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Pharmacology Biochemistry and Behavior, Vol. 64, No. 1, pp. 41–52, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00108-2

Involvement of Cholinergic and GABAergic Systems in the Reversal of Memory Disruption by NS-105, a Cognition Enhancer

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Received 14 August 1998; Revised 19 January 1999; Accepted 15 February 1999

OGASAWARA, T., Y. ITOH, M. TAMURA, T. MUSHIROI, Y. UKAI, M. KISE AND K. KIMURA. *Involvement of cholinergic and GABAergic systems in the reversal of memory disruption by NS-105, a cognition enhancer.* PHARMACOL BIOCHEM BEHAV **64**(1) 41–52, 1999.—The effects of (+)-5-oxo-D-prolinepiperidinamide monohydrate (NS-105) on the scopolamine-, electrolytic lesion of the nucleus basalis magnocellularis (NBM)-, AF64A-, baclofen-, cerebral ischemia- and electroconvulsive shock (ECS)-induced memory disruption in the passive avoidance response or radial arm maze tasks were investigated in rats. The effects of NS-105 were compared with those of aniracetam, bifemelane, idebenone, and indeloxazine in two tasks of the passive avoidance response. Furthermore, effects of NS-105 on in vivo release of acetylcholine (ACh) in the cerebral cortex, high-affinity choline uptake (HACU) of the cerebral cortex in rats with lesion of NBM, HACU of the hippocampus in rats treated with pentobarbital and activity of choline acetyltransferase (ChAT) of the cerebral cortex in rats with lesion of NBM were examined. NS-105 showed antiamnestic actions in a variety of animal models of cholinergic dysfunction employed in this study. Aniracetam improved memory disruption caused by scopolamine, but bifemelane, idebenone, and indeloxazine did not. NS-105 (10 mg/kg) showed the increase of ACh release from the cerebral cortex and the enhancement of HACU both in the cerebral cortex and hippocampus, but showed no change in activity of ChAT. NS-105 also reversed memory disruption induced by baclofen, a potent $GABA_B$ receptor agonist, but all of reference drugs did not. These results suggest that antiamnestic action of NS-105 is due to the facilitation of cholinergic neuronal activity and the suppression of $GABA_B$ receptor-mediated responses. © 1999 Elsevier Science Inc.

NS-105 Scopolamine NBM AF64A Baclofen Brain ischemia ECS Passive avoidance response Radial arm maze Rat

CHOLINERGIC dysfunction in the central nerve system has been implicated in the pathophysiology of senile dementia (45). Biochemical evidence has shown that NS-105 stimulates central cholinergic activity: this compound reversed the decrease in high-affinity choline uptake (HACU), both in the cerebral cortex after electrolytic lesion of the nucleus basalis magnocellularis (NBM) and in the hippocampus after pentobarbital anesthesia in rats. Besides the cholinergic mechanism, central GABAergic systems have been reported to participate in the process of learning and memory (8,9,46). Baclofen, a potent $GABA_B$ receptor agonist (7), has been reported to interfere with memory consolidation or retention in rodents (9,46) as well as humans, particularly when it was given in combination with anticholinergic drugs (42). Biochemical evidence has shown that repeated administration of NS-105 increases the number of $GABA_B$ receptors implicating the upregulation mechanism, but shows no affinity for $GABA_B$ receptors in the rat cerebral cortex without affecting binding properties for β-adrenergic and 5-hydroxytryptamine$_2$ receptors (44).

To further clarify the possible roles of acetylcholine (ACh) and $GABA_B$ receptor-mediated responses in the antiamnestic actions of NS-105, we examined and compared the effects of NS-105 and reference drugs clinically used as cognition en-

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hancers: aniracetam, bifemelane, idebenone, and indeloxazine, on learning and memory deficits elicited by scopolamine, electric NBM lesion, baclofen, AF64A, cerebral ischemia, or electroconvulsive shock (ECS) using a passive avoidance response and radial arm maze tasks.

METHOD

Animals

In all experiments, 5–13-week-old male Wistar rats (Japan SLC Inc., Hamamatsu, Japan; Charles River Japan Inc., Yokohama, Japan) were used. Rats were housed in a group of five, with free access to food (Clea Japan Inc., Tokyo, Japan), and water in an air-conditioned room maintained at 21–25°C, with a relative humidity of 45–65% and a 12 L:12 D cycle (lights automatically on at 0800 h). Experiments were carried out in accordance with the Guideline for the Care and Use of Laboratory Animals written by the Japanese Pharmacological Society.

Chemicals and Drugs

Chemicals used in the present experiments were as follows: NS-105 [(+) 5-Oxo-D-prolinepiperidinamide monohydrate], aniracetam, bifemelane hydrochloride, idebenone, indeloxazine hydrochloride, and AF64A were synthesized in our laboratories. The following chemicals and drugs were obtained from commercial sources: scopolamine hydrochloride, physostigmine salicylate, ACh chloride, and tetraphenyl boron (Sigma Chemical, St. Louis, MO); (±) baclofen (Funakoshi, Tokyo); methylcellulose (SM-400, Shin-etsu Chemical, Tokyo); pentobarbital sodium (Abbott, Chicago, IL); isopropylhomocholine chloride (Eicom, Kyoto, Japan); sodium-1-decanesulfonate (Tokyo Kasei Kogyo, Tokyo); tetramethylammonium chloride, choline chloride, and TDTA-2Na (Nacalai Tesque, Kyoto); [methyl-^{3}H] choline chloride (2.96 GBq/mol, Amersham, UK); Soluene (Soluene-350, Packard Instrument, Downers Gove, IL); ampicillin sodium-cloxacillin sodium (Viccillin-S, Meiji Seika, Tokyo).

AF64A, the aziridinium specie of AF64, was formed by dissolving AF64 (as the picrate salt) in physiological saline and adjusting the pH to 7.4 with solid $NaHCO_3$. This solution was allowed to stand at room temperature for at least 60 min before injection into animals to achieve maximal conversion of the mustard analog to the aziridinium form. AF64A obtained in this manner was used (13). NS-105 and reference drugs were dissolved or suspended in an appropriate volume of 0.5% methylcellulose in physiological saline and administered orally in a volume of 5 ml/kg. Scopolamine, pentobarbital, and baclofen were dissolved in physiological saline and injected intraperitoneally.

Experiment I: Effect of NS-105 on Scopolamine-Induced Memory Disruption of a Passive Avoidance Response

The passive avoidance response of rats was examined in the step-down type situation, as previously described (10). Briefly, 5-week-old rats were first trained to remain on a rubber platform (15 × 15 × 0.5 cm) placed at a corner of the Skinner box (25 × 30 × 33 cm) that was equipped with an electrifiable grid floor. When the rats were moved away from the safety zone, they were exposed to continuous electroshocks (0.8 mA) from the grid floor. The training session of a passive avoidance response was composed of five consecutive trials. Each trial was carried out every 1 min. The normal reaction of naive animals was to jump back onto the platform at the first and/or second trial. All rats used never stepped down onto the grid floor after third trial, and learned not to step down onto the grid floor until the final trial of training session. However, 10% of these control animals showed lack of accurate response in the retention test. NS-105 was administered orally 1 h before the training (acquisition) session of a passive avoidance response. Scopolamine (0.3 mg/kg) was injected IP 30 min prior to the training session. The retention test was carried out 24 h after training session. In the retention test, the latent period of step-down onto the grid floor was measured up to 180 s.

