Pharmacology Biochemistry & Behavior, Vol. 39, pp. 997–1002. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Naftidrofuryl Oxalate, Nootropic Effects on the Scopolamine- and the Basal Forebrain Lesion-Induced Amnesia in Rats

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Received 15 August 1989

OGAWA, S-I., T. KAMEYAMA AND T. NABESHIMA. *Naftidrofuryl oxalate, nootropic effects on the scopolamine- and the basal forebrain lesion-induced amnesia in rats.* PHARMACOL BIOCHEM BEHAV 39(4) 997–1002, 1991.—We studied the effects of naftidrofuryl oxalate on scopolamine- and basal forebrain (BF) lesion-induced amnesia using passive avoidance and multiple T-maze tasks, in comparison with Ca-hopantenate and physostigmine in rats. In the passive avoidance task, one-week treatment with naftidrofuryl oxalate (12.5 and 25 mg/kg, IP) ameliorated BF lesion-induced amnesia. The multiple T-maze task was done with two training sessions per day for five continuous days. We measured the number of errors made from start box to goal box. Naftidrofuryl oxalate (12.5 mg/kg, IP, b.i.d.) and physostigmine (0.1 mg/kg, IP, b.i.d.) attenuated scopolamine- and BF lesion-induced amnesia. However, treatment with naftidrofuryl oxalate for one week failed to inhibit the decrease of the choline acetyltransferase induced by the BF lesion. Ca-hopantenate did not show attenuation of amnesia induced by the scopolamine or BF lesion. These results suggest that naftidrofuryl oxalate enhances the storage of spatial information, and that the nootropic effects of naftidrofuryl oxalate may be produced by an indirect activation of the cholinergic system through serotonergic neuronal systems.

Basal forebrain (BF) lesion Maze Memory and learning Naftidrofuryl oxalate Passive avoidance Rats Scopolamine

IN recent years, numerous studies have indicated that age-related cholinergic deficits may partially account for the memory loss of senile dementia (SD). A marked decrease in choline acetyltransferase (CAT) activity is observed over a large part of the cortex and hippocampus in patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT). Whitehouse et al. (27) demonstrated that cholinergic neurons are lost from the basal forebrain (BF) areas of such patients. In rats, biochemical and histochemical studies show that electrolytic or excitotoxic BF lesion produces a marked reduction in CAT and acetylcholine esterase activity (8,20).

Thus a dysfunction of cholinergic neuronal transmission may be an important factor in causing the cognitive and memory deficits observed in SD. However, the results of clinical use of direct cholinergic agonists have been controversial and unconvincing.

Naftidrofuryl oxalate (LS-121) is a compound, synthesized by Szarvasi et al. (25). It is the acid oxalate of ethyl-N-diethylamine-2'''-(napthtyl-1)-3-(tetrahydrofuryl-2'')-2-propionate. It has been reported that LS-121 increases cerebral blood flow in humans (28), and improves cerebral metabolism as measured by changes in glucose utilization in mice (15). Glucose and ATP levels are increased, but the lactate level is reduced. Moreover, LS-121 favorably affects acute ischemic stroke, and is an effective and safe drug for the treatment of cerebrovascular disorders (18). In addition, Takeo et al. have shown that LS-121 protects cerebral mitochondria from the effects of cerebral embolism (26). Furthermore, LS-121 attenuates bicuculline ($GABA_A$ antagonist)-, picrotoxin (nonselective GABA antagonist)-, and cycloheximide (protein synthesis inhibitor)-induced amnesia in mice (11). Recently, we have reported that LS-121 may have a therapeutic effect on patients with AD or SDAT, since scopolamine- and BF lesion-induced amnesia were alleviated in rats after a single treatment (12.5 and 25 mg/kg, IP) (10).

Therefore, it is of interest to evaluate the effects of LS-121 on models of AD. We now report a nootropic effect of one-week treatment with LS-121 on BF lesion-induced amnesia accompanied by a decrease of CAT activity in a passive avoidance task. Moreover, the effects of LS-121 on scopolamine- and BF lesion-induced amnesia were investigated in comparison with Ca-hopantenate and physostigmine, which are nootropic drugs for the clinical field in Japan and cholinesterase inhibitor, respectively, using a multiple T-maze.

