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Halothane and Diazepam Inhibit Ketamine-induced c-fos Expression in the Rat Cingulate Cortex

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Background: **Ketamine, a noncompetitive N-methyl-D-aspartate antagonist, has psychotomimetic side effects. Recent studies have shown that noncompetitive N-methyl-D-aspartate antagonists cause morphologic damage to the cingulate and retrosplenial cortices and induce c-fos protein (c-Fos) in the same regions. Although benzodiazepines are effective in preventing these side effects, the neural basis of the drug interactions has not been established.**

Methods: **The effects of diazepam and halothane on c-Fos expression induced by ketamine were studied. Diazepam (1 and 5 mg/kg) or vehicle were administered subcutaneously, followed 7 min later by 100 mg/kg ketamine given intraperitoneally. Halothane (1.0 and 1.8%), was administered continuously from 10 min before ketamine administration until brain fixation. Two hours after ketamine injection, rats were perfused and their brains fixed and extracted. Brain sections were prepared in a cryostat and c-Fos expression was detected using immunohistochemical methods.**

Results: **Ketamine induced c-Fos–like immunoreactivity in the cingulate and retrosplenial cortices, thalamus, and neocortex. Diazepam suppressed the ketamine-induced c-Fos–like immunoreactivity in the cingulate and retrosplenial cortices in a dose-dependent manner, leaving the thalamus and neocortex less affected. Halothane suppressed the ketamine-induced c-Fos–like immunoreactivity in the cingulate and retrosplenial cortices and the neocortex in a dose-dependent manner, leaving the thalamus relatively unaffected.**

Conclusion: **Halothane and diazepam inhibited ketamine-induced c-Fos expression in the cingulate and retrosplenial cortices, leaving the thalamus relatively unaffected. (Key words: Anesthetics, intravenous: ketamine. Anesthetics, volatile: halothane. Brain: neurotoxicity. Molecular biology, immediate early gene: c-fos protein. Immunohistochemistry).**

KETAMINE, a noncompetitive N-methyl-D-aspartate (NMDA) antagonist, induces undesirable psychological reactions, such as vivid dreams and hallucinations.[1,2] Although ketamine is an anesthetic, Mori and associates[3] showed that there is not a total depression but rather an activation of the central nervous system (CNS) electrical activities. Studies of regional cerebral metabolism of glucose (CMR_{Glu}) provided divergent views of activation and suppression on the action of ketamine, except on hippocampal formation, where enhanced CMR_{Glu} has been confirmed.[4–6] Mori and associates[3] also postulated that the psychological phenomena in humans were not dreams but hallucinations in that, in cats, the CNS electrical activity after ketamine administration could not be related to a dream sleep (*i.e.*, the paradoxical phase of sleep). They noted a marked similarity of the CNS electrical activities to that observed after hallucinogens, such as LSD-25 and mescaline. Wide clinical experience shows that benzodiazepines prevent these ketamine-induced hallucinations.[7–9]

Olney and colleagues[10] and Sharp and associates[11] reported recently that noncompetitive NMDA antagonists, such as phencyclidine, ketamine, and MK801, damaged the cingulate and retrosplenial (which is often called the posterior cingulate,[12] but because nomenclature is complicated, it is called a cingulate) corti-

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ces, and that the damage was prevented by either anticholinergic drugs by blocking the muscarinic M_1 receptor or diazepam and barbiturates by activating the γ-aminobutyric acid (GABA) receptor complex.[13] Olney and colleagues[10,13] also postulated that these regions might be responsible for the phencyclidine- and ketamine-induced psychotomimetic effects.

On the other hand, we have found that ketamine induced c-fos, a proto-oncogene, protein (c-fos), in various brain areas such as the cerebral cortices (especially in the cingulate cortex) and the thalamic nuclei but not in the hippocampus.[14] c-Fos expression is induced in activated states, such as convulsions,[15-18] and is widely considered a high-resolution metabolic marker in the brain.[15,19] It also acts as a transcriptional modulator and initiates a sequence of biochemical events leading to long-term adaptive changes in the neurons, such as participating in plastic changes.[20-22] This indicates that ketamine induces CNS activation and also some long-lasting postanesthetic effects.

In addition to a generalized depressant action, halothane has an inhibitory action by increasing Cl^- influx at the GABA receptor complex[23,24] and facilitates diazepam binding to its receptor complex.[25] Thus we might expect that halothane inhibits NMDA antagonist-induced psychological and neurologic side effects.

