

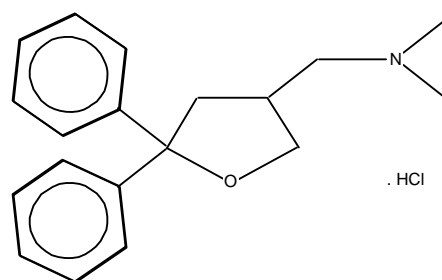
Involvement of the σ_1 receptor in the anti-amnesic effects of the aminotetrahydrofuran derivative AE14

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Introduction

Tetrahydro-N, N-dimethyl-5, 5-diphenyl-3-furanmethanamine hydrochloride (AE14) is a new tetrahydrofuranic compound showing prominent anti-amnesic, anticonvulsant and anti-depressant potentials (Vamvakides, 2002). Preliminary experiments revealed that AE14, at 0.3-30 mg/kg per os (p.o.), improved the mnemonic capacities of mice submitted to a step-down type passive avoidance procedure; at 19-45 mg/kg p.o., the compound antagonized the pentylenetetrazol- or electroshock-induced tonic seizures; and, at 10 mg/kg intraperitoneally (i.p.) or 30 mg/kg p.o., it shortened the immobility duration in the forced swimming test (Vamvakides, 2002; unpublished data). Several pharmacological targets have been identified for AE14. In particular, functional tests in the rabbit vas deferens, guinea pig atria and ileum revealed that the drug is a potent M1 muscarinic acetylcholine receptor agonist and M2/M3 antagonist (unpublished data). The compound also inhibited the [³H]batrachotoxin binding to the sodium channel site 2 and [³H](+)-pentazocine binding to the σ_1 receptor, in the rat brain, with low micromolar affinities. AE14 may therefore present a very attractive pharmacological profile with unique characteristics. The drug may simultaneously act as an agonist on postsynaptic M1 receptors and as an antagonist on presynaptic M2 autoreceptors. It may also present potent pro-mnesic or anti-amnesic properties by activating membrane-bound M1 receptors and intracellular σ_1 receptor mainly located on the endoplasmic reticulum (Hayashi et al., 2000). Both receptors have been shown to activate phospholipase C (PLC) and protein kinase C (PKC)-dependent and mitogen-activated protein kinase (MAPK) pathways (Fisher, 2000; Morin-Surun et al., 1999). Consequently, synergistic mechanisms could be expected regarding AE14 effects on M1 and σ_1 receptors.

The aim of the present study was to firmly establish whether AE14 readily interacts with muscarinic and σ_1 receptors in vivo. The mnemonic effects of AE14 alone or the anti-amnesic effects of the drug in combination with scopolamine or dizocilpine were examined in mice submitted to short-term memory tests (spontaneous alternation) or spatial or contextual memory tests (place learning in the water-maze and passive avoidance, respectively).

Materials and Methods

Animals & Drugs

Male Swiss mice weighing 35 ± 2 g were used. All animal procedures were conducted in strict adherence of European Union Directive of 24 November 1986. Tetrahydro-N, N-dimethyl-5, 5-diphenyl-3-furanmethanamine hydrochloride (AE14) was synthesized in the laboratory (ANAVEX, Greece). N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine hydrochloride (BD1047) was kindly provided by Dr Wayne D. Bowen (Brown University, Providence, RI, USA). Other drugs were purchased from Sigma-Aldrich.

Design and administration of oligodeoxynucleotides

Based on the mouse cDNA sequences for the σ_1 receptor, 16-mer phosphorothioate-modified oligodeoxynucleotide (ODN) sequences were designed, as previously described (Maurice et al., 2001) : antisense ODN (aODN) = 5'-CGCGGCCACGGCATT-3', mismatch ODN (mODN) = 5'-CACGTCCCTCTCCATT-3' (Eurobio labs). Mice were implanted with an icv cannula under pentobarbital anesthesia and injected into the right ventricle. ODN (1 μ l) were injected twice per day, at 12 h time interval, during 3 days. They were used for behavioral observations, 10 h after the last injection, i.e., 4 days after cannulation.

Spontaneous alternation performances

Spatial working memory was examined through the measure of spontaneous alternation performance in the Y-maze, as previously described by Maurice et al. (2001).

