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Effects of S-8510, a novel benzodiazepine receptor partial inverse agonist, on basal forebrain lesioning-induced dysfunction in rats

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Abstract

We investigated the effects of a novel benzodiazepine partial inverse agonist, S-8510 (2-(3-isoxazolyl)-3,6,7,9-tetrahydroimidazo [4,5-d] pyrano [4,3-b] pyridine monophosphate monohydrate), on the impairment of spatial memory, decreased high-affinity choline uptake and acetylcholine release in basal forebrain-lesioned rats. S-8510 (3 and 5 mg/kg, p.o. 30 min before each training session) significantly ameliorated the basal forebrain-lesion-induced impairment of spatial memory in water maze task. In vivo brain microdialysis studies showed that systemic administration of S-8510 at 3 and 10 mg/kg significantly increased the release of acetylcholine in the front-parietal cortex in basal forebrain-lesioned rats. Further, repeated administration of S-8510 (3 and 10 mg kg^{-1} day^{-1} for 5 days) reversed the decrease in cortical high-affinity choline uptake induced by basal forebrain lesion. Thus, S-8510 improved the spatial memory impairment induced by lesion of the basal forebrain in rats. In addition, it increased acetylcholine release and high-affinity choline uptake from the cortex, a region closely associated with memory, in basal forebrain-lesioned rats. These results indicate that S-8510 has cognition enhancing and cholinergic-activating effects in the basal forebrain-lesioned rats, suggesting that this agent may be useful for the treatment of mild to moderate senile dementia including Alzheimer's disease. © 1998 Elsevier Science B.V.

Keywords: Benzodiazepine receptor; Partial inverse agonist; Basal forebrain lesion; Learning; Memory; Acetylcholine release; Choline uptake, high affinity

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disorder, which is characterized clinically by large numbers of neurofibrillary tangles and senile plaques in the brain (Hardy and Allsop, 1991; Kosik, 1991). A variety of neurotransmitter systems have been shown to be degenerated in patients with Alzheimer's disease (Palmer et al., 1978; Sims et al., 1983; Hardy et al., 1985). Cholinergic dysfunction has also been found (Whitehouse et al., 1982; Coyle et al., 1983), which is related to the severity of the cognitive dysfunction and memory loss in patients with Alzheimer's disease (Perry et al., 1978; Sims et al., 1983). Furthermore, several recent studies have shown that amyloid precursor protein is regulated by cholinergic mechanisms and that cholinomimetic treatments possibly inhibit amyloid deposition (Buxbaum et al., 1992; Nitsch et al., 1992; Felder et al., 1993; Lahiri et al., 1994). Thus, dysfunction of the cortical cholinergic system seems to correlate with the cognitive dysfunction in Alzheimer's disease patients (Perry et al., 1978).

Benzodiazepine receptor agonists, the clinically effective anxiolytic, anticonvulsant and sedative drugs, decrease vigilance and impair cognitive processes (Thiebot, 1985; Jensen et al., 1987). In contrast, benzodiazepine receptor inverse agonists produce various pharmacological effects which are the opposite of those induced by benzodiazepine receptor agonists and enhance vigilance (Koelega, 1989) and cognitive processes (Venault et al., 1986; Kumar et al., 1988; Miller et al., 1992). These cognition-enhancing effects of benzodiazepine receptor inverse agonists result from the inhibition of γ-aminobutyric acid (GABA)ergic neurotransmission via the $GABA_A$ receptor complex in the brain. $GABA_A$/benzodiazepine receptor binding sites have been detected in the basal forebrain (Sarter and Schneider, 1988). Cortical cholinergic neurons of the basal forebrain appear to be regulated by GABAergic afferents (Bigl et al., 1982; Zaborski et al., 1986; Ingham et al., 1988). The

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administration of benzodiazepine receptor agonists decreases, and the administration of benzodiazepine receptor inverse agonists increases, cortical acetylcholine measured by microdialysis in freely moving animals (Moore et al., 1993). These previous reports suggested that the effects of the basal forebrain in the cortical cholinergic system may be accelerated by the use of benzodiazepine receptor ligands which inhibit GABAergic transmission, and benzodiazepine receptor inverse agonists may enhance cognition because of their ability to selectively disinhibit cholinergic neurons in the basal forebrain (Sarter and Bruno, 1997).

