly (i.c.v.; 20 and 40 nmol/mouse) to the mice. One hour (i.p.) or 30 min (i.c.v.) after the administration, the mice were placed individually in the home cage equipped with a running wheel (shown in the photograph), and the wheel-running count was measured for 15 hr. The results are expressed as the mean ± S.E. of 5 (i.p.) or 9 (i.c.v.) animals. *p < 0.05, significantly different from the control (Dunnett test).

Experiments on Normal Mice —— In the intraperitoneal administration experiment, QUIN (100 mg/kg) or saline (control group) was administered intraperitoneally to the mice. One hour after the administration, the mice were placed individually in the home cage equipped with a running wheel (Fig. 1; wheel diameter, 20 cm; cage size, 22 × 9 × 8 cm; TK-48B; Toyo-riko Co., Ltd., Tokyo, Japan). Subsequently, the wheel-running count indicated by the counter, with 1 count corresponding to 1 revolution, was measured for 15 hr (18:00 until 9:00) under conditions that allowed the mice to take food and water and to rest freely. In the intracerebroventricular administration experiment, the mice were anesthetized with ether, and the scalp was incised. Subsequently, QUIN (20 and 40 nmol/10 µl per mouse) or saline (control group) was administered intracerebroventricularly using a Hamilton 710LT-type syringe attached to a needle with a stopper (needle size: 27 G, 3.0 mm length; TOP Corporation, Tokyo, Japan) at the coordinates of −0.2 mm caudal, +1.0 mm lateral to bregma and 3.0 mm depth from the skull surface. Thirty minutes after the administration, the wheel-running count was measured for 15 hr (18:00 until 9:00) using the above method.

Experiments on Stress-Loaded Mice —— Mice were individually subjected to restraint stress by placing them in a 50-ml polypropylene conical tube with multiple punctures for ventilation. After 1 hr of restraint, QUIN (10, 50 and 100 mg/kg) or saline (control group) was administered intraperitoneally to the mice, and then mice were restrained again for 1 hr. Subsequently, the mice were placed individually in the home cage equipped with a running wheel, and the wheel-running count was measured for 15 hr (18:00 until 9:00) using the method described above. At the end of the wheel-running measurement, the mice were sacrificed by exsanguination under ether anesthesia, and the brain was removed. The brain was immediately used for the measurement of QUIN as described below. MEM (30 mg/kg) was administered intraperitoneally just before the first restraint.

Experiments on LPS-Treated Mice

Effects of LPS on Wheel-running Activity and Blood and Brain QUIN Levels: LPS (0.2, 0.6 and 2.0 mg/kg) or saline (control group) was administered intraperitoneally to the mice. Six hours after the administration, the mice were placed individually in the home cage equipped with a running wheel, and the wheel-running count was measured for 15 hr (18:00 until 9:00) using the method described above. Three hours after the end of the wheel-running measurement, blood was collected from the carotid artery under ether anesthesia, and then mice were sacrificed by exsanguination. Subsequently, the brain was removed, and QUIN levels
of serum and brain were immediately measured using the method described below. MEM (30 mg/kg) was administered intraperitoneally twice: 30 min before and 6 hr after the LPS treatment, with the second administration occurring just before the wheel-running measurement.

Effects of LPS on Blood TNF-α Levels: LPS (0.6 mg/kg) or saline (control group) was administered intraperitoneally to the mice. Blood was collected from the carotid artery under ether anesthesia, and then the serum TNF-α level was measured using the Mouse TNF-α ELISA Kit (Thermo Fisher Scientific Inc., Rockford, IL, U.S.A.). The serum TNF-α level was measured 2 hr after LPS treatment because of its rapid elevation in the blood after LPS treatment. MEM (30 mg/kg) was administered intraperitoneally 30 min before LPS treatment.