METHOD

Animals

Male Kbl Wistar rats (Kyoto Institute, Kitayama Laboratories Co. Ltd., Kyoto, Japan) of 270~320 g body weight, 10

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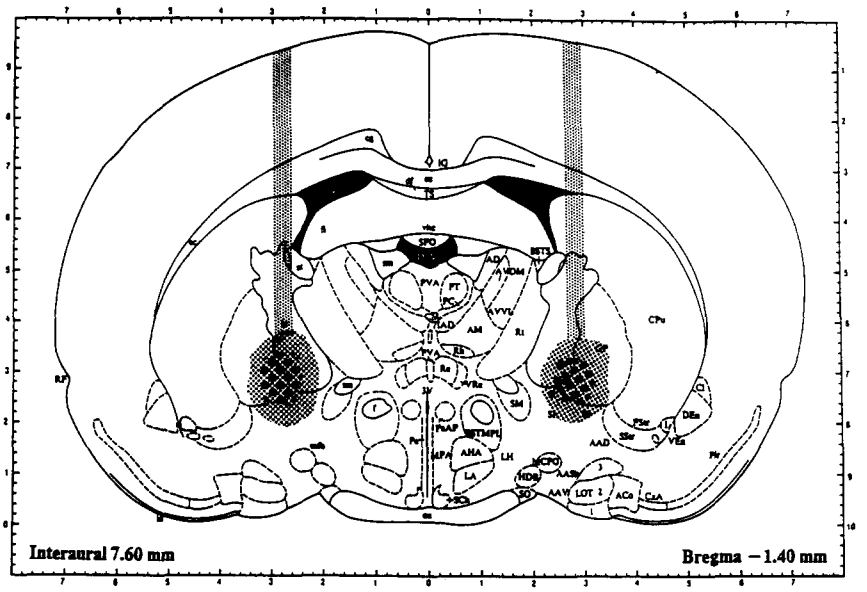

FIG. 1. Diagram representing the location and extent of bilateral electrolesion of the basal forebrain.

weeks old at the start of the experiments, were housed in a temperature- and light-controlled room (23 ± 1 °C, 12-h light cycle starting at 0800). Approximately 7 days after BF lesions, food was reduced for 3 or 4 days, and the animals were maintained at 85% of free-feeding weight, with free access to tap water throughout the experiments.

Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg IP) and fixed on a stereotaxic apparatus. Bilateral electrolytic lesions were made by passing an anodal DC current (2.0 mA, 20 s) through the uninsulated tip (0.8 mm) of a stainless-steel electrode (1.0 mm in diameter) inserted into the BF (10), according to the Paxinos and Watson (19) atlas of the rat brain (1.5 mm anterior to bregma, 3.0 mm bilateral, 7.2 mm below dura). A histological representation of lesion sites is shown in Fig. 1. Sham-operated rats underwent the same surgical procedure, but without the electric current. The experiments were performed 14 days after surgery.

Passive Avoidance Task

The apparatus consisted of two compartments, one light (25 cm long, 15 cm wide and 15 cm high) and one dark (25 cm long, 15 cm wide and 15 cm high), connected via a guillotine door (10).

On day 1, just before the acquisition trial, each rat was placed in the light compartment and then allowed to enter the dark compartment. The time in seconds (s) taken to do so was recorded. Rats having latencies greater than 60 s were eliminated, as they were outside the normal range. The same procedure was carried out using the rats within normal range. Once the rat was in the dark compartment, the guillotine door was closed and an electric shock (5 mA for 3 s) was delivered to the animal via the foot. The animal was then put back into the home cage until the retention trial. The retention trial was carried out 24 h after the acquisition trial. At that time, we returned the rat to the light compartment and recorded the time taken to enter the dark compartment (retention latency). An extended retention latency indicated that the animal had learned the association between the shock and dark compartment. A maximum latency of 300 s was used during the retention trial.