Experiment II: Effects of NS-105 and Reference Drugs on Scopolamine-Induced Memory Disruption of a Passive Avoidance Response

The passive avoidance response of rats were examined in the step-through type situation, as previously described (1). Briefly, on the day 1, 5-week-old rats were adapted for 5 min to the apparatus that consisted of a dark compartment equipped with an electrifiable grid floor and an illuminated Plexiglas compartment. The two compartments were separated by a solenoid-operated guillotine door. On day 2, the adaptation was followed by a single acquisition trial in which animals were placed in the illuminated compartment, and after 30 s delay, allowed to enter the dark compartment by raising the guillotine door. When the rat moved completely into the dark compartment, the door was closed, and it received a foot shock (1.3 mA) for 3 s through the grid floor, delivered by an electric generator. Immediately after receiving the shock, the rat was removed from the dark compartment and returned to its home cage. The retention test was carried out 24 h after receiving the shock (acquisition trial). It was produced in a manner similar to the acquisition trial, except that the guillotine door did not close when the rat entered the dark compartment and the shock was not applied to the grid floor. The time spent (latency time) without moving into the dark compartment after raising the guillotine door was measured. During the retention test, rats were provided access to the dark compartment for 300 s. NS-105, aniracetam, bifemelane, idebenone, and indeloxazine were administered orally 1, 2, 2, 0.5, and 1 h before the acquisition trial, respectively.

Experiment III: Effect of NS-105 on NBM Lesion-Induced Memory Disruption of a Passive Avoidance Response

Eight- to 9-week-old rats were anesthetized with pentobarbital (50 mg/kg, IP) and fixed on a stereotaxic apparatus. Bilateral electrolytic lesions were made by passing an anodal DC current (1.5 mA, 25 s) through the uninsulated tip (0.5 mm) of a platinum electrode inserted stereotaxically into the NBM (1.2 mm posterior to bregma, 2.6 mm lateral to midline, 6.5 mm below dura matter), according to the atlas of König and Klippel (29). The circuit was completed by attaching a cathode to the wound edge. In the sham-operated rats, the tip of an electrode was inserted 1 mm above the lesion site without delivery of current. After the operation, Viccillin-S (50 mg/kg), an antibiotic was injected IM.

NS-105 was administered orally 1 h before the training session of a passive avoidance response using a step-down type task as described in Experiment I, which was conducted 14 days after the NBM lesion. The retention test was carried out 24 h after the training session. All animals used in the retention test stepped down onto the grid floor after third trial of five consecutive training trials. The latent period of step-down onto the grid floor was measured up to 180 s.

At the end of the experiment, all rats with an NBM lesion were anesthetized with pentobarbital (50 mg/kg, IP) and were perfused with 30 ml of physiological saline followed by 50 ml of 10% formalin through the left cardiac ventricle. The brain was removed and kept in 10% formalin for at least 7 days, and serial 50 μm-thick coronal brain sections were made and stained with cresyl violet to verify the location of the lesioned areas. In the case of incomplete lesions of the bilateral NBM, data from the animal were excluded from the experiments.

Experiment IV: Effect of NS-105 on AF64A-Induced Memory Disruption of a Radial Arm Maze

Ten-week-old rat were anesthetized with pentobarbital (50 mg/kg, IP) and fixed on a stereotaxic apparatus. A sagittal incision was made in the scalp, and two holes were drilled through the skull for placement of the infusion cannulae into the lateral cerebral ventricle (0.7 mm posterior to bregma, 1.6 mm lateral to midline, 4.0 mm below dura matter), according to the atlas of König and Klippel (29). Rats were bilaterally infused with 2.5 nmol of a cholinergic neurotoxic agent, AF64A (i.e., 5 nmol per brain) or physiological saline using an injection cannulae. A total volume of 2.5 μl of solution was infused into each lateral ventricle at a rate of 0.5 μl/min. The injection cannulae was left in place for 2 min after injection to allow for diffusion of the solution into the cerebrospinal fluid. The cannula was then removed, and the scalp was sutured. Three weeks later, training of the radial arm maze task was started. Throughout the training, rats were individually housed with free access to water, but were deprived food and maintained at 80% of free-feeding body weight.

Training of the radial arm maze task was carried out according to the method of Olton et al. (38), with slight modifications. After adapting to the experimental apparatus, the rat received one training trial per day for 14 days. A piece of cheese (25 to 30 mg) as a reward was placed in each food well, and the rat was allowed to choose between them freely for 10 min after the start of the trial. When the rat entered the unselected arm and ate the reward, this selection was considered as a correct choice, while if the rat reentered a previously selected arm, this selection was defined as an error choice. NS-105 was administered orally 1 h before every training trial.

Experiment V: Effects of NS-105 and Reference Drugs on Baclofen-Induced Memory Disruption of a Passive Avoidance Response

The passive avoidance response of 5-week-old rats was examined in the step-through type situation as described in Experiment II. The latent period to enter the dark compartment was measured up to 300 s. NS-105, aniracetam, bifemelane, idebenone, and indeloxazine were administered orally 40, 120, 120, 30, and 60 min before the acquisition trial, respectively. Baclofen (8 mg/kg) was administered IP 10 min before the acquisition trial.

Experiment VI: Effect of NS-105 on Cerebral Ischemia-Induced Memory Disruption of a Passive Avoidance Response

The passive avoidance response of 10-week-old rats were examined in the step-through type situation as well as the method of Experiment II. The transient forebrain ischemia was induced by four-vessel occlusion (41,43). Briefly, rats were anesthetized with pentobarbital (50 mg/kg, IP), and the vertebral arteries were permanently occluded by electrocautery at the first cervical vertebra. Twenty-four hours later, the training session of a passive avoidance response was carried out. NS-105 was administered orally 1 h before the training session. Immediately after the training session, common carotid arteries were occluded for a 5-min period by small, temporary clips (Sugita aneurysm clip, Mizuho Ikakogyo, Tokyo). After that, the clips were removed, free flow through common carotid arteries was ascertained by microscopic examination, and wounds were closed. Animals were returned to their cages. The retention test of a passive avoidance response was carried out 24 h after training session.

Experiment VII: Effect of NS-105 on Cerebral Ischemia-Induced Memory Disruption of a Radial Arm Maze

The transient forebrain ischemia was induced by four-vessel occlusion (41,43). Briefly, 10-week-old rats were anesthetized with pentobarbital (50 mg/kg, IP), and the vertebral arteries were permanently occluded by electrocautery at the first cervical vertebra. On the next day, common carotid arteries were occluded for a 15-min period by the identical method for Experiment VI. The sham-operated rats were subjected to no occlusion of the vertebral arteries and common carotid arteries. Three weeks later, training of the radial arm maze task as previously described was started with slight modifications. Throughout the training periods, rats were individually housed with free access to water, but were food deprived and maintained at 80% of free-feeding body weight. In this task, a 10-s delay was imposed between consumption of first four and second four rewards. Each rat was underwent two trials (1 session) per day for 14 days. NS-105 was administered orally 1 h before daily session. From the total number of choices until rats ate four rewards after a 10-s interval delay in the two trials, the ability of acquisition was judged.