Passive avoidance procedures

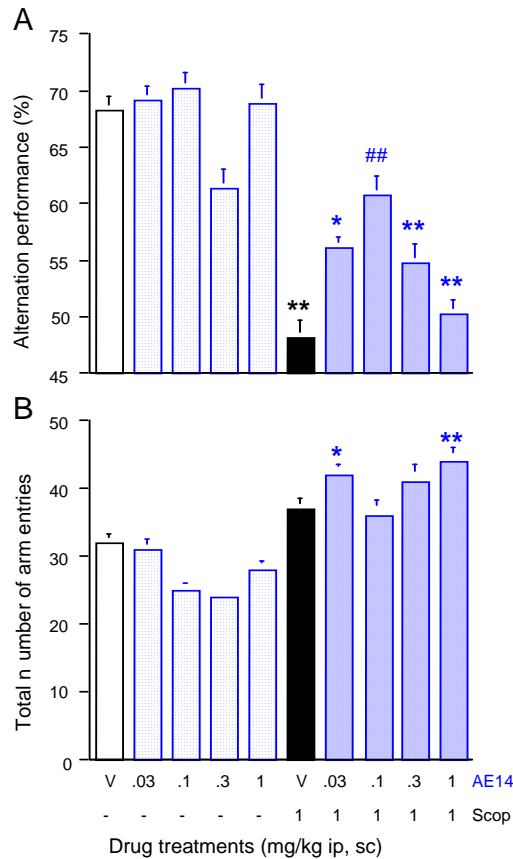
Contextual long-term memory was measured using the step-down type or step-through type passive avoidance tests, as previously described (Maurice et al., 2001; Meunier et al. 2006). During the retention session, performed 24 h after training, the step-down or step-through latency was determined. In the step-down procedure, the animals-to-criterion value shows the percentage of animals showing a retention latency three fold higher than the training latency.

Place learning in the water-maze

Spatial long-term memory was assessed using place learning in the water-maze. Reference memory procedure: training consisted in 3 swimings per day during 5 days (20 min ITI), with a fixed platform location. On day 5, 1 h after the last swimming, animals were submitted to a probe test. Working memory procedure : animals were submitted to 4 swimings per day during 3 days (2 min ITI), with a daily changing platform location.

Figure 1

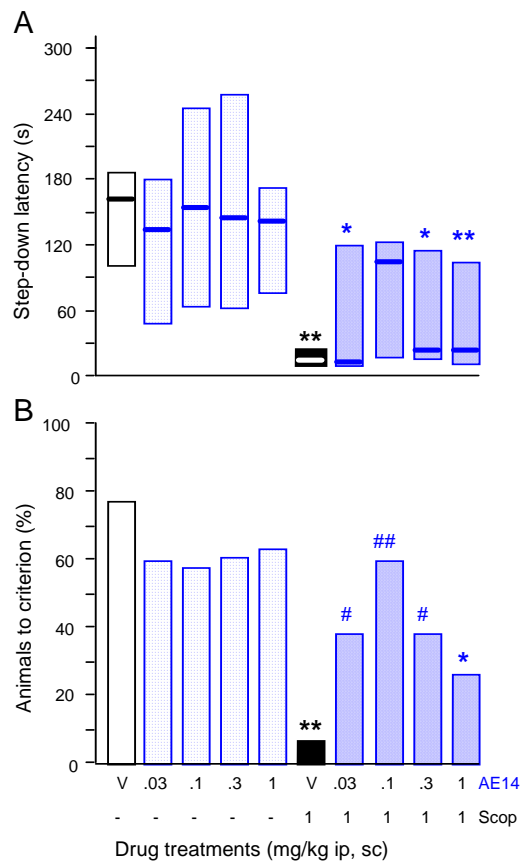
AE14 dose-dependently attenuated the scopolamine-induced spontaneous alternation deficits in mice: (A) alternation performances, (B) total number of arm entries



AE14 (0.03 - 1 mg/kg i.p.), or vehicle solution (V), was administered 30 min before the test, or 10 min before scopolamine (Scop, 1 mg/kg s.c.), which was administered 20 min before the test. $n = 11-16$ per group; $F_{(9,119)} = 8.62$, $p < 0.0001$ in (A); $F_{(9,119)} = 7.04$, $p < 0.0001$ in (B). * $p < 0.05$, ** $p < 0.01$ vs. the V-treated group; ## $p < 0.01$ vs. the Scop-treated group; Dunnett's test.

