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Involvement of NMDA receptor in the regulation of plasma interleukin-6 levels in mice

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Abstract

MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cyclopepten-5,10-imine maleate), a non-competitive NMDA receptor antagonist (0.01–1 μg), injected intracerebroventricularly (i.c.v.) dose dependently increased the baseline levels of plasma interleukin-6 in mice. In the 1-h immobilization-stressed animals, MK-801 (1 μg) administered i.c.v. produced an additive increase of plasma interleukin-6. NMDA (*N*-methyl-D-aspartate) (3, 10 ng) administered i.c.v. attenuated dose dependently the 1-h immobilization stress-induced rise in plasma interleukin-6 level. Neither 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) (0.01–0.5 μg) nor α-methyl-4-carboxyphenylglycine (MCPG) (1–20 μg), antagonists of non-NMDA and metabotropic glutamate receptors, respectively, i.c.v. administered, affected the basal and stress-induced plasma interleukin-6 levels. These data indicate that NMDA receptors may be involved in the suppressive regulation of the plasma interleukin-6 levels.

Keywords: Interleukin-6, plasma; MK-801; NMDA (*N*-methyl-D-aspartate); Immobilization stress; (Mouse)

1. Introduction

Interleukin-6 is a multifunctional cytokine involved in the regulation of immune responses, hematopoiesis and acute-phase reactions (for review, see Hirano, 1994). Recently, it has been observed that several experimental stressors, such as open field, foot shock, immobilization and hemorrhage, induce an increase of plasma interleukin-6 level in rats (LeMay et al., 1990; Zhou et al., 1993; Komaki et al., 1994; Takaki et al., 1994). Although the central catecholaminergic system was suggested to play critical roles in the plasma interleukin-6 elevation induced by immobilization stress (Takaki et al., 1994), the involvement of other central neurotransmitter systems in the regulation of plasma interleukin-6 levels is obscure.

Glutamate is a major excitatory neurotransmitter in the brain, acting through the receptors generally divided into NMDA, non-NMDA, and metabotropic subtypes. There is considerable evidence that glutamatergic systems are activated in response to stressful stimuli. Immobilization stress increases glutamate uptake and release in limbic forebrain regions (Gilad et al., 1990; Moghaddam, 1993). NMDA receptor antagonists block the immobilization stress-induced increases in hippocampal glucose utilization (Schasfoort et al., 1988), and in the dopamine metabolism of the medial prefrontal cortex (Morrow et al., 1993). Additionally, immobilization stress induces changes in NMDA and non-NMDA receptor levels in the hippocampus and hypothalamus (Tocco et al., 1991; Yoneda et al., 1994; Bartanusz et al., 1995). However, the possible involvement of the glutamatergic system in the stress-induced elevation of plasma interleukin-6 levels has not been investigated. Thus, the effects of intracerebroventricular (i.c.v.) injection of (+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cyclopepten-5,10-imine maleate (MK-801), 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), and α-methyl-4-carboxyphenylglycine (MCPG), antagonists of NMDA, non-NMDA, and metabotropic receptors, respectively, on the basal and immobilization stress-induced plasma interleukin-6 levels were examined in the present study.

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2. Materials and methods

2.1. Animals

Male ICR mice weighing 25–30 g were used for all the experiments. The animals were housed 5 per cage in a room maintained at 22 ± 1°C with an alternating 12-h light-dark cycle. Food and water were available ad libitum.

2.2. Drugs

(+)-MK-801 hydrogen maleate, *N*-methyl-D-aspartic acid (NMDA), 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), and (±)-α-methyl-4-carboxyphenylglycine (MCPG) were purchased from Research Biomedicals International (Natick, MA, USA).

