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N-Methyl-D-aspartate receptor agonists and antagonists partially affect the duration of ketamine anesthesia in the rat

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Abstract: The effects of intracerebroventricular injection of excitatory amino acids which act on the N-Methyl-D-aspartate (NMDA) receptor complex on the duration of loss of righting reflex (DLRR) induced by intravenous injection to ketamine (20 mg/kg) were investigated in rats. Ketamine-induced DLRR was 10.3 min, but NMDA receptor agonists D-alanine (200 μg) or NMDA (0.15 μg) did not change DLRR. However, D-alanine combined with NMDA significantly shortened DLRR (7.7 min). The NMDA receptor antagonist 7-chlorokynurenic acid (10 μg) alone prolonged DLRR significantly (16.2 min), but not when combined with D-alanine. These data suggest that NMDA receptor blockade contributes at least partially to the mechanism of ketamine anesthesia.

Key words: NMDA receptor, N-Methyl-D-aspartate, 7-Chlorokynurenic acid, D-Alanine, Ketamine anesthesia

Introduction

Ketamine is a widely used anesthetic derived from phencyclidine. In 1985, Martin and Lodge showed that ketamine blocked the *N*-methyl-D-aspartate (NMDA) receptor, an excitatory amino acid receptor, in a non-competitive manner in the frog spinal cord [1]. In 1990, Yamamura et al. suggested that the NMDA receptor complex was a site of action of ketamine anesthesia in the isolated lamprey spinal cord [2]. The NMDA receptor complex is supposed to be a receptor-ion channel complex consisting of at least three distinct subcomponents: 1) the NMDA recognition site, 2) the glycine modulatory site, and 3) the cation channel. Since ketamine binds to the phencyclidine binding site inside the cation channel [3], some of its pharmacological properties may arise from blockade of the NMDA receptor-gated ion channel [4]. In this study, we investigated how agonists and antagonists of the NMDA receptor complex could affect the duration of ketamine anesthesia in vivo, and attempted to clarify the involvement of the NMDA receptors in ketamine anesthesia.

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Materials and methods

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee of The University of Kansas Medical Center, Kansas City, Kansas. We used male Wistar rats (Harlan Sprague Dawley, Indianapolis, IN, USA) weighing 200–250 g. The animals were housed at 22.0° ± 0.5°C in a humidity-controlled room under a 12-h light/dark cycle (lights on at 6:00 a.m.). The animals were allowed food and water ad libitum. Five to 7 days before the experiment, the animals were anesthetized with an intraperitoneal (i.p.) injection of pentobarbital sodium (35–40 mg·kg^{-1}), placed onto a stereotaxic instrument (Narishige Scientific Instrument Lab., Tokyo, Japan), and stainless-steel guide cannulae (25 gauge) were aseptically implanted in both sides of the cerebral cortex. The lower tip of the cannula was placed 2.0 mm from the center of each lateral ventricle (AP: −1.0 mm, L: ±1.5 mm, V: +2.0 mm from the bregma; according to the atlas of Paxinos and Watson [5]). On the day of the experiment, injection needles (OD: 0.2 mm, ID; 0.08 mm) were inserted through the guide cannulae into the lateral ventricles (2.0 mm beyond the guide cannula). Phosphate buffered saline (saline, pH 7.4) with or without the agents listed below were simultaneously injected into both lateral ventricles in a total volume of 10 μl at a rate of 1.0 μl·min^{-1} for 10 min, through the injection needles

using a microsyringe pump (EP-60, EICOM, Kyoto, Japan).

The 96 rats were divided into 12 groups of 8 each. The control group received 20 μl of saline. The other animals received 0.15 μg of *N*-methyl-D-aspartate (NMDA, Research Biochemicals, Natick, MA, USA), an agonist of the NMDA recognition site in the NMDA receptor complex, 200 μg of D-alanine (Nacalai Tesque, Kyoto, Japan), an agonist of the glycine modulatory site in the NMDA receptor complex, 200 μg of L-alanine (Nacalai Tesque, Kyoto, Japan), stereoisomer of D-alanine or 10 μg of 7-chlorokynurenic acid (7-Cl-KYNA, Research Biochemicals), an antagonist of the glycine modulatory site in the NMDA receptor complex, dissolved in 20 μl saline. In addition, combinations of D-alanine and NMDA, D-alanine and 7-Cl-KYNA, L-alanine and NMDA, or L-alanine and 7-Cl-KYNA at the same doses as above, dissolved in 20 μl of saline, were also administered. Five minutes after intracerebroventricular (i.c.v.) administration of these amino acids, 20 mg·kg^{-1} ketamine HCl (Ketalar, Parke-Davis, Morris Plains, NJ, USA) was injected into the tail vein. When the rats were anesthetized and fell over, they were turned on their backs on a wood plate and the duration of loss of righting reflex (DLRR; the duration from the time when rats fell over and placed on their back until the time when they spontaneously reverted completely to the prone position) was measured. After the experiment, the rats were anesthetized with pentobarbital sodium (50 mg·kg^{-1}, i.p.) and injected with 40 μl of 0.5% methylene blue through the guide cannulae into the brain. Then the rats were decapitated, and their brains were removed and checked to see if the i.c.v. injections had been precisely made.