S-8510 has the properties of a low-efficacy benzodiazepine receptor inverse agonist (Kawasaki et al., 1996). Briefly, this compound was found to potently displace [^{3}H]flunitrazepam binding and to show a low intrinsic activity similar to that of CGS8216 (2-phenylpyrazolo(4,3c)quinolin-3 (5*H*)-one). S-8510 itself produces no sign of anxiety or convulsion in mice and rats. S-8510 has also been found to increase the excitability of hippocampal neurons and to antagonize the effects of scopolamine in a water maze task in rats. On the basis of these data, the present study was designed to clarify the cognition-enhancing effects of S-8510 on impairment of spatial memory and dysfunction of the cholinergic system in basal forebrain-lesioned rats, in order to elucidate its effects on the brain dysfunction associated with Alzheimer's disease.

2. Materials and methods

2.1. Animals

Ten-week-old male Wistar rats were obtained from Japan SLC. The initial free-feeding weights were 280–300 g. The rats were housed in groups of three per cage at a constant temperature (23 ± 2°C) and maintained under a 12-h light/dark cycle with lights on at 0700. Experiments were conducted during the light portion of the day.

2.2. Surgery

The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and fixed on a stereotaxic apparatus with the incisor bar set 3.3 mm below the interaural line. In all surgical procedures, stereotaxic coordinates refer to the atlas of Paxinos and Watson (1986). Bilateral neurotoxic lesions of the basal forebrain were induced by injection of ibotenic acid (Sigma) through the cannula on each side with an interval of 2 days. A stainless steel injection needle (outer diameter 0.25 mm) connected via polyethylene tubing to a 10-μl microsyringe was inserted into the basal forebrain. The coordinates of the basal forebrain were: -0.8 mm posterior to bregma, 2.8 mm lateral to the midline and 8 mm ventral from the skull surface. Ibotenic acid was dissolved in 0.1 M phosphate buffer (pH 7.2) at a concentration of 10 μg/μl, and then infused in a volume of 1 μl over a period of 3 min. The injection needle was left in place for a further 5 min to ensure that the drug had diffused away from the needle tip. Sham-operated rats received 0.1 M phosphate buffer in the same way. The behavioral test was started 14 days after the operation. The injection sites of ibotenic acid are shown schematically in Fig. 1.

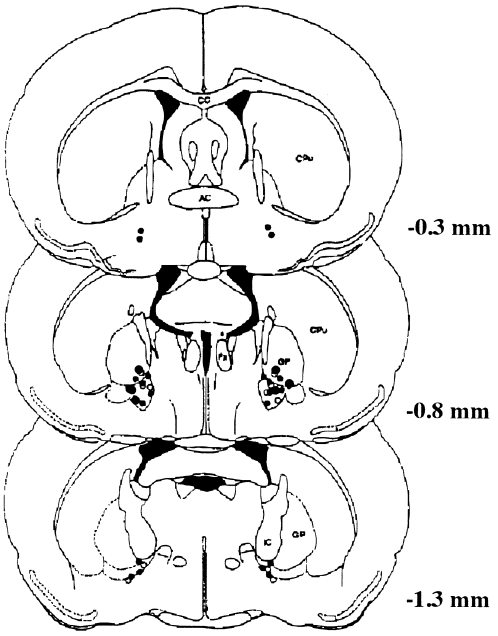

Fig. 1. Schematic drawing of the position of the basal forebrain lesion in a coronal section approximately at the level of 0.8 mm posterior from bregma according to the atlas of Paxinos and Watson (1986). basal forebrain lesion: the stippled area indicates the injection sites of ibotenic acid.

2.3. Water maze task

The apparatus and experimental procedure for the water maze task were described previously (Abe et al., 1997). Briefly, a circular water tank 178 cm in diameter was used in this study. The tank was filled with water at approximately 23°C. A transparent platform, 10 cm in diameter, was located at a constant position in the center of one quadrant inside the tank. The upper surface of the platform was 3 cm below the surface of the water. During each trial, we recorded the time it took a rat to escape onto the platform within 200 s (escape latency). If the rat failed to find the platform within 200 s, it was placed on it for 30 s. The training session was terminated and a maximum score of 200 s was assigned. The behavioral trace and the distance of swimming in the water maze were monitored with a TV camera and analyzed using a computer system (AXIS 60, Neuroscience, Tokyo, Japan). The latency to locate the platform was also recorded. Behavioral tests were performed in rats 14 days after the basal forebrain lesion. Each rat was tested with one trial/day for 5 days.

