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High-dose ketamine does not induce c-Fos protein expression in rat hippocampus

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The effects of high-dose ketamine on the c-*fos* protein (c-Fos) expression were investigated in rat by an immunohistochemical technique. The administration of 100 mg/kg ketamine i.p. induced seizure-like activity (limbic seizure). No c-Fos immunoreactivity was observed in hippocampus, piriform cortex and amygdala, while it was observed in neocortex and thalamus. These findings disagree with the reports that ketamine depresses the neuronal function of the neocortex and thalamus, while it stimulates the limbic system.

Recent studies have shown that proto-oncogene c-*fos* mRNA is induced in central nervous system (CNS) following convulsions, brain injuries, and noxious stimuli [4, 5, 11, 18]. Therefore, the c-*fos* protein (c-Fos) expression in brain has been thought to be a metabolic marker and an indicator of neuronal plasticity [6, 18]. Ketamine is an intravenous general anesthetic, the primary site of CNS action of which is thought to be the thalamo-neocortical projection system [15]. It selectively depresses neuronal function of the neocortex and thalamus, while it simultaneously stimulates the limbic system including the hippocampus and sometimes induces seizure activity (usually limbic seizure) [9, 12, 14–16, 23]. Moreover, 2-deoxyglucose (2-DG) studies have documented that ketamine increases cerebral metabolism especially in the hippocampus, while it depresses most of the neocortex [2, 3, 17]. However, the effects of ketamine on the c-Fos expression in the hippocampus were unknown.

Here, with an immunohistochemical technique, we demonstrated that ketamine-induced seizure does not induce the c-Fos expression in the rat hippocampal areas, while it induces the apparent one in the neocortex and thalamus.

The study protocol was approved by the Animal Committee of the Medical Faculty, Kyoto University. All experiments were performed on male Wistar rats weighing 280–330 g. The drugs used were dissolved in distilled water and were administered through the intraperitonal route. The rats were administered 100 mg/kg of ketamine (n = 3, at each time) or 10 mg/kg of kainic acid (n = 3, at each time) for comparison. Another six rats were administered vehicle alone as a control group. One h, 2 h and 4 h after administration of drugs, rats were anesthetized with a 100 mg/kg of sodium pentobarbital and were perfused through the heart with 100 ml of 0.01 M phosphate buffered saline (PBS, pH 7.4), followed by the fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 and 0.2% picric acid) containing 0.35% glutaraldehyde. Then, the brain was removed, post-fixed one day in the same fixative solution, and placed in 15% sucrose in 0.1 M phosphate buffer (pH 7.4) containing 1 g/l sodium azide for more than 1 day at least until they sank. After that, frozen slices (20 μm thick) were cut with cryostat and immersed in 0.01 M PBS. The slices were then incubated with the primary c-Fos antibody (Cambridge Research Biochemical, Cambridge, UK) at dilutions of 1/1,000, 1/2,000, 1/5,000, 1/8,000 and 1/10,000 by 0.1 M PBST (0.1 M PBS containing 0.3% Triton X-100) at 4°C for 4 days. The slices were again washed 3 times with PBST, 10 min per wash, and incubated with biotinylated anti-sheep antibody (1/1000 dilution in PBST, Vector Laboratories, Burlingame, CA) at room temperature for 1 h, using the ABC technique as described by Hsu et al. [10]. After washing slices 3 times in PBST, they were incubated with the ABC solution (Vector Laboratories, Burlingame, CA) at room temperature for 1 h, and then washed 3 times in PBST. The ABC staining was

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visualized using 3,3′-diaminobenzidine tetrahydrochloride, NiCl and H_2O_2 in 0.05 M Tris-HCl (pH 7.6) for 5 min. To control for specificity of immunostaining, some slices were incubated with c-Fos antibody pre-absorbed with the peptide against which the antibody was raised.

At higher titers (1/1,000 and 1/2,000 dilution) of the c-Fos antibody, dark blue nuclear staining was found in hippocampal pyramidal neurons and granule cells of the dentate gyrus or other areas of the rat brain in control group. However, at the titer of 1/5,000 or lower, specific staining was reduced to almost background levels in control group. Thus, we defined c-Fos expression responding to some stimuli as positive in case the specific immunoreactivity of c-Fos was detectable at the 1/5,000 dilution.

After the administration of ketamine, all rats rapidly became sedated, cataleptic-like unresponsiveness, though exhibited sporadic behavioral signs of seizure activity (muscle twitching), followed by a side-to-side head rocking, and eventually frantic, agitated hyperactivity. In EEG study, epileptoid discharges characterized by high voltage spikes (>100 μV) were seen (data not shown). On the other hand, the rats administered kainic acid were manifested in limbic seizures accompanied by immobilization, staring and wet-dog shakes, followed by generalized convulsion, as reported before [13].