In preliminary experiments, ketamine increased DLRR dose-dependently up to 30 mg·kg^{-1} i.v. When ketamine (30 mg·kg^{-1}) was administered with 7-Cl-KYNA (2–50 μg i.c.v.), half of the rats died from respiratory depression. Thus, we chose 20 mg·kg^{-1} i.v. of ketamine for the experiments. The dose of 7-Cl-KYNA (10 μg) was determined as the minimal dose that showed a significant difference from the control. The dose of D-alanine (200 μg) was determined as the minimal dose that significantly inhibited DLRR induced by ketamine 20 mg·kg^{-1} i.v. and 7-Cl-KYNA 50 μg i.c.v. (from 32.0 ± 11.3 to 21.3 ± 6.4 min, mean ± SD). The dose of D-alanine (200 μg) was the same as the dose used in the in vivo study by Tanii et al. [6]. A single i.c.v. administration of 7-Cl-KYNA 10 μg or D-alanine 200 μg did not induce loss of righting reflex. In addition, the dose of NMDA (0.15 μg) was determined as the maximum dose which did not induce excitation in rats. The data were analyzed using one-way ANOVA followed by the Tukey-Kramer test.

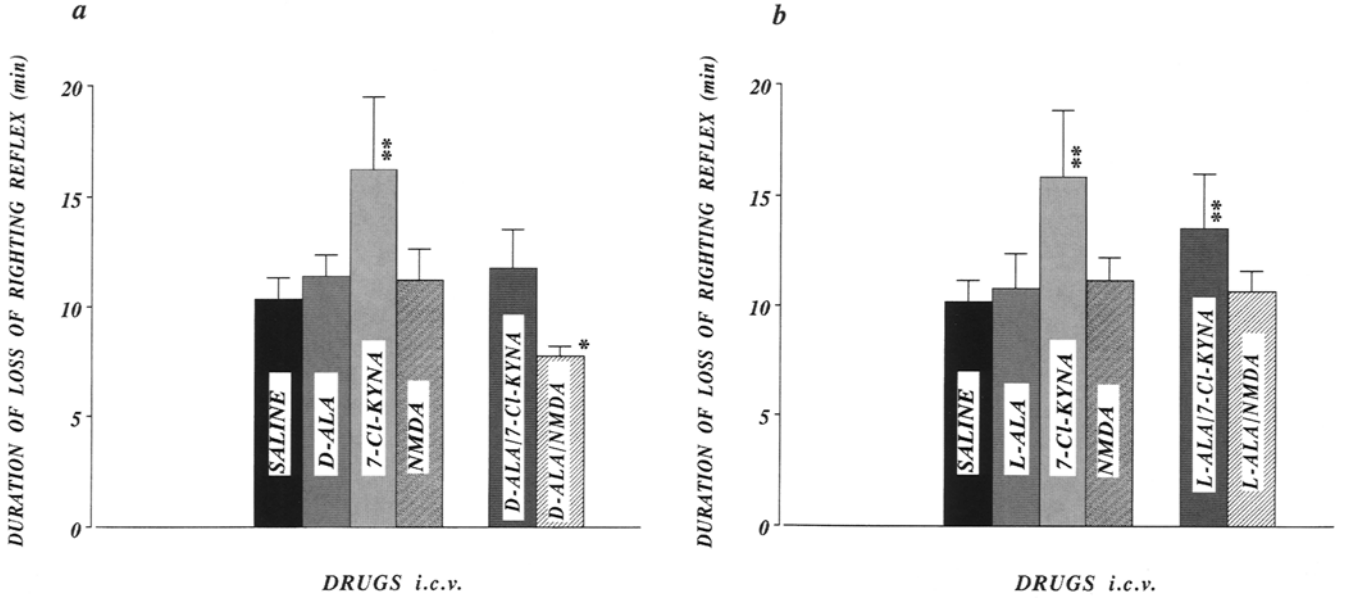

Fig. 1a. Effects of intracerebroventricular (*i.c.v.*) administration of D-alanine (*D-ALA*) (200 μg), *N*-methyl-D-aspartate (*NMDA*) (0.15 μg), and 7-chlorokynurenic acid (*7-Cl-KYNA*) (10 μg) on the duration of loss of righting reflex induced by ketamine (20 mg·kg^{-1} i.v.) **b** Effects of i.c.v. administration of L-alanine (*L-ALA*) (200 μg), NMDA (0.15 μg) and 7-Cl-KYNA (10 μg) on the duration of loss of righting reflex induced by ketamine (20 mg·kg^{-1} i.v.). These drugs or saline were injected into both lateral ventricles at a rate of 1.0 μl·min^{-1} for 10 min. Five minutes later, ketamine was injected into the tail vein, and the duration from loss to initiation of righting reflex was measured. Values are mean ± SD (min) of 8 rats. *$P < 0.05$, **$P < 0.01$ saline