2.4. Brain microdialysis procedure

The rats were habituated to the testing environment and to handling for at least one week before surgery. Under sodium pentobarbital anesthesia, a stainless steel guide cannula to insert the probe was implanted into the left fronto-parietal cortex and permanently fixed to the skull with dental cement. Experiments were started 24 h after implantation of the dialysis cannula. On each test day, the rats were allowed to adapt to the environment for 1 h before the dialysis probe was inserted. After probe insertion, the rats were dialyzed with Ringer solution consisting of 4 mM KCl, 147 mM NaCl, 2.3 mM $CaCl_2$, and 1 μM physostigmine at a flow rate of 2 μl/min by means of a microinfusion pump. Samples were collected 2 h after probe insertion to allow the baseline acetylcholine release to stabilize and were then collected every 10 min. The average concentration of acetylcholine in the last three samples prior to drug administration was taken as 100%. All subsequent post-treatment values were expressed as percentages of basal values. Each dialysis sample was added to ethylhomocholine (Eicom, Kyoto, Japan) as an internal standard and the levels of acetylcholine were measured using high performance liquid chromatography (HPLC) with an electrochemical detector (Eicom, Kyoto, Japan) and an immobilized enzyme column (Eicom, Kyoto, Japan).

2.5. High-affinity choline uptake

The rats were killed by decapitation 30 min following the last administration of S-8510. The brains were rapidly removed and dissected on ice into frontal, parietal and occipital cortexes and hippocampus. High-affinity choline uptake was assessed in synaptosomes according to the method of Simon et al. (1976). Briefly, each tissue was weighed and homogenized in 20 volumes of 0.32 M sucrose. Synaptosomes were prepared by differential centrifugation. The pellets (P2) were resuspended in the original volume of sucrose. Aliquots (50 μl) of this suspension were then added to 450 μl of ice-cold buffer containing 0.2 μM choline, 0.25 μCi [^{3}H]choline, 126 mM NaCl, 9.6 mM KCl, 4.2 mM $MgSO_4$, 2.4 mM $CaCl_2$-H_2O, 252 mM dextrose and 40.0 mM Tris base. After incubation for 4 min at 37°C, the tubes were quenched immediately by the addition of 3 ml of ice-cold buffer. Tissue was collected on glass fiber filters Type A/E (Gelman) by vacuum filtration. After two washings with 3 ml of ice-cold buffer, the filters were placed in scintillation vials and 10 ml of scintillation fluid was added to each. Vials were assayed for radioactivity in a scintillation counter. [^{3}H]Choline chloride was obtained from NEN Du Pont and had a specific activity of 80.0 Ci/mmol. Protein determination (Bradford, 1976) was performed using a commercially available kit (Bio-Rad, Richmond, CA, USA).

2.6. Choline acetyltransferase activity

Changes in the brain choline acetyltransferase activity in the sham-operated and basal forebrain-lesioned groups were assayed. The rats were killed 21 days after basal forebrain lesioning, and the brain was rapidly removed and dissected on ice into frontal, parietal and occipital cortexes and the hippocampus. The tissues were stored at −80°C until choline acetyltransferase assay. Choline acetyltransferase activity was measured according to the radioenzymatic method of Fonnum (1975) using [^{14}C]acetyl coenzyme A (Amersham), and is expressed as nmol acetylcholine/h/mg protein. Protein determination (Bradford, 1976) was performed using a commercially available kit (Bio-Rad, Richmond, CA).

2.7. Drugs

S-8510 (2-(3-isoxazolyl)-3,6,7,9-tetrahydroimidazo[4,5-d]pyrano[4,3-b]pyridine monophosphate monohydrate) was synthesized in Shionogi Research Laboratories. S-8510 was dissolved in distilled water and injected p.o. in a volume of 1 ml/kg. The rats were treated with S-8510 30 min before each training trial in the water maze. Flumazenil was suspended in saline with a drop of Tween-80, and was administered i.p. in a volume of 1 ml/kg.

2.8. Statistics

Data for the water maze task are expressed as medians, and were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney's *U*-test (two-tailed). Biochemical data are expressed as means ± S.E.M. Statistical significance of changes in brain neurochemistry was calculated with Student's *t*-test (two-tailed) when comparing two groups, and one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used when three or more groups were to be compared.