Experiment VIII: Effect of NS-105 on ECS-Induced Memory Disruption of a Passive Avoidance Response

The passive avoidance response of rats were examined in the step-down type situation. Briefly, 5-week-old rats were trained according to the method of Experiment I. Immediately after the training session, the rats received ECS (45 mA, 2 s) through the ears and were subsequently administered orally with NS-105. Three hours later, their retention test of a passive avoidance response was carried out. The latent periods of step-down onto the grid floor were measured up to 180 s.

Experiment IX: Effect of NS-105 on the Extracellular Concentration of ACh in the Cerebral Cortex of Conscious and Freely Moving Rats

Intracerebral microdialysis. The experiment was performed, as described previously (26). Briefly, 10–12-week-old rats were anesthetized with pentobarbital (50 mg/kg, IP) and placed on a stereotaxic apparatus (Narishige Scientific Instrument, Tokyo). The skull was exposed, and a burr hole was bored to implant a stainless steel guide cannulae. The stereotaxic coordinates for the frontal cortex were 3.0 mm lateral and 3.7 mm anterior to bregma, and 4.0 mm below the surface of dura mater, according to the atlas of Paxinos and Watson (39). The guide cannulae was fixed to the skull using dental cement and anchor screw, and a dummy cannulae was inserted into the guide cannulae. The dummy cannulae was replaced by a microdialysis prove 24 h after surgery. The microdialysis probe used in the present experiment was I-shaped with 3-mm long cellulose membrane tubing (BDP-I-4-03, Eicom, Kyoto, Japan), the lower tip of which was placed 3

mm below the end of the guide cannulae after insertion. Molecular weight cutoff value of the cellulose membrane was approximately 50,000 dalton. Ringer's solution containing 5 μM neostigmine was then perfused at a flow rate of 2 μl/min, using a microinfusion pump (CMA/100, Carnegie Medicine, Stockholm, Sweden). Microdialysates were collected every 20 min into polyethylene microtubes containing an appropriate amount of isopropylhomocholine as the internal standard, 3 h after start of perfusion. At the end of experiment, brains were perfused with 50 ml of 10% formalin-physiological saline solution via carotid artery, and serial 50-μm thick of coronal sections were made and stained with cresyl violet to verify the location of the tip of the dialysis probe.

Determination of ACh. ACh concentration in microdialysates was determined by HPLC with electrochemical detection, according to the method of Damsma et al. (11) with modifications (26). Briefly, the HPLC system was composed of a pump (L-6200, Hitachi, Tokyo) equipped with a degassor (DG-100, Eicom), damper (PL-100, Eicom), sample injector with a 100-μl sampling loop (model 7125, Rheodyne, CA), column system, electrochemical detector (LC-4B, BAS, Osaka, Japan) fitted with a flow cell unit (CB-100, Eicom) with a platinum working electrode, the potential of which was set at 0.45 V vs. an Ag/AgCl reference electrode and a recorder (Chromatopac C-R4A, Shimazu, Kyoto). The column system consisted of a guard column (5.0 × 4.0 mm inside diameter) packed with 5C18 silica resin (AC-ODS, Eicom), a reversed-phase separation column (AC-Gel; 150 × 6.0 mm inside diameter, Eicom), and an enzyme reactor column containing covalently bound acetylcholinesterase and choline oxidase (AC-Enzympak; 5.0 × 4.0 mm inside diameter). The column temperature was 34°C. The mobile phase was 0.1 M sodium phosphate buffer (pH 8.5) containing 0.82 mM sodium-1-decanesulfonate, 0.59 mM tetramethylammonium chloride and 10 μM disodium ethylenediamine tetraacetate. The flow rate was 1.0 ml/min. The amount of ACh was calculated from the ratio of the peak height of ACh to that of isopropylhomocholine.

Experiment X: Effect of NS-105 on the Reduction of the HACU of Synaptosomes From the Cerebral Cortex in NBM-Lesioned Rats

Nine-week-old rats were used. NBM lesion of rats were carried out as well as the method of Experiment III. NS-105 was administered orally 1 h before sacrifice by decapitation. Measurement of HACU was carried out according to the method of Haga and Noda (21). Brains were quickly removed and dissected into the cerebral cortex and other tissues. The cerebral cortex was weighed and homogenized in 10 vol of ice-cold 0.32 M sucrose with a Teflon glass homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min, the pellet discarded and the supernatant recentrifuged at 17,500 × *g* for 20 min to obtain a crude mitochondrial pellet. The resultant pellet was resuspended in the original volume of 0.32 M sucrose, and was then utilized in uptake studies. Aliquots (100 μl; 0.25–0.5 mg of protein) were added in 1.9 ml of a Krebs–Ringer phosphate buffer in polyethylene tubes. [Methyl-^{3}H]choline chloride (80.0 Ci/mmol) was added to the tubes to obtain a final concentration of 0.5 μM equivalent to 0.4 μCi per sample. The Krebs–Ringer phosphate buffer had the following composition expressed in mM: NaCl 126, KCl 4.8, $CaCl_2$ 1.3, Na_2HPO_4 15.8, $MgCl_2$ 1.4, D-glucose 11.1, pH 7.4. Samples were incubated for 10 min at 30°C. The incubation was terminated by adding 50 μl of 0.4 μM choline, and the tubes were transferred to an ice bath. The particulate fraction in each sample was collected by centrifugation at 9000 × *g* for 15 min. The remaining medium was aspirated and the pellets were surface washed twice with 3 ml of saline. After removing the saline, the pellets were digested by adding 0.5 ml of soluene. After digestion was completed, the soluene mixture was transferred to vials containing 10 ml of scintillator fluid and the radioactivity was counted using a liquid scintillation counter (Packard TRI-CARB model 460/4640).

Experiment XI: Effect of NS-105 on the Reduction of the HACU of Synaptosomes From the Hippocampus in Pentobarbital-Treated Rats

Nine-week-old rats were used. Fifteen minutes after NS-105 administration (IP), pentobarbital at a dose of 50 mg/kg was given IP. Thirty minutes later, animals were sacrificed by decapitation. Then brains were quickly removed and dissected into the hippocampus and other tissues. The hippocampus were weighed, and experimental procedures and measurement of HACU were examined as well as the method of Experiment XI.

Experiment XII: Effect of NS-105 on the Reduction of Cholineacetyltransferase (ChAT) Activity of the Cerebral Cortex in NBM-Lesioned Rats

Ten- to 13-week-old rats were used. NBM lesion of rats were carried out as well as the method of Experiment III. NS-105 was administered orally 1 h before sacrifice by decapitation. Measurement of ChAT was carried out according to the method of Fonnum (17). Brains were quickly removed and dissected into the cerebral cortex and other tissues. The cerebral cortex was frozen at −80°C. On the day of the measurement of ChAT activity, the frozen tissue was thawed and weighed, and homogenized in 20 vol of ice-cold Tris-phosphate buffer (pH 7.4) obtained 0.5% Triton X-100 and 10 mM EDTA for 30 s with a Polytoron homogenizer. The homogenate with the incubation mixture was incubated for 20 min at 37°C in polyethylene tubes. The incubation mixture contained (final conc.): 0.2 mM [1-^{14}C]acetyl-CoA (125,000 d.p.m.; New England Nuclear Corp., Boston, MA, 300 mM NaCl, 50 mM sodium phosphate buffer (pH 7.4), 8 mM choline bromide, 20