Effects of LPS on Hippocampal Neurons: LPS (0.6 mg/kg) or saline (control group) was administered intraperitoneally to the mice. Three days after the administration, the mice were anesthetized with ether, and then the brain was perfused with 30 ml of 0.1 M phosphate buffer (pH 7.0) followed by 30 ml of 4% paraformaldehyde solution from the left ventricle of the heart. Subsequently, the brain was removed and fixed by immersing it in 4% paraformaldehyde solution for over 24 hr, and then it was embedded in paraffin. The paraffin block was sliced into 3-µm thick sections, and the slices were treated with cresyl violet (Nissl) staining. Using these histological preparations, the number of pyramidal neurons with a clear cell body in a 50×100 µm area of the CA1 and CA3 regions of the hippocampus was measured microscopically, and then the cell number per 1 mm³ was calculated. MEM (30 mg/kg) was administered intraperitoneally twice: 30 min before and 6 hr after LPS treatment, with the second administration occurring just before wheel-running measurement.

Measurement of Blood and Brain QUIN Levels: The cerebral cortex was immediately dissected from the removed brain on an ice-cold dish, and 10 mg was weighed out. Twenty-five volumes (w/v) of pure water were added to it, and the mixture was suspended by sonication. Subsequently, QUIN concentrations in this sample were measured according to the method of Yu et al.24 using GC/MS (GC/MS-QP2010; Shimadzu Corporation, Kyoto, Japan), and the brain QUIN level was calculated from the resulting value. In the blood sample, serum QUIN concentration was measured using the same method after the serum was appropriately diluted with pure water.

Statistical Analysis: The results were expressed as the mean ± S.E. Statistical differences were assessed using the Dunnett test (comparison with the control group only) or the Tukey-Kramer test (comparison between each pair of groups). StatView Version 5.0 software (SAS Institute Japan Ltd., Tokyo, Japan) was used for the analysis, and the difference was judged significant when the p value was less than 0.05 (p < 0.05).

RESULTS

Experiments on Normal Mice

In the normal mice, there were no differences between the wheel-running count of the group administered QUIN at 100 mg/kg intraperitoneally and that of the control group. However, the wheel-running count of the group administered QUIN intracerebroventricularly at 40 nmol/mouse was significantly decreased by about 50% compared to the control group (p < 0.05). The count of the group administered QUIN at 20 nmol/mouse did not differ from that of the control group (Fig. 1).

Experiments on Stress-Loaded Mice

In the restraint stress-loaded mice, the wheel-running counts of the groups administered QUIN at 10, 50 and 100 mg/kg intraperitoneally were lower than that of the control group. In contrast, brain QUIN levels of the groups administered QUIN were higher than that of the control group. Both changes were dependent on the dose of QUIN and were significantly different from the control group at 100 mg/kg (Fig. 2).

Then, the effect of MEM on the decrease in wheel-running activity induced by intraperitoneal administration of QUIN at 100 mg/kg was examined. MEM significantly inhibited the QUIN-induced decrease in wheel-running activity (p < 0.01), although it did not affect wheel-running activity by itself (Fig. 3).

Experiments on LPS-Treated Mice

The wheel-running counts of the mice administered LPS intraperitoneally at 0.2, 0.6 and 2.0 mg/kg were significantly lower than that of the control mice; the size of the effect was dependent on the dose of LPS. In contrast, serum and brain QUIN
The Effect of QUIN on Wheel-Running Activity and Brain QUIN Level in Restraint Stress-Loaded Mice

Mice were restrained individually by placing them in a 50-ml polypropylene conical tube. After 1 hr of restraint, QUIN (10, 50 and 100 mg/kg) was administered intraperitoneally to the mice, and then the mice were restrained again for 1 hr. Subsequently, the mice were placed individually in the home cage equipped with a running wheel, and the wheel-running count was measured for 15 hr. At the end of the wheel-running measurement, the mice were sacrificed, and the brain was removed. QUIN levels in the brain (cerebral cortex) were measured using GC/MS. The results are expressed as the mean ± S.E. of 6 (wheel-running) or 6 (brain QUIN) animals. *p < 0.05, **p < 0.01, significantly different from the control (Dunnett test).