3. Results

3.1. Effects of S-8510 on performance of water maze task in basal forebrain-lesioned rats

Basal forebrain-lesioned rats showed a significant increase in escape latency on the 2nd, 3rd, 4th and 5th trials of training sessions when compared to the sham-operated group. Swimming speed during the 5 trials did not differ among groups. S-8510 did not cause any changes in general behavior of basal forebrain-lesioned rats up to the highest dose used (10 mg/kg). S-8510 at 3 mg/kg significantly decreased escape latency on the 4th trial, while 5 mg/kg showed a significantly decreased escape latency on the 4th and 5th trials of the training session when compared to the basal forebrain-lesioned group, indicating that

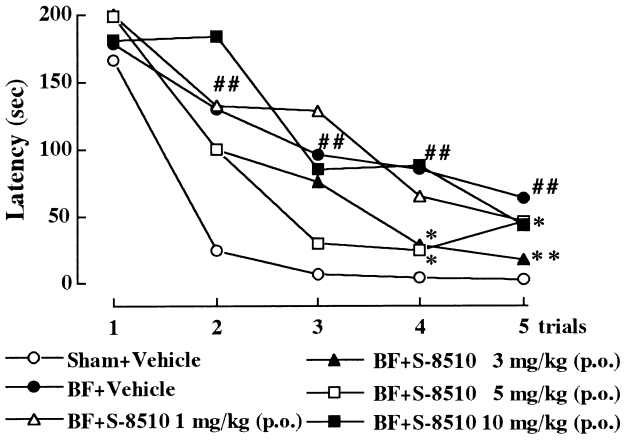

Fig. 2. Effects of S-8510 on memory impairment induced by the basal forebrain lesion in rats, as assessed in the water maze task. Values represent the median latency. S-8510 was given orally 30 min before the training trials. $n = 7–9$ per group. $^{\#\#}P < 0.01$ vs. Sham + Vehicle, $* P < 0.05$, $** P < 0.01$ vs. basal forebrain-lesioned group (BF) + Vehicle (Mann–Whitney's *U*-test).

S-8510 ameliorated the memory impairment induced by basal forebrain lesion. On the other hand, S-8510 at 10 mg/kg did not improve the memory impairment induced by basal forebrain lesions (Fig. 2). Furthermore, the benzodiazepine receptor antagonist, flumazenil, 1 mg/kg, blocked the improving effect of S-8510, 5 mg/kg, on the memory impairment induced by basal forebrain lesion (Fig. 3).

3.2. Effects of S-8510 on the release of acetylcholine in basal forebrain-lesioned rats

Basal forebrain lesioning had no effect the basal acetylcholine release from the fronto-parietal cortex (mean of 3 fractions: basal forebrain-lesioned group, 8.2 ± 1.8 pmol/60 min, $n = 7$; sham group, 11.5 ± 1.5 pmol/60 min, $n = 8$). S-8510 (3 and 10 mg/kg, p.o.) dose dependently increased acetylcholine release from the fronto-parietal cortex in basal forebrain-lesioned rats. S-8510 at 3 mg/kg increased acetylcholine release by 140% 20 min after administration. S-8510 at 10 mg/kg increased acetylcholine release by 160%. The effect reached a maximum 40–50 min after administration, and continued for about 90 min (Fig. 4). Flumazenil (1 mg/kg, i.p.) administered 5 min after S-8510 blocked the increase in acetylcholine release induced by S-8510 at 3 mg/kg (Fig. 4).

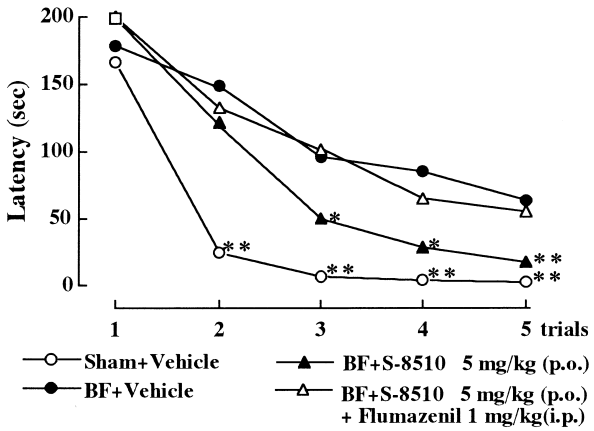

Fig. 3. Antagonism by flumazenil of memory improving effects of S-8510 in the basal forebrain-lesioned rats. Values represent the median latency. S-8510 (5 mg/kg) was administered orally 30 min before the training trials. $n = 8–9$ per group. Flumazenil (1 mg/kg, i.p.) was administered 5 min after S-8510 (10 mg/kg). $* P < 0.05$, $** P < 0.01$ vs. basal forebrain-lesioned group (BF) + Vehicle (Mann–Whitney's *U*-test).