Multiple T-Maze Task

All behavioral training was carried out in an automated multiple T-maze. The correct path through the wooden maze consisted of 4 sections, each one meeting the one following it at a right angle. The entire maze (105 cm long, 105 cm wide and 30 cm high) was subdivided into 32 subsections. One section: 15 cm long, 15 cm wide and 30 cm high, consisted of 17 correct subsections and 15 wrong subsections, with start and goal boxes (Fig. 2). Rats received a 250-mg food pellet in a metal cup on the floor of the goal box. Access to both the start and goal boxes was controlled by cord-operated wooden guillotine doors. The rat's location in the maze was observed by an infrared camera 2 m above the apparatus. The maze was located in a sound-attenuated room and lit with four 75-W bulbs (70 ± 2 lux). The room was filled with a variety of materials that provided numerous spatial cues.

Behavioral training was done in 2 stages. In the first, each rat was confined in the goal box of the maze, and allowed to explore it for 15 min. A 250-mg food pellet was placed in the goal box in the first stage to accustom the rats to the reward. In the second stage, each rat was placed into the start box of the T-maze for 5 s. Then a guillotine door was raised to permit access to the maze. The training ended when the rat entered the goal box and got the pellet. Each rat received 2 training sessions per day for 5 days. The intertrial interval was 6 h. Throughout the experiment, the number of errors was recorded automatically (PC-9801, NEC, Tokyo, Japan). The number of errors was the number of times that the rat entered wrong sections and of reentering correct sections.

In order to reduce food and odor cues, the apparatus was

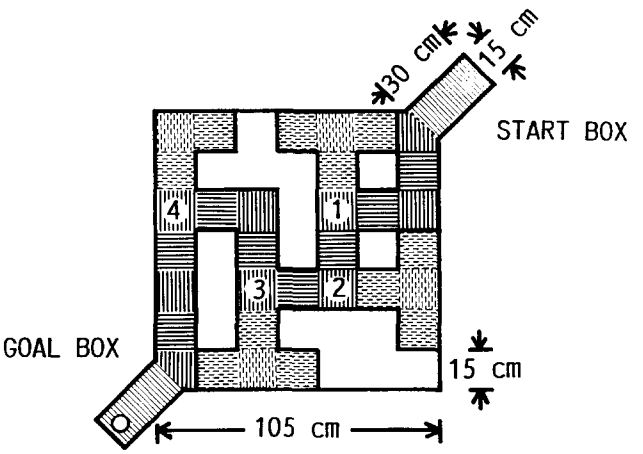

FIG. 2. The experimental apparatus of the multiple T-maze. The correct path through the wooden maze consisted of 4 sections, each one meeting the one following it at a right angle; the entire maze was subdivided into 32 subsections with start and goal boxes.

cleaned after every training session.

CAT Activity

Rats were decapitated at one day after the retention trial of the passive avoidance task, and the brain was removed rapidly and dissected into various areas including the fronto-parietal cortex (9). The tissue was stored at −80°C for assay at a later time.

CAT activity was measured by the method of Fonnum (7). The tissue was homogenized (4% w/v) in cold 50 mM phosphate-buffer (pH 7.4). Triton X-100 (0.5% v/v) was added to homogenates to ensure release of enzyme (enzyme solution). Substrate mixture {0.35 mM [^{3}H]-acetyl-Co A (4.0 mCi/mmol), 300 mM NaCl, 50 mM phosphate-buffer (pH 7.4), 8 mM choline chloride, 20 mM EDTA, and 0.1 mM physostigmine} of 125 μl was added to the 75 μl of enzyme solution in a scintillation vial and incubated at 37°C for 30 min. After the incubation, 1 ml of cold 50 mM phosphate-buffer, 0.5 ml of acetonitrile containing 2.5 mg of tetraphenylboron and 1.5 ml of toluene were added to the scintillation vial. The vials were shaken lightly and allowed to sit overnight before counting. Protein was measured by the method of Lowry et al. (13).

