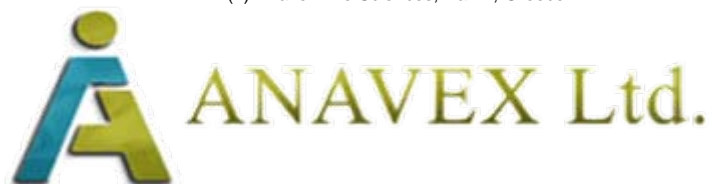


Anti-amnesic and neuroprotective potentials of aminotetrahydrofuran derivatives, mixed muscarinic/ σ_1 ligands, against amyloid β_{25-35} peptide toxicity in mice

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INTRODUCTION

Chronic neurodegenerative diseases are characterized by the pathologic aggregation of proteins responsible of synaptic and neuronal loss. In Alzheimer's disease (AD), the senile plaque core is formed of aggregated amyloid- β ($A\beta$) proteins. Cholinergic systems are highly sensitive to the amyloid toxicity and present strategies target central cholinergic systems. Nicotinic and muscarinic receptor ligands or inhibitors of acetylcholinesterase showed neuroprotective activity in AD animal models and in AD patients. Recently, we reported that σ_1 receptor agonists are also neuroprotective drugs in AD animal models, putatively due to the σ_1 receptor modulatory role on calcium mobilization and signal transduction pathways.

Tetrahydro-N,N-dimethyl-5,5-diphenyl-3-furanmethanamine (ANAVEX1-41) and tetrahydro-N,N-dimethyl-2,2-diphenyl-3-furanmethanamine (ANAVEX2-73) are new potent muscarinic ligands which showed a high to moderate affinity for the σ_1 receptor (46 and 860 nM, respectively). Their mixed cholinergic/ σ_1 pharmacological actions suggest possibly important neuroprotective efficacy in AD.

We here analyzed the anti-amnesic and protective effects of ANAVEX1-41 and ANAVEX2-73 in a nontransgenic mouse model of AD. Central injection of $A\beta_{25-35}$ peptide into the mouse brain induces within 7 days histological and biochemical changes, oxidative stress and learning deficits, highly reminiscent of AD. Injection of scrambled $A\beta$ peptide was used as control. Drugs were injected, in the 1-1000 $\mu\text{g}/\text{kg}$ dose range, either 7 days after $A\beta_{25-35}$ and 20 min before the tests, to assess their anti-amnesic effects, or 20 min before $A\beta_{25-35}$ peptide and 7 days before the tests, to assess their neuroprotective efficacy. Neuroprotective activity was also examined in, hippocampus extracts by measuring the level of lipid peroxidation and induction of the pro-apoptotic caspase-3 protease. Moreover, pre-injection of the σ_1 receptor antagonist BD1047 or the M1 muscarinic antagonist scopolamine before the drugs was used to analyze their pharmacological mode of action.

METHODS

Animals & Drugs

Male Swiss mice, aged 5-7 weeks were used according to the usual CompAn behavioral phenotyping facility procedures, in strict adherence of EC Council Directive 86-609.

ANAVEX1-41 and ANAVEX2-73 were provided by Anavex. N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine (BD1047) by Dr Wayne D. Bowen (Brown University, Providence, RI, USA). The amyloid β_{25-35} peptide ($A\beta_{25-35}$, SC489) and scrambled $A\beta$ peptide (Sc $A\beta$, SC492) were from NeoMPS. Other chemical reagents, were from Sigma-Aldrich. Drugs were injected i.p. in 100 μ l/20 g body weight.

Spontaneous alternation performance in the Y maze

Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The series of arm entries, including possible returns into the same arm, were checked visually. An alternation was defined as entries into all three arms on consecutive occasions and the percentage of alternation was calculated as (actual alternations / maximum alternations) x 100.

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. In the step-through, the time animals spent to re-exit the dark compartment was recorded as escape latency.