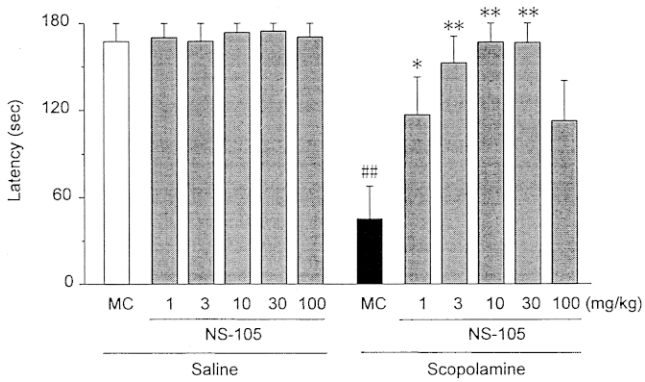

FIG. 1. Effect of NS-105 on the scopolamine-induced disruption of a step-down passive avoidance response in rats. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. Significantly different from control; ##$p < 0.01$ vs. (saline + MC); * $p < 0.05$, ** $p < 0.01$ vs. (scopolamine + MC). MC: 0.5% methylcellulose.

mM EDTA (pH 7.4), and 0.1 mM physostigmine. The incubation was terminated by an ice-cold water bath. After the solution was mixed with a buzzer, it was centrifuged. The radioactivity was counted using a scintillator counter (Pachard TRICARB model 460/4640) after adding an Emulsifier scintillator 299 (Packard, Meriden, CT) in the supernatant.

Statistical Analysis

Data of latency were analyzed by using nonparametric Kruskal–Wallis test followed by a Wilcoxon rank sum test, and other data were analyzed by an ANOVA test followed by Dunnett's test to compare multiple means.

RESULTS

Experiment I: Effect of NS-105 on Scopolamine-Induced Memory Disruption of a Passive Avoidance Response

Results are shown in Fig. 1. In saline-treated rats, NS-105 showed no significant effects on the latency of a passive avoidance response at all doses employed. Scopolamine (0.3 mg/kg)

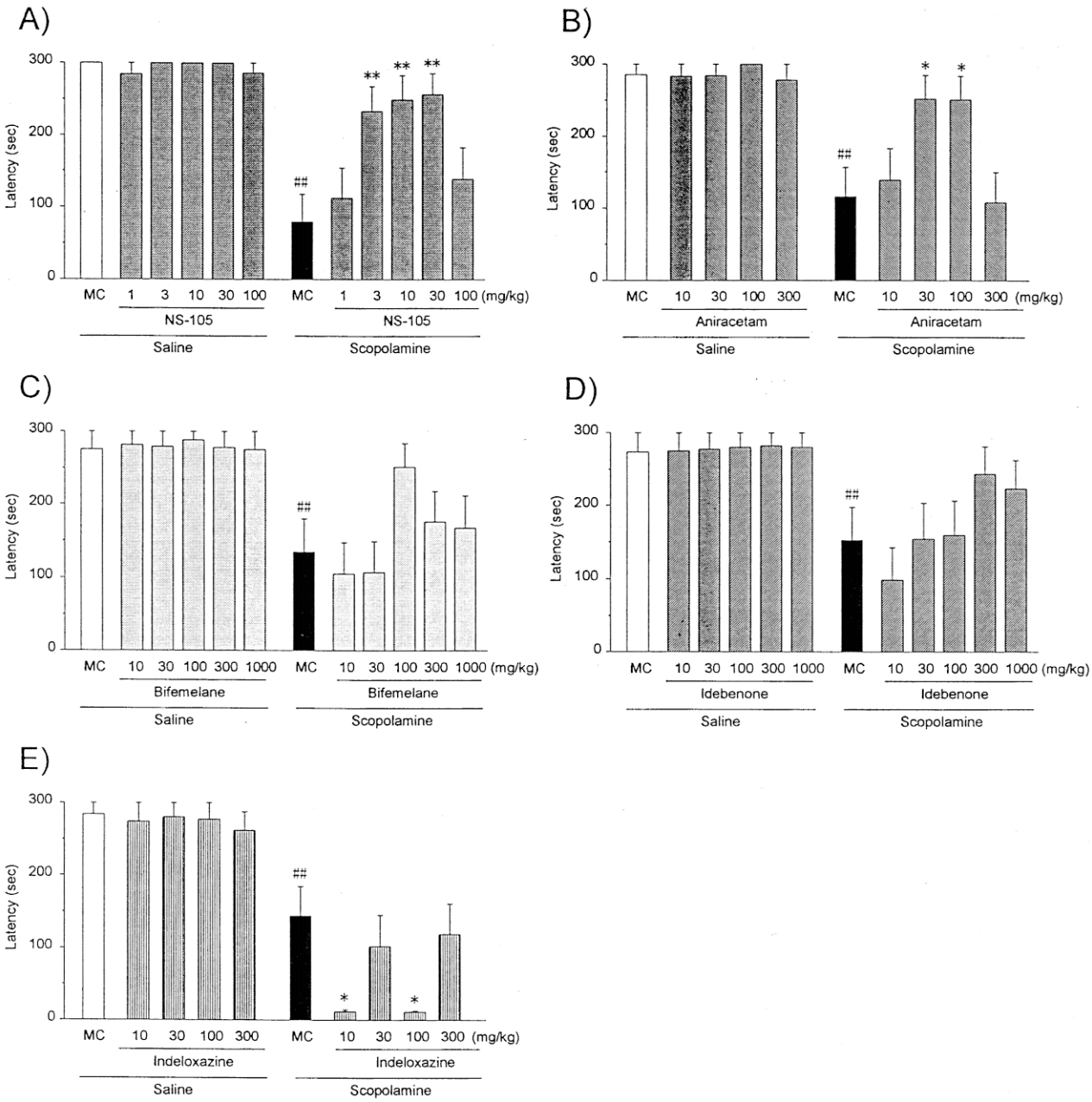

FIG. 2. Effects of NS-105 and reference drugs on the scopolamine-induced disruption of a step-through passive avoidance response in rats. Each value represents the mean ± SE of the latency to enter the dark compartment in a group of 10 animals. For further explanations, see Fig. 1.

produced a marked reduction of the latent period of step-down onto the grid floor. NS-105, at doses of 3–30 mg/kg, prevented the scopolamine-induced memory disruption of a passive avoidance response. The dose–response curve showed a bell shape, with no significant increases of latent periods at the highest dose (100 mg/kg) tested.

Experiment II: Effects of NS-105 and Reference Drugs on Scopolamine-Induced Memory Disruption of a Passive Avoidance Response

Results are shown in Fig. 2. In saline-treated rats, NS-105, aniracetam, bifemelane, idebenone, and indeloxazine showed no significant effect on the memory of a passive avoidance response at any dose employed. Scopolamine (0.3 mg/kg) produced a marked reduction of the latent period until the rat entered the dark compartment. NS-105 (3–30 mg/kg) and aniracetam (30 and 100 mg/kg) prevented the scopolamine-induced memory disruption of a passive avoidance response. However, bifemelane, idebenone, and indeloxazine failed to prevent this at any dose used. The dose–response curve for NS-105 and aniracetam showed bell-shaped relationships, with no significant increase of latent periods at the highest doses (100 mg/kg for NS-105 or 300 mg/kg for aniracetam) tested.

