AG-4:A NICOTINIC AGONIST ENDOWED WITH ANTIAMNESIC PROPERTIES

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Research Article

AG-4: A Nicotinic Agonist Endowed With Antiamnesic Properties

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Strategy, Management and Health Policy				
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ABSTRACT The effect of the nicotinic agonist AG-4 on memory processes was evaluated in the mouse passive avoidance test. AG-4 (100 μg per mouse icv) prevented amnesia induced by scopolamine (1.5 mg kg^{-1} ip), mecamylamine (20 mg kg^{-1} ip), and dihydro-β-erythroidine (10 μg per mouse icv). In the same experimental conditions, AG-4 (100 μg per mouse icv) also prevented baclofen (2 mg kg^{-1} ip), clonidine (0.125 mg kg^{-1} ip), and diphenhydramine (20 mg kg^{-1} ip) amnesia in mice. AG-4 exerted an antiamnesic effect comparable to that produced by nicotine (2 mg kg^{-1} ip), physostigmine (0.2 mg kg^{-1} ip), and the nootropic drug piracetam (30 mg kg^{-1} ip). At the active dose, AG-4 did not impair mice motor coordination and spontaneous motility as revealed, respectively, by Rota-rod test and Animex apparatus. Present results evidence the antiamnesic activity of the nicotinic agonist AG-4 suggesting a potential employment of this compound in the symptomatic treatment of cognitive impairments. Drug Dev. Res. 51:191–196, 2000.

Key words: AG-4; amnesia; learning and memory; passive avoidance; nicotinic agonist

INTRODUCTION

Nicotine enhances cognitive function in normal rats [Levine et al., 1990; Levin, 1992] and attenuates memory deficits produced by destruction of cholinergic input to the cortex and hippocampus [Tilson et al., 1988; Decker et al., 1992; Hodges et al., 1992], an effect shared by some other nicotinic acetylcholine receptor (nAChR) agonists [Decker et al., 1993]. In addition, nicotine improves short-term memory performance in both young and aged monkeys [Elrod et al., 1988; Buccafusco and Jackson, 1991]. The involvement of nicotinic neurotransmission in cognitive function processes is further substantiated by observed deficits in cognitive performances following administration of mecamylamine, an nAChR channel blocker, to rodents [Oliverio, 1966; Levine et al., 1987; Riekkinen et al., 1990], monkeys [Elrod et al., 1988], and humans [Newhouse et al., 1992]. Strong evidence for the potential of central nicotinic stimulation to enhance cognitive performance in Alzheimer disease patients derives from the clinical observation that nicotine infused intravenously significantly reduced the number of errors in cognitive tests [Newhouse et al., 1988].

The beneficial effect of nicotine was also observed with other agents that act at nAChRs. (-)-Lobeline [Decker et al., 1993] and ABT-418 [Decker et al., 1994] have both been reported to ameliorate spatial learning deficits in rats with septal lesions. Similarly, GTS-21 improved avoidance learning in rats with nucleus basalis magnocellularis lesions and reduced age-related learning deficits in both rats and rabbits [Arendash et al., 1995; Woodruff-Pak et al., 1994]; DBO-83 also prevented pharmacological-induced amnesia in the passive avoidance test [Ghelardini et al., 1998].

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The compound AG-4 (2-methyl-5-N,N-dimethyl-aminomethyl-cyclopentan-1-one methyl iodide) has been reported by Giannella et al. [1980] to be an agonist selective for the nicotinic receptors. It has also been reported that AG-4 enhanced the pain threshold in rodents without eliciting any side effect [Ghelardini et al., 1997]. Within the framework of research for new nicotinic agonists acting as cognitive enhancers, it was decided to investigate the pharmacological profile of AG-4 as a compound endowed with antiamnesic properties. In order to delineate the AG-4 procognitive action, its potential antiamnesic activity was investigated in the mouse passive avoidance test.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (24–26 g) from Morini (San Polo d'Enza, Italy) were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1°C on a 12-h light/dark cycle, lights on at 7AM.