The Effect of MEM on the Wheel-Running Activity in Restraint Stress-Loaded Mice Treated with QUIN

Mice were restrained individually by placing them in a 50-ml polypropylene conical tube. After 1 hr of restraint, QUIN (100 mg/kg) was administered intraperitoneally to the mice, and then mice were restrained again for 1 hr. Subsequently, the mice were placed individually in the home cage equipped with a running wheel, and the wheel-running count was measured for 15 hr. MEM (30 mg/kg) was administered intraperitoneally just before the first restraint. The results are expressed as the mean ± S.E. of 7 to 8 animals. *p < 0.05, **p < 0.01, significantly different (Tukey-Kramer test).

DISCUSSION

In the present study, we measured the wheel-running activity of mice under several conditions using the home cage equipped with a running wheel. In the experiments on normal mice, no differences...
The Effect of LPS on Wheel-Running Activity and Blood and Brain QUIN Levels in Mice

LPS (0.2, 0.6 and 2.0 mg/kg) was administered intraperitoneally to the mice. Six hours after the administration, the mice were placed individually in the home cage equipped with a running wheel, and the wheel-running count was measured for 15 hr. Three hours after the end of the wheel-running measurement, blood was collected, and the brain was removed. QUIN levels in the serum and brain (cerebral cortex) were measured using GC/MS. The results are expressed as the mean ± S.E. of 7 to 8 animals. *p < 0.05, **p < 0.01, significantly different from the control (Dunnett test).

The Effect of MEM on the Wheel-Running Activity and Blood and Brain QUIN Levels in LPS-Treated Mice

LPS (0.6 mg/kg) was administered intraperitoneally to the mice. Six hours after the administration, the mice were placed individually in the home cage equipped with a running wheel, and the wheel-running count was measured for 15 hr. Three hours after the end of the wheel-running measurement, blood was collected, and the brain was removed. QUIN levels in the serum and brain (cerebral cortex) were measured using GC/MS. MEM (30 mg/kg) was administered intraperitoneally twice: 30 min before and 6 hr after LPS treatment. The results are expressed as the mean ± S.E. of 8 animals. *p < 0.05, **p < 0.01, significantly different (Tukey-Kramer test).

in wheel-running activity were observed between the mice administered QUIN intraperitoneally and the control mice. It is thought that the reason for this lack of effect is that QUIN does not enter the brain because it can hardly pass through the blood-brain barrier (BBB).\textsuperscript{25,26} However, the wheel-running activity of the mice administered QUIN (40 nmol/mouse) intracerebroventricularly was significantly lower than that of the control mice. These results suggest that QUIN decreases wheel-running activity through its direct action in the brain.

In the restraint stress-loaded mice, the wheel-running activity of the mice administered QUIN intraperitoneally was lower than that of the control mice, and this effect was dependent on the dose of QUIN. In contrast, the levels of QUIN in the brains of QUIN-treated mice were higher than that of the control mice. As mentioned above, QUIN can hardly enter the brain through the intact BBB when it is administered systemically. However, it is known that QUIN enters the brain in BBB-undeveloped juvenile rats\textsuperscript{27} or in BBB-disrupted
adult rats,\textsuperscript{28} even if QUIN is administered systemically. In addition, stress reportedly increases the permeability of the BBB.\textsuperscript{29,30} Therefore, in stress-loaded mice, it is thought that the decrease in wheel-running activity is caused by QUIN that enters the brain through the weakened BBB after it is administered intraperitoneally. Indeed, in the present study, extravasation of Evans blue into the brain was observed in the restraint stress-loaded mice when Evans blue was administered intravenously just before the restraint (data not shown). MEM, a representative NMDA receptor antagonist, significantly inhibited the QUIN-induced decrease in wheel-running activity in stress-loaded mice, although it did not affect wheel-running activity by itself. These results suggest that QUIN decreases wheel-running activity by activating NMDA receptors in the brain.

