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NS-3, a TRH-Analog, Reverses Memory Disruption by Stimulating Cholinergic and Noradrenergic Systems

TAKASHI OGASAWARA, YOSHINORI ITOH, MASARU TAMURA, YOJIRO UKAI,[1] YOSHIAKI YOSHIKUNI AND KIYOSHI KIMURA

Research Laboratories, Nippon Shinyaku Co., Ltd., Nishiohji Hachijo, Minami-ku, Kyoto 601, Japan

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OGASAWARA, T., Y. ITOH, M. TAMURA, Y. UKAI, Y. YOSHIKUNI AND K. KIMURA. *NS-3, a TRH-analog, reverses memory disruption by stimulating cholinergic and noradrenergic systems.* PHARMACOL BIOCHEM BEHAV 53(2) 391-399, 1996.—The effects of a TRH-analog, N[[(3R,6R)-6-methyl-5-oxo-3-thiomorpholinyl]carbonyl]-L-histidyl-L-prolinamide tetrahydrate (NS-3, CG3703, montirelin hydrate) were compared with those of physostigmine on learning and memory disruption in the passive avoidance response (PAR) induced by either electrolytic lesion of the nucleus basalis magnocellularis (NBM) or by treatment with the noradrenergic neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) in rats. a) In NBM-lesioned rats, both NS-3 and physostigmine significantly reversed disruption of memory consolidation examined 15 min after the training session when these drugs were injected IP immediately after the training session. In addition, reversal by NS-3 (0.1 mg/kg) of the disruption of memory was observed even in the retention test conducted 24 h after the training session. b) NS-3 (0.5 mg/kg) significantly reversed the disruption of memory retrieval, when the drug was administered 15 min before the test session. c) DSP4 (50 mg/kg IP) caused memory disruption when the retention tests were conducted between 1 and 48 h after the acquisition session. NS-3 (0.1 mg/kg), but not physostigmine, significantly reversed the disruption of memory induced by DSP4 treatment. These findings suggest that the consistent antiamnestic action of NS-3 is due to the enhancement of both central cholinergic and noradrenergic systems, possibly via facilitation of the release of these transmitters.

NS-3 (CG3703) Amnesia Passive avoidance response Nucleus basalis magnocellularis (NBM) DSP4 Rat

THYROTROPIN-releasing hormone (TRH) containing neurons are highly concentrated in the hypothalamus, nucleus accumbens, septum, and various nuclei of the brain stem (26,42), while TRH binding sites are distributed in the amygdala, septum, and hippocampus in the rat brain (9,43,47).

TRH has been demonstrated to exert a wide variety of neurochemical and behavioral effects independent of its hormonal actions, i.e., TSH or prolactin release (35,55). TRH and its analogs are known to produce antiamnestic actions in rodents (52-54). However, it has some clinical disadvantages, such as the transient action due to the short biological half-life, because the peptide is rapidly metabolized by pyroglutamylaminopeptidase and prolylendopeptidase, and it poorly penetrates the blood-brain barrier (6,25,31).

NS-3 (CG3703, montirelin hydrate) substitues a thiomorpholin for the pyroglutamyl moiety of TRH, thereby conferring resistance to degradation by pyroglutamylaminopeptidases (7,15,16).

Learning and memory processes are markedly disrupted after central cholinergic dysfunction induced either by the administration of centrally acting anticholinergic agents, such as scopolamine (1,10,18), or by lesion of the nucleus basalis magnocellularis (NBM), which projects cholinergic neurons to the cortical areas (12,23,24,37,48), or by lesion of the fimbria-fornix, which contains a large majority of cholinergic inputs to the hippocampus (12,36). The disruption of learning and memory has also been reported to be induced by a reduction of central noradrenergic neuronal activities (4,5,44,50). NS-3 has been shown to reverse scopolamine- or electroconvulsive shock (ECS)-induced memory disruption, possibly by an enhancement of cholinergic neuronal activities (30,41).

Neurochemical evidence has demonstrated that NS-3 and

[1] To whom requests for reprints should be addressed.

TRH increases the in vivo release of ACh and NA in the rat brain as measured by microdialysis (27,28).

To further clarify the possible roles of ACh and NA neurons in the antiamnestic actions of NS-3, we examined and compared the effects of NS-3 and physostigmine on learning and memory deficits elicited by electrolytic NBM lesion or administration of the noradrenergic neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4).

METHOD

Subjects

In all experiments, male Wistar rats (Japan SLC Inc., Shizuoka, Japan) weighing 180–250 g 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.

Procedure for a Passive Avoidance Task

The 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 (PAR) was composed of five consecutive trials. Each trial was carried out every 1 min. All animals used in the present experiment learned not to step down onto the grid floor until the final trial of training session. In the retention test, the latent period of step-down onto the grid floor was measured up to 180 s.

Experiment I: Effects of NS-3 and Physostigmine Administration on NBM Lesion-Induced Disruption of a PAR

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 (32). 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) was injected IM.