Passive Avoidance

The test was performed according to the step-through method described by Jarvik and Kopp [1967]. The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. As soon as mice entered the dark compartment they received a punishing electrical shock (0.15 mA, 1 sec). The latency times for entering the dark compartment were measured in the training test and after 24 h in the retention test. The maximum entry latency allowed in the training and retention session was 60 and 180 sec, respectively. The memory degree of received punishment was expressed as increase in seconds between the latencies recorded in the training and retention sessions.

Rota-Rod Test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a nonslippery surface. This rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed according to Kuribara et al. [1977] on the basis of endurance time of the animals on the rotating rod. One day before the test the animals were trained twice. On the day of the test only the mice that were able to stay balanced on the rotating rod between 70 and 120 sec (cut-off time) were selected for testing. The performance time was measured before and at various times after treatment.

Spontaneous Activity Meter (Animex)

Locomotor activity in mice was quantified using an Animex activity meter Type S (LKB, Farad, Sweden) set to maximum sensitivity. Every movement of mice, which were placed on top of the Animex activity meter, produced a signal due to variation in inductance and capacity of the apparatus resonance circuit. Then signals were automatically converted to numbers. On the day of the experiment the mice were treated and then the cage, containing five mice, was put on the measuring platform. Activity counts were made every 15 min for 45 min starting immediately after injection of the drug. Because of the arbitrary scale adopted to quantify movements, drug-treated mice were always compared with saline-treated ones.

ICV Injection Technique

Intracerebroventricular (icv) administration was performed under ether anesthesia with isotonic saline as solvent, according to the method described by Haley and McCormick [1957]. During anesthesia, mice were grasped firmly by the loose skin behind the head. A hypodermic needle (0.4 mm external diameter) attached to a 10 μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse, where 5 μl of solution was administered. The injection site was 1 mm to the right or left from the midpoint on a line drawn through to the anterior base of the ears. Injections were performed randomly into the right or left ventricle. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice were injected with 5 μl of diluted 1:10 India ink and their brains examined macroscopically after sectioning. The accuracy of the injection technique was evaluated, with 95% of injections being correct.

Reagents and Compounds

The following compounds were used: AG-4 (2-methyl-5-N,N-dimethylaminomethyl-cyclopentan-1-one methyl iodide) was a kind gift of Prof. Giannella (University of Camerino); diphenhydramine hydrochloride (De Angeli); mecamylamine hydrochloride, clonidine hydrochloride (RBI, Natick, MA), nicotine dihydrogentartrate, dihydro-β-erythroidine (Fluka, Buchs, Switzerland); baclofen, piracetam, physostigmine hemisulphate, scopolamine hydrobromide (Sigma, St. Louis, MO).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution and concentrations were prepared in such a way that the necessary dose could be administered in a volume of 5 μl per mouse by icv injection and 10 ml kg^{-1} by ip injection

Statistical Analysis

All experimental results are given as the mean ± SEM. ANOVA, followed by Fisher's Protected Least Significant Difference procedure for post-hoc comparison, were used

to verify significance between two means. Data were analyzed with the StatView software for the Macintosh (1992). *P*-values of less than 0.05 were considered significant.

RESULTS

Antiamnesic Activity of AG-4

The nicotinic agonist AG-4 prevented amnesia induced by the antimuscarinic drug scopolamine (1.5 mg kg^{-1} ip) in the mouse passive avoidance test (Fig. 1). AG-4, at the doses of 10 and 25 μg per mouse icv, was completely ineffective, whereas at the dose of 100 μg per mouse icv it prevented antimuscarinic amnesia, reaching entrance latency values comparable to those produced by saline-treated mice.

The antiamnesic effect of AG-4 was also equal to that produced by nicotine (2 mg kg^{-1} ip) and the well-known nootropic drug piracetam (30 mg kg^{-1} ip) (Fig. 1). However, at the active dose, AG-4 did not enhance the entrance latency in unamnesic mice in comparison with the control group (Fig. 1). There were no differences observed in the various entrance latencies of every group in the training session of the passive avoidance test (Fig. 1).