Experiment III: Effect of NS-105 on NBM Lesion-Induced Memory Disruption of a Passive Avoidance Response

Results are shown in Fig. 3. In sham-operated rats, NS-105 showed no effect on the memory of a passive avoidance response at any dose employed. The NBM lesion produced a marked reduction of the latent period of step-down onto the grid floor. NS-105 (10–300 mg/kg) prevented the NBM lesion-induced memory disruption of a passive avoidance response.

Experiment IV: Effect of NS-105 on AF64A-Induced Memory Disruption of a Radial Arm Maze

Results are shown in Fig. 4. In sham-operated rats, the number of correct choices along with repeated training was increased, but AF64A-treated rats failed to increase the number of correct choices for 14 sessions. A significant difference was observed between the sham-operated group and AF64A-treated control group. NS-105 (10–100 mg/kg) reversed memory disruption caused by AF64A.

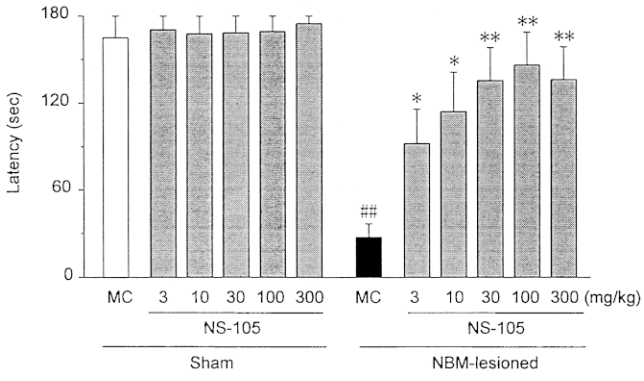

FIG. 3. Effect of NS-105 on the disruption of a step-down passive avoidance response in NBM-lesioned rats. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. Significantly different from control; ## $p < 0.01$ vs. (sham + MC); *$p < 0.05$, **$p < 0.01$ vs. (NBM-lesioned + MC). NBM: nucleus basalis magnocellularis, MC: 0.5% methylcellulose.

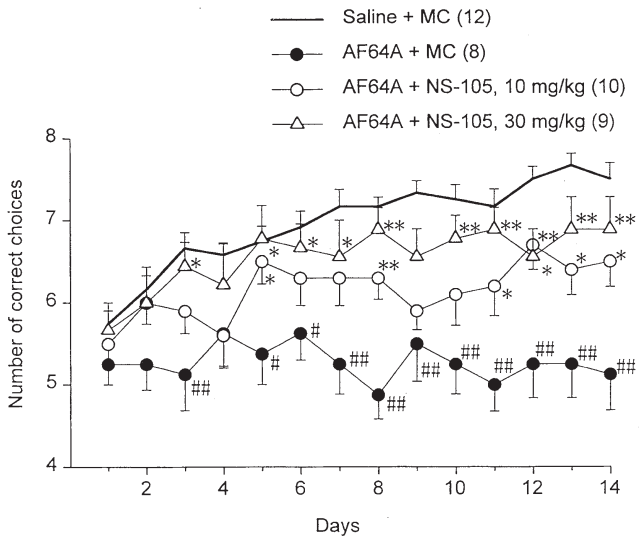

FIG. 4. Effect of NS-105 on the reduction in correct choices induced by AF64A (5 nmol ICV) during the first eight selections in radial arm maze task in rats. Each point represents the mean ± SE of 8 to 12 animals. Numbers in parentheses represent numbers of animals tested. Significantly different from control; # $p < 0.05$, ## $p < 0.01$ vs. (saline + MC); *$p < 0.05$, **$p < 0.01$ vs. (AF64A + MC). MC: 0.5% methylcellulose.

Experiment V: Effects of NS-105 and Reference Drugs on Baclofen-Induced Memory Disruption of a Passive Avoidance Response

Results are shown in Figs. 5 and 6. Baclofen (4 and 8 mg/kg) caused memory disruption of a passive avoidance response. On the basis of these results, baclofen—at a dose of 8 mg/kg—

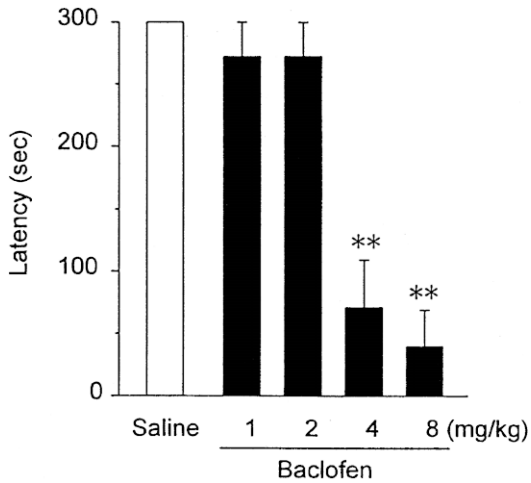

FIG. 5. Effect of baclofen on a step-through passive avoidance response in rats. Each value represents the mean ± SE of the latency to enter the dark compartment in group of 10 animals. Significantly different from control; ** $p < 0.01$ vs. saline.