Figure 2

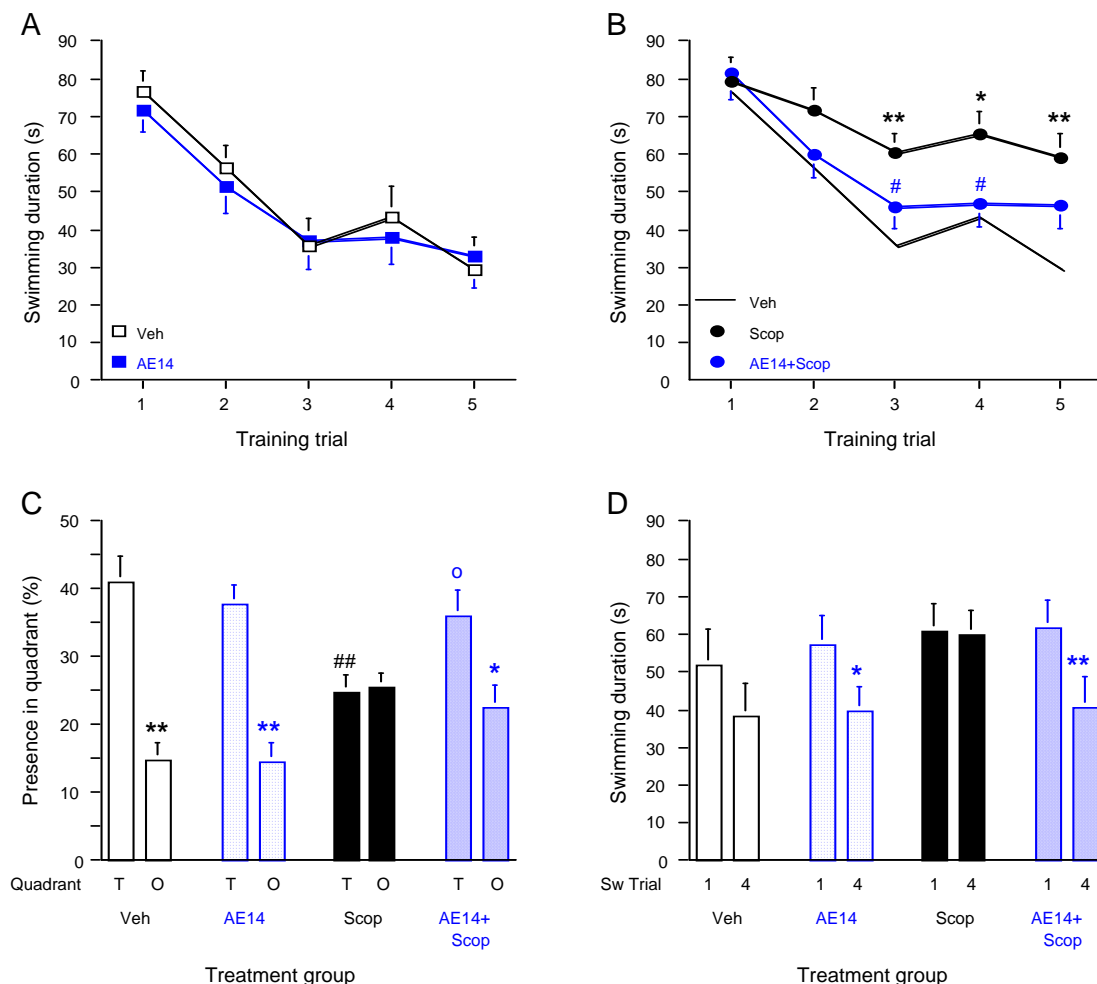
AE14 dose-dependently attenuated the scopolamine-induced passive avoidance deficits: (A) step-down latency, (B) percentage of animals to criterion



AE14 (0.03 - 1 mg/kg i.p.) , or vehicle solution (V), was administered 30 min before the first training, or 10 min before scopolamine (Scop, 1 mg/kg s.c.), which was administered 20 min before the first training. A second training was repeated 90 min after, and retention was examined after 24 h. In (A), results show the median and interquartile range. $n = 10-16$ per group; $KW = 41.70$, $p < 0.0001$. * $p < 0.05$, ** $p < 0.01$ vs. the V-treated group; # $p < 0.05$ vs. the Scop-treated group; Dunn's test in (A) and χ^2 test in (B).

Figure 3

AE14 attenuated the scopolamine-induced impairments of place learning in the water-maze: (A,B) acquisition profiles, (C) probe test performances and (D) working memory performances