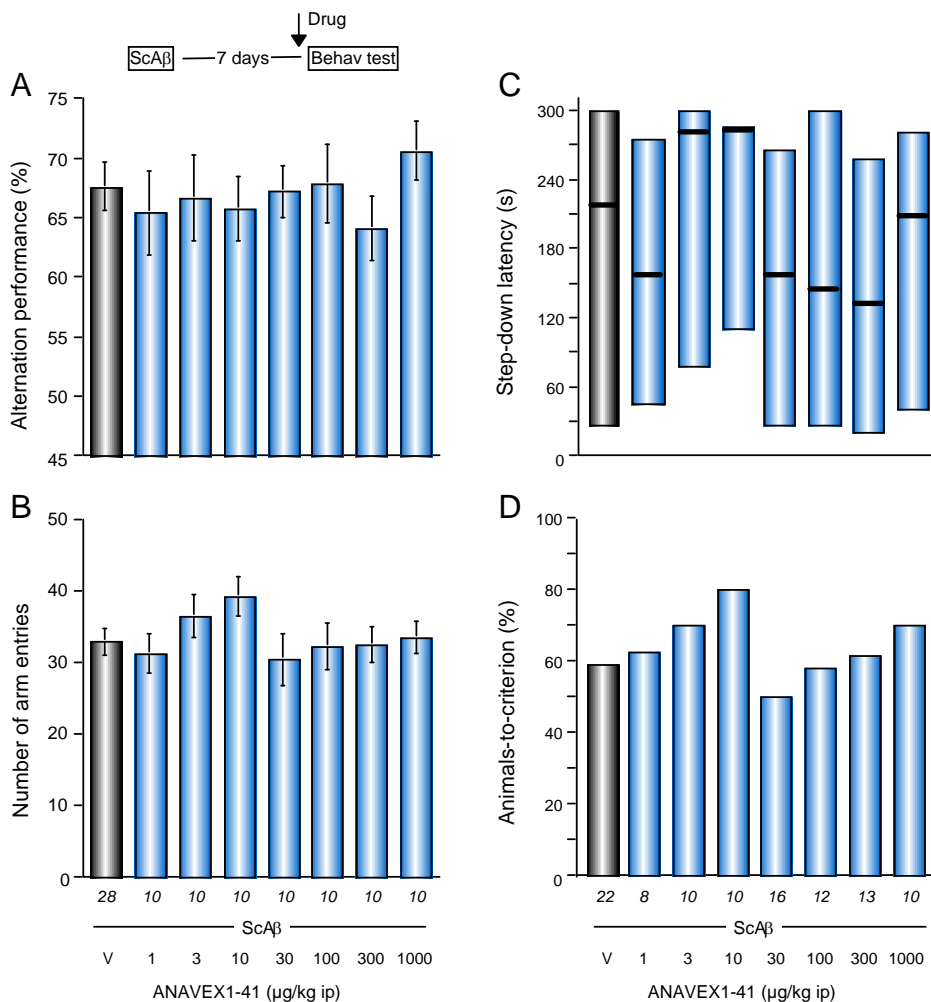
Lipid peroxidation measure

The quantification of lipid peroxidation in hippocampus preparations was performed using the modified FOX assay, based on Fe(III) xylene orange complex formation, according to Meunier et al. (2006).

