Serotonin Depletion Enhances the Intracerebroventricularly Administered MK-801-Induced Plasma Interleukin-6 Levels in Mice

Do-Hoon Kim,a Jun-Sub Jung,b Yoo-Sun Moon,c Hong-Won Suh,b and Dong-Keun Song*a,b

*Department of Psychiatry, College of Medicine, Hallym University; bDepartment of Pharmacology, College of Medicine, Institute of Natural Medicine, Hallym University; and cDepartment of Family Medicine, College of Medicine, Hallym University; Chunchon, Kangwon-Do, 200–702, South Korea. Received December 12, 2002; accepted January 21, 2003

To investigate the effect of serotonin depletion on the intracerebroventricularly (i.c.v.) administered MK-801-induced plasma interleukin-6 (IL-6) levels, we pretreated mice with parachlorophenylalanine (PCPA, 300 mg/kg, i.p.) 3 d before an i.c.v. injection of MK-801 (1 µg). The i.c.v. MK-801-induced rise of plasma IL-6 level was markedly enhanced in the PCPA-pretreated mice. However, serotonin depletion by PCPA pretreatment did not affect the i.c.v. MK-801-induced increase in plasma corticosterone level. These results suggest that serotonergic system is involved in the suppressive regulation of MK-801-induced plasma IL-6 level.

Key words interleukin-6; corticosterone; serotonin; parachlorophenylalanine (PCPA); MK-801

Glutamate, a major excitatory amino acid, has been found to play an important role in neuroendocrine regulation of plasma corticosterone1–3) and interleukin-64) (IL-6) levels, mainly acting via the N-methyl-D-aspartate (NMDA) receptor. Recently, we have shown that an intracerebroventricularly (i.c.v.) injection of MK-801, an NMDA antagonist, increases plasma IL-6 level, which suggests that there is a tonic inhibitory control mechanism in the brain via NMDA receptors for the regulation of the plasma IL-6 level.4) However, the exact mechanism by which the NMDA receptor system suppresses the plasma IL-6 level is currently obscure. A possible mechanism is that NMDA receptors might interact with other neurotransmitters systems that could affect the plasma IL-6 level. On the other hand, plasma corticosterone level is also increased by MK-801.5,6)

Recently, it has been reported that serotonin (5-hydroxytryptamine, 5-HT) appears to be closely involved in the modulation of neuronal functions in a diverse regions of the brain by changing activities of the glutamatergic system.7) However, little is known about the regulatory effect of serotonergic modulation on plasma IL-6 or corticosterone levels altered by MK-801. Thus, we examined the effects of serotonin depletion on MK-801-induced plasma IL-6 and corticosterone levels in the present study.

MATERIALS AND METHOD

Animals and Drugs Male ICR mice weighing 25—30 g, supplied by Myung-Jin, Inc. (Seoul, Korea), 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. DL-p-Chlorophenylalanine methyl ester hydrochloride (PCPA) and MK-801 were purchased from Research Biomedicals International (Natick, NA, U.S.A.). PCPA was dissolved in 0.1 N NaOH and diluted to the appropriate volume with 0.2 N HCl. MK-801 was dissolved in normal saline solution (0.9% NaCl) for intracerebroventricular (i.c.v.) or intraperitoneal (i.p.) injection. The doses of all drugs represent the salt.

i.c.v. injection The i.c.v. administration followed the method described by Laursen and Belknap.8) Briefly, the animal was injected at bregma with a 50 µl Hamilton syringe fitted with 26-ga. 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.

Blood Sampling and IL-6 and Corticosterone Assays Four hundred microliters of blood was collected by puncturing the retro-orbital venous plexus. Plasma was separated by centrifugation and stored at −70 °C until assayed. Plasma IL-6 level was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Genzyme, Cambridge, MA, U.S.A.). Assays were performed exactly as described by the manufacturers. The detection limit of the assay was 5 pg/ml. Plasma corticosterone level was determined by the fluorometric determination method.9)

Monoamine Assays Mice were killed by decapitation and the hypothalamus was dissected out. Hypothalami were frozen, and stored at −70 °C until assayed for 5-HT and 5-hydroxyindole acetic acid (5-HIAA). Concentrations of 5-HT and 5-HIAA were quantified using a high-performance liquid chromatography (HPLC) with electrochemical detection (at a potential of 0.85 V). The hypothalami were weighed and homogenized in 0.1 M perchloric acid containing 0.1 M sodium metabisulfite and 5-hydroxy-N-methyltryptamine as an internal standard. Following centrifugation at 12000×g for 2 min, the supernatant was filtered through a 0.45 m Millipore HV-4 filter. Ten microliters of sample was injected onto a C18 Bondapak column (Waters, Milford, U.S.A.). As a mobile phase, 0.1 M KH₂PO₄ (adjusted to pH 3.8) containing sodium octanesulfonic acid (0.25 mM), disodium EDTA (0.1 mM) and acetonitrile (9% v/v) was used. The flow rate was 1 ml/min and the oxidation potential was 1 V.