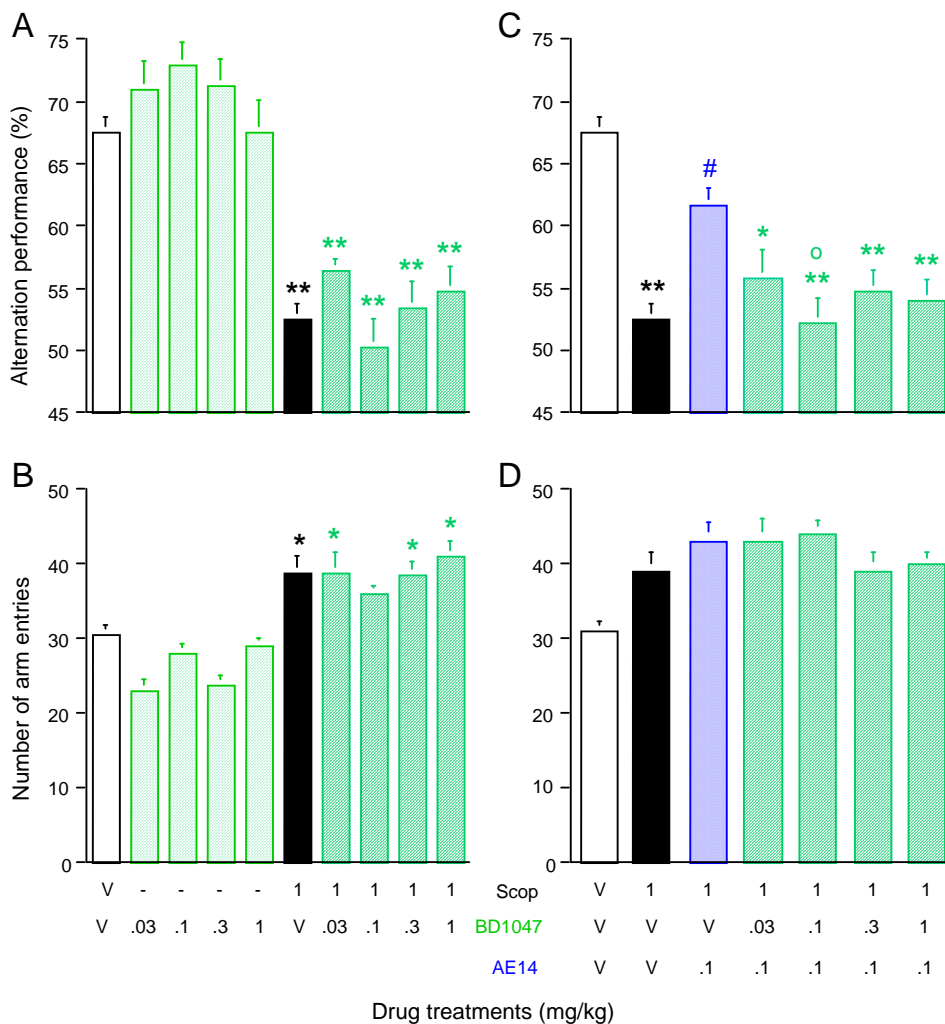


Animals were administered with vehicle solution (Veh i.p.), AE14 (0.3 mg/kg i.p.) and/or scopolamine (Scop, 1 mg/kg s.c.) 20 min before the first trial and submitted during 5 days to 3 swims per day. (A, B) Acquisition profiles. Repeated measures ANOVA: $F_{r} = 25.06$, $p < 0.0001$, $n = 17$ for the Veh-treated group; $F_{r} = 11.70$, $p < 0.05$, $n = 18$ for the Scop-treated group; $F_{r} = 20.69$, $p < 0.001$, $n = 18$ for the AE14-treated group; $F_{r} = 27.37$, $p < 0.0001$, $n = 22$ for the (AE14+Scop)-treated group; * $p < 0.05$, ** $p < 0.01$ vs. latencies shown by the Veh-treated group during the same training day; # $p < 0.05$ vs. latencies shown by the Scop-treated group; Dunn's test. (C) Probe test. The presence in each quadrant was measured and presence in the training (T) and opposite (O) quadrants are presented. ** $p < 0.01$ vs. time spent in the T quadrant for the same experimental group; # $p < 0.05$, ### $p < 0.01$ vs. time spent in the T quadrant for the Veh-treated group; Dunnett's test. (D) Working memory procedure. The swimming duration measured for the 1st and 4th swimming trial are presented. * $p < 0.05$, ** $p < 0.01$ vs. trial 1; Dunn's test.

Figure 4

The σ_1 receptor antagonist BD104 blocked the AE14 (0.1 mg/kg i.p.) beneficial effect on alternation deficits induced by scopolamine:

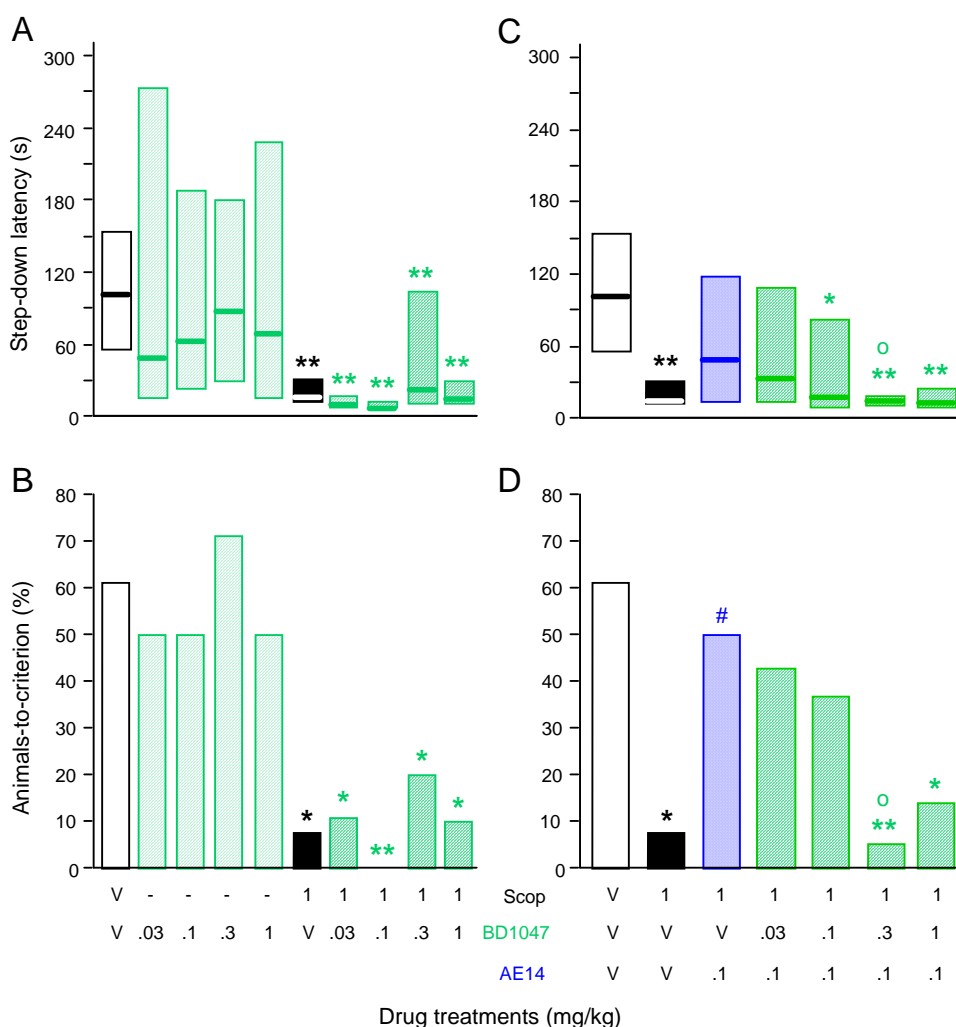
(A,C) alternation percentages, (B,D) total number of arm entries. (A,B) Lack of effect of BD1047 alone or in combination with scopolamine; (C,D) Antagonism studies



BD1047 (0.03 – 1 mg/kg i.p.), AE14 (0.1 mg/kg i.p.), or vehicle solution (V), was administered 30 min before the test, or 10 min before scopolamine (Scop, 1 mg/kg s.c.), which was administered 20 min before the test. $n = 9-14$ in (A, B) and $n = 8-14$ in (C, D); $F_{(9,92)} = 5.68$, $p < 0.0001$ in (A), $F_{(9,92)} = 4.07$, $p < 0.001$ in (B), $F_{(6,77)} = 3.18$, $p < 0.01$ in (C), $F_{(6,77)} = 1.52$, $p > 0.05$ in (D). * $p < 0.05$, ** $p < 0.01$ vs. the V-treated group; # $p < 0.05$ vs. the Scop-treated group; ° $p < 0.05$ vs. the (AE14+Scop)-treated group; Dunnett's test.

Figure 5

BD1047 blocked the AE14 (0.1 mg/kg i.p.) beneficial effect on scopolamine-induced passive avoidance deficits: (A,C) step-down latency, (B,D) percentage of animals to criterion. (A,B) Lack of effect of BD1047 alone or in combination with scopolamine; (C,D) Antagonism studies