Drug Preparation and Administration

LS-121 (Nippon Roussel, K.K.), Ca-hopantenate (Tanabe Pharmaceutical Co.), physostigmine (Wako Chemicals) and scopolamine hydrobromide (Tokyo Kasei) were dissolved in physiological saline.

In the passive avoidance task, rats were pretreated with LS-121 (12.5 or 25 mg/kg, IP) once a day for one week. The drug was also administered IP 20 min prior to the training session.

In the multiple T-maze task, rats received scopolamine hydrobromide (0.5 mg/kg, IP) 40 min prior to each training session. LS-121 (12.5 mg/kg), Ca-hopantenate (100 mg/kg) or physostigmine (0.1 mg/kg) were given IP 20 min before each training session. The doses of Ca-hopantenate and physostigmine were selected from those used in a previous study (10).

The drugs were administered in a volume of 2 ml/kg. Sham-operated rats received an equivalent volume of saline.

Data Analysis

In the passive avoidance task, data were expressed in terms of medians and interquartile ranges and analyzed using a Kruskal-Wallis test and Tukey's test.

In the multiple T-maze task, the effects of the drugs were analyzed with a 2-way analysis of variance (ANOVA), followed by Tukey's test. Due to detailed evaluation for drug effects on training, effects of drugs were analyzed at the first half (the 1st–5th training session) and the latter half (the 6th–10th training session) of training sessions.

The CAT activity data were analyzed using a one-way analysis of variance, followed by Tukey's test.

RESULTS

Bilateral BF lesions produced aphagia and ataxia for 2~3 days after the surgery. None of the rats died from the lesions, and the aphagia was ameliorated by providing water and small amounts of food inside the home cage. In no cases did these effects persist beyond 4 days after surgery.

Effect of LS-121 on BF Lesion-Induced Amnesia in the Passive Avoidance Task

Effects of LS-121 on the retention of a passive avoidance response are shown in Table 1. There was a significant difference between the sham-operated and BF-lesioned groups. Lesioned rats showed an impaired response on the test day compared to the sham-operated group ($p<0.05$). Repeated administration of LS-121 (12.5 and 25 mg/kg) significantly increased retention latencies ($p<0.05$).

Effect of LS-121, Ca-Hopantenate and Physostigmine on Scopolamine-Induced Amnesia in the Multiple T-Maze Task

The mean number of errors in each training session of each of the 5 groups is shown in Table 2. The number of errors in the control group decreased rapidly with repeated training. By the end of training, rats in the control group made approximately 4 errors per training session, while those in the scopolamine-treated group made approximately 17 errors per training session. In the 1st and 2nd session, the number of errors in the control group was larger than that in the scopolamine-treated group. In

TABLE 1

EFFECT OF NAFTIDROFURYL OXALATE ON BASAL FOREBRAIN LESION-INDUCED AMNESIA IN RATS IN THE PASSIVE AVOIDANCE TASK

Groups	Step-Through Latency (s)	
	Training	Retention
Sham-operated	11 (7–14)	300 (300–300)
Basal forebrain-lesioned	9 (5–12)	59 (4–128)*
Basal forebrain-lesioned + naftidrofuryl oxalate (12.5 mg/kg)	14 (6–17)	136 (97–241)*†
Basal forebrain-lesioned + naftidrofuryl oxalate (25 mg/kg)	13 (4–14)	197 (54–300)‡

The drug treatment schedule was described in the Method section. Each group consisted of 10 rats.

*$p<0.05$ vs. sham-operated group.

†$p<0.05$, ‡$p<0.01$ vs. basal forebrain-lesioned group.