The administration of AG-4, at the dose of 100 μg per mouse icv, antagonized also the memory disruption produced by the two nicotinic antagonists: mecamylamine (20 mg kg^{-1} ip; Fig. 2) and dihydro-β-erythroidine (10 μg per mouse icv; Fig. 3). The antiamnesic effect of AG-4 was comparable to that exerted by nicotine (2–5 mg kg^{-1} ip; Figs. 2, 3), by the anticholinesterase inhibitor physostigmine (0.2 mg kg^{-1} ip; Fig. 2) and by the nootropic drug piracetam (30 mg kg^{-1} ip; Fig. 3).

AG-4, at the dose of 100 μg per mouse icv, was able to prevent amnesia induced by the α_2 agonist clonidine

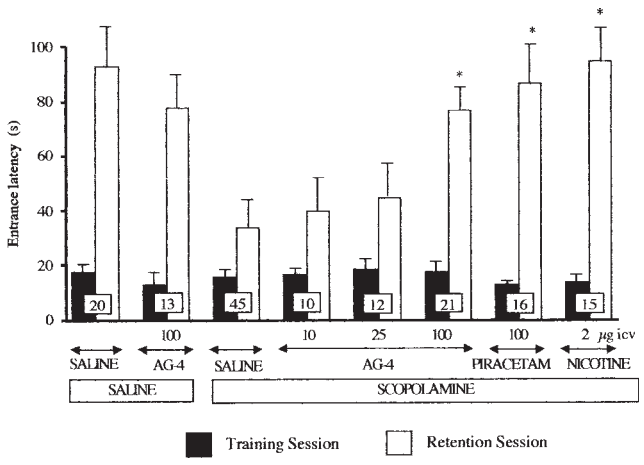

Fig. 1. Dose–response curve of AG-4 in comparison with piracetam and nicotine on amnesia induced by scopolamine (1.5 mg kg^{-1} ip) in the mouse passive avoidance test. AG-4 (icv), piracetam (icv), and nicotine (icv) were administered 20 min before the training session, while scopolamine was injected immediately afterwards. The number of mice is inside the column. *$P < 0.01$ in comparison with scopolamine-treated mice.

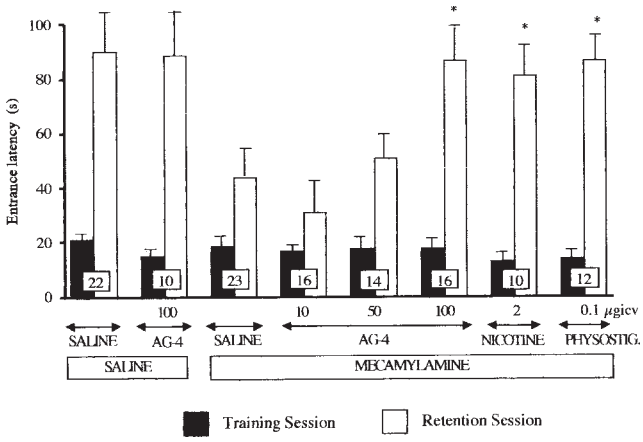

Fig. 2. Dose–response curve of AG-4 in comparison with nicotine and physostigmine on amnesia induced by mecamylamine (20 mg kg^{-1} ip) in the mouse passive avoidance test. AG-4 (icv), nicotine (icv), and physostigmine (icv) were administered 20 min before the training session, while mecamylamine was injected immediately afterwards. The number of mice is inside the column. *$P < 0.01$ in comparison with mecamylamine-treated mice.

(0.125 mg kg^{-1} ip), the $GABA_B$ agonist baclofen (2 mg kg^{-1} ip) and the H_1 antagonist diphenhydramine (20 mg kg^{-1} ip) (Fig. 4) in the mouse passive avoidance test. This antiamnesic effect was comparable to that exerted by piracetam (30 mg kg^{-1} ip; Fig. 4).