ANAVEX1-41

FIGURE 1

ANAVEX1-41 failed to affect the mnemonic capacities by itself in mice

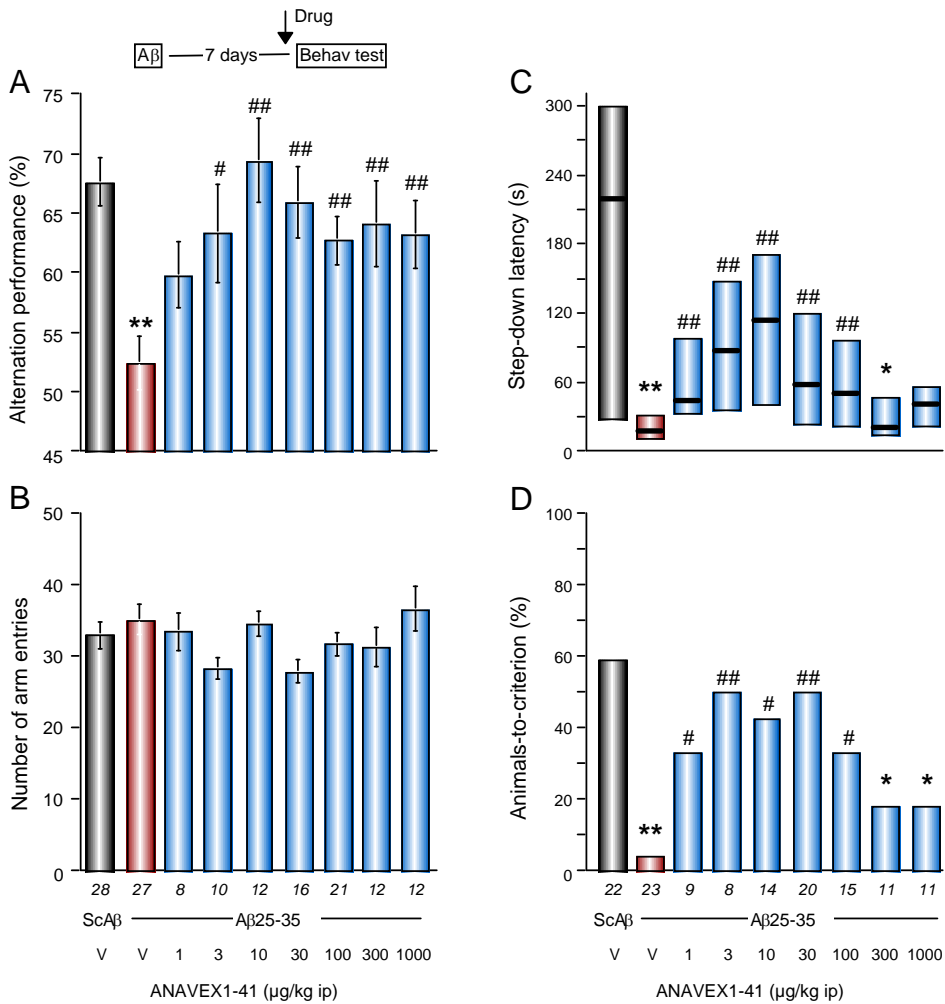


Spontaneous alternation test: (A) alternation percentage [$F < 1$] and (B) number of arm entries [$F(7,97) = 1.02, P > 0.05$]. Step-down passive avoidance test: (C) step-down latency ($KW = 2.98, P > 0.05$) and (D) percentage of animals to criterion.

ANAVEX1-41

FIGURE 2

ANAVEX1-41 dose-dependently reversed the A β ₂₅₋₃₅ peptide-induced memory deficits in mice