TABLE 2

EFFECTS OF NAFTIDROFURYL OXALATE, Ca-HOPANTENATE AND PHYSOSTIGMINE ON THE SCOPOLAMINE-INDUCED AMNESIA IN TERMS OF THE NUMBER OF ERRORS IN THE MULTIPLE T-MAZE TASK IN RATS

	The Number of Training Sessions									
Group	1	2	3	4	5	6	7	8	9	10
CONT	86.1	62.5	28.7	13.9	11.5	13.1	14.2	6.2	6.1	3.5
	(19.5)	(16.9)	(7.1)	(5.2)	(1.5)	(2.3)	(3.8)	(1.9)	(1.5)	(0.6)*
SCOP	59.3	45.6	46.8	43.6	38.1	39.0†	36.1†	31.9†	25.5†	17.4
	(23.9)	(10.5)	(13.7)	(9.7)	(8.2)	(6.4)	(11.8)	(5.9)	(5.5)	(1.8)
			N.S.				$F(1,90)=41.67$, $p<0.01$			
SCOP+LS	50.7	48.2	47.4	36.1	34.1	22.7	23.3	17.2‡	14.3	7.4‡
	(12.9)	(14.9)	(10.3)	(9.4)	(8.0)	(2.6)	(5.9)	(2.4)	(3.1)	(1.1)
			N.S.				$F(1,90)=14.67$, $p<0.01$			
SCOP+HO	79.4	81.3	50.7	71.7	31.8	35.3	32.2	26.9	19.6	17.1
	(20.7)	(40.5)	(13.1)	(19.2)	(4.4)	(14.0)	(6.7)	(9.3)	(4.4)	(2.9)
			N.S.					N.S.		
SCOP+PH	69.8	56.5	44.3	43.3	24.0	19.9‡	12.6‡	9.0‡	10.1‡	7.0‡
	(10.2)	(28.5)	(8.2)	(18.1)	(4.9)	(6.1)	(1.5)	(1.4)	(1.6)	(1.6)
			N.S.				$F(1,90)=28.38$, $p<0.01$			

CONT: control group; SCOP: scopolamine (0.5 mg/kg, IP)-treated group; SCOP+LS: scopolamine (0.5 mg/kg, IP)- and naftidrofuryl oxalate (12.5 mg/kg, IP)-treated group; SCOP+HO: scopolamine (0.5 mg/kg, IP)- and Ca-hopantenate (100 mg/kg, IP)-treated group; SCOP+PH: scopolamine (0.5 mg/kg, IP)- and physostigmine (0.1 mg/kg, IP)-treated group.

Each group consisted of ten rats.

Values for the control and other groups were not significantly different on the first training session.

*Standard error means (SEM).

†$p<0.05$ vs. control group.

‡$p<0.05$ vs. scopolamine-treated group.

N.S.: not significant.

later sessions, the scopolamine-treated group made more errors. The difference between the control- and scopolamine-treated groups decreased daily. However, scopolamine treatments impaired performance in a multiple T-maze [Table 2; $F(1,90)=41.67$, $p<0.01$ at the 6th–10th session].

LS-121 (12.5 mg/kg) and physostigmine (0.1 mg/kg) attenuated scopolamine-induced amnesia as measured by the number of errors [Table 2; LS-121; $F(1,90)=14.67$, $p<0.01$ at the 6th–10th session, physostigmine; $F(1,90)=28.38$, $p<0.01$ at the 6th–10th session]. There was no effect of Ca-hopantenate.

Effect of LS-121, Ca-Hopantenate and Physostigmine on BF Lesion-Induced Amnesia in the Multiple T-Maze Task

The mean number of errors in each training session for each of the 5 groups is shown in Table 3. By the end of training, sham-operated rats made approximately 2 errors per session, while the BF-lesioned rats made approximately 38 errors per session. Even in the last sessions, BF-lesioned rats made many errors, and their performance was significantly impaired [Table 3; $F(1,50)=21.73$, $p<0.01$ at the 1st–5th session, $F(1,50)=49.06$, $p<0.01$ at the 6th–10th session].

On the other hand, LS-121 (12.5 mg/kg) attenuated BF lesion-induced amnesia [Table 3; $F(1,50)=10.42$, $p<0.01$ at the 6th–10th session]. Physostigmine (0.1 mg/kg) decreased the number of errors in the BF-lesioned group [$F(1,50)=12.45$, $p<0.01$ at the 1st–5th session, $F(1,50)=33.27$, $p<0.01$ at the 6th–10th session]. Ca-hopantenate did not affect the number of errors.