The primary purpose of this study was to assess the interaction of halothane and diazepam with ketamine in the cingulate cortex, which is a possible cortical representation of the psychotomimetic action of ketamine.[10,13] The cingulate cortex belongs to the limbic structures,[26] and thus the drugs' effects on the neocortex and thalamus were studied comparatively.

Materials and Methods

The study was approved by the Animal Research Committee of the Kyoto University Faculty of Medicine. All experiments were performed on male Wistar rats weighing 280 to 330 g. Fifty rats were used. Forty rats were randomly assigned to eight groups, groups 1 to 8, and an additional ten rats were assigned to two groups, groups 9 and 10.

In group 1 (control for groups 2 and 3; n = 5), the rats received the vehicle of diazepam (0.015 ml benzyl alcohol, 0.4 ml propylene glycol, 0.1 ml ethanol, and 42.8 mg benzoic acid in 1 ml water) subcutaneously followed 7 min later by 100 mg/kg of ketamine given intraperitoneally.

In group 2 (n = 5), the rats received 1 mg/kg diazepam subcutaneously followed 7 min later by 100 mg/kg ketamine given intraperitoneally.

In group 3 (n = 5), the rats received 5 mg/kg diazepam subcutaneously, followed 7 min later by 100 mg/kg ketamine given intraperitoneally.

In group 4 (control for groups 5 and 6; n = 5), the rats received 100 mg/kg ketamine intraperitoneally.

In group 5 (n = 5), the rats were first anesthetized with 3% halothane in 50% nitrogen and 50% oxygen in a rectangular plastic box for 3 min and transferred to a plastic cylinder (7 cm × 30 cm) continuously insufflated with 1% halothane in 50% nitrogen and 50% oxygen. Ten minutes later, the rats received 100 mg/kg ketamine intraperitoneally.

In group 6 (n = 5), the experimental condition was the same as in group 5, but the halothane concentration was maintained at 1.8%.

In group 7 (n = 5), the rats received 5 mg/kg diazepam subcutaneously.

In group 8 (n = 5), the experimental condition was the same as in group 6, but the rats received equal volumes of saline instead of ketamine. Rats in groups 1 to 4 and 7 were placed in cages with sawdust bedding, and those in groups 5, 6, and 8 were placed in a plastic cylinder insufflated with halothane until brain fixation.

In group 9 (n = 5), rats were first anesthetized with 3% sevoflurane in 60% nitrous oxide and 40% oxygen. The right femoral artery was cannulated to measure arterial blood pressure and to sample blood for gas analysis. The tail vein was cannulated to administer fluids and drugs. Tracheostomy was performed and mechanical ventilation was instituted using an animal respirator (SN-480-7, Shinano, Tokyo) and with the aid of pancuronium. End-tidal carbon dioxide was maintained at 30 to 35 mmHg throughout the experiment. After cessation of sevoflurane, 20 μg/kg fentanyl was administered; at least 40 min later, the rats received 100 mg/kg ketamine intraperitoneally. This dose of fentanyl was administered to reduce the stress of animals under light nitrous oxide-oxygen anesthesia. A pilot study showed that light sevoflurane, isoflurane, and urethane anesthesia all suppressed the ketamine-induced c-Fos expression in the cingulate cortex considerably, and finally a mixture of nitrous oxide and fentanyl of this dose was confirmed to have little effect on c-Fos expression.

In group 10 (n = 5), the experimental condition was

the same as in group 9, but 1.8% halothane was administered continuously from 10 min before the ketamine injection to brain fixation. Mean arterial blood pressure was maintained at more than 110 mmHg with an infusion of phenylephrine in groups 9 and 10. Halothane concentration was monitored continuously using an Anesthetic Gas Monitor (Type 1304; Brüel & Kjær, Denmark). A rectal thermometer was inserted and the animals' temperature was maintained at 37 to 38°C using a warm-water mattress and a heating lamp.

Tissue Preparation

Two hours later, the rats were deeply anesthetized with 100 mg/kg pentobarbital given intraperitoneally. They were perfused transcardially, initially with ice-cold 0.01 M phosphate-buffered saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4) and subsequently with a fixative solution containing 4% paraformaldehyde, 0.2% picric acid, and 0.35% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was quickly removed from the skull and immersed for 1 day in a postfixative solution containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer at 4°C. The brain was placed in 0.1 M phosphate buffer containing 15% sucrose and 0.1% sodium azide at least until it sank. The brain was frozen and cut into 20-μm-thick coronal sections in a cryostat. The sections were immersed in 0.1 M phosphate-buffered saline at 4°C. Thirty coronal sections per animal were made at the plane of approximately interaural 6.2 mm.[27] From these sections, three sections were selected at every five sections and subjected to the following immunohistochemical procedure.