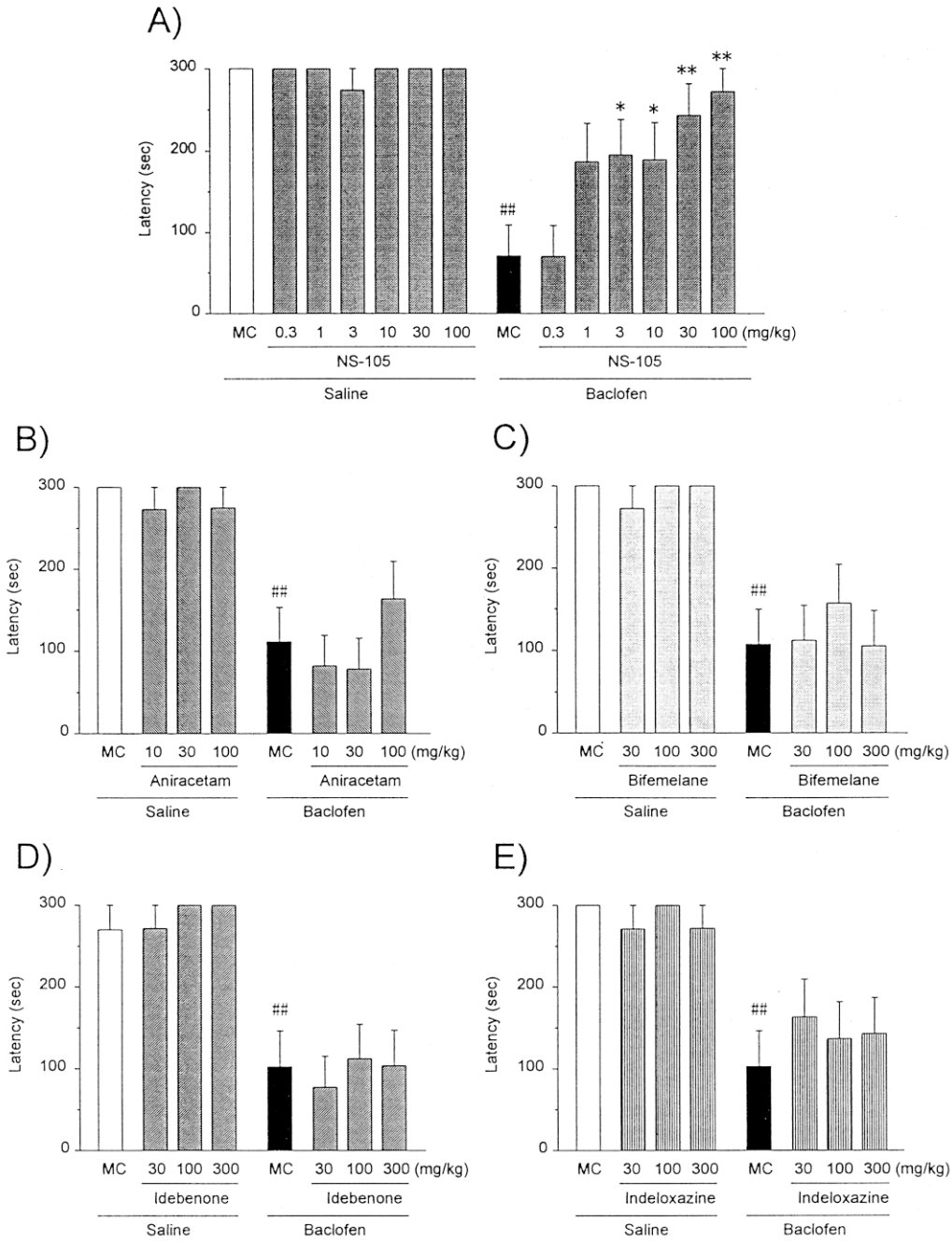

FIG. 6. Effects of NS-105 and reference drugs on the baclofen-induced disruption of a step-through passive avoidance response in rats. Each value represents the mean ± SE of the latency to enter the dark compartment in a group of 10 animals. Significantly different from control; ##$p < 0.01$ vs. (saline + MC); *$p < 0.05$; **$p < 0.01$ vs. (baclofen + MC). MC: 0.5% methylcellulose.

was used for producing the memory disruption in this study. In saline-treated rats, NS-105, aniracetam, bifemelane, idebenone, and indeloxazine showed no significant effects on the memory of a passive avoidance response at all doses employed. NS-105 (1–100 mg/kg) significantly prevented baclofen-induced memory disruption, but aniracetam, bifemelane, idebenone, or indeloxazine at doses of 30–300 mg/kg did not.

Experiment VI: Effect of NS-105 on Cerebral Ischemia-Induced Memory Disruption of a Passive Avoidance Response

Results are shown in Fig. 7. In sham-operated rats, NS-105 showed no effects on the memory of a passive avoidance response at any dose employed. Cerebral ischemia caused memory disruption of a passive avoidance response. NS-105 (3 mg/

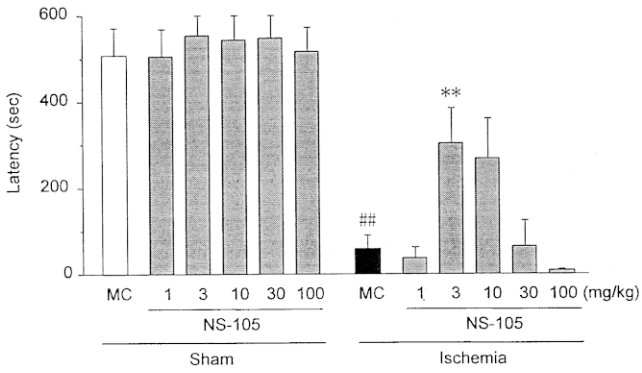

FIG. 7. Effect of NS-105 on the disruption of a step-through passive avoidance response induced by cerebral ischemia in rats. Training and test trials were carried out 24 and 48 h after the insult of the transient cerebral ischemia, respectively. Each value represents the mean ± SE of the latency to enter the dark compartment in a group of 10 animals. Significantly different from control; ## $p < 0.01$ vs. (sham + MC); **$p < 0.01$ vs. (ischemia + MC). MC: 0.5% methylcellulose.

kg) prevented memory disruption induced by cerebral ischemia. NS-105 showed a bell-shaped dose–response curve without causing a significant increase of latent periods at doses of 10–100 mg/kg.

Experiment VII: Effect of NS-105 on Cerebral Ischemia-Induced Memory Disruption of a Radial Arm Maze

Results are shown in Fig. 8. In sham-operated rats, there was a decrease in the mean number of error choices until they ate four rewards in the second trial after a 10-s interval delay. Rats treated with cerebral ischemia showed a decrease in the mean number of error choices. NS-105 (30mg/kg) significantly reversed memory disruption caused by cerebral ischemia.

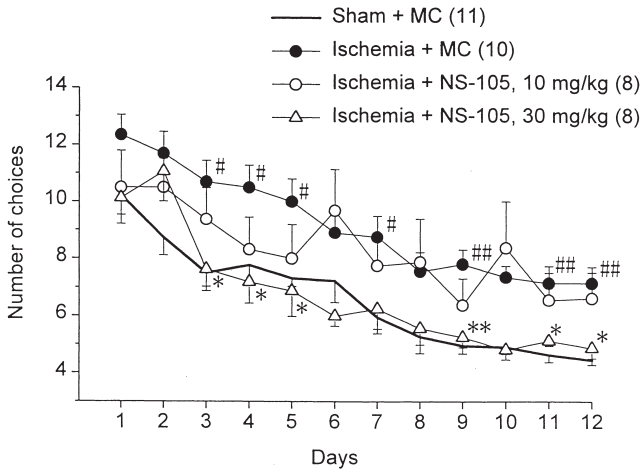

FIG. 8. Effect of NS-105 on the disruption of radial arm maze task treated with cerebral ischemia. Test sessions were started 3 weeks after the insult of the transient cerebral ischemia. Each point represent the mean ± SE of 8 to 11 animals. Numbers in parentheses represent number of animals tested. Significantly different from control; # $p < 0.05$, ## $p < 0.01$ vs. (sham + MC); * $p < 0.05$ ** $p < 0.01$ vs. (ischemia + MC). MC: 0.5% methylcellulose.

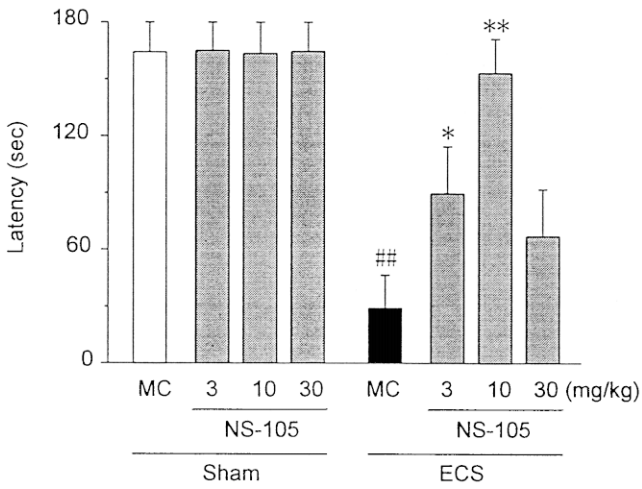

FIG. 9. Effect of NS-105 on the ECS-induced disruption of a step-down passive avoidance response in rats. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. Significantly different from control. ## $p < 0.01$ vs. (sham + MC); * $p < 0.05$, ** $p < 0.01$ vs. (ECS + MC). MC: 0.5% methylcellulose.