Histologically, the lesions destroyed many of the cholinergic neurons in the BF, such as the nucleus basalis magnocellularis and small parts (less than 1 mm in diameter) of the globus pallidas (Fig. 1).

Effect of LS-121 on the Decrease of CAT Activity Induced by BF Lesions

There was a significant decrease in CAT activity in the fronto-parietal cortex of the BF-lesioned group in comparison with the sham-operated group (Table 4), but not in the striatum, hippocampus, and occipital cortex (data not shown). LS-121 did not reverse the decrease.

DISCUSSION

The central cholinergic system has been reported to modulate performance of a variety of behavioral tasks involving learning and memory. Considerable evidence suggested that impairment of the central cholinergic system is releated to deficits in the retention of newly acquired information (3, 4, 21, 27). In AD, there are major losses of cortical ACh production and choline acetyltransferase (CAT) activity and secondary loss of cell bodies in the magnocellular cholinergic neurons of the BF area (2,24). Drugs that block the acetylcholine (ACh) muscarinic receptor have been known to disrupt higher cognitive functions, and to induce a transient amnesic state (12). Scopolamine produces a pattern of memory dysfunction in young, healthy people similar to that observed in AD (5). In rats and mice, scopolamine also impairs acquisition in the radial arm maze and the passive avoidance tasks (6). Destroying BF produces a significant decrease in cholinergic marker in the neocortex (20).

In the present experiments, we used the scopolamine- and BF

TABLE 3

EFFECTS OF NAFTIDROFURYL OXALATE, Ca-HOPANTENATE AND PHYSOSTIGMINE ON THE BASAL FOREBRAIN LESION-INDUCED AMNESIA IN TERMS OF THE NUMBER OF ERRORS IN THE MULTIPLE T-MAZE TASK IN RATS

	The Number of Training Sessions									
Group	1	2	3	4	5	6	7	8	9	10
SHAM	38.2	26.0	19.8	14.7	11.3	7.7	6.8	5.8	3.7	1.7
	(12.1)	(8.5)	(12.7)	(8.4)	(4.6)	(3.8)	(4.7)	(2.8)	(1.3)	(0.6)*
BF	96.2	73.8†	68.5†	66.7†	61.0†	51.5†	50.0†	51.2†	41.2†	38.3†
	(43.4)	(7.7)	(14.0)	(16.4)	(12.1)	(9.6)	(11.9)	(20.4)	(2.7)	(12.8)
			$F(1,50)=21.73$, $p<0.01$				$F(1,50)=49.20$, $p<0.01$			
BF+LS	77.0	69.7	54.0	35.5	48.0	34.3	30.1	27.3	16.0‡	22.0
	(24.4)	(17.4)	(12.5)	(11.0)	(13.9)	(8.3)	(8.4)	(5.1)	(0.8)	(4.0)
			N.S.				$F(1,50)=10.42$, $p<0.01$			
BF+HO	95.5	67.2	67.3	55.8	48.3	45.5	43.8	40.0	34.7	9.8
	(20.7)	(40.5)	(13.1)	(19.2)	(4.4)	(14.0)	(6.7)	(9.3)	(4.4)	(2.9)
			N.S.					N.S.		
BF+PH	66.8	32.5‡	27.0	21.7‡	27.2	19.5	13.5	13.2	9.2‡	8.2
	(16.5)	(6.6)	(5.1)	(4.0)	(5.0)	(3.0)	(4.0)	(2.8)	(1.8)	(3.1)
			$F(1,50)=12.45$, $p<0.01$				$F(1,50)=32.81$, $p<0.01$			

SHAM: sham-operated group; BF: basal forebrain-lesioned group, BF+LS: basal forebrain-lesioned naftidrofuryl oxalate (12.5 mg/kg, IP)-treated group; BF+HO: basal forebrain-lesioned Ca-hopantenate (100 mg/kg, IP)-treated group; BF+PH: basal forebrain-lesioned physostigmine (0.1 mg/kg, IP)-treated group.

Each group consisted of six rats.