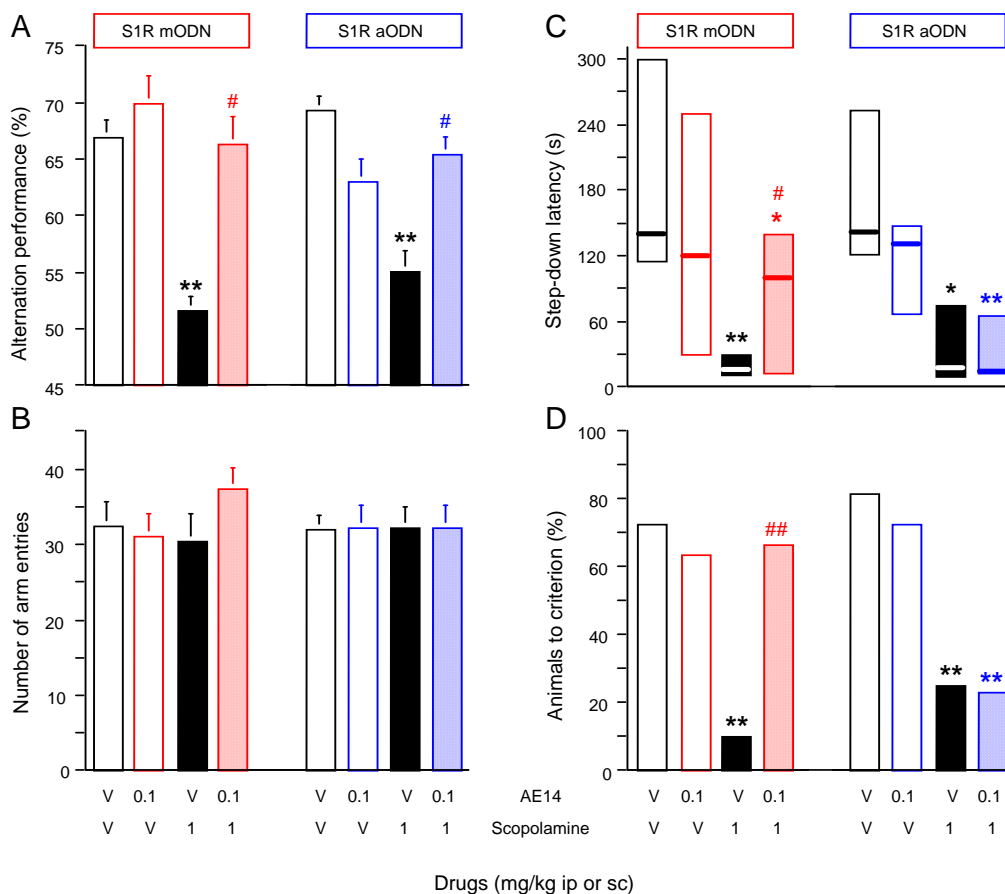


BD1047 (0.03 – 1 mg/kg i.p.), AE14 (0.1 mg/kg i.p.), or vehicle solution (V), was administered 30 min before the first training, or 10 min before scopolamine (Scop, 1 mg/kg s.c.), which was administered 20 min before the first training. In (A, C), results show the median and interquartile range. $n = 10-14$ in (A, B) and $n = 12-21$ in (C, D); $KW = 40.64$, $p < 0.0001$ in (A); $KW = 21.95$, $p < 0.01$ in (C). * $p < 0.05$, ** $p < 0.01$ vs. the V-treated group; # $p < 0.05$ vs. the Scop-treated group; ° $p < 0.05$ vs. the (AE14+Scop)-treated group; Dunn's test in (A, C) and χ^2 test in (B, D).

Figure 6

σ_1 receptor antisense ODN treatments blocked the anti-amnesic effect of AE14 against the scopolamine-induced passive avoidance impairments:

(A) alternation performances and (B) number of arm entries in the Y-maze; (C) latencies and (D) percentage of animals to criterion during retention in the passive avoidance test

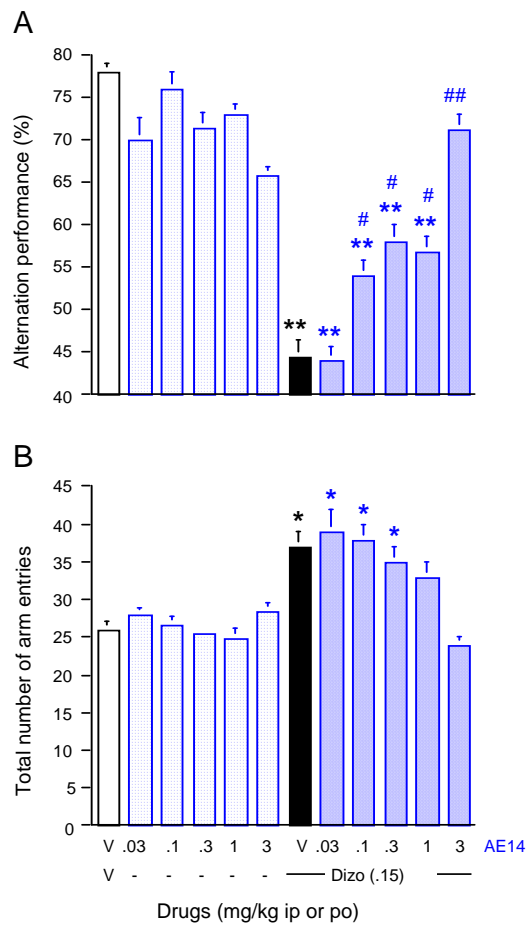


The σ_1 receptor antisense ODN (S1R aODN) or mismatch control ODN (S1R mODN) were administered i.c.v. every 12 h during 3 days. AE14 (0.1 mg/kg i.p.) was administered 10 min before scopolamine (1 mg/kg s.c.) which was given 20 min before the Y-maze session or the first passive avoidance training session. $n = 8-11$ in (A, B), $n = 9-13$ in (C, D); $F_{(3,34)} = 4.98$, $p < 0.01$ for S1R mODN and $F_{(3,38)} = 3.00$, $p < 0.05$ for S1R aODN in (A); $F_{(3,34)} = 0.84$, $p > 0.05$ for S1R mODN and $F_{(3,38)} = 0.00$, $p = 1.0$ for S1R aODN in (B); $KW = 11.32$, $p < 0.05$ for S1R mODN and $KW = 14.38$, $p < 0.01$ for S1R aODN in (C). * $p < 0.05$, ** $p < 0.01$ vs. the (V+V)-treated group; # $p < 0.05$, ## $p < 0.01$ vs. the (V+Scop)-treated group; Dunnett's test in (A), Dunn's test in (C), χ^2 test in (D).