Experiment VIII: Effect of NS-105 on ECS-Induced Memory Disruption of a Passive Avoidance Response

Results are shown in Fig. 9. ECS produced memory disruption of a passive avoidance response. NS-105 (3–10 mg/kg) significantly prevented memory disruption induced by ECS. NS-105 showed a bell-shaped dose–response curve without causing a significant increase of latent periods at a dose of 30 mg/kg.

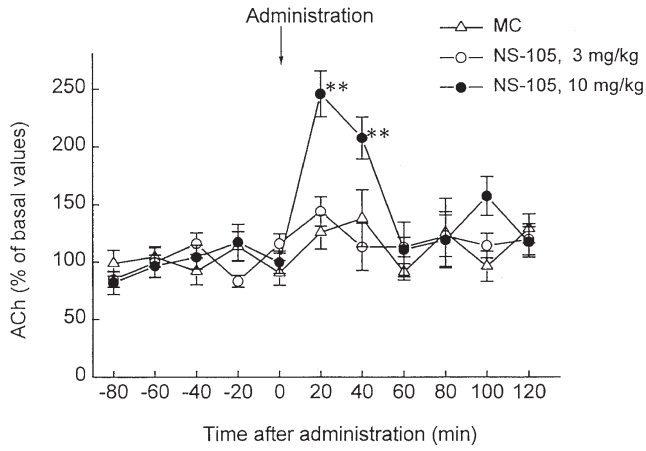

FIG. 10. Effect of NS-105 on the extracellular of ACh in the cerebral cortex of conscious rats as measured by intracerebral microdialysis. In conscious and freely moving rats with prior implantation of stainless steel guide cannula into the cerebral cortex, a microdialysis probe was inserted and Ringer's solution was perfused at a flow rate of 2 ml/min. Microdialysates were collected every 20 min 3 h after start of perfusion. MC or NS-105 (3 or 10 mg/kg) was administered orally. Each point represents the mean ± SE of five to six experiments. Significantly different from mean basal values: ** $p < 0.01$. MC: 0.5% methylcellulose.

Experiment IX: Effect of NS-105 on the Extracellular Concentration of ACh in the Cerebral Cortex of Conscious and Freely Moving Rats

Results are shown in Fig. 10. In conscious and freely moving rats, the basal ACh release from the cerebral cortex measured in the present experiment was almost constant at least for 3 h, and the value was 2.1–2.6 pmol/20 min. NS-105 (10 mg/kg) significantly increased ACh release from the cerebral cortex, although the compound at 3 mg/kg had no significant effect on the ACh release. The maximal effect (245% of basal value) was observed during the first 20 min after injection, and the significant effect lasted for 40 min.

Experiment X: Effect of NS-105 on the Reduction of the HACU of Synaptosomes from the Cerebral Cortex in NBM-Lesioned Rats

Results are shown in Fig. 11. In NBM-lesioned rats, the HACU in the cerebral cortex was significantly reduced. NS-105 (30 mg/kg) restored the reduced HACU significantly.

Experiment XI: Effect of NS-105 on the Reduction of the HACU of Synaptosomes from the Hippocampus in Pentobarbital-Treated Rats

Results are shown in Fig. 12. In pentobarbital-treated rats, the HACU in the hippocampus was significantly reduced. NS-105 (10 mg/kg) restored the reduced HACU significantly.

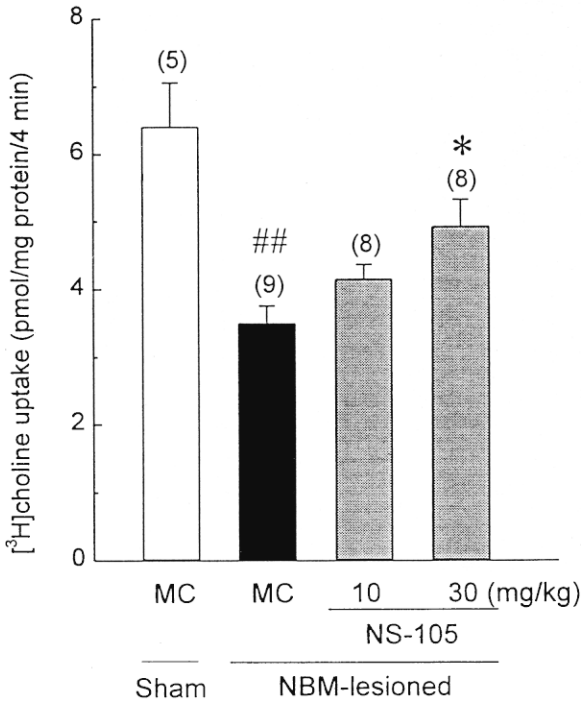

FIG. 11. Effect of NS-105 on the reduction of the HACU of synaptosomes from the cerebral cortex in NBM-lesioned rats. Electrical lesion of bilateral NBM was made, and 2 weeks later the experiment on HACU was conducted. Animals were sacrificed 1 h after oral administration of NS-105 and synaptosomes were prepared from the cerebral cortex. Each value represents the mean ± SE of five to nine experiments. Numbers of experiments are shown in parentheses. Significantly different from control; ## $p < 0.01$ vs. (sham + MC); * $p < 0.05$ vs. (NBM-lesioned + MC). MC: 0.5% methylcellulose.

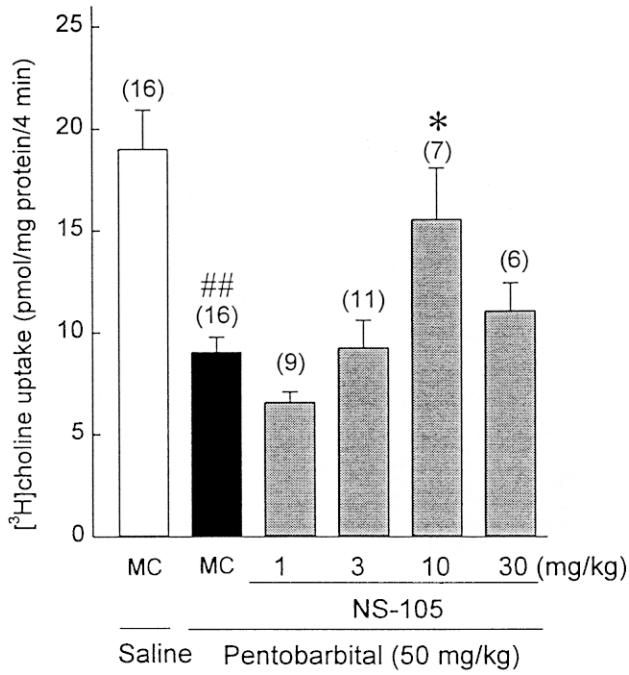

FIG. 12. Effect of NS-105 on the reduction of the HACU of synaptosomes from the hippocampus in pentobarbital treated rats. Rats were sacrificed 30 min after pentobarbital (50 mg/kg, IP) and synaptosomes were prepared from the hippocampus. NS-105 was administered IP 15 min before pentobarbital. Each value represents the mean ± SE of 6 to 16 experiments. Numbers of experiments are shown in parentheses. Significantly different from control; ## $p < 0.01$ vs. (saline + MC); *$p < 0.05$ vs. (pentobarbital-treated + MC). MC: 0.5% methylcellulose.