2.3. Intracerebroventricular (i.c.v.) injection

The i.c.v. administration was performed following the method described by Laursen and Belknap (1986). Briefly, each mouse in conscious state was injected at bregma with a 50 μl Hamilton syringe (No. 705) fitted with 26-gauge needle of which the tip was adjusted to be inserted 2.4 mm deep. The i.c.v. injection volume was 5 μl and injection sites were verified by injecting the same volume of 1% methylene blue and then observing the distribution of the injected drugs or dye in the ventricular space. The dye injected i.c.v. was found to be distributed in the ventricular spaces and ventral surface of the brain and in the upper cervical portion of the spinal cord.

2.4. Immobilization stress and plasma interleukin-6 assay

The stress procedure consisted of restraint of each animal for 1 h in a 50-ml Corning tube, with the nose of the mouse at the tip of the tube. Adequate ventilation was provided by means of a hole at the tip of the tube. Blood was collected by puncture of the retro-orbital venous plexus. Plasma was separated by centrifugation of the freshly drawn blood and stored at −80°C until assayed. The plasma interleukin-6 level was determined with an enzyme-linked immunosorbent assay (ELISA) kit (Genzyme, Cambridge, MA, USA). Assays were performed exactly as described by the manufacturers.

2.5. Experimental protocol

In the experiment on the time course of plasma interleukin-6 rise under continuous stress, blood was collected from intact mice after various durations (0.5, 1, 2, 4 h) of immobilization stress. In the experiment for kinetic analysis of changes in plasma interleukin-6 after exposure to 1-h immobilization stress, blood was collected from intact mice at various intervals (0, 0.5, 1, 2, 4, 6, 8 h) after termination of immobilization. Each mouse was bled once and killed. In the experiment for the involvement of glutamatergic system in the regulation of plasma interleukin-6 levels, the mice were pretreated i.c.v. with saline (5 μl), various doses of MK-801 (0.01–1 μg), NMDA (3, 10 ng), CNQX (0.01–0.5 μg), or MCPG (1–20 μg) for 10 min before the start of the immobilization stress, and blood was collected after 1-h immobilization stress. For the non-stressed control mice, blood was collected at 70 min after the i.c.v. injection of vehicle or drugs.

2.6. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) with post-hoc test. P values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Profiles of immobilization stress-induced increase of plasma interleukin-6 in mice

To disclose the time course of plasma interleukin-6 rise under continuous stress, the plasma interleukin-6 level was assayed after various durations of immobilization stress (Fig. 1). The plasma interleukin-6 level was elevated from the baseline value of 5 ± 1 pg/ml as the duration of stress increased and reached a maximum of 81 ± 11 pg/ml after 2 h of restraint. Subsequent studies were done with 1-h immobilization stress. The kinetic analysis of changes in plasma interleukin-6 after exposure to 1-h immobilization stress is shown in Fig. 2. The plasma interleukin-6 level was measured immediately, or 0.5, 1, 2, 4, 6, 8 h after termination of the stress procedure. The increase in plasma

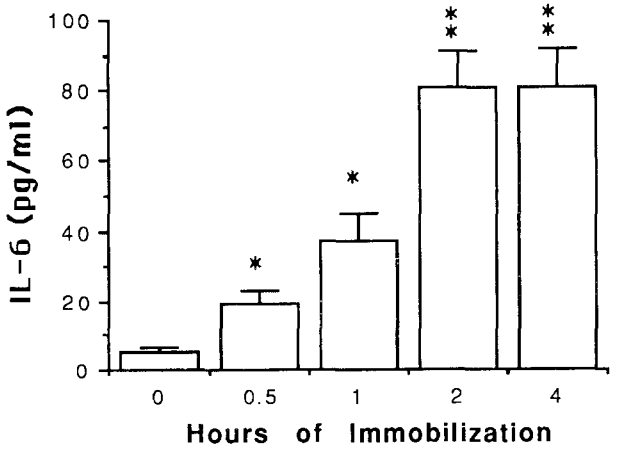

Fig. 1. Effect of duration of immobilization on plasma interleukin-6 (IL-6) levels. Mice were immobilized for the times indicated and blood samples were obtained immediately after completion of the procedure. The data are means ± S.E.M. ($n = 15$). * $P < 0.01$; ** $P < 0.001$, significantly different from non-stressed control.