At the end of the experiment, all rats with NBM lesion were anesthetized with pentobarbital (50 mg/kg, IP), and were perfused with 30 ml of 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 then sectioned to verify the lesioned areas (Fig. 1).

Pretraining administration of drugs. The test drugs were injected IP 15 min before the training (acquisition) session of a PAR, which was conducted 14 days after the NBM lesion. In the dose-response study of NS-3 and physostigmine, the retention test was conducted 15 min after the acquisition session.

In another set of experiment, to determine the effect of NS-3 on memory retention, the retention test was performed at intervals of 15 min, 6 h, 24 h, and 48 h after the acquisition session. In all experiments, separate groups of rats at each time point were used.

Posttraining administration of drugs. The test drugs were injected IP immediately after the acquisition session of a PAR, which was conducted 14 days after the NBM lesion. In the dose-response study of NS-3 and physostigmine, the retension test was conducted 24 h after the acquisition session.

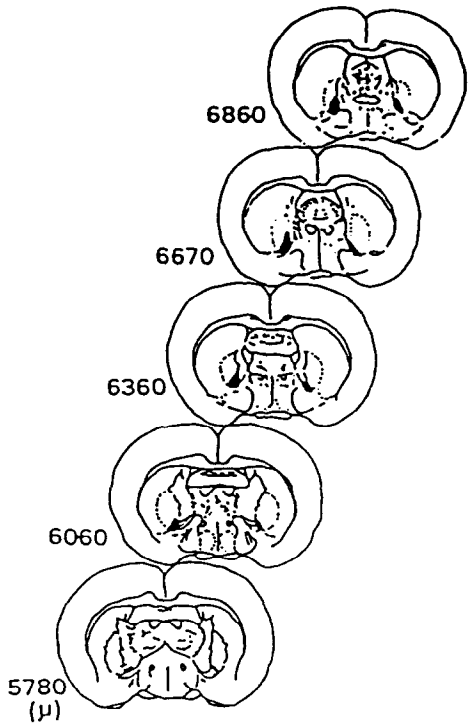

FIG. 1. Schematic drawings of the neuronal degeneration of the nucleus basalis magnocellularis (NBM) in rat. Five coronal sections are presented with numbers referring to distances anterior to the intracranial line according to the atlas of König and Klippel (32). The lesioned area is shown in black.

In another set of experiment, to determine the effect of NS-3 on memory retention, the retension test was performed at intervals of 15 min, 6 h, 24 h, and 48 h after the acquisition session. In all experiments, separate groups of rats at each time point were used.

Pretest administration of NS-3. The acquisition session of a PAR was conducted 14 days after the NBM lesion, followed 48 h later with the retrieval test. NS-3 was injected IP 15 min before the retrieval test.

Determination of sodium-dependent high-affinity choline uptake (SDHACU). In other set of experiment, electrolytic NBM lesion was performed in rats to examine the degree of decrease in cortical cholinergic activity. Animals were killed by decapitation 15 days after the NBM lesion, and SDHACU in the brain was measured according to the method of Haga and Noda (20). The brain was quickly removed and the cerebral cortex and hippocampus dissected according to the method of Glowinski and Iversen (19). Brain tissues were weighed and homogenized in 10 vol of ice-cold 0.32 M sucrose in a Teflon glass homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min. The pellet was then discarded and the supernatant was centrifuged at 17500 × *g* for 20 min to obtain a crude mitochondrial pellet (P_2). The resultant pellet was resuspended in the original volume of 0.32 M sucrose,

and was then utilized in uptake studies. Portions of aliquot (100 μl; 0.25–0.5 mg of protein) were added in 1.9 ml of a Krebs-Ringer phosphate buffer in 5 ml 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. The tubes were incubated for 10 min at 30°C. The incubation was terminated by the addition of 50 μl of 0.4 M choline and the tubes were transferred into an ice bath. The particulate matter in each sample was collected by centrifugation at 9000 × *g* for 15 min. The remaining media were aspirated and the pellets were surface washed twice with 3 ml of saline. After removing the saline, the pellets were digested by the addition of 0.5 ml of Soluene-350 (Packard, USA). After digestion was completed, the Soluene mixture was transferred to vials containing 10 ml of scintillation fluid and radioactivity was counted using a liquid scintillation counter (Packard TRI-CARB model 460/4640).

Experiment II: Effects of NS-3 and Physostigmine Administration on the Disruption of Memory Induced by the Treatment of DSP4

Posttraining administration of drugs. The acquisition session of a PAR was conducted 14 days after the injection of DSP4 (50 mg/kg IP). The retention of a PAR was tested in separate groups of rats at intervals of 15 min, 1 h, 6 h, 24 h, and 48 h after the acquisition session.

In another set of experiment, to determine the effect of NS-3 and physostigmine on the disruption of a PAR, the drugs were injected IP immediately after the acquisition session and retention tested 24 h thereafter.