Fig. 1 shows the c-Fos-like immunoreactivity in the hippocampus after ketamine or kainic acid administration. No c-Fos-like signal was observed in hippocampus after the ketamine administration, while a marked c-Fos-like staining was observed after the kainic acid administration. Fig. 2 shows the c-Fos-like immunoreactivity in the neocortex and thalamus after ketamine administration.

We reported that in cats subanesthetic doses (10–20 mg/kg, i.p.) of ketamine produced apparent CNS excitation, coupled with severe ataxia and catatonic behavior, large doses (20–50 mg/kg, i.p.) produced profound catatonia and anesthesia and at doses sufficient to produce near-apnea (40–50 mg/kg i.v.) bursts of polyspike-wave complex activity interrupted by postictal depression were recorded [16]. In this experiment, we used a

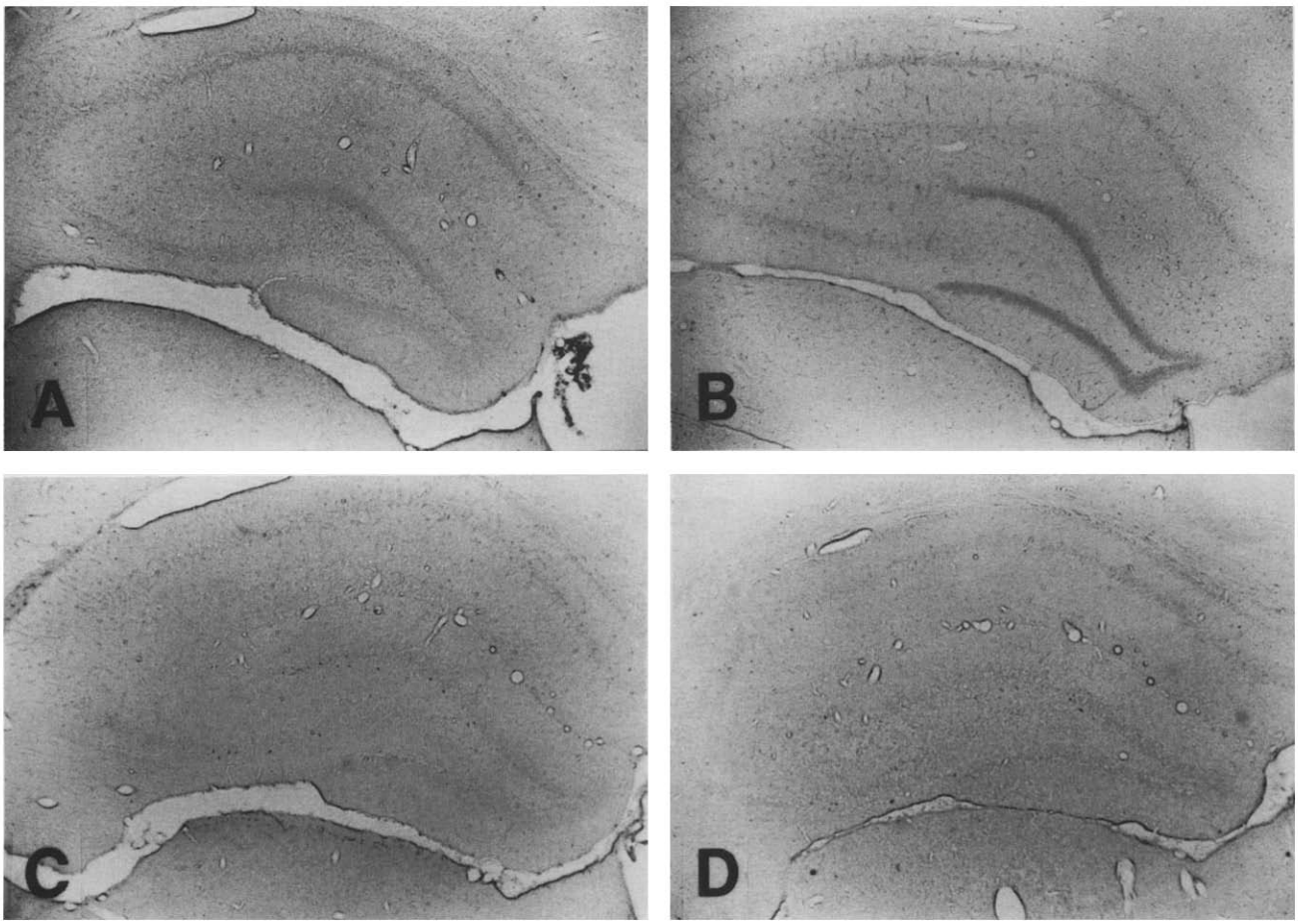

Fig. 1. Representative c-Fos expression in hippocampal region of rat brain following ketamine or kainic acid administration at a titer of c-Fos antibody, 1/5,000. The data are photomicrographs of coronal sections at approximately the same position in hippocampus. A: control (×40), B: 2 h after the administration of kainic acid (×40). C and D: 1 h and 2 h after the administration of ketamine, respectively (×40).