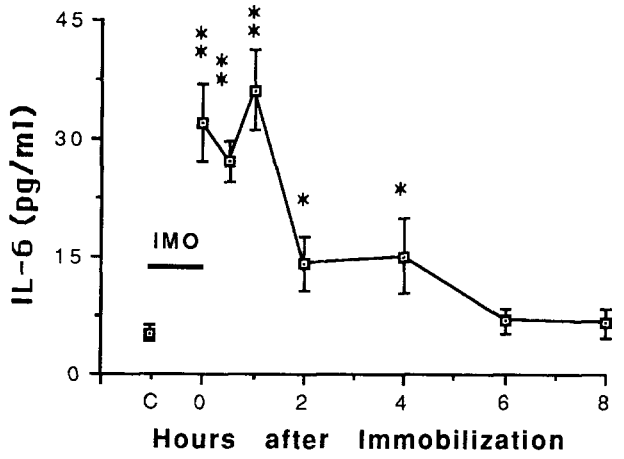

Fig. 2. Time course of the effect of a 1-h immobilization (IMO) on the level of plasma interleukin-6 (IL-6). Blood samples were obtained from one group of animals immediately after completion of the procedure (value at time point 0), whereas other groups of animals were allowed to rest for the indicated intervals before blood samples were obtained. C represents the intact control. The data are means ± S.E.M. ($n = 15$). * $P < 0.05$; ** $P < 0.001$, significantly different from non-stressed control.

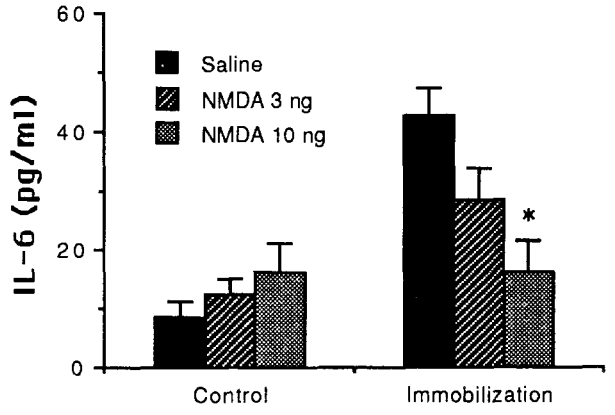

Fig. 4. Effect of *N*-methyl-D-aspartate (NMDA) injected intracerebroventricularly (i.c.v.) on plasma interleukin-6 (IL-6) levels in non-stressed control and immobilization-stressed mice. Either saline or various doses of NMDA (3, 10 ng) were given as pretreatment i.c.v. 10 min before the start of immobilization stress. Mice were immobilized for 1 h and blood samples were obtained immediately after completion of the procedure. The data are means ± S.E.M. ($n = 15$). * $P < 0.01$, significantly different from saline-treated stressed animals.

interleukin-6 caused by the 1-h restraint persisted for 1 h, then rapidly decreased, and by 6 h the level was not significantly higher than in the non-stressed control animals.

3.2. Effects of i.c.v. injections of MK-801 or NMDA on the basal and stress-induced plasma interleukin-6 levels

Next, we investigated the possible involvement of NMDA receptors in the regulation of both baseline and 1-h immobilization stress-induced plasma interleukin-6 levels. Different doses (0.01–1 μg) of MK-801, a non-competitive NMDA blocker, were injected i.c.v. 10 min prior to the start of 1-h immobilization. Plasma interleukin-6 levels in the non-stressed control mice injected i.c.v. saline (5 μl) were 5 ± 1, 12 ± 1, 22 ± 3, 20 ± 4 pg/ml ($n = 19$–44, mean ± S.E.M.) at 0, 1, 2 and 4 h after injection, respectively. As shown in Fig. 3, MK-801 injected i.c.v. dose dependently increased the baseline level of plasma interleukin-6 in non-stressed control mice. In the stressed animals, MK-801 (1 μg) administered i.c.v. produced an additive increase in plasma interleukin-6 level. To examine the possibility that stimulation of NMDA receptor by NMDA could also affect the immobilization stress-induced rise of plasma interleukin-6, different doses (3, 10 ng) of NMDA were injected i.c.v. 10 min prior to the start of 1-h immobilization. As shown in Fig. 4, NMDA dose dependently inhibited the stress-induced rise in plasma interleukin-6 levels. NMDA injected i.c.v. showed a tendency to increase the basal plasma interleukin-6 levels in non-stressed control animals, but the values did not reach statistical significance (Fig. 4).