Determination of Monoamines

In the other set of experiment, rats were injected with DSP4 to examine changes in the contents of brain monoamines. The rats were killed by decapitation 15 days after a single injection of DSP4 and the brain was quickly removed. The cerebral cortex, hippocampus, and brain stem were dissected on ice. Brain tissues were homogenized with more than 10 vol of 0.2 N perchloric acid containing an appropriate amount of 3,4-dihydroxybenzylamine as an internal standard. The monoamine contents were determined by the method of Warnhoff (49), with modifications. Briefly, after centrifugation of the tissue homogenate, a portion of the supernatant was injected directly onto the HPLC.

The potential was set at a 0.6 V vs. an Ag/AgCl reference electrode. The mobile phase was a mixture of 0.1 M citrate-sodium acetate buffer (pH 3.8) containing 0.1 mM EDTA-2Na and 1.3 mM sodium octanesulfonate as an ion-pair reagent, and methanol (90:10, v/v). The flow rate was 1.0 ml/min.

Drugs

The drugs used in the present experiment were NS-3 (Grünenthal GmbH, Aachen, Germany), physostigmine salicylate (Tokyo Kasei, Japan), DSP4 (Sigma, St. Louis, MO), pentobarbital Na (Abbott, Chicago, IL) and antibiotics (Viccillin-S; Meiji Seika, Japan). All drugs were dissolved in an appropriate volume of saline. NS-3, physostigmine and pentobarbital Na were injected IP, and Viccillin-S (50 mg/kg) was injected IM in a volume of 1 ml/kg. DSP4 solution was prepared immediately before use.

Statistical Analysis

Statistical analysis was performed with the two-tailed Student's *t*-test for the means of two groups or with the analysis of variance followed by Dunnett's test to compare multiple means.

RESULTS

Acquisition of a Passive Avoidance Task

In the acquisition session, all rats, regardless of the NBM lesion or DSP4 treatment, jumped back onto the platform at the first or the second trial and never stepped down onto the grid floor at the final trial.

More than 80% of the sham-operated or vehicle-injected rats retained a PAR when tested between 15 min and 48 h after the training session.

Experiment I: Effects of NS-3 and Physostigmine Administration on NBM Lesion-Induced Disruption of a PAR

Effect of NBM lesion on SDHACU in the rat brain. In NBM-lesioned rats, the SDHACU in the cerebral cortex was significantly decreased ($p < 0.01$). The values (pmol/mg protein/4 min) for sham-operated and NBM-lesioned rats in the cerebral cortex were 8.1 ± 0.9 (mean ± SE $n = 12$) and 4.4 ± 0.4 ($n = 17$), respectively. On the other hand, the lesion did not significantly alter SDHACU in the hippocampus. The values for sham-operated and NBM-lesioned rats in the hippocampus were 13.5 ± 1.7 ($n = 13$) and 11.3 ± 1.3 ($n = 17$), respectively.

Pretraining administration of drugs. Results are shown in Figs. 2 and 3. In sham-operated rats, NS-3 and physostigmine showed no significant effects on the memory of a PAR at all doses employed. The NBM lesion produced a marked reduction of the latent period of step-down onto the grid floor.

NS-3 (0.03–0.3 mg/kg) significantly prevented the NBM

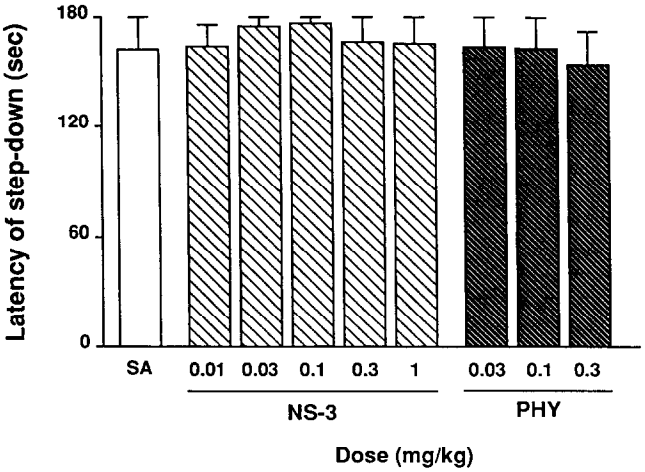

FIG. 2. Effects of NS-3 and physostigmine on the memory of a PAR in sham-operated rats. The test drugs were injected IP 15 min before the acquisition session of a PAR, which was conducted 14 days after sham operation. Fifteen minutes after the acquisition session, the retension test of a PAR was conducted. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. SA: saline, PHY: physostigmine.