Values for the control and other groups were not significantly different on the first training session.

*Standard error means (SEM).

†$p<0.05$ vs. sham-operated group.

‡$p<0.05$ vs. basal forebrain-lesioned group.

N.S.: not significant.

lesion-induced models of amnesia to compare the effects of LS-121 with Ca-hopantenate and physostigmine. Recent experiments in our laboratory have indicated that a single injection of LS-121 (25 and 50 mg/kg, IP), Ca-hopantenate (200 and 400 mg/kg, IP) or physostigmine (0.1 and 0.2 mg/kg, IP) attenuates scopolamine-induced amnesia. Injection of LS-121 and physostigmine also attenuates BF lesion-induced amnesia in the passive avoidance task (10). But in the presence of scopolamine, repeated administration of LS-121 (50 mg/kg) and Ca-hopantenate (400 mg/kg) had side effects such as stretching (LS-121; effect of oxalate?) and even death (Ca-hopantenate). Therefore, we used low doses of LS-121 (12.5 mg/kg, IP, b.i.d.) and Ca-hopantenate (100 mg/kg, IP, b.i.d.). These doses were equivalent to 25 mg/kg/day and 200 mg/kg/day, respectively. In this study, seven days of treatment with LS-121 also ameliorated the memory deficit induced by BF lesion on a passive avoidance task. Furthermore, LS-121 attenuated both scopolamine- and BF lesion-induced amnesia on a multiple T-maze task, as well as physostigmine.

TABLE 4

CHOLINE ACETYLTRANSFERASE (CAT) ACTIVITY IN THE FRONTO-PARIETAL CORTEX

Groups	CAT Activity (nmol acetylcholine/h/mg protein)
Sham-operated	81.4 ± 7.2
Basal forebrain-lesioned	60.5 ± 3.9 (74.3%)*
Basal forebrain-lesioned + naftidrofuryl oxalate (12.5 mg/kg)	62.7 ± 3.5 (77.0%)*
Basal forebrain-lesioned + naftidrofuryl oxalate (25 mg/kg)	64.9 ± 4.8 (79.7%)*

Each group consisted of ten rats.

*$p<0.05$ vs. sham-operated group.

Biochemical study has shown that LS-121 most effectively inhibits the binding of a specific ligand [^{3}H]ketanserin to the serotonin-2 receptor (14). In a behavioural study, head-twitch response induced by 5-hydroxytryptophan and pargyline was inhibited by LS-121 (17). Quirion et al. (22) have reported that lesioning of BF markedly decreased the binding of [^{3}H]ketanserin in lamina IV of the anterior and middle cortex, suggesting the existence of a serotonin receptor on the cholinergic nerve terminal. Concerning the involvement of serotonergic neurons in the process of memory, the enhancement of the retrieval of a previously learned aversive habit is observed with the use of serotonergic receptor antagonists, such as ketanserin, methysergide and mianserin in mouse (1). From these reports, they suggested that at least a certain proportion of serotonin-2 receptor binding sites is located on cholinergic nerve terminals in the cortex, and serotonin neurons interact with cholinergic afferents to the frontal cortex by the inhibiting cholinergic function (23). From these results, although the exact mechanism by which LS-121 exerts its effects against scopolamine and BF lesion is not known, an indirect activation of the cholinergic neuronal system induced by

the antagonistic effect of LS-121 on the serotonergic neuronal system may be responsible for its antiamnesic action. Antiamnesic effects of physostigmine may be related to its antiacetylcholine esterase activity. Ca-hopantenate increases release of Ach (16), but failed to attenuated the scopolamine- and BF lesion-induced amnesia. The lack of efficacy of Ca-hopantenate should be interpreted only in relation to the dose and the duration of treatment. Although LS-121 failed to attenuate the decrease of CAT activity induced by BF lesion, these results suggest that successive administration of LS-121 should be done to get positive effects.

ACKNOWLEDGEMENTS

We are grateful to Nippon Roussel K.K. for its supply of LS-121 and Ca-hopantenate. We would like to thank Dr. Akira Katoh for his statistical advice.

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