The wheel-running activity of the mice administered LPS was significantly lower than that of the control mice, and this effect was dependent on the dose of LPS. In contrast, the serum and brain QUIN levels of LPS-treated mice were higher than those of the control mice. These results are consistent with the results of Heyes et al.,\textsuperscript{31,32} who reported that LPS increased brain QUIN levels in mice. It is well known that LPS enhances the production of cytokines that induce IDO, such as TNF-\(\alpha\) and IFN-\(\gamma\).\textsuperscript{11,12} Among them, TNF-\(\alpha\) is more likely to be responsible for LPS-induced induction of IDO than IFN-\(\gamma\).\textsuperscript{11} In addition, it has been reported that IFN-\(\gamma\) enhances LPS-induced production of TNF-\(\alpha\) in rat brain.\textsuperscript{33} In the present study, the serum TNF-\(\alpha\) level was markedly increased by LPS treatment. Therefore, it is hypothesized that QUIN is increased after LPS treatment through induction of IDO that is mainly mediated by TNF-\(\alpha\). Such IDO induction and subsequent QUIN production predominantly occur in peripheral extrahepatic organs,\textsuperscript{8,9} but TNF-\(\alpha\) is also known to weaken the BBB.\textsuperscript{34,35} Taken together, these facts suggest

**Fig. 6.** The Effect of MEM on the Blood TNF-\(\alpha\) Levels in LPS-Treated Mice

LPS (0.6 mg/kg) was administered intraperitoneally to the mice. Two hours after the administration, blood was collected, and the serum TNF-\(\alpha\) level was measured using the Mouse TNF-\(\alpha\) ELISA Kit. MEM (30 mg/kg) was administered intraperitoneally 30 min before LPS treatment. The results are expressed as the mean \(\pm\) S.E. of 8 animals. \(^{\ast\ast}p < 0.01,\) significantly different (Tukey-Kramer test).

**Fig. 7.** The Effect of MEM on the Number of Hippocampal Neurons in LPS-Treated Mice

LPS (0.6 mg/kg) was administered intraperitoneally to the mice. Three days after the administration, the mice were sacrificed, and the brain was removed. After the fixation of brain with paraformaldehyde, the brain was embedded in paraffin. The paraffin block was sliced into 3 \(\mu\)m-thick sections, and the slices were treated with cresyl violet (Nissl) staining. Using these histological preparations, the number of pyramidal neurons with a clear cell body in the CA1 and CA3 regions of the hippocampus was measured microscopically, and then the cell number per 1 mm\(^3\) was calculated. MEM (30 mg/kg) was administered intraperitoneally twice: 30 min before and 6 hr after LPS treatment. The results are expressed as the mean \(\pm\) S.E. of 7 to 8 animals. \(^{\ast}p < 0.05,\) \(^{\ast\ast}p < 0.01,\) significantly different (Tukey-Kramer test).
LPS (0.6 mg/kg) was administered intraperitoneally to the mice. Three days after the administration, the mice were sacrificed, and the brain was removed. After the fixation of brain with paraformaldehyde, the brain was embedded in paraffin. The paraffin block was sliced into 3 µm-thick sections, and the slices were treated with cresyl violet (Nissl) staining. MEM (30 mg/kg) was administered intraperitoneally twice: 30 min before and 6 hr after LPS treatment. The pyramidal layers of the CA1 and CA3 regions of the hippocampus were observed microscopically. As shown with arrows in a photograph, neuronal cell loss and dispersion of the pyramidal layer are observed slightly but extensively in both regions of LPS-treated mice. However, such changes are apparently inhibited by MEM treatment. Scale bar: 100 µm.

that QUIN is produced in peripheral organs and increased in the blood of LPS-treated mice, and then it enters the brain through the weakened BBB.

The brain QUIN levels of LPS-treated mice whose wheel-running activity decreased were much lower than the amount of QUIN required to induce the decrease in wheel-running activity in normal mice by intracerebroventricular injection. It is thought that the reason for this inconsistency is that some factors which potentiate QUIN actions arise in the brain besides the increase in QUIN after LPS treatment. For instance, LPS reportedly causes neuroinflammation in the brain, in which proinflammatory cytokines such as TNF-α, interleukin (IL)-1β and IL-6 are implicated. Therefore, it is thought that the amount of QUIN required to decrease wheel-running activity is smaller in LPS-treated mice than in mice injected QUIN directly into the brain, because brain neurons become vulnerable due to such neuroinflammation after LPS treatment.