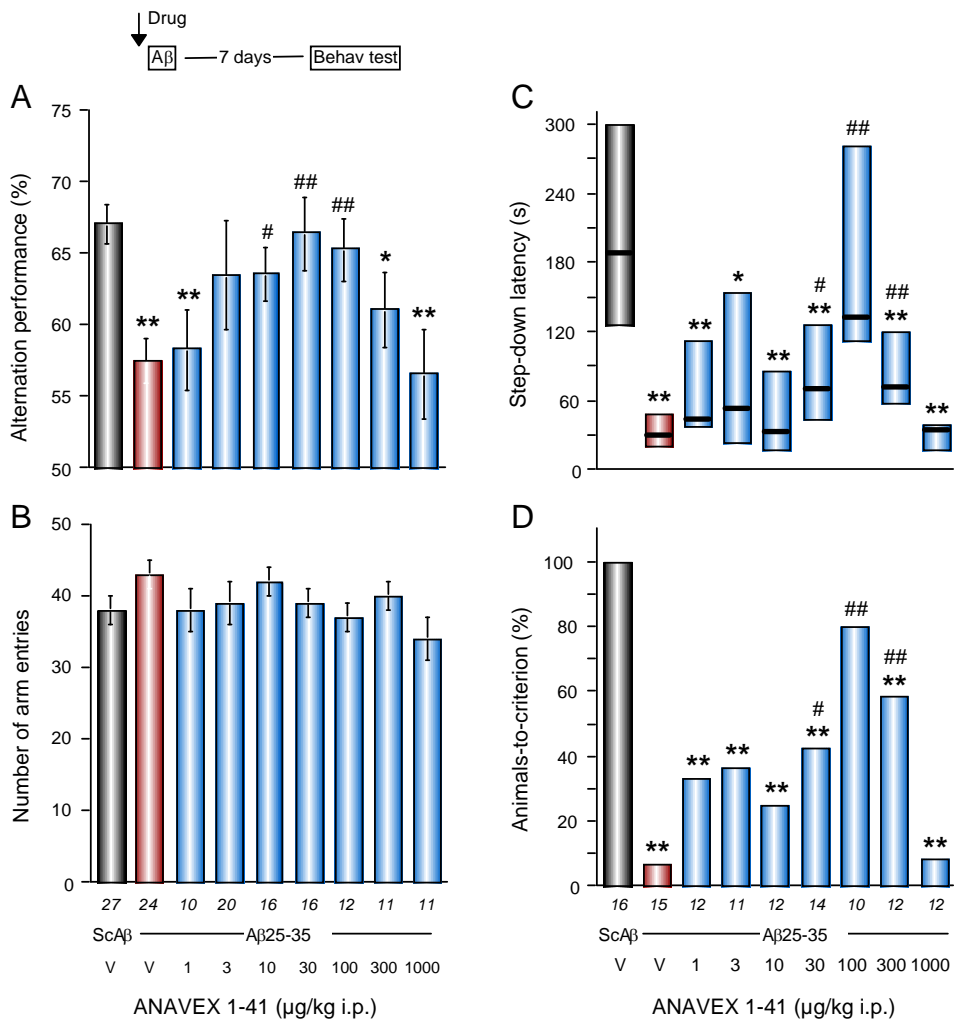


Spontaneous alternation test: (A) alternation percentage [F(8,145) = 4.41, P < 0.0001] and (B) number of arm entries [F(8,145) = 1.62, P > 0.05]. Step-down passive avoidance test: (C) step-down latency (KW = 27.72, P < 0.001) and (D) percentage of animals to criterion.

ANAVEX1-41

FIGURE 3

ANAVEX1-41 dose-dependently protected against the A β_{25-35} peptide-induced memory deficits in mice