Immunohistochemical Procedure

The sections were incubated with a polyclonal anti-c-Fos antibody (Oncogene Science, Uniondale, NY) at a dilution of 1:1,000 in 0.1 M PBST (0.1 M phosphate-buffered saline containing 0.3% Triton X-100) at 4°C for 4 days. The sections were washed three times with 0.1 M PBST, 10 min per wash, and incubated with biotinylated anti-rabbit antibody (1:1,000 dilution in 0.1 M PBST; Vector Laboratories, Burlingam, CA) at room temperature for 1.5 h. After washing, the sections were incubated with an avidin-biotin-peroxidase complex (1:800 dilution in 0.1 M PBST; Vector Laboratories) as described by Hsu and coworkers[28] at room temperature for 1.5 h. The sections were reacted with a solution containing 0.0045% H_2O_2, 0.02% 3,3′-diaminobenzidine 4HCl, and 0.3% nickel ammonium sulfate in 0.05 M Tris-HCl, pH 7.6, at room temperature for 5 min. Immunohistochemically detected nuclear-associated reaction product was referred to as c-Fos-like immunoreactivity (c-Fos-LI). To confirm the specificity of immunostaining, some sections were incubated with anti-c-Fos antibody preabsorbed with an excess of the peptide against which the antibody was raised and confirmed to yield no cellular-specific reaction product.

Image Analysis

C-Fos expression was quantified with respect to the number of c-Fos-LI-positive boutons in approximately the same three brain regions (retrosplenial cortex as a representative of cingulate cortex, paraventricular thalamic nuclei as representatives of the thalamus, and parietal cortex as a representative of the neocortex) per section using a computer-assisted image analyzer (Nexus 6400, Osaka, Japan) attached to a light microscope at 40 times magnification and a high-resolution color video camera, as described previously.[29] The images of purple-blue-stained c-Fos-LI-positive boutons were transferred to a collar monitor screen through the light microscope. An analysis field (0.894 mm^2) was fixed on the screen. The image was digitized semiautomatically, depending on the intensity of positive color staining, by gray brightness level ranging from 0 to 256. An appropriate gray-level threshold was chosen to selectively label the positive boutons. Usually the threshold was set on the lightest stained nucleus in each section. "Ignored minimum area" of the positive staining was first set to exclude stain of dust (in the present study, 2 μm^2). Minor errors such as pseudopositive dots, which were apparently not nuclei (*i.e.*, those that were irregularly shaped, and those that were too large), and overlapping boutons, were all manually corrected using the erasing function of the analyzer.

Data Analysis

We counted the c-Fos-LI-positive boutons of the three sections per animal, the mean of which represented the number for the individual animals. The number of c-Fos-LI-positive boutons of each group was expressed as mean ± SEM (n = 5 in each group). Statistical comparisons among groups 1 to 3 and 4 to 6 were performed by one-way analysis of variance, followed by independent Bonferroni modification of the *t* test. Statistical comparisons between the two control

groups (1 and 4), and between groups 9 and 10 were made using the unpaired *t* test.

Statistical comparison of physiologic variables between groups 9 and 10 was made by unpaired t test.

Differences at $P < 0.05$ were considered statistically significant.