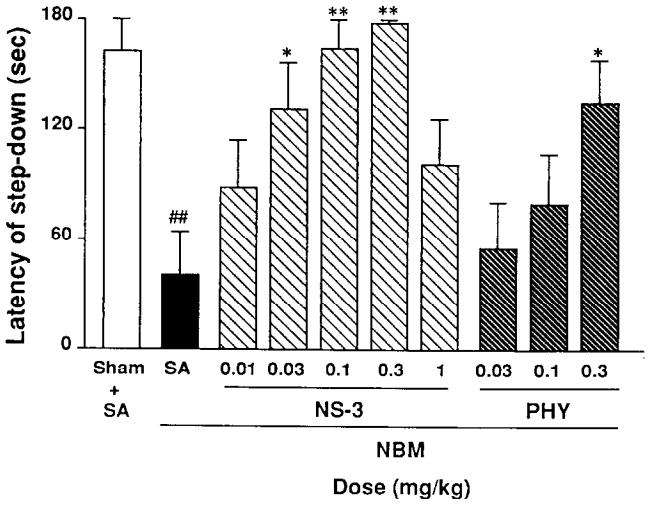

FIG. 3. Effects of NS-3 and physostigmine on the disruption of memory of a PAR in NBM-lesioned rats. The test drugs were injected IP 15 min before the acquisition session of a PAR, which was conducted 14 days after NBM lesion. Fifteen minutes after the acquisition session, the retention test of a PAR was conducted. 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 + SA); *$p < 0.05$, **$p < 0.01$, vs. (NBM + SA) NBM: nucleus basalis magnocellularis lesion, SA: saline, PHY: physostigmine.

lesion-induced memory disruption of a PAR. The dose-response relationship showed a bell shape, with no significant increase at the highest dose (1 mg/kg) tested.

Physostigmine significantly prevented memory disruption of a PAR, although only at a high dose (0.3 mg/kg).

In another set of experiment, results are shown in Figs. 4

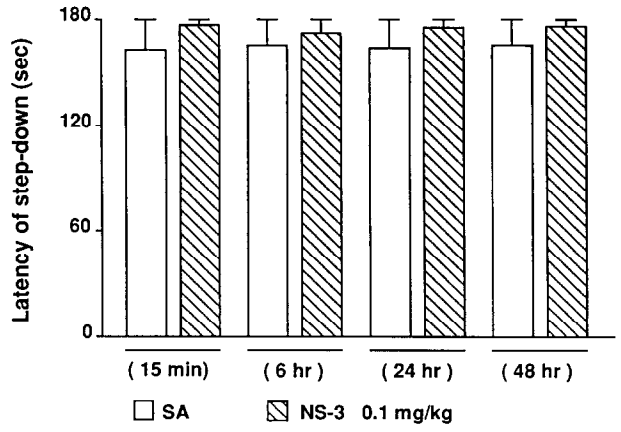

FIG. 4. Effects of NS-3 on the memory of a PAR in the retention test performed at various periods after the acquisition session in sham-operated rats. NS-3 was injected IP 15 min before the acquisition session of a PAR, which was conducted 14 days after sham operation. The retention test of a PAR was conducted 15 min, 6 h, 24 h, and 48 h after the acquisition session. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. Time in parentheses indicates the period between the acquisition and retention test. SA: saline.

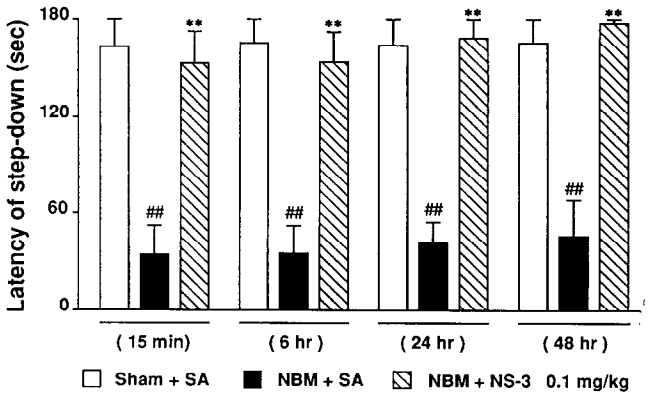

FIG. 5. Effects of NS-3 on the disruption of memory of a PAR in the retention test performed at various periods after the acquisition session in NBM-lesioned rats. NS-3 was injected IP 15 min before the acquisition session of a PAR, which was conducted 14 days after NBM lesion. The retention test of a PAR was conducted 15 min, 6 h, 24 h, and 48 h after the acquisition session. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. Time in parentheses indicates the period between the acquisition and retention test. Significantly different from control; ##$p < 0.01$, vs. (Sham + SA); **$p < 0.01$ vs. (NBM + SA) SA: saline.

and 5. In sham-operated rats, NS-3 (0.1 mg/kg) showed no significant effects on the memory of a PAR, which was conducted at intervals of 15 min, 6 h, 24 h, and 48 h after the acquisition session.

While NS-3 (0.1 mg/kg) prevented memory disruption significantly even when the retention test was performed 48 h after the acquisition session.