Effect of AG-4 on Mouse Rota-Rod Test and Mouse Animex Apparatus

It should be noted that AG-4 elicited its modulatory effect on cognitive processes without changing either gross behavior or motor coordination and spontaneous motility

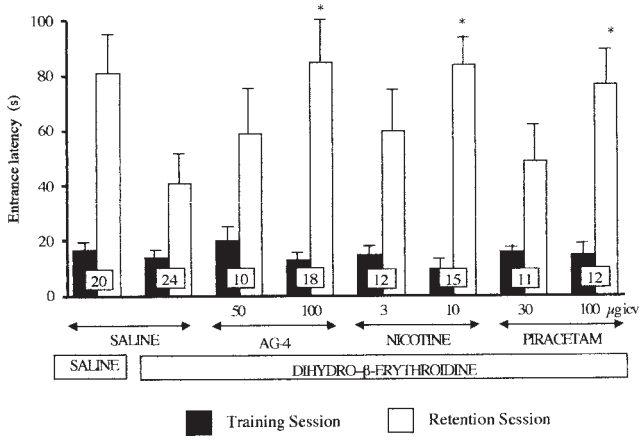

Fig. 3. Dose–response curve of AG-4 in comparison with nicotine and piracetam on amnesia induced by dihydro-β-erythroidine (10 μg per mouse icv) in the mouse passive avoidance test. AG-4 (icv), nicotine (icv), and piracetam (icv) were administered 20 min before the training session, while dihydro-β-erythroidine was injected immediately afterwards. The number of mice is inside the column. *$P < 0.01$ in comparison with dihydro-β-erythroidine-treated mice.

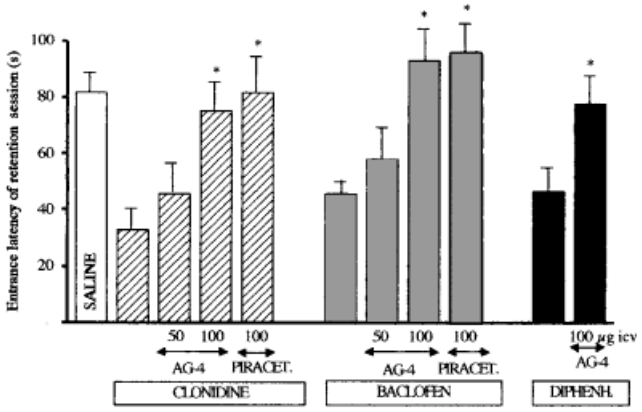

Fig. 4. Dose–response curve of AG-4 in comparison with piracetam on amnesia induced in the mouse by clonidine (0.125 mg kg $^{-1}$ ip), baclofen (2 mg kg $^{-1}$ ip), and diphenhydramine (20 mg kg $^{-1}$ ip) in the passive avoidance test. AG-4 (icv) and piracetam (icv) were administered 20 min before the training session, clonidine 60 min before, baclofen and diphenhydramine immediately afterwards. Each column represents the mean of at least 10 mice. *$P < 0.01$ in comparison with amnesic drug-treated animals.

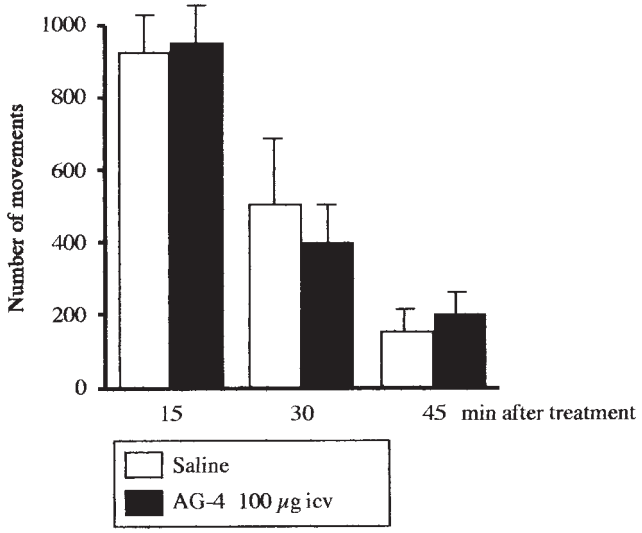

Fig. 5. Lack of effect of AG-4 on mouse spontaneous motility. Each column represents the mean of 15 mice.

as revealed, respectively, by the mouse Rota-rod test (Table 1) and the rat Animex apparatus (Fig. 5). AG-4, administered at the active dose, did not reduce the endurance time on the rotating rod in comparison with saline-treated mice (Table 1).The spontaneous motility of mice was unmodified by AG-4 administration (100 µg per mouse icv) as revealed by the Animex apparatus in comparison with saline-treated rats (Fig. 5).