Results

DLRR induced by ketamine was 10.3 ± 1.0 min in saline-pretreated rats (control group). Neither D-alanine nor NMDA changed DLRR induced by ketamine (11.4 ± 1.0, 11.2 ± 1.4 min, respectively). However, the combination of D-alanine and NMDA significantly shortened DLRR to 7.7 ± 0.4 min. 7-Cl-KYNA significantly prolonged DLRR to 16.2 ± 3.2 min. The DLRR in D-alanine and 7-Cl-KYNA combination group (11.8 ± 1.8 min) showed no significant difference from DLRR in the control group (Fig. 1a). On the other hand, L-alanine administered either alone or in combination with 7-Cl-KYNA or NMDA did not change DLRR (Fig. 1b).

Discussion

Our results show that agonists and antagonists of the NMDA receptor complex can affect the duration of ketamine anesthesia. Although a single administration of D-alanine, an agonist at the glycine modulatory site, or NMDA, an agonist at the NMDA recognition site, did not affect the duration of ketamine anesthesia, the combination of D-alanine and NMDA significantly shortened it. These results appear to be consistent with in vitro observations [7–9]. It was reported that glycine alone (also an agonist at the glycine modulatory site) or NMDA alone could not activate the NMDA receptor-gated channel sufficiently in cultured brain neurons from mouse embryos [7], while the combination of D-alanine as well as glycine or D-serine, another agonist at the glycine modulatory site, with NMDA was demonstrated to open the channel in the NMDA receptor expressed in *Xenopus* oocytes [8]. In neocortical slices from adult rats, glycine was found to enhance the NMDA receptor-mediated excitatory postsynaptic potentials (EPSP) [9]. Therefore, it is assumed that a pronounced activation of the ion channel of the NMDA receptor complex in the brain, enough to antagonize ketamine anesthesia, can be achieved only by stimulation of both the glycine modulatory site and the NMDA recognition site.

On the other hand, 7-Cl-KYNA, an antagonist at the glycine modulatory site can prolong the duration of ketamine anesthesia. 7-Cl-KYNA is supposed to inactivate the glycine modulatory site. It is reported that the ion channel in the NMDA receptor complex cannot function sufficiently without activation of the glycine modulatory site [7–9]. Therefore, it is likely that 7-Cl-KYNA depressed the NMDA receptor function by inactivation of the glycine modulatory site, leading to enhancement of ketamine anesthesia. In our study, D-alanine competed with the augmenting effect of 7-Cl-KYNA on DLRR induced by ketamine, presumably at the glycine modulatory site. This explanation can be supported by in vitro evidence that either glycine or D-serine, which is also an agonist of the glycine modulatory site, completely reversed the effect of 7-Cl-KYNA on the intracellular free calcium level in cultured hippocampal neurons [10]. In contrast, L-alanine, which is known to be inactive on the glycine modulatory site, did not alter the effects of NMDA or 7-Cl-KYNA on the duration of ketamine anesthesia. Our results indicate that the effect on the duration of ketamine anesthesia was stereoselective between D-alanine and L-alanine. These data are consistent with an in vivo study of Tanii et al. on stereoselective inhibition by D- and L-alanine of phencyclidine-induced locomotor stimulation in rats [6].

Noncompetitive antagonists of the NMDA receptor complex, MK-801 and phencyclidine, which bind to the phencyclidine site inside the ion channel like ketamine, are reported to increase the potency of general anesthetics [11]. MK-801 has also been demonstrated to reduce volatile anesthetic requirements [12]. On the other hand, a competitive antagonist of the NMDA recognition site, CGS-19755, was found to show anesthetic effects similar to ketamine. France et al. suggested that the anesthetic effects of CGS-19755 resulted from the competitive blockade of the NMDA receptor complex [13]. These findings and the present data suggest that NMDA receptor blockade is involved at least partially in the mechanism of ketamine anesthesia. Thus, our findings are likely to support the hypothesis of MacDonald et al., based on an electrophysiological experiment, that the mechanism of ketamine anesthesia is a pharmacologically specific interaction with ion channels in the NMDA receptor complex [14].

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