Experimental Protocol For depletion of 5-HT, PCPA (300 mg/kg, i.p.) was injected 3 d before MK-801 injection (1 µg, i.c.v.). To prove the PCPA-induced 5-HT depletion in the brain, levels of 5-HT and its metabolite, 5-HIAA, in hypothalamus were measured 3 d after the PCPA injection. Blood was collected 60 min after the MK-801 injection for assays of plasma IL-6 and corticosterone levels.
RESULTS

Effects of Pretreatment with PCPA on 5-HT and 5-HIAA Levels in Hypothalamus A single injection of PCPA (300 mg/kg, i.p.) markedly decreased 5-HT or 5-HIAA levels in hypothalamus to 17.8% and 16.7% of control values respectively, measured 3 d after the injection (Table 1). However, PCPA did not affect norepinephrine (NE) and dopamine (DA) levels in hypothalamus (data not shown).

Effect of Pretreatment with PCPA on i.c.v. MK-801-Induced Plasma IL-6 Level To study the involvement of serotonergic system in the NMDA antagonist-induced increase in plasma IL-6 level, we pretreated animals with PCPA (300 mg/kg, i.p.) 3 d before MK-801 injection (1 µg, i.c.v.). The i.c.v. MK-801-induced rise of plasma IL-6 level was markedly increased in the PCPA-pretreated animals (Fig. 1).

Effect of Pretreatment with PCPA on i.c.v. MK-801-Induced Plasma Corticosterone Level To investigate the involvement of serotonergic system in the NMDA antagonist-induced increase in plasma corticosterone level, we pretreated animals with PCPA (300 mg/kg, i.p.) 3 d before MK-801 injection (1 µg, i.c.v.). Pretreatment with PCPA did not affect i.c.v. MK-801-induced increase in plasma corticosterone level (Fig. 2).

DISCUSSION

The present study showed that i.c.v. MK-801-induced increase of plasma IL-6 level was markedly enhanced in animals pretreated with PCPA. However, serotonin depletion by PCPA pretreatment did not affect i.c.v. MK-801-induced increase in plasma corticosterone level.

Previously, we proposed that central nervous system regulation of plasma IL-6 level is modulated by, at least in part, two opposing influences: stimulation by the central cholinergic system and inhibition by the central NMDA system.4) From the results of the present study, we suggest that serotonergic system in the brain may be involved in the suppressive regulation of plasma IL-6 level by the NMDA receptor system. However, it is not clear by what mechanism(s) serotonin depletion can augment MK-801-induced increase in plasma IL-6 level. In our previous report, the MK-801-induced increase in plasma IL-6 level was inhibited by stimulation of γ-aminobutyric acid (GABA) receptors and we suggested that central GABA receptors play important roles in the suppressive modulation of plasma IL-6 level induced by NMDA receptor blockade.10) The interaction between serotonergic and GABA systems has been intensively reported.11) A recent study also showed that serotonin depletion by PCPA decreases [3H]muscimol (GABA A agonist) binding in the brain.12) Thus, it can be speculated that central GABA receptors might mediate important roles of serotonergic neuron in the regulation of MK-801-induced plasma IL-6 level. However, we cannot rule out the involvement of other neurotransmitters systems that may be affected by serotonin depletion. Serotonin depletion by PCPA did not affect basal plasma IL-6 level in the present study. This result is in line with reports that have shown that serotonin depletion by tryptophan-free diet does not affect plasma IL-6 level in healthy human.13,14) Thus, it is supposed that serotonin is not involved in the tonic regulation of basal plasma IL-6 level. Serotonin reportedly increases IL-6 synthesis in human vascular smooth muscle cells.15) To our knowledge, the present study revealed for the first time that serotonergic system is involved in the regulation of plasma IL-6 level. However, mechanism and exact site involved remain to be investigated.

In contrast, serotonin depletion did not affect i.c.v. MK-801-induced increase in plasma corticosterone level. Although the mechanism of MK-801-induced increase in
plasma corticosterone level is obscure, some studies have suggested that the action sites of i.c.v. MK-801 in the regulation of plasma corticosterone level would be PCP binding receptors in hippocampus rather than hypothalamus because PCP receptors are rare in hypothalamus.\textsuperscript{5,6} Thus, it is speculated that serotonin depletion may not affect PCP receptors in hippocampus, but further study is necessary to verify the speculation.

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REFERENCES