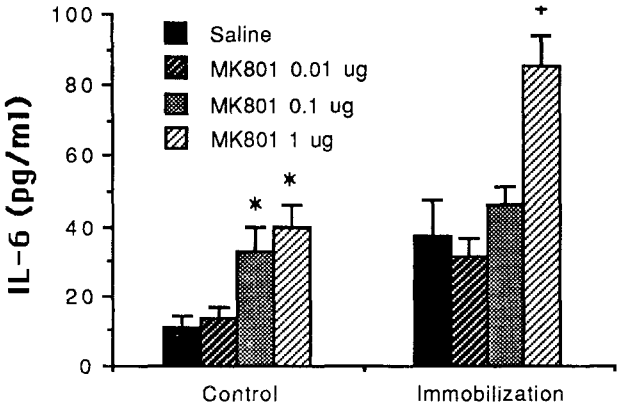

Fig. 3. Effect of MK-801 injected intracerebroventricularly (i.c.v.) on plasma interleukin-6 (IL-6) levels in non-stressed control and immobilization-stressed mice. Either saline or various doses of MK-801 (0.01–1 μg) were given as pretreatment i.c.v. 10 min before the start of immobilization stress. Mice were immobilized for 1 h and blood samples were obtained immediately after completion of the procedure. The data are means ± S.E.M. ($n = 15$). * $P < 0.01$, + $P < 0.01$, significantly different from saline-treated non-stressed and saline-treated stressed animals, respectively.

3.3. Effects of i.c.v. injections of CNQX or MCPG on the basal and stress-induced plasma interleukin-6 levels

To determine if non-NMDA- and metabotropic glutamate receptors are involved in the modulation of the plasma interleukin-6 level, various doses of CNQX (0.01–0.5 μg; a non-NMDA glutamate receptor antagonist), or MCPG (1–20 μg; a metabotropic glutamate receptor antagonist) were given as pretreatment i.c.v. 10 min before the start of immobilization stress. Basal and stress-induced