Results

After 100 mg/kg ketamine given intraperitoneally, rats became quiet and lay down within 5 min. After 1 mg/kg diazepam given subcutaneously, rats became quiet and moved slower, and after 5 mg/kg all rats lay down within 7 min. Ketamine (100 mg/kg given intraperitoneally) induced marked c-Fos-LI in the cingulate cortex (figs. 1a, 3a), thalamus, and the neocortex. Diazepam reduced the expression of the c-Fos-LI in the cingulate cortex in a dose-dependent manner (fig. 1). The computer-assisted count of the c-Fos-LI-positive boutons in the control cingulate cortex (group 1) was 270 ± 21, which was reduced by pretreatment with 1 mg/kg and 5 mg/kg diazepam to 75 ± 15 and 18 ± 5, respectively ($P < 0.001$; fig. 2). In the thalamus and neocortex, both 1 mg/kg and 5 mg/kg diazepam significantly reduced the number of c-Fos-LI-positive boutons from the control values ($P < 0.05$). These reductions, however, were not related to the dose of diazepam (fig. 2). The number of c-Fos-LI-positive boutons in group 1 (control for the diazepam experiments) was slightly greater than that in group 4 (control for the halothane experiments) in all areas we observed (270 ± 21 *vs.* 202 ± 23 in cingulate cortex, 268 ± 39 *vs.* 238 ± 20 in thalamus, 307 ± 33 *vs.* 228 ± 14 in neocortex), which may be due to the pain induced by the vehicle injection in group 1. However, there were no significant differences among them. Halothane also significantly reduced the number of c-Fos-LI-positive boutons in a dose-dependent manner, not only in the cingulate cortex (fig. 3) but also in the neocortex. The computer-assisted count of the c-Fos-LI-positive boutons in the control cingulate cortex (group 4) was 202 ± 23 and was reduced by 1% halothane and 1.8% halothane to 111 ± 28 ($P < 0.05$) and 40 ± 11 ($P < 0.001$), respectively (fig. 4). In the neocortex, the computer-assisted count of the c-Fos-LI-positive boutons in controls was 228 ± 14 and was reduced by 1% halothane and 1.8% halothane to 117 ± 28 ($P < 0.01$) and 32 ± 6 ($P < 0.001$), respectively (fig. 4). In contrast, halothane did not reduce the number of c-Fos-LI-positive boutons significantly in the thalamus (fig. 4). Figures 5 and 6 show the c-Fos-LI of group 7 and 8. The computer-assisted counts of the c-Fos-LI-positive boutons of group 7, in the cingulate cortex, thalamus, and the neocortex, were 0, 17 ± 3, and 0, respectively. The computer-assisted counts of the c-Fos-LI-positive boutons of group 8, in the cingulate cortex, thalamus, and the neocortex, were 0, 58 ± 12, and 6 ± 2, respectively. The computer-assisted counts of the c-Fos-LI-positive boutons of group 9 in the cingulate cortex was 181 ± 8, and was reduced by 1.8% halothane to 0 ($P < 0.001$).

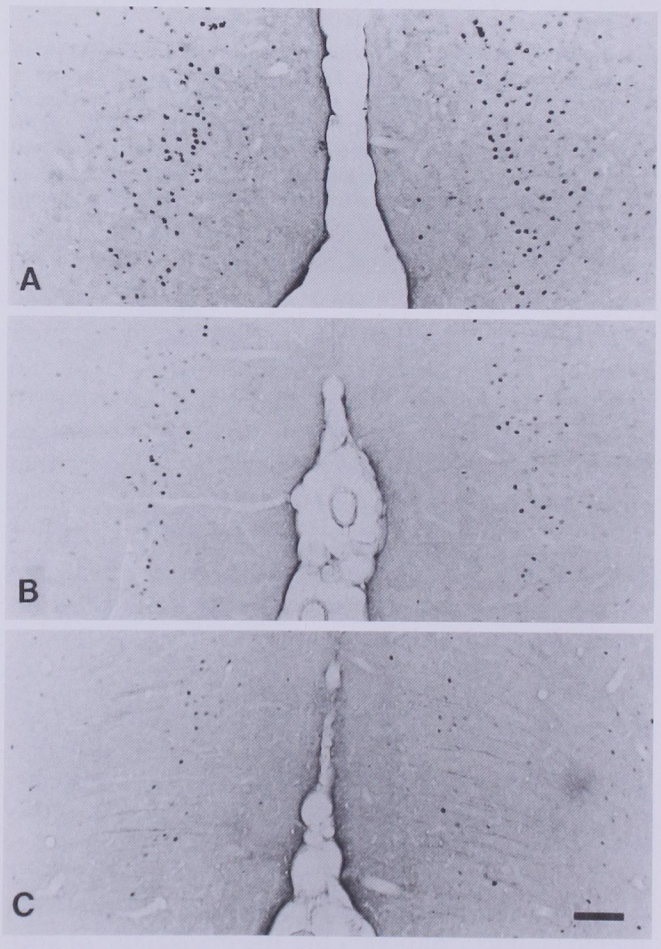

Fig. 1. Photomicrographs showing c-Fos–LI in rat cingulate cortex 2 h after intraperitoneal administration of 100 mg/kg ketamine. (*A*) Vehicle pretreated 7 min before ketamine injection (group 1). (*B*) Diazepam (1 mg/kg) pretreated 7 min before ketamine injection (group 2). (*C*) Diazepam (5 mg/kg) pretreated 7 min before ketamine injection (group 3). Scale bar = 100 μm.