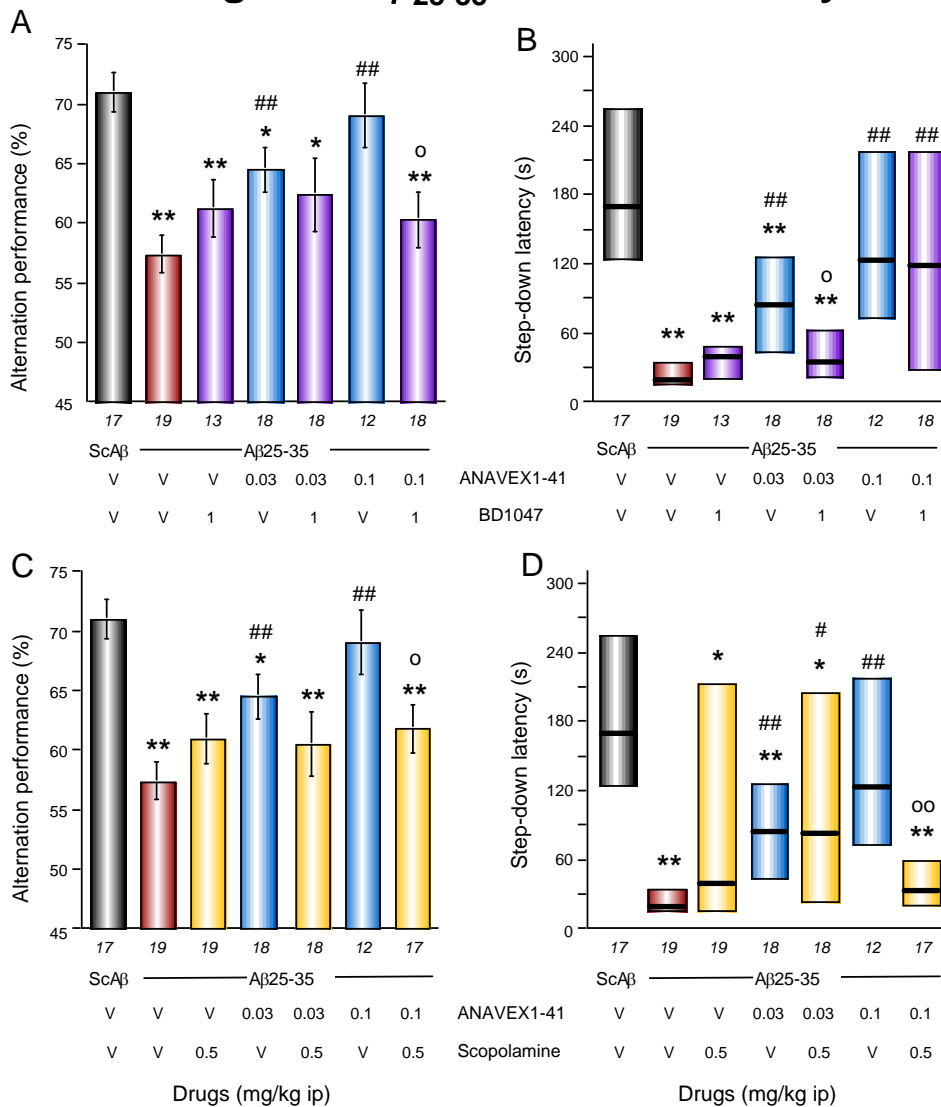


(A) $F(8,145) = 3.40, P < 0.01$; (B) $F(8,145) = 1.64, P > 0.05$; (C) $KW = 45.2, P < 0.0001$.

ANAVEX1-41

FIGURE 4

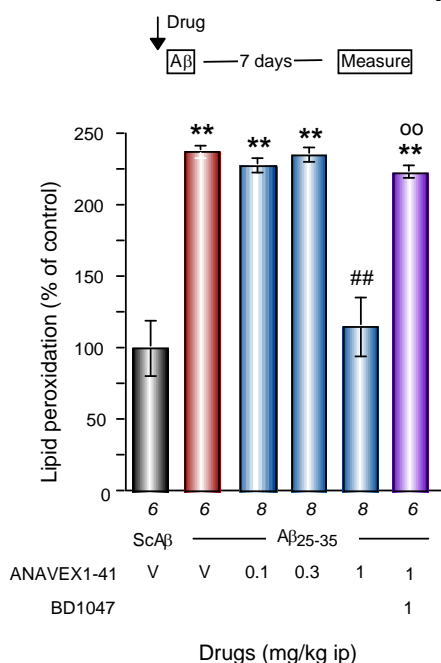
Pre-administration of the σ_1 antagonist BD1047 or the muscarinic antagonist scopolamine blocked the effect of ANAVEX1-41 against $A\beta_{25-35}$ -induced memory deficits



(A) $F(6,114) = 4.55$, $P < 0.001$; (B) $KW = 39.7$, $P < 0.0001$; (C) $F(6,119) = 5.14$, $P = 0.0001$; (D) $KW = 30.6$, $P < 0.0001$.

FIGURE 5

ANAVEX1-41 dose-dependently blocked the induction of lipid peroxydation in the hippocampus of $A\beta_{25-35}$ -treated mice