Posttraining administration of drugs. Results are shown in Figs. 6 and 7. In sham-operated rats, NS-3 and physostigmine showed no significant effects on the memory of a PAR

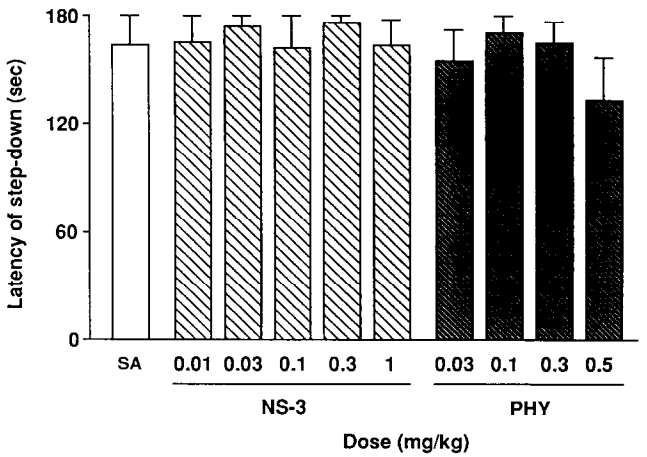

FIG. 6. Effects of NS-3 and physostigmine on the memory of a PAR in sham-operated rats. The acquisition session of a PAR was conducted 14 days after sham operation. The test drugs were injected IP immediately after the acquisition session. Twenty four hours after, they were subjected to the retention test of a PAR. For further explanations, see Fig. 2.

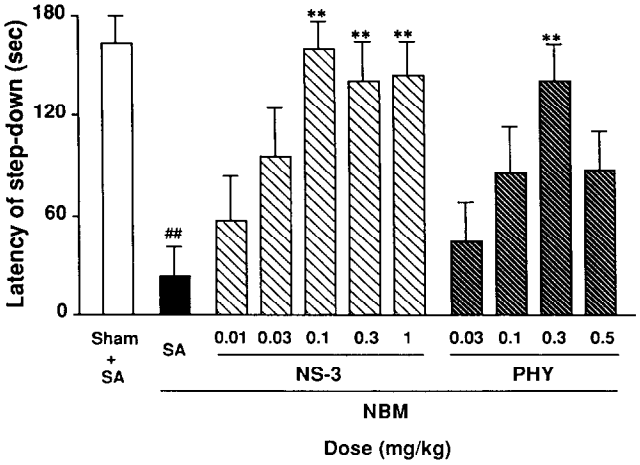

FIG. 7. Effects of NS-3 and physostigmine on the disruption of memory of a PAR in NBM-lesioned rats. The acquisition session of a PAR was conducted 14 days after NBM lesion. The test drugs were injected IP immediately after the acquisition session. Twenty four hours after the acquisition session, they were subjected to the retention test of a PAR. For further explanations, see Fig. 3.

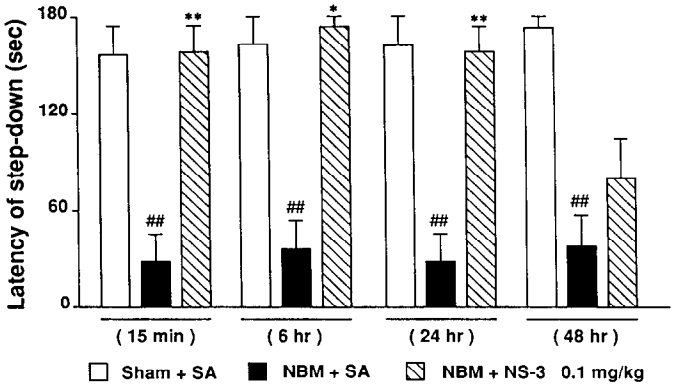

FIG. 9. Effects of NS-3 on the disruption of memory of a PAR in the retention test performed at various periods after the acquisition session in NBM-lesioned rats. The acquisition session of a PAR was conducted 14 days after NBM lesion. Immediately after the acquisition session, NBM-lesioned rats were injected IP with NS-3 and returned to their cages. The retention test of a PAR was conducted 15 min, 6 h, 24 h, and 48 h after the acquisition session. For further explanations, see Fig. 5.

at all doses employed. NS-3 (0.1–1 mg/kg), when administered immediately after the training session, significantly prevented the NBM lesion-induced memory disruption of a PAR.

Physostigmine only at 0.3 mg/kg significantly prevented memory disruption of a PAR. The dose–response relationship of physostigmine showed a bell shape, with no significant increase at 0.5 mg/kg.

In another set of experiment, results are shown in Figs. 8 and 9. In sham-operated rats, NS-3 (0.1 mg/kg) showed no significant effects on the memory of a PAR, which was conducted at intervals of 15 min, 6 h, 24 h, and 48 h after the acquisition session.

While NS-3 (0.1 mg/kg) significantly prevented memory disruption of a PAR that was conducted at intervals between 15 min and 24 h after the acquisition session.

Pretest administration of NS-3. Results are shown in Figs. 10 and 11. In sham-operated rats, NS-3 showed no significant effects on the retrieval of memory of a PAR at all doses employed. The retrieval of memory of a PAR was disrupted significantly in NBM-lesioned rats. NS-3 (0.5 mg/kg) significantly prevented NBM lesion-induced disruption of retrieval of a PAR, although the dose–response relationship was bell shaped, with no significant effect being observed at 1 mg/kg.