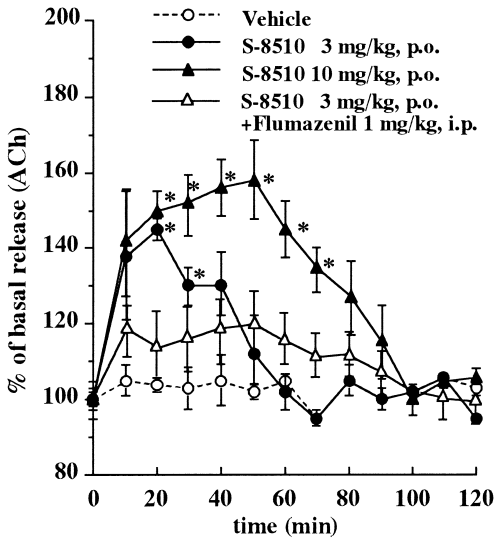

Fig. 4. Effects of S-8510 on cortical acetylcholine release in basal forebrain-lesioned rats. The results are expressed as percentages (means ± S.E.M.) of basal values. Animals were given S-8510 at 3 (closed circles) or 10 (closed triangles) mg/kg p.o., or vehicle (open circles). Antagonism by flumazenil (1 mg/kg, i.p.) of the increase in acetylcholine release after S-8510 (3 mg/kg) (open triangles). $n = 8$ per group. $* P < 0.05$ vs. basal forebrain-lesioned group + Vehicle (Dunnett's multiple comparison test).

3.3. Effects of S-8510 on the high-affinity choline uptake in basal forebrain-lesioned rats

The results for the regional high-affinity choline uptake are shown in Table 1. Basal forebrain lesioning resulted in decreases of 28 and 24% in high-affinity choline uptake in the frontal and parietal cortexes, respectively. Repeated administration of S-8510 3 mg/kg but not 1 mg/kg reversed the decrease in high-affinity choline uptake induced in the parietal cortex by the basal forebrain lesions. At 10 mg/kg, S-8510 ameliorated the decrease in high-affinity choline uptake in the frontal and parietal cortexes. In addition, S-8510, 3 and 10 mg/kg, significantly increased the hippocampal high-affinity choline uptake in basal forebrain-lesioned rats.

Table 1
Effects of S-8510 on high-affinity choline uptake in basal forebrain-lesioned rats

Treatment	Brain region			
	Frontal cortex	Parietal cortex	Occipital cortex	Hippocampus
Sham + Vehicle	4.71 ± 0.18[a]	4.41 ± 0.19[a]	4.70 ± 0.16	4.73 ± 0.44
BF + Vehicle	3.41 ± 0.12	3.36 ± 0.05	3.96 ± 0.17	4.78 ± 0.19
+ S-8510 1 mg/kg	3.56 ± 0.40	3.35 ± 0.13	4.05 ± 0.49	4.74 ± 0.30
+ S-8510 3 mg/kg	4.08 ± 0.46	4.51 ± 0.31[a]	4.96 ± 0.48	6.60 ± 0.19[a]
+ S-8510 10 mg/kg	4.86 ± 0.23[aa]	5.23 ± 0.19[a]	5.08 ± 0.16	6.58 ± 0.43[a]

High-affinity choline uptake (pmol/4 min per mg protein) was determined 5 days after treatment with S-8510.
Values represent means ± S.E.M., $n = 8$.
[a] $P < 0.05$ vs. basal forebrain-lesioned group (BF) + Vehicle (Dunnett's multiple comparison test).