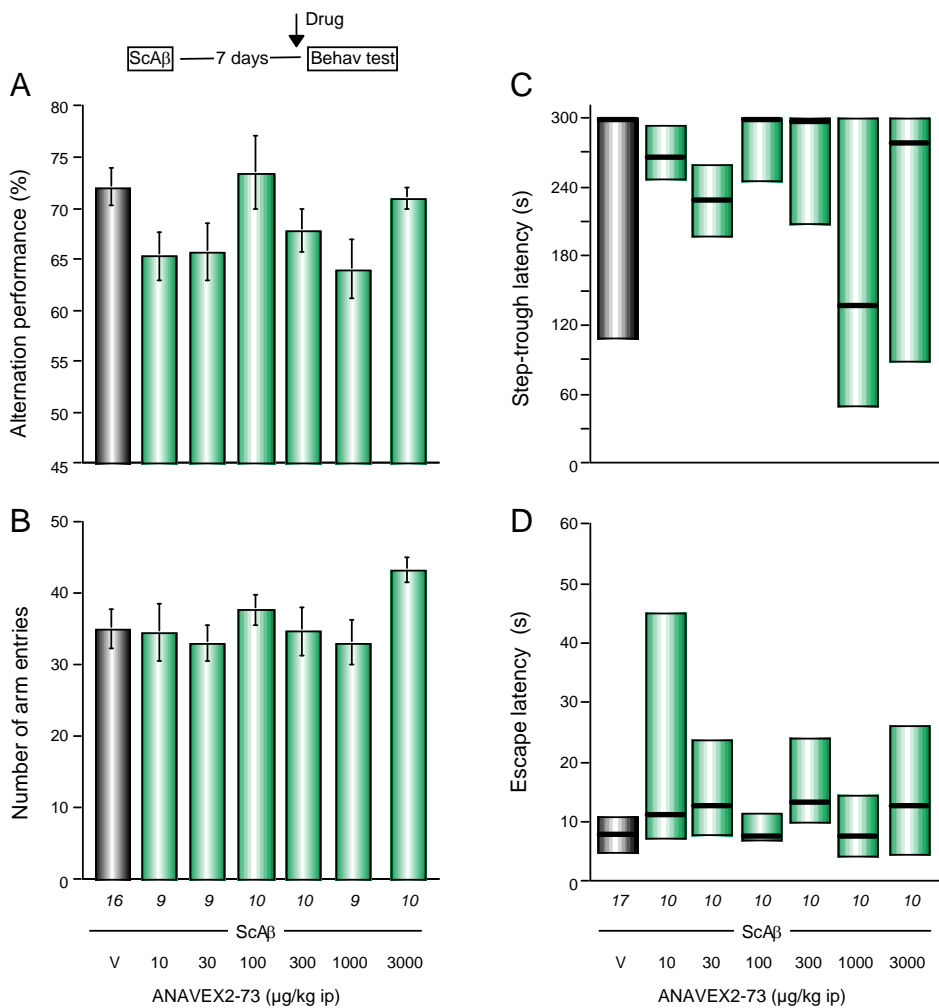
COMMENTS

ANAVEX1-41 failed to affect the memory capacities in control (ScA β -treated) mice, but dose-dependently reversed the learning deficits induced by $A\beta_{25-35}$ peptide. The compound was effective in both short-term and long-term memory tests, with active doses about 30 μ g/kg. When injected before the peptide, i.e., 7 days before the tests, ANAVEX1-41 was effective in preventing the appearance of learning deficits and lipid peroxydation in the hippocampus, measuring the $A\beta_{25-35}$ peptide-induced oxidative stress. The compound therefore showed a potent neuroprotective activity, with active doses between 100 and 1000 μ g/kg. Pretreatment with the σ_1 receptor selective antagonist BD1047 or the muscarinic M1 selective antagonist scopolamine differentially blocked the ANAVEX1-41 effects on short- or long-term memory tests, suggesting that the compound acts as a mixed muscarinic and σ_1 receptor agonist.