MEM significantly inhibited the decrease in wheel-running activity induced by LPS. Therefore, it is thought that wheel-running activity is decreased through the activation of NMDA receptors by QUIN that enters the brain in LPS-treated mice. The inhibitory effect of MEM on the LPS-induced decrease in wheel-running activity is exclusively due to the prevention of the activation of NMDA receptors by QUIN; it is not due to the inhibition of QUIN and TNF-α production because MEM did not affect the LPS-induced increase in serum and brain QUIN levels or the serum TNF-α level.

In the present study, the neurons of hippocampus were observed microscopically after LPS treatment because NMDA receptors are abundant in this region. Moreover, it is suggested that hippocampal NMDA receptors are closely related to the causes of various psychiatric disorders such as anxiety and depression as well as the functions of learning and memory. The number of pyramidal neurons in the hippocampal CA1 and CA3 regions of LPS-treated mice was slightly but significantly lower than that of the control mice 3 days after administration, suggesting that wheel-running activity is decreased by the damage to brain neurons after LPS treatment. However, no differences were observed between the number of hippocampal neurons (× 10^5 cells/mm^3) in the control group (CA1: 23.3 ± 0.5; CA3: 26.9 ± 0.6) and those in the LPS-treated group (CA1: 22.2 ± 0.4; CA3: 25.3 ± 0.7) 24 hr after LPS treatment when the decrease in wheel-running activity and the increases in blood and brain QUIN levels were observed. Thus, the
hippocampal neuronal damage induced by LPS occurred after both the behavioral and the biochemical changes. The causes of these phenomena are unclear. It is reported that transient cerebral ischemia causes neuronal damage in the CA1 region of hippocampus of the mouse or the rat after 48 hr of the end of ischemia.\textsuperscript{40,41} NMDA receptor antagonists prevent such delayed neuronal damage after ischemia, but this effect is observed only when they are administered immediately after or 15 min after the end of ischemia.\textsuperscript{40,41} Therefore, in the case of cerebral ischemia, it is supposed that some functional disorders involving the activation of NMDA receptors (e.g., excessive calcium entry into neurons) occur in neurons early after ischemia, and morphological damage of neurons develops late afterwards.\textsuperscript{40,42} In the present study, both the increase in brain QUIN levels and the decrease in wheel-running activity were observed 24 hr after LPS treatment (Fig. 5), whereas the decrease in hippocampal neurons was observed 3 days after LPS treatment (Figs. 7 and 8). However, not only the decrease in wheel-running activity but also the hippocampal neuronal damage was inhibited when MEM was administered 30 min before and 6 hr after LPS treatment. These results suggest that NMDA receptors are first activated by QUIN and early functional disorders of neurons are induced thereby, and morphological damage of neurons develops laterly alike the case of cerebral ischemia mentioned above. On the other hand, it is thought that MEM inhibits the decrease in wheel-running activity by protecting neurons from the functional disorders induced by QUIN early after LPS treatment. The hypothesis that MEM protects neurons via its inhibitory effect on QUIN-induced activation of NMDA receptors is supported by the results of Keilhoff and Wolf\textsuperscript{43} and Misztal \textit{et al.}\textsuperscript{44} which showed protective effects of MEM against QUIN-induced neuronal damage.

In conclusion, the present study at least partially supports our hypothesis that the increase in brain QUIN and subsequent neuronal damage participates in the causes of mental fatigue. However, the significance of the morphological changes in the neurons in the causes of mental fatigue must be further investigated using chronic models. There are some reports to date that serotonin, a metabolite of TRP in the serotonin pathway, participates in the causes of central or mental fatigue,\textsuperscript{45,46} but this is the new report that QUIN, a metabolite of TRP in another pathway, participates in the causes of mental fatigue. The present results also suggest that NMDA receptor antagonists may become therapeutic drugs for mental fatigue, although more detailed studies are required in the future to confirm such efficacy of these drugs. The decrease in wheel-running activity in the present study reflects a decrease in mental activity but not a decrease in physical endurance because a stay in the home cage was prolonged in the mice whose wheel-running activity was decreased. However, there are no specific data so far to show that such staying in the home cage originates from mental fatigue. Therefore, in the future, other methods besides those using the home cage equipped with a running wheel are required to evaluate mental activity in mice more exactly.

\section*{REFERENCES}