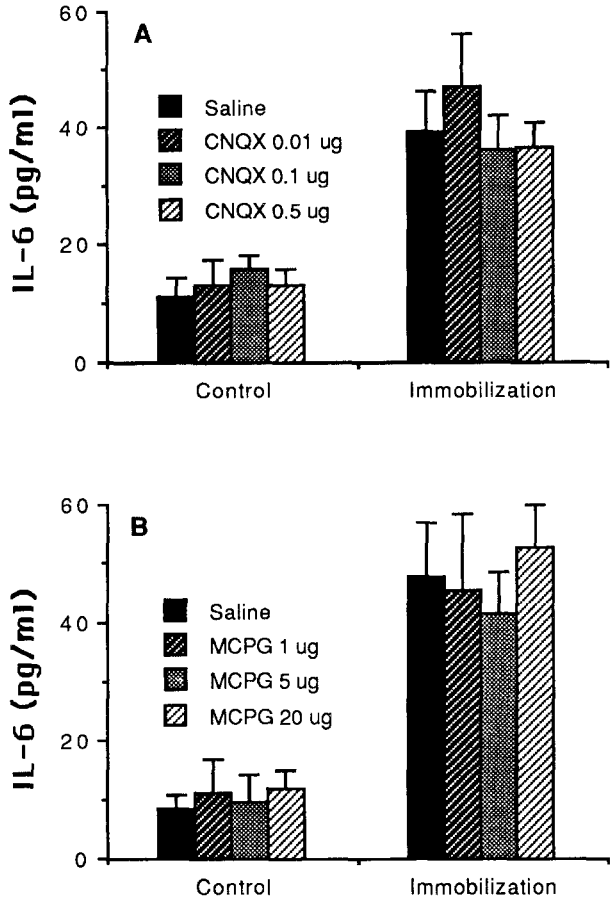

Fig. 5. Effect of 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) (A) and (±)-α-methyl-4-carboxyphenylglycine (MCPG) (B) injected intracerebroventricularly (i.c.v.) on plasma interleukin-6 (IL-6) levels in non-stressed control and immobilization-stressed mice. Either saline or various doses of CNQX (0.01–0.5 μg, i.c.v.) and MCPG (1–20 μg, i.c.v.) were given as pretreatment i.c.v. 10 min before the start of immobilization stress. Mice were immobilized for 1 h and blood samples were obtained immediately after completion of the procedure. The data are means ± S.E.M. ($n = 15$ (A) or 6 (B)).

plasma interleukin-6 levels were not altered by either CNQX or MCPG (Fig. 5).

4. Discussion

The major finding in the present study was that i.c.v. injection of MK-801, unexpectedly, increased the baseline level of plasma interleukin-6, suggesting that there is a tonic inhibitory control mechanism via the NMDA receptor for the regulation of the basal plasma interleukin-6 level. The effect of MK-801 on the basal plasma interleukin-6 levels may not be specific to i.c.v. injection of the drug. Intraperitoneal injection of MK-801 at the dose of 0.25 mg/kg induced an increase in plasma interleukin-6 levels, comparable to the effect of MK-801 (0.1 μg) administered i.c.v. (unpublished observation). Furthermore, we found that i.c.v. injection of NMDA can attenuate the immobilization stress-induced rise in plasma interleukin-6 level, while i.c.v. injection of MK-801 additively increased the stress-induced plasma interleukin-6 elevation. Additionally, neither CNQX, a non-NMDA glutamate receptor antagonist, nor MCPG, a metabotropic glutamate receptor antagonist, administered i.c.v., affected the basal and stress-induced plasma interleukin-6 levels, suggesting that non-NMDA and metabotropic glutamate receptors may not be involved in the regulation of the plasma interleukin-6 levels.

Therefore, from these results it is suggested that central glutamatergic systems may not be involved in the immobilization stress-induced elevation of plasma interleukin-6 levels, although considerable evidence indicates that glutamatergic systems are activated during stress (Schasfoort et al., 1988; Gilad et al., 1990; Moghaddam, 1993; Morrow et al., 1993). On the contrary, these results suggest that the central NMDA receptor system has a suppressive regulatory role in the basal and in the immobilization-induced plasma interleukin-6 levels. Given the evidence of involvement of central catecholaminergic system in the immobilization stress-induced increase in plasma interleukin-6 levels (Takaki et al., 1994), it is tempting to propose a hypothesis that central nervous system regulation of plasma interleukin-6 levels is modulated by, at least in part, two opposing influences: stimulation by the central catecholaminergic system and inhibition by the central NMDA system. Although the exact site of action and mechanism by which the NMDA receptor system suppresses the plasma interleukin-6 levels remain to be clarified, one possible mechanism could be that NMDA receptors may exert a tonic facilitatory effect on other neurotransmitters, which inhibit plasma interleukin-6 levels or the central catecholaminergic system.

To our knowledge, all the previous studies of the immobilization-induced elevation of plasma interleukin-6 have been done with rat models (Zhou et al., 1993; Takaki et al., 1994). We found in the present study that an increase of plasma interleukin-6 levels by immobilization stress also occurs in mice. The magnitude and the time course of the immobilization stress-induced changes in the plasma interleukin-6 levels found in mice appear to be quite similar to those obtained in rats (Zhou et al., 1993; Takaki et al., 1994).

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