ANAVEX2-73

FIGURE 6

ANAVEX2-73 failed to affect the mnemonic capacities by itself in mice

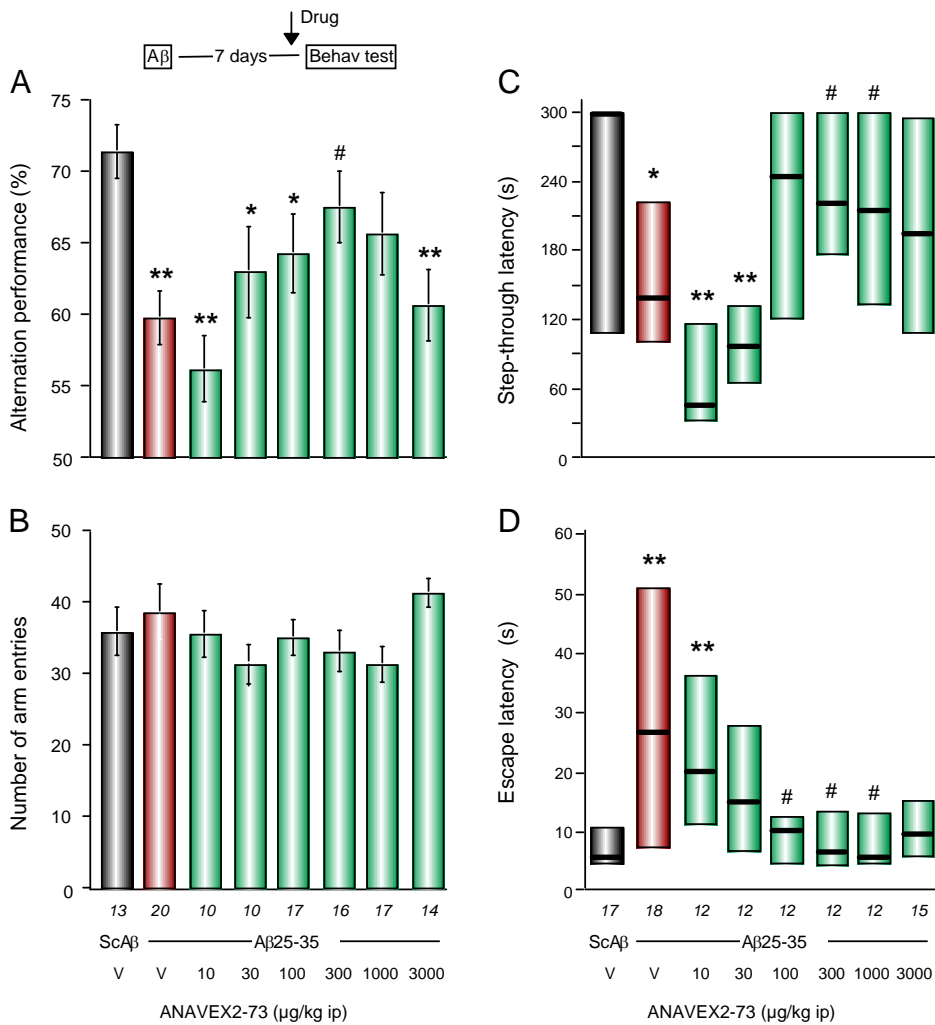


Spontaneous alternation test: (A) alternation percentage [$F(6,72) = 1.78, P > 0.05$] and (B) number of arm entries [$F(6,72) = 1.43, P > 0.05$]. Step-through passive avoidance test: (C) step-through latency [$KW = 5.05, P > 0.05$] and (D) escape latency [$KW = 7.93, P > 0.05$].

ANAVEX2-73

FIGURE 7

ANAVEX2-73 dose-dependently reversed the A β_{25-35} peptide-induced memory deficits in mice

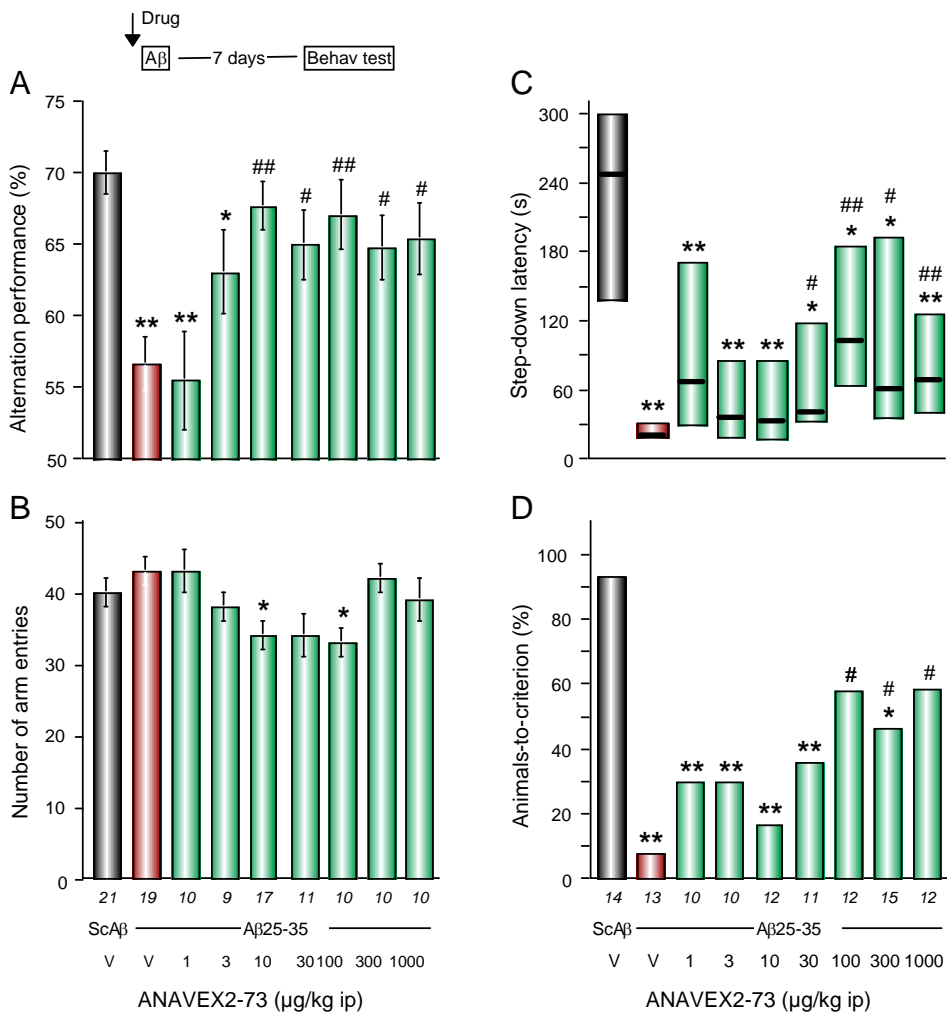


Spontaneous alternation test: (A) alternation percentage [F (7,116) = 3.19, P < 0.01] and (B) number of arm entries [F(7,116) = 1.26, P > 0.05]. Step-through passive avoidance test: (C) step-through latency [KW = 24.8, P < 0.001] and (D) escape latency [KW = 16.4, P < 0.05].