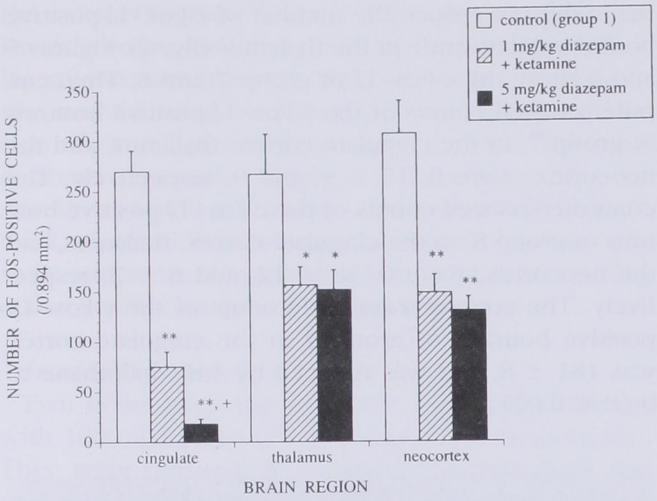

Fig. 2. Effects of diazepam on ketamine-induced c-Fos–LI-positive boutons in the rat brain. Data are expressed as means ± SEM; n = 5. *$P < 0.05$, **$P < 0.001$: Significant difference in control group (group 1) compared with the 1 mg/kg diazepam-pretreated (group 2) and the 5 mg/kg diazepam-pretreated (group 3) group. +$P < 0.05$: Significant difference in 1 mg/kg diazepam-pretreated group compared with 5 mg/kg diazepam-pretreated group.

Mean arterial blood pressures 30 min and 2 h after ketamine administration were 122 ± 10 and 124 ± 6 in group 9, and 116 ± 7 and 117 ± 6 in group 10 (mean ± SD), respectively. Blood gas analysis of groups 9 and 10 revealed arterial pH and Pa_{CO_2} were within physiologic range (pH: 7.41 ± 0.06 *vs.* 7.45 ± 0.08; Pa_{CO_2}: 34 ± 7 mmHg *vs.* 33 ± 5 mmHg; Pa_{O_2}: 141 ± 21 mmHg *vs.* 147 ± 20 mmHg; means ± SD). There were no significant differences in these variables between groups 9 and 10.

Discussion

This study confirmed our hypothesis that halothane, as well as diazepam, suppressed ketamine-induced c-Fos expression in the cingulate cortex.

The brain was fixed 2 h after administration of ketamine. Two hours is the maximum c-Fos-LI expression after ketamine.[14] Although the 100-mg/kg dose of ketamine was supraclinical, it was chosen to detect the most marked c-Fos-LI expression.[14] We did not monitor arterial blood pressure, Pa_{CO_2}, and Pa_{O_2} in groups 1 to 8, because these measurements require surgical manipulations, such as skin incision and tracheotomy, which are noxious and require anesthesia. It has been reported that not only noxious stimulation[30,31] but also anesthetics[32] induce c-Fos expression. Therefore, we confirmed that neither diazepam nor halothane induced c-Fos-LI in the cingulate cortex (figs. 5 and 6). In groups 9 and 10, however, to eliminate the effects of hemodynamic and respiratory changes on c-Fos-LI expression, we monitored and maintained these variables. The result was the same as for groups 4 to 6: Halothane reduced c-Fos-LI expression in the cingulate cortex. We assessed the c-Fos-LI expression only in the cingulate cortex in groups 9 and 10, because