Figure 7

AE14 dose-dependently blocked the dizocilpine-induced alternation deficits:

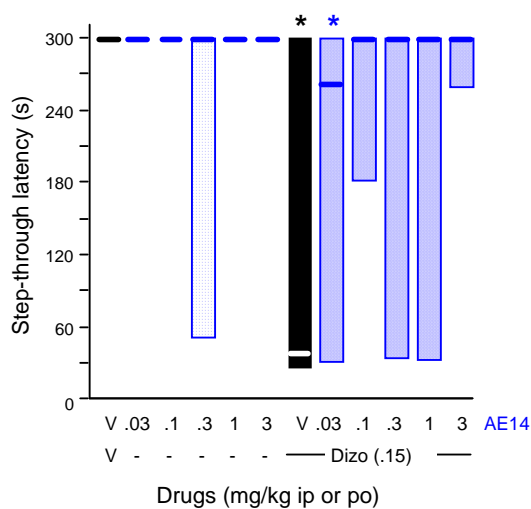
(A) alternation performances and (B) total number of arm entries



AE14 (1, 3 mg/kg p.o.) or vehicle solution (V), was administered 60 min before the test, or 40 min before dizocilpine (dizo, 0,15 mg/kg i.p.), which was administered 20 min before the test. The number of mice per group was $n = 8-15$. $F_{(11,132)} = 10,435$, $p < 0,0001$ in (A); $F_{(11,132)} = 2,6014$, $p = 0,0035$ in (B). * $p < 0.05$, ** $p < 0.01$ vs. the V-treated group; # $p < 0.05$, ## $p < 0.01$ vs. the dizo-treated group; Dunnett's test.

Figure 8

Effect of AE14 on the dizocilpine induced amnesia in the step-through passive avoidance test