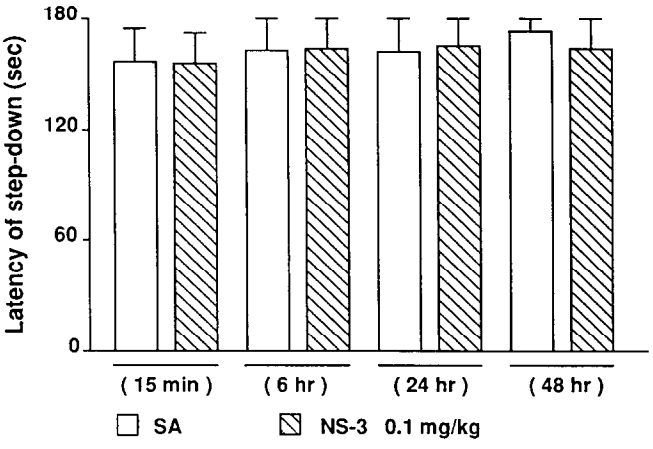

FIG. 8. Effects of NS-3 on the memory of a PAR in the retention test performed at various periods after the acquisition session in sham-operated rats. The acquisition session of a PAR was conducted 14 days after sham operation. Immediately after the acquisition session, sham-operated rats were injected IP with NS-3 and returned to their cages. For further explanations, see Fig. 4.

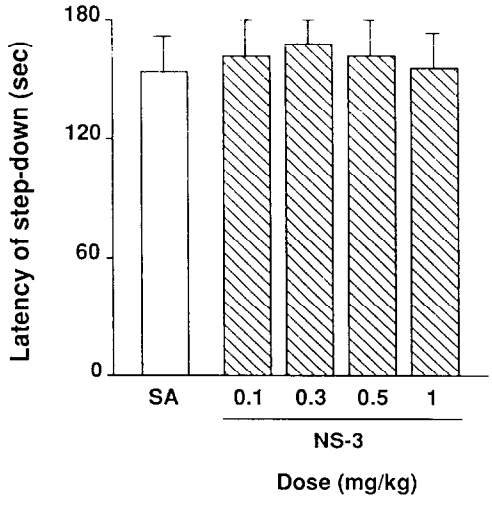

FIG. 10. Effects of NS-3 on the retrieval of memory of a PAR in sham-operated rats. The acquisition session of a PAR was conducted 14 days after sham operation. Forty eight hours after the acquisition session, the retention test was conducted. NS-3 was injected IP 15 min before the retention test. For further explanations, see Fig. 2.

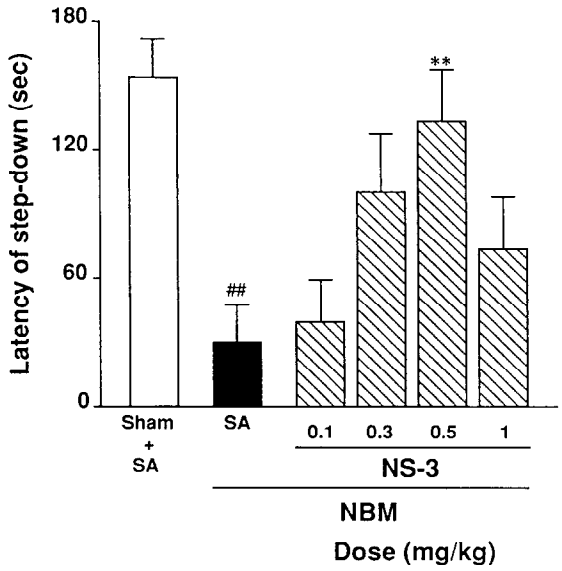

FIG. 11. Effects of NS-3 on the memory disruption of the retrieval of a PAR in NBM-lesioned rats. The acquisition session of a PAR was conducted 14 days after NBM lesion. Forty eight hours after the acquisition session, the retention test was conducted. NS-3 was injected IP 15 min before the retention test. For further explanations, see Fig. 3.

Experiment II: Effects of NS-3 and Physostigmine Administration on the Disruption of Memory Induced by the Treatment of DSP4

Effect of DSP4 treatment on monoamine contents in the rat brain. Results are shown in Table 1. DSP4 treatment markedly reduced the NA level in all regions determined, but the effects were more pronounced in telencephalic regions, such as the cerebral cortex and hippocampus (90.4–96.7% decrease) than in the brain stem (48.6% decrease). On the other hand, DSP4 treatment hardly affected the concentrations of dopamine (DA) and serotonin (5-HT), although it slightly but significantly lowered the 5-HT content in the cerebral cortex.

Posttraining administration of drugs. Results are shown in Fig. 12. When the retention test was conducted between 1 and 48 h after the acquisition session, DSP4 disrupted the memory of a PAR, but not when the interval between the acquisition and retention tests was short (15 min).