DISCUSSION

The present results describe acute effects observed with AG-4 on experimentally impaired memory in mice. AG-4 has been demonstrated to prevent amnesia induced by pharmacological treatments in the passive avoidance test.

That stimulation of the nicotinic system improves cognitive processes has long been observed (see Introduction). On the other hand, the administration of nicotinic ACh receptor antagonists, such as mecamylamine, produces a dose-dependent impairment of performance in the passive avoidance test [Elrod and Buccafusco, 1991]. The administration of scopolamine, an unselective muscarinic ACh receptor antagonist, results in impaired learning and memory in humans [Frumier et al., 1976] and animals [Dilts and Berry, 1967; Levin and Bowman, 1986].

Amnesia can also be obtained by modulating neurotransmitter systems different other than the cholinergic. GABA is the main inhibitory neurotransmitter in the brain and it plays an important role in learning and memory. The activation of $GABA_A$ receptors impairs memory performances [Jerusalinsky et al., 1994] and the stimulation of $GABA_B$ receptors by baclofen disrupts memory after systemic, intra-amygdala or intraseptal administration [Swartzwelder et al., 1987; Castellano et al., 1989; Stackman and Walsh, 1994]. The antihistaminics are known to exert a variety of effects on the central nervous system. Central depression usually accompanies therapeutic doses of the H_1 antagonists, which appears to be related to occupancy of cerebral H_1 receptors; impairment of cognitive functions is a common manifestation [Simons and Simons, 1994]. Furthermore, the administration of the cerebral H_1 antagonist diphenhydramine induces amnesia also in animals [Kamei et al., 1990; Galeotti et al., 1998]. It has also been reported that activation of α_2 receptors modulates memory processes. The administration of the α_2 receptor agonist clonidine induces amnesia [Lazarova-Bakarova et al., 1991; Varonina et al., 1991; Genkova-Papazova et al., 1997].

AG-4 was able to prevent amnesia induced by the administration of scopolamine, mecamylamine, β-eryth-

TABLE 1. Effect of AG-4 in Comparison With Nicotine in the Mouse Roda- Test

	Endurance time on Rota-rod (s)			
	Before treatment	After treatment		
		15 min	30 min	45 min
Saline icv	96.5 ± 7.2 (10)	91.2 ± 7.9 (10)	102.3 ± 8.6 (10)	95.4 ± 7.6 (10)
AG-4 100 µg icv	96.8 ± 7.2 (9)	88.9 ± 6.7 (9)	94.7 ± 9.2 (9)	89.5 ± 8.3 (9)
Nicotine 10 µg icv	98.7 ± 8.2 (8)	63.5 ± 8.1* (8)	75.4 ± 0.1** (8)	94.3 ± 8.8 (8)

**$P < 0.05$; *$P < 0.01$ in comparison with the corresponding saline controls. The number of mice is shown in parentheses.

roidine, clonidine, baclofen, and diphenhydramine. Thus, AG-4 counteracts amnesia not only induced by nicotinic antagonists, but also that obtained independently from a nicotinic blockade.

AG-4 is also endowed with antinociceptive properties and the time course of the antiamnesic activity of AG-4 was equal to that observed for its antinociceptive action, reaching its maximum between 15 and 30 min after injection [Ghelardini et al., 1997]. Therefore, in the learning and memory experiments AG-4 was administered 20 min before the training session.

The evaluation of the behavioral parameters in mice indicates that AG-4 exerted its antiamnesic activity without producing any behavioral side effect. AG-4, at the active dose, nor did impair motor coordination as revealed by the Rota-rod test or modify spontaneous motility, as indicated by the Animex apparatus. Therefore, AG-4 shares many of the positive CNS attributes (e.g., memory-enhancing action, antinociception) of nicotine, but exhibits a reduced propensity to elicit the typical side effects, such as tremors, increase of spontaneous motility produced by injection of nicotine, a limit for its potential use as a safe treatment of cognitive dysfunctions. In other words, AG-4 is able to counteract amnesia in a physiological manner.

In summary, AG-4 appears to have cognitive-enhancing properties in the presence of pharmacologically induced amnesia. On these bases, AG-4 could be considered a new potential antiamnesic drug useful in the treatment of cognitive disorders.

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