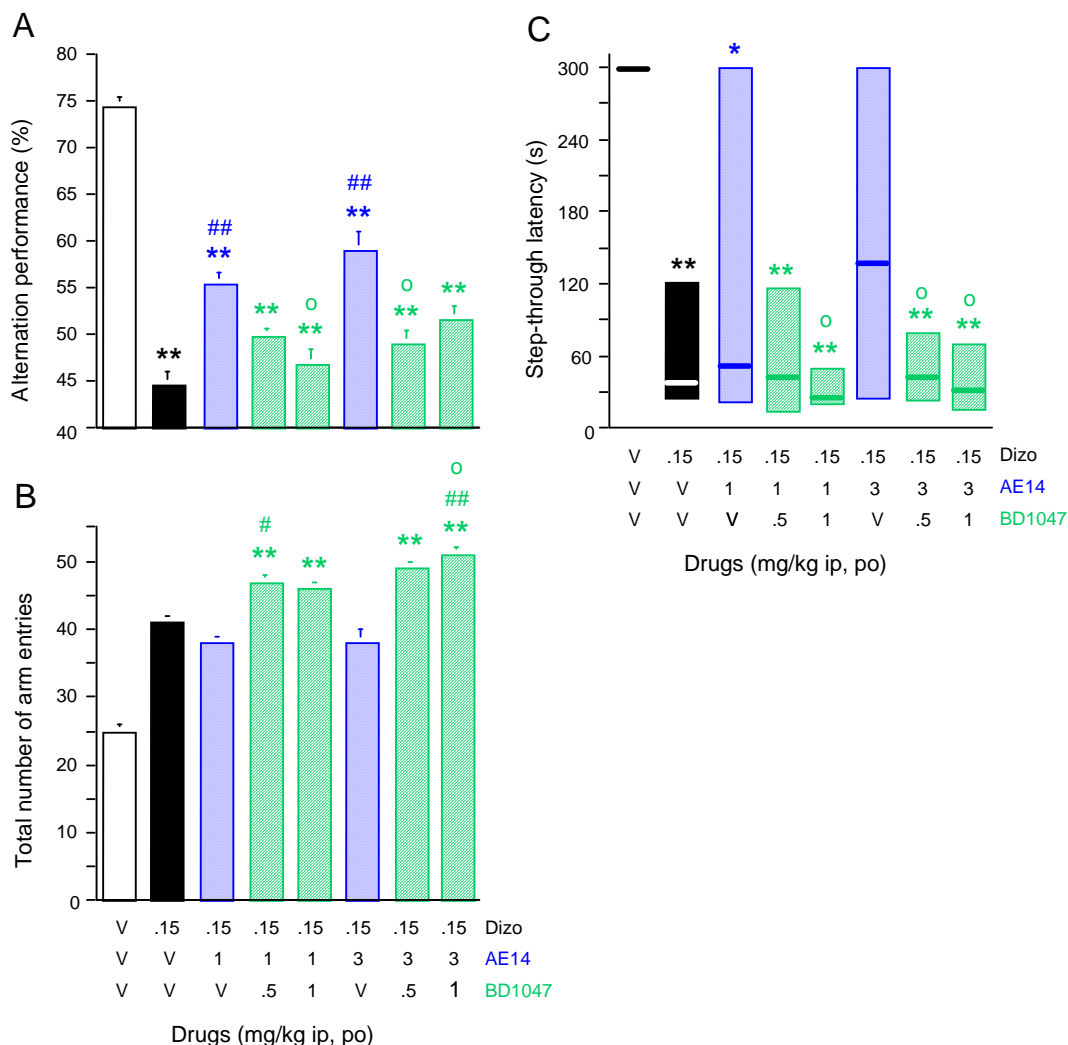


AE14 (1, 3 mg/kg p.o.) or vehicle solution (V), was administered 60 min before the test, or 40 min before dizocilpine (dizo, 0,15 mg/kg i.p.), which was administered 20 min before the test. The number of mice per group was $n = 8-15$. $KW = 27,242$, $p = 0,0042$, * $p < 0.05$ vs. the V-treated group; Kruskal-Wallis' test.

Figure 9

Blockade by BD1047 of the AE14 (1 and 3 mg/kg p.o.) beneficial effects on dizocilpine-induced learning impairments:

(A) alternation performances and (B) total number of arm entries in the Y-maze test; (C) step-through passive avoidance latency



AE14 (1, 3 mg/kg p.o.), or vehicle solution (V), was administered 60 min before the test, or 40 min before dizocilpine (dizo, 0,15 mg/kg i.p.), which was administered 20 min before the test. BD1047 (0.5, 1 mg/kg i.p.) was administered simultaneously with AE14. The number of mice per group was $n = 19-25$ in (A, B) and $n = 18-25$ in (C); $F_{(7,165)} = 13,10$, $p < 0.0001$ in (A), $F_{(7,165)} = 2,9937$, $p = 0,0056$ in (B), $KW = 45,24$, $p < 0.0001$ in (C). * $p < 0.05$, ** $p < 0.01$ vs. the V-treated group; # $p < 0.05$, ## $p < 0.01$ vs. the Dizo-treated group; ° $p < 0.05$ vs. the (AE14+Dizo)-treated group; Dunnett's test in (A,B), Kruskal-Wallis' test in (C).

Comments

This study showed that:

- AE14 failed to affect mnemonic capacities by itself in the dose-range tested (0,03-3 mg/kg, i.p. or p.o.). This was checked using behavioral tests measuring spatial working memory (spontaneous alternation in the Y-maze, place learning in the water-maze), contextual long-term memory (passive avoidance tests) or spatial reference memory (place learning in the water-maze).

- AE14 attenuated in a bell-shaped manner the scopolamine-induced amnesia, with a significant effect at 0.1 mg/kg i.p. observable in all behavioral procedures. This effect involved an interaction with the σ_1 receptor, since it was blocked by the σ_1 receptor antagonist BD1047.

- Interestingly, down-regulation of the σ_1 receptor expression by an in vivo antisense strategy blocked the anti-amnesic effects of AE14 on the long-term memory impairments induced by scopolamine, but not on working memory deficits.

- AE14 attenuated in a dose-response manner the doxycipine-induced amnesia, with significant effects at doses higher than 0.1 mg/kg p.o. The effects were observable on both short- and long-term memory tests and blocked by BD1047, suggesting a marked involvement of the σ_1 receptor.

In conclusion, the tetrahydrofuranic compound presents a mixed pharmacological activity involving muscarinic and σ_1 components, that may explain its efficacy as an anti-amnesic drug. The observation that a blockade of the σ_1 receptor by BD1047 resulted in a full blockade of the behavioral activity has been previously made using another mixed cholinergic/ σ_1 drug, the acetylcholinesterase inhibitor donepezil (*Meunier et al., 2006*). It suggests that σ_1 receptor activation, downstream to the activation of cholinergic membrane receptors modify their transduction mechanism. In other words, mixed muscarinic/ σ_1 activities maybe synergic rather than purely additive. This hypothesis, leading to very active and promising compounds, must be further investigated in the future.

Acknowledgements

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Fisher, *Jpn J Pharmacol* 84, 101-112 (2000); Hayashi et al., *J Pharmacol Exp Ther* 293, 788-798 (2000); Maurice et al., *Br J Pharmacol* 134, 1731-1741 (2001); Meunier et al., *Br J Pharmacol*, in press (2006); Morin-Surun et al., *Proc Natl Acad Sci USA* 96, 8196-8199 (1999); Vamvakides, *Ann Pharmaceut Franç*, 61, 207-210 (2003).