NS-3 (0.1 mg/kg) significantly reversed the DSP4-induced disruption of memory retention at 24 h after the acquisition trial, whereas physostigmine (0.3 mg/kg) was without effect (Fig. 13).

DISCUSSION

Electrolytic or chemical lesion of NBM in rats leads to a substantial reduction of choline acetyltransferase activity and SDHACU in the cerebral cortex and a concomitant disruption of memory consolidation (2,8,14,17,29,34,37,51). The electrolytic NBM lesion performed in the present experiment may cause the damage not only to ACh neurons but also to other axons passing through or adjacent to NBM. However, in the present experiment, the impairment of learning and memory induced by the electrolytic NBM lesion was almost completely reversed by physostigmine, thereby indicating that the memory deficit after electrolytic NBM lesion results predominantly from the central cholinergic dysfunction. Electrolytic NBM lesion has been reported to produce a rapid disruption of the memory of a PAR in rats, although they gradually acquired the PAR when they were repeatedly trained, indicating that these rats were capable of acquiring or consolidating the PAR (37). An NBM lesion in rats has also been demonstrated to lead to the disruption of substantial long-term or reference memory rather than short-term or working memory deficit (37,38). SDHACU, which reflects cholinergic neuronal activity, has been reported to be elevated in the frontal cortex of mice 15 min after reference memory testing in an eight-arm radial maze (13). In particular, the NBM–cortical cholinergic neuronal pathway is activated for a longer time following reference memory testing than after working memory procedures (13).

In the present study, the effect of NS-3 on the disruption of memory in NBM-lesioned rats was examined in various drug treatment schedules, such as pretraining, posttraining, and pretest administration, to detect whether acquisition and/or retention processes of memory are affected by this compound.

NBM lesion markedly disrupted the learning and memory of a PAR conducted at intervals between 15 min and 48 h after the acquisition session. NS-3 consistently reversed the disruption of learning and memory of a PAR, regardless of the above treatment regimen. Therefore, NS-3 may reverse the learning and memory deficit by acting throughout the memory process, including the acquisition and retention.

Physostigmine also showed reversal of the disruption of learning and memory in NBM-lesioned rats when it was injected before or immediately after the acquisition session, which is in agreement with the findings reported by other investigators (21,45). The physostigmine-induced reversal of the disruption of learning and memory may solely be due to

TABLE 1

THE CONCENTRATIONS OF NA, 5-HT AND DA IN THE CEREBRAL CORTEX, HIPPOCAMPUS, AND BRAIN STEM 15 DAYS AFTER THE ADMINISTRATION OF DSP4 (50 mg/kg, IP)

		Cerebral Cortex			Hippocampus			Brain Stem		
	N	NA	5-HT	DA	NA	5-HT	DA	NA	5-HT	DA
Control	12	351.2 ± 7.3	421.2 ± 14.6	26.3 ± 1.7	469.1 ± 12.2	401.8 ± 40.8	N.D.	761.3 ± 12.9	687.0 ± 42.3	54.1 ± 0.9
DSP4	6	33.7 ± 6.7*	255.9 ± 14.8*	26.9 ± 1.8	15.7 ± 0.5*	273.2 ± 37.0	N.D.	391.6 ± 11.6*	659.5 ± 19.6	51.5 ± 1.7

Each value represents the mean ± SE expressed in ng/g tissue.
Significantly different from control; *$p < 0.01$ (Student's t-test).
N.D. represents not determined.

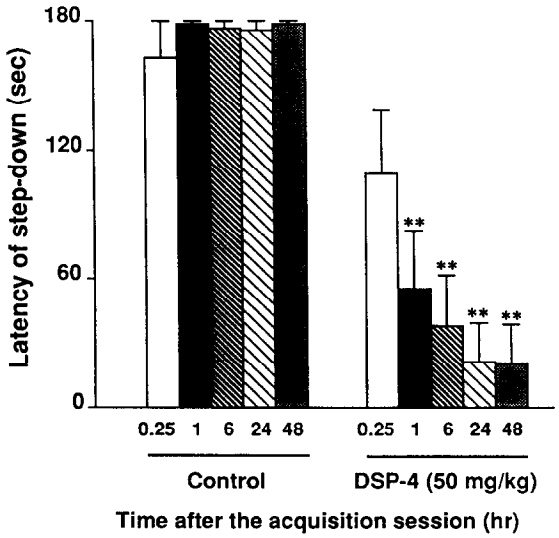

FIG. 12. Time course of the inhibitory effect of DSP4 (50 mg/kg, IP) on the PAR in rats. The acquisition session of a PAR was conducted 14 days after the injection of DSP4. The retention test of a PAR was conducted 15 min, 1 h, 6 h, 24 h, and 48 h after the acquisition session. Each value represents the mean ± SE of the latency of step-down onto the grid floor in a group of 10 animals. Significantly different from control; $^{**}p < 0.01$.

an enhancement of central cholinergic neuronal activity in NBM-lesioned rats.