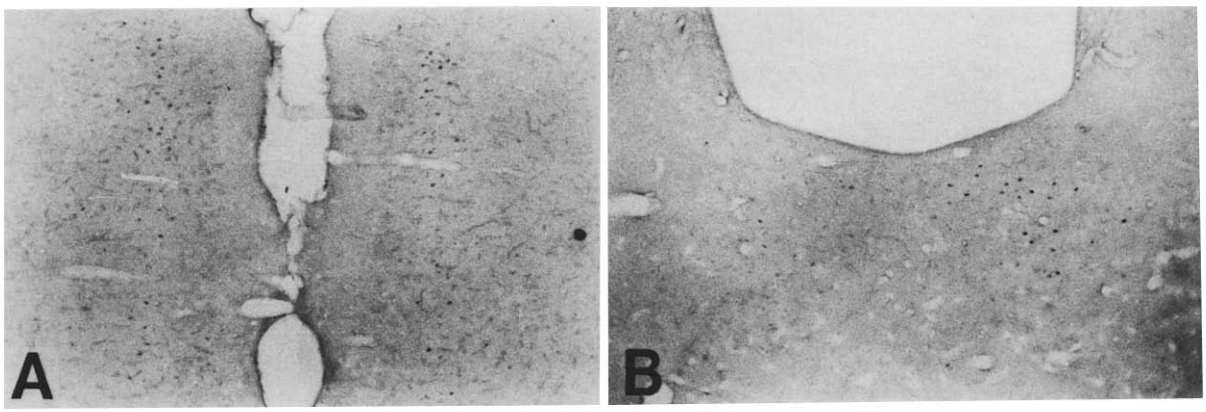

Fig. 2. Photomicrographs showing the pattern of c-Fos induction in rat neocortical region (cingulate cortex) (A) and thalamus (B), respectively (×100).

rather high-dose ketamine (100 mg/kg, i.p.) because there were some reports that the glucose utilization was most stimulated during withdrawal from a high-dose ketamine [3] and that Dragunow et al. used this dose to prevent c-*fos* mRNA expression after brain injury [7]. Ketamine is known to increase cerebral metabolic rate (CMR) [21]. The 2-DG studies showed that the increases in CMR occur in hippocampus simultaneously with modest change or depression in cortical structures [2, 3, 17]. Furthermore, ketamine produces limbic seizures [9, 14, 24]. Therefore, ketamine possibly induces the c-Fos expression in the hippocampus. However, the c-Fos induction did not occur in that region. This means there was a mismatch between the increase in CMS and c-Fos expression. Although there exist some reports on mismatches between 2-DG mapping and c-Fos expression [6], to our best knowledge, nobody has ever pointed out the mismatch of them with ketamine-induced seizure. On the other hand, kainic acid first induces limbic seizure like ketamine, followed by generalized convulsion. During this period, diffuse labeling of c-Fos was detectable in the dentate gyrus and the entorhinal cortex [13]. This was confirmed in our study.

Ketamine acts as a noncompetitive NMDA antagonist [1, 22], and it is reported that the high-dose ketamine (100 mg/kg, i.p.) completely prevented c-*fos* mRNA and protein induction in neurons after focal brain injury [7]. The evidence that ketamine is a NMDA antagonist may concern the lack of the c-Fos expression in hippocampus and olfactory cortex with ketamine-induced seizure. Recently, Dragunow and Faull reported that MK-801 itself produced a marked induction of c-Fos in the neocortex and thalamus despite the lack of c-Fos induction in the hippocampus and piriform cortex [8]. They assumed the mechanism of this activation in the neocortex and thalamus is due to the diversity of the NMDA agonist binding site and antagonist binding site, and consequently, the inhibition of inhibitory cells via NMDA antagonism. However, in the current time, we have no way to explain the discrepancy between the electrophysiological and 2-DG studies and c-Fos expression with ketamine and the evidence that ketamine, a NMDA antagonist, induced c-Fos expression in neocortex and thalamus. To rule out the effect of intracranial pressure increase of ketamine on c-Fos expression, some rats were mechanically ventilated and $PaCO_2$ was kept around 30 mm Hg; the results obtained were the same. Other properties of ketamine to interact with other receptors, such as opiate receptors and central cholinergic receptors or to act as 'general anesthetics' may explain the mechanism of the above mentioned evidences [20, 22]. Anyhow, further studies will be required for the explanation.

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