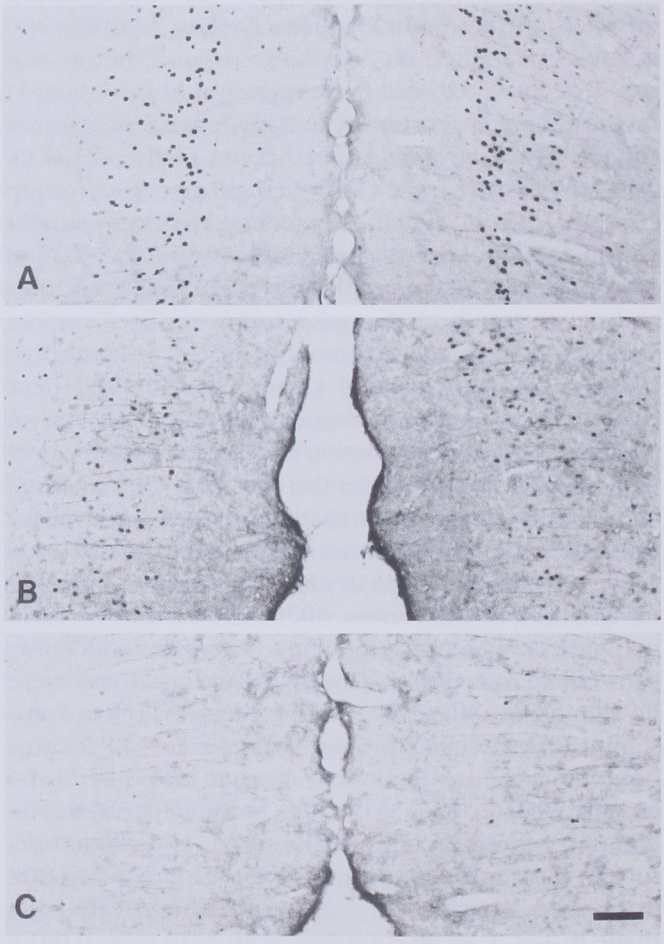

Fig. 3. Photomicrographs showing c-Fos–LI in rat cingulate cortex 2 h after 100 mg/kg ketamine was give intraperitoneally. (*A*) 100 mg/kg ketamine injection (group 4). (*B*) 1% halothane insufflated continuously from 10 min before ketamine injection until brain fixation (group 5). (*C*) 1.8% halothane continuously insufflated from 10 min before ketamine injection until brain fixation (group 6). Scale bar = 100 μm.

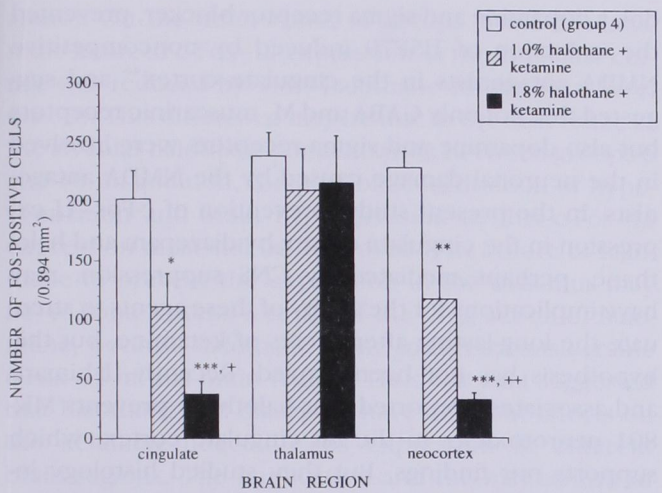

Fig. 4. Effects of halothane on ketamine-induced c-Fos–LI-positive boutons in the rat brain. Data are expressed as means ± SEM; n = 5. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: Significant difference in control group (group 4) compared with 1% halothane-insufflated (group 5) and 1.8% halothane-insufflated (group 6) groups. +$P < 0.05$, ++$P < 0.01$: Significant difference in 1% halothane-insufflated (group 5) compared with 1.8% halothane-insufflated (group 6) groups.

the thematic brain region of this study is the cingulate cortex.

C-Fos is expressed rapidly and transiently in response to various extracellular stimuli[15-17,30,31,33-35] and acts as a "third messenger" molecule in signal transduction systems, where it couples short-term signals elicited by cell-surface stimulation to long-term adaptive modifications by regulating the pattern of gene expression. Extracellular stimuli activate the intracellular signal transduction (*i.e.*, intracellular increases in "second messengers"), including Ca^{2+} influx, which results in transcription activation of c-fos and c-jun.[36-38] Fos and Jun form a heterodimeric protein complex that binds to a nucleotide sequence motif known as the AP-1 binding site and regulates gene transcription.[20,21] In *in vivo* studies, it is expressed after convulsions,[15-17] which are associated with maximum CNS cell activation and cellular damage of various origins, such as hypoxia[34,35] and mechanical injury.[33] Although there is some controversy,[39,40] most researchers agree that it can be used as an index of plastic changes of regional brain functions,[20-22] cell excitation,[18,19] and, in some cases, the process of cell damage.[33-35]

The action of systemic ketamine on the CNS is activation; in large doses it produces generalized electrographic seizures with slight myoclonic jerkings.[3] In contrast, both halothane and diazepam suppress CNS cell activation through nonspecific suppression[41,42] and GABA receptors.[23-25] The marked expression of c-Fos-LI induced in the cingulate cortex by ketamine may reflect CNS activation. Olney and colleagues[10,13] showed that NMDA antagonists, such as phencyclidine, ketamine, and MK801, induced neuronal vacuole formation in the cingulate cortex, and that muscarinic M_1 antagonists or diazepam and barbiturates blocked this neuronal damage. Although the precise mechanism for the NMDA antagonist-induced