We recently reported using intracerebral microdialysis that both NS-3 and TRH enhance the ACh release from the cerebral cortex and hippocampus of rats (27). An intracerebral microdialysis study has also shown that both NS-3 and TRH stimulate the release of NA in the rat cerebral cortex via acting on the locus coeruleus (LC) (28), where the cell bodies of noradrenergic neurons projecting to the cerebral cortex are localized (3). In the present experiment, bilateral electrolytic NBM lesions caused only 46% decrease in cortical SDHACU, while systemic DSP4 injection produced a depletion of NA by 49% in the brain stem. Therefore, it is likely that NS-3 facilitates the release of cortical ACh and NA from neurons that were saved from electrolytic NBM lesion or DSP4 treatment, respectively. Such actions of NS-3 may explain its antiamnestic action observed in rats with electrolytic NBM lesion or DSP4 injection.

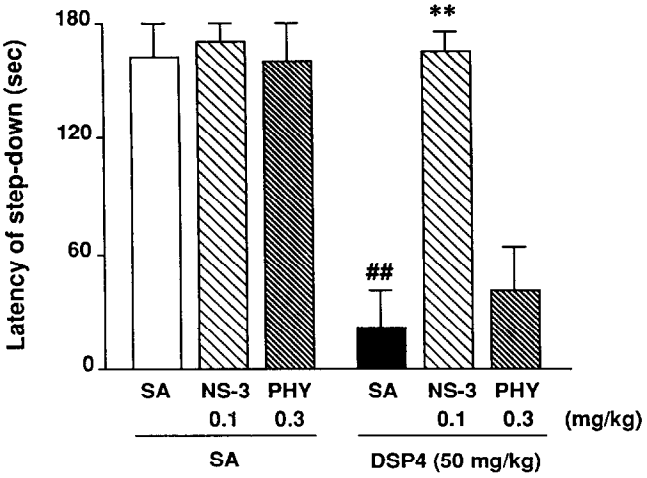

FIG. 13. Effects of NS-3 and physostigmine on the memory of a PAR in saline- or DSP4-treated rats. The acquisition session of a PAR was conducted 14 days after the IP injection of saline or DSP4 (50 mg/kg). Immediately after the acquisition session, saline- or DSP4-treated rats were injected IP with the test drugs and returned to their cages. Twenty four hours after the acquisition session, they were subjected to the retention test of a PAR. 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. (SA + SA); $^{**}p < 0.01$ vs. (SA + DSP4 50 mg/kg) SA: saline, PHY: physostigmine.

Electrophysiological studies have demonstrated that noradrenergic neuronal activity in the LC is proportional to the level of vigilance and attention, thereby suggesting an involvement of LC noradrenergic neurons in learning and memory processes (33,46). Therefore, when injected before the training session, NS-3 may reverse the disruption of the memory processes from the acquisition of learning to its consolidation, partly via activation of LC noradrenergic neurons.

To confirm the possible involvement of noradrenergic neurons in the antiamnestic action of NS-3, we examined its effect on memory disruption caused by DSP4 treatment. Fifteen days after DSP4 (50 mg/kg, IP) injection, NA levels in the cerebral cortex and hippocampus almost completely disappeared, while that in the brain stem was moderately lowered.

When the interval between the acquisition and test sessions was short (15 min), DSP4-treated rats showed no significant disruption of memory of a PAR. However, when the interval was longer, DSP4-treated rats showed a significant time-dependent disruption of memory of a PAR. The present findings are consistent with those reported by Wenk et al. (50), who showed in rats that DSP4 caused no impairment of choice accuracy at short delay, but it impaired the response at long delays in the T-maze paradigm. These findings strongly suggest that the noradrenergic neuronal system plays an important role in the long-term memory process rather than in the short-term memory of the PAR.

NS-3 significantly reversed the disruption of memory induced by the treatment of DSP4, but physostigmine did not. These findings suggest that the disruption of long-term memory induced by treatment with DSP4 is independent of the activity of the cholinergic system.

Therefore, we assume that the reversal by NS-3 of DSP4-induced amnesia is mediated by the activation of the central noradrenergic system, possibly via an action on the LC. Several investigators have examined the interactions between cholinergic and noradrenergic systems on learning and memory in rats (11,22,50). For example, the scopolamine-induced reduction of correct choices in the radial arm maze task was enhanced by NA depletion (12). These previous studies indicate that the ascending noradrenergic system may regulate the activity of cholinergic neurons in the forebrain of rats.

In conclusion, NS-3 improved the disruption of both short-term and long-term memory of a PAR mediated by the enhancement of cholinergic and noradrenergic neuronal activities in the cerebral cortex. NS-3, therefore, is a promising drug for clinical use in ameliorating the cognitive impairment caused by cerebral cholinergic or adrenergic dysfunction, i.e., in patients with senile dementia of the Alzheimer's type.

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