Table 2
Effects of S-8510 on choline acetyltransferase activity in basal forebrain-lesioned rats

Treatment	Brain region			
	Frontal cortex	Parietal cortex	Occipital cortex	Hippocampus
Sham + Vehicle	39.8 ± 3.0[a]	40.1 ± 2.1[a]	40.6 ± 2.3[a]	52.3 ± 4.1
BF + Vehicle	26.2 ± 2.5	24.9 ± 2.5	31.5 ± 1.7	50.1 ± 3.3
+ S-8510 1 mg/kg	26.8 ± 3.3	26.9 ± 1.7	35.3 ± 1.7	50.4 ± 2.7
+ S-8510 3 mg/kg	30.2 ± 1.2	28.5 ± 1.9	36.5 ± 1.7	49.4 ± 2.3
+ S-8510 10 mg/kg	32.3 ± 3.1	36.3 ± 3.4[a]	35.4 ± 1.3	51.5 ± 3.6

Values represent the means ± S.E.
Choline acetyltransferase activity (nmol/h per mg protein) was determined 5 days after treatment with S-8510, $n = 6–8$.
[a] $P < 0.05$ vs. basal forebrain-lesioned group (BF) + Vehicle (Dunnett's multiple comparison test).

3.4. Effects of S-8510 on the choline acetyltransferase activity in basal forebrain-lesioned rats

Basal forebrain lesions caused marked reductions in choline acetyltransferase activity in the frontal, parietal and occipital cortexes, but not in the hippocampus or striatum, as shown in Table 2. Lesions of the basal forebrain decreased choline acetyltransferase activity by an average of 30% in the frontal cortex, 30% in the parietal cortex and 19% in the occipital cortex, but not in the hippocampus, as compared to sham-operated animals. S-8510 at 1 and 3 mg/kg did not affect the choline acetyltransferase activity in the frontal, parietal and occipital cortexes, but 10 mg/kg improved the decreased choline acetyltransferase activity induced in the parietal cortex by basal forebrain lesions.

4. Discussion

The present study demonstrated that S-8510, a benzodiazepine receptor partial inverse agonist, significantly improved the spatial memory impairment induced by basal forebrain lesion. Furthermore, S-8510 enhanced cholinergic transmission in the cortex, a region that is closely associated with learning and memory, in basal forebrain-lesioned rats.

Previous experiments have provided evidence for the beneficial behavioral effects of weak or selective benzodiazepine receptor inverse agonists in humans and animals pretreated with scopolamine (Sarter et al., 1988a,b; Holley et al., 1992) and in animals with basal forebrain lesions (Hodges et al., 1989; Sarter and Stecker, 1989; Holley et al., 1993; Mazurkiewicz et al., 1992). S-8510, 3 and 5 mg/kg, significantly improved the basal forebrain lesion-induced impairment of spatial memory in a water maze task. These previous results and our present findings showed that S-8510 has clinical usefulness for memory deficits induced by dysfunction of the cholinergic neuronal system. High doses (10 mg/kg) of S-8510, however, could not improve the cognitive deficits induced by basal forebrain lesions in this experiment. It has been reported that low doses of inverse agonists such as β-carboline-3-carboxylate monomethylamide (FG7142) and methyl-6,7-dimethyl-4-ethyl-β-carboline-3-carboxylate (DMCM) potentiate acquisition in passive avoidance behavior, but at high doses these drugs disrupt acquisition (File and Pellow, 1988). This means that the optimal dose must be used to obtain improvement of cognitive disruption. The balance between multiple neurotransmitters may also play an important role in the performance of higher brain functions such as spatial memory. In fact, S-8510 at 10 mg/kg has been shown to increase cortical noradrenaline and dopamine release in intact rats (Kawasaki et al., 1996). This evidence indicates that high doses of S-8510 might induce an imbalance of multiple neurotransmitter interactions. Thus, S-8510 at the optimal dose was found to attenuate the memory impairment induced by basal forebrain lesions when tested with a water maze task. These

observations cannot explain why the effects of S-8510, a benzodiazepine receptor inverse agonist, in basal forebrain-lesioned rats were related to the effects on cholinergic transmission. Therefore, we examined the effects of S-8510 on cholinergic transmission in basal forebrain-lesioned rats to clarify its cholinergic-enhancing effects.