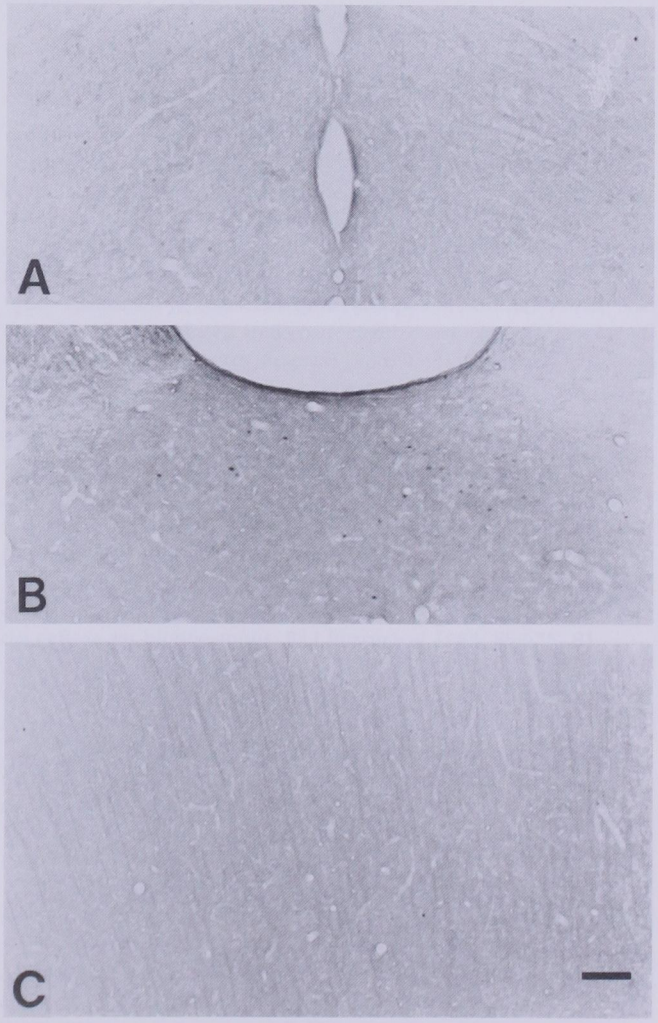

Fig. 5. Photomicrographs showing c-Fos–LI in rat cingulate cortex, thalamus, and neocortex 2 h after 5 mg/kg diazepam was given subcutaneously (group 7). (*A*) Cingulate cortex. (*B*) Thalamus (paraventricular thalamic nuclei). (*C*) Neocortex (parietal cortex). Scale bar = 100 μm.

neuronal damage is unclear, they proposed a wiring diagram to explain the neurotoxicity in the cingulate cortex: The cingulate neuron is glutamatergic with an axon collateral feeding back to an NMDA receptor on a GABAergic neuron to maintain tonic inhibitory control over the release of acetylcholine at an M_1 muscarinic receptor on the cingulate neuron's surface. Blockade of the NMDA receptor abolishes the inhibitory control over acetylcholine release and subjects the cingulate neuron to a state of persistent cholinergic hyperstimulation.[13] Sharp and associates[43-45] showed that haloperidol, a dopamine and sigma receptor blocker, prevented the induction of HSP70 induced by noncompetitive NMDA antagonists in the cingulate cortex[46] and suggested that not only GABA and M_1 muscarinic receptors but also dopamine and sigma receptors were involved in the neuronal damage caused by the NMDA antagonists. In the present study, prevention of c-Fos-LI expression in the cingulate cortex by diazepam and halothane, perhaps mediated by CNS suppression, may have implications for the ability of these agents to attenuate the long-lasting after-effects of ketamine, but this hypothesis has not been proved. Recently, Ishimaru and associates[47] reported that halothane prevents MK-801 neurotoxicity in the rat cingulate cortex, which supports our findings. But they studied histology instead of c-Fos and they used MK-801 instead of ketamine. Furthermore, they neither described the halothane concentration nor studied dose-dependent effects of halothane, and they did not publish the data in which hemodynamic and respiratory variables were monitored and maintained. Olney and colleagues[10,13] also suggested that the cingulate cortex was responsible for the psychotomimetic actions of phencyclidine and ketamine, and they postulated a correlation between the protection of the damage and the prevention of psychotomimetic actions. In contrast, the precise implication and mechanism of ketamine-induced c-Fos expression, except in the cingulate cortex, have not been clarified.