Experiment XII: Effect of NS-105 on the Reduction of ChAT Activity of the Cerebral Cortex in NBM-Lesioned Rats

Results are shown in Fig. 13. In NBM-treated rats, the ChAT activity in the cerebral cortex was significantly reduced. NS-105 (10–30 mg/kg) did not restored.

DISCUSSION

In the present experiments, NS-105 reversed memory disruption of passive avoidance response or radial arm maze tasks caused by scopolamine (2,10,19,35), or by NBM lesion (3,5,14,16,34), or by AF64A (22,31,32), or by ECS (12,20,30), which produce dysfunction or hypoactivity of the cerebral cholinergic system. NS-105, at doses of 3 to 30 mg/kg, reversed memory disruption induced by scopolamine or NBM lesion. Furthermore, NS-105 (30 mg/kg) reversed memory disruption of the radial arm maze caused by cerebral ischemia, which produces disruption of the cholinergic system (27,28). In the present biochemical experiments, the ACh release measured in the cerebral cortex of conscious and freely moving rats is assumed to depend mostly on the cholinergic neuronal activity. NS-105 produced a significant elevation of ACh release at a dose of 30 mg/kg that reversed memory deficits caused by the dysfunction of central cholinergic neurons. In this study, NS-105 stimulated the central cholinergic activity: this compound reversed the decrease in the HACU, both in the cerebral cortex after an electrolytic lesion of NBM and in the hippocampus after pentobarbital anesthesia in rats

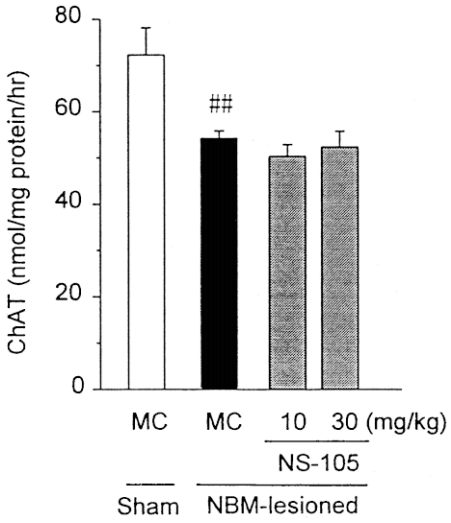

FIG. 13. Effect of NS-105 on the reduction of ChAT activity of the cerebral cortex in NBM-lesioned rats. Electrical lesion of the bilateral NBM was made, and 2 weeks later the experiment on ChAT activity was conducted. Animals were sacrificed 1 h after oral administration of NS-105 and synaptosomes were prepared from the cerebral cortex. Significantly different from control; ## $p < 0.01$ vs. (sham + MC). MC: 0.5% methylcellulose.

without affecting ChAT activity in the cerebral cortex after the NBM lesion. Therefore, it is suggested that NS-105 stimulates the central cholinergic neurons, which may contribute to its antiamnestic actions. A reference drug of NS-105, aniracetam, also reversed scopolamine-induced memory disruption of a PAR, but bifemelane, idebenone, and indeloxazine did not. The antiamnestic effect of aniracetam has been reported to due to the enhancement of cholinergic neurons in the hippocampus (18).

Besides cholinergic mechanisms, central GABAergic systems have been reported to participate in the process of learning and memory (8,9,46). Baclofen, a potent $GABA_B$ agonist (7), has been demonstrated to interfere with memory consolidation or retention in rodents (9,46) as well as in humans, particularly when it was given in combination with anticholinergic drugs (42). $GABA_B$ receptors are negatively coupled with adenylate cyclase via pertussis toxin-sensitive GTP-binding proteins (4, 47, 49). Several lines of evidence have shown that intracellular cycle AMP signal transduction pathway is involved in the processes of learning and memory: stimulation of postsynaptic cyclic AMP pathway facilitates learning and memory (33). Recently, Wu et al. (50) have reported in type I adenylate cyclase mutant mice that these animals show an impairment of spatial memory in the Morris water maze task. In addition, long-term potentiation (LTP) is perturbed in the hippocampal CAl subfield of the mutant mice (50). It has also been demonstrated that the postsynaptic A kinase pathway is important for the induction of LTP (6,15,23,48). Therefore, the inhibition of adenylate cyclase activity may contribute to the baclofen-induced learning and memory disruption. In the present experiment, NS-105 produced a marked reversal of the impairment of a passive avoidance response induced by baclofen. In our recent biochemical study, NS-105 antagonizes baclofen-induced inhibition of adenylate cyclase activity in slices of the rat cerebral cortex (36). Therefore, the behavioral antibaclofen action of NS-105 observed in the present experiment may result, at least in part, from the reversal of suppressive action of baclofen on the intracellular second-messenger systems such as cyclic AMP production. However, it is unlikely that the biochemical antibaclofen action of NS-105 is mediated by the blockade of $GABA_B$ receptors, because this compound has negligible affinity for $GABA_B$ receptors labeled with [^{3}H]GABA in the presence of isoguvacine, a $GABA_A$ receptor ligand (36). We have also found that NS-105 stimulates metabotropic glutamate receptors (mGluRs) to modulate adenylate cyclase activity in membranes of the rat cerebral cortex as well as in primary cultured neurons of fetal mouse cerebral cortex (36,37). Moreover, an mGluRs agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid similarly reverses baclofen-induced inhibition of adenylate cyclase activity (36). Taken together, it is suggested that NS-105 may block baclofen response by stimulating mGluRs in neurons where crosstalk between mGluRs and $GABA_B$ receptors exists.

On the other hand, aniracetam, bifemelane, idebenone, and indeloxazine produced no reversal of the impairment of a passive avoidance response induced by baclofen in this study. Isaacson and Nicoll (24) reported that aniracetam, a drug to enhance glutamate responses (25), reduces glutamate receptor desensitization. Aniracetam may facilitate AMPA receptor transmission directly (40). However, it is not reported that aniracetam affects the $GABA_B$ receptor-mediated responses.

$GABA_B$ receptors have been considered to be implicated in the pathophysiology of depressive illness. We evaluated the antidepressant activity of NS-105 in the forced swim and learned helplessness in rats (44). Orally administered NS-105 (1–100 mg/kg) significantly decreased immobility time in forced swimming, an effect being comparable to that of desipramine. Repeated administration of NS-105 also reversed escape failure in the shuttle-box test in rats with prior exposure to inescapable footshock. Biochemical data showed that repeated administration of NS-105 increased the number of $GABA_B$ receptors in the rat cerebral cortex without affecting properties for β-adrenergic and 5-hydroxytryptamine$_2$ receptors (44). In contrast to other antidepressants, NS-105 did not inhibit monoamine uptake in vitro, nor did it change monoamine concentrations in brain tissues or extracellular fluids (unpublished data). These findings suggest that NS-105 has potent antidepressant activity, in which upregulation of $GABA_B$ receptors after repeated administration may be involved.

In conclusion, antiamnestic action of NS-105 may be due to the facilitation of the cholinergic system and the suppression of $GABA_B$ receptor-mediated responses. Furthermore, repeated treatment with NS-105 shows antidepressant effects induced by upregulation of $GABA_B$ receptors. NS-105, therefore, is a promising drug for clinical use in ameliorating the cognitive impairment in patients with senile dementia of the Alzheimer's and/or cerebrovascular types.

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