Systemic administration of S-8510 (3 and 10 mg/kg) dose dependently increased the release of acetylcholine from the fronto-parietal cortex in basal forebrain-lesioned rats. Repeated administration of S-8510 at 3 and 10 mg/kg reversed the decrease in cortical high-affinity choline uptake induced by basal forebrain lesions. Results of several studies have indicated that benzodiazepine receptor inverse agonists can indirectly enhance several markers of cholinergic function (e.g., hemicholinium binding, high-affinity choline uptake, acetylcholine level) in intact animals (Simon et al., 1976; Richter et al., 1982; Miller and Chmielewski, 1990; Moore et al., 1993). Sarter and Bruno (1997) have demonstrated that the effects of these agonists may be related to their ability to enhance cognition. Furthermore, intra-basal and -septal administration of benzodiazepine receptor inverse agonists increases the acetylcholine release and high-affinity choline uptake from the cortex and hippocampus (Walsh et al., 1993; Imperato et al., 1994). These facts suggest that the cognition-enhancing effect of S-8510 is accompanied by cholinergic activation. Moreover, these previous data indicate that these effects of S-8510 do not result from a direct action on cholinergic nerve terminals, but an effect on the cholinergic cell bodies in the basal forebrain. Thus, our results indicate that S-8510 acts by disinhibiting the surviving damaged basal forebrain cholinergic neurons in basal forebrain-lesioned rats. Indeed, basal forebrain lesions in our experiments caused a decrease of about 30% in cortical choline acetyltransferase activity and of about 20% in high-affinity choline uptake. Therefore, it is tempting to speculate that disinhibition of the remaining basal forebrain cholinergic neurons may sufficiently increase the levels of acetylcholine in cortical synapses. On the other hand, repeated administration of S-8510 (1, 3 mg/kg) had no effect on the decreases in choline acetyltransferase activity in the frontal and occipital cortexes. High doses (10 mg/kg) of S-8510 significantly ameliorated the basal forebrain lesion-induced reduction of choline acetyltransferase activity in the parietal cortex. The site of action and the mechanisms by which S-8510 induced improvements in choline acetyltransferase activity in the parietal cortex of basal forebrain-lesioned rats are not yet clear. Single administration of S-8510 at the same dose had no effect on choline acetyltransferase activity (data not shown), suggesting that the ameliorating effect was not attributable to a direct interaction with the enzyme. Therefore, further studies are required to clarify the effect of S-8510 on decreased choline acetyltransferase activity.

Thus, the enhancement of cognition by S-8510, a benzodiazepine receptor partial inverse agonist, may represent a useful approach to treat cognitive disorders that are characterized by cholinergic hypofunction such as Alzheimer's disease. Cholinomimetic drugs such as muscarinic agonists and cholinesterase inhibitors directly stimulate the non-selective postsynaptic cholinergic receptor, but inverse agonists act via disinhibitory mechanisms in the presynaptic cholinergic system. Therefore, S-8510 might be able to amplify the ongoing rhythmic activity of cholinergic neurons by restricting the inhibitory influence of $GABA_A$/benzodiazepine receptors (Sarter et al., 1990). A potential limitation of these compounds is that they can produce anxiogenic and proconvulsant effects. However, S-8510 is little likely to produce anxiety or convulsion by itself (Kawasaki et al., 1996). Binding studies showed that the GABA ratio for S-8510 was close to the value for the benzodiazepine receptor antagonist, flumazenil. Flumazenil has been shown to enhance memory, and to improve the memory impairments induced by anticholinergic drugs or lesions to the basal forebrain (Sarter et al., 1988b; Brioni et al., 1991). These previous data suggest that endogenous benzodiazepine ligands might inhibit the ongoing activity of brain mechanisms involved in memory (Braestrup et al., 1984; Izquierdo and Medina, 1991). Therefore, S-8510 may antagonize the effect of endogenous benzodiazepine ligands. However, flumazenil at 1 mg/kg had no effect on a memory impairment induced by basal forebrain lesion(data not sown). Our observation that flumazenil at 1 mg/kg, a dose that alone had no effect on the release of acetylcholine, antagonized the improving effect of S-8510 suggests that, at this dose, 1 mg/kg flumazenil has a purely antagonistic effect. Wolfman et al. (1991) have reported that endogenous benzodiazepine ligands are altered in a regionally specific and task-dependent manner. Flumazenil is not a neutral benzodiazepine receptor antagonist, but the direction of its intrinsic activity (agonist to inverse agonist) may vary according to the experimental model, receptor state and, often, the dose administered (File and Pellow, 1986; Wolfman et al., 1991). Therefore, to fully know these compounds it will be important to determine their range of activities in various models of dementia and the relationship between their behavioral effects (anxiogenesis and memory enhancement) and cholinergic activity.

In conclusion, the present study demonstrated that S-8510 significantly ameliorated a memory impairment by activating the remaining cholinergic neurons in basal forebrain-lesioned rats, suggesting that S-8510 may be useful in the treatment of Alzheimer's disease.

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