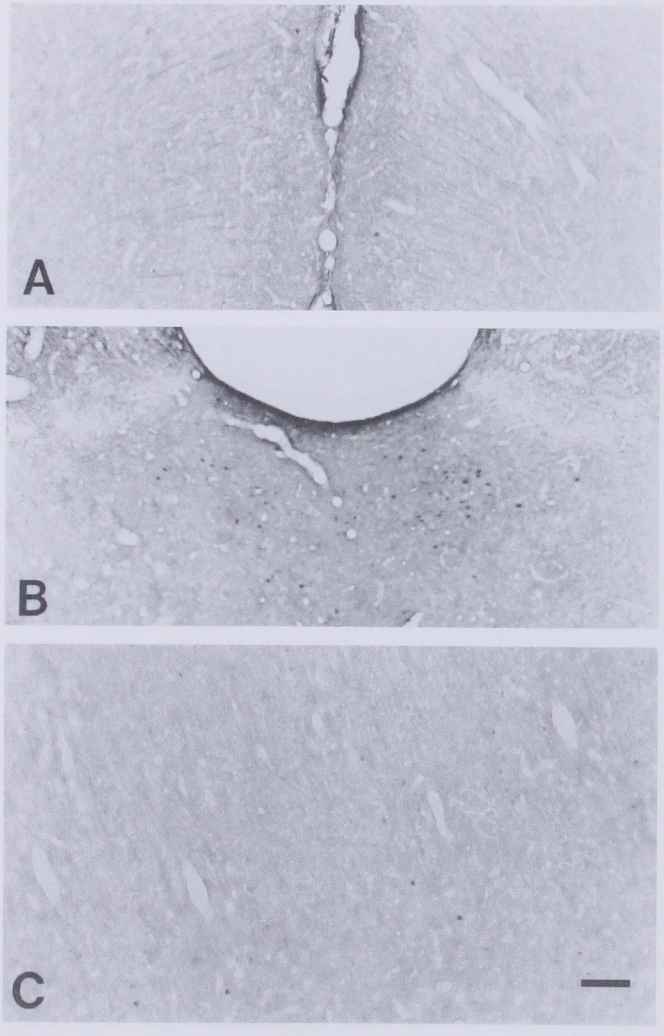

Fig. 6. Photomicrographs showing c-Fos–LI in rat cingulate cortex, thalamus, and neocortex 2 h after continuous insufflation of 1.8% halothane (group 8). (*A*) Cingulate cortex. (*B*) Thalamus (paraventricular thalamic nuclei). (*C*) Neocortex (parietal cortex). Scale bar = 100 μm.

Halothane suppressed the c-Fos-LI expression induced by ketamine in the cingulate cortex, but it did not affect its expression in the thalamus. Although the brain areas involved were divergent by different drugs, Takayama and coworkers[52] recently reported that halothane and other related drugs, such as urethane, α-chloralose, pentobarbital, and fentanyl-midazolam, induced c-Fos expression in various brain regions, including the thalamic nuclei in rats. However, they did not identify the drug effects on the cingulate cortex. We specified the action of halothane: It induced c-Fos-LI in the thalamus and neocortex but not in the cingulate cortex. Furthermore, we observed that diazepam also induced c-Fos-LI in the thalamus but not in the cingulate cortex and neocortex (figs. 5 and 6). In groups 1 to 8, in which hemodynamics and arterial blood gases were not controlled, both diazepam and halothane, at the concentrations used in the current study, did not reduce ketamine-induced c-Fos-LI to the basal level (*i.e.*, the level produced by halothane or diazepam

alone). On the other hand, in groups 9 and 10, ketamine-induced c-Fos-LI expression in the cingulate cortex was reduced by 1.8% halothane to the basal level (*i.e.*, 0). This difference may be due to the difference of the alveolar halothane concentration between groups 6 and 10. In addition, the possible contribution of hypoventilation and brain hypoperfusion to this c-Fos-LI expression must still be disproved. The failure of halothane to prevent the expression in the thalamus may be, to some extent, a result of the drug action of halothane, because halothane alone induced considerable c-Fos-LI in this brain region. Thus our data suggested that halothane and diazepam have different effects on the ketamine-induced c-Fos expression in different brain regions. The implication and the mechanism of c-Fos expression by halothane and diazepam have not been clarified and further studies are needed to clarify these differences.

Our results in experimental animals suggest that halothane and diazepam suppress the effects of ketamine on the cingulate cortex, which is very sensitive to NMDA antagonists and is postulated to be the origin of psychotomimetic actions of NMDA antagonists.

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