ANAVEX2-73

FIGURE 8

ANAVEX2-73 dose-dependently protected against the A β_{25-35} peptide-induced memory deficits in mice

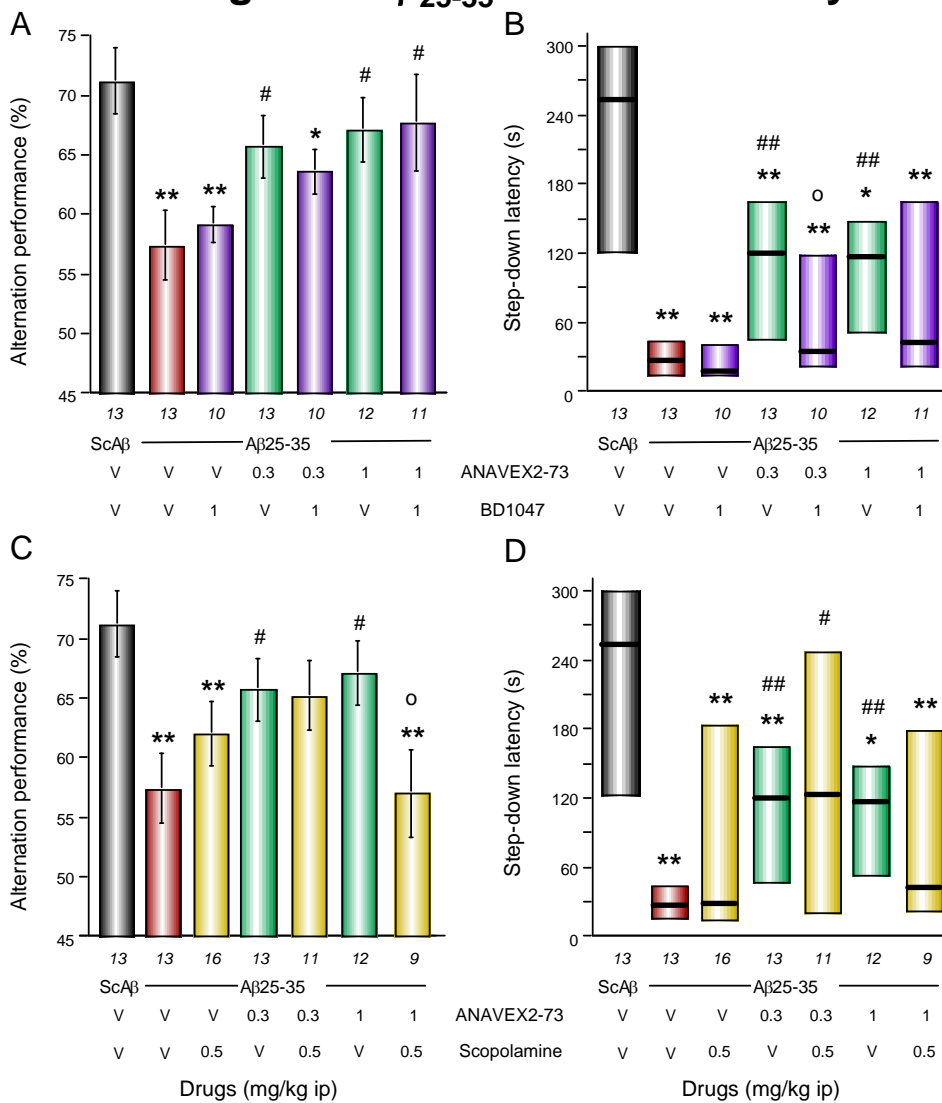


(A) $F(8,116) = 5.61, P < 0.0001$; (B) $F(8,116) = 2.658, P < 0.05$; (C) $KW = 26.82, P < 0.001$.

ANAVEX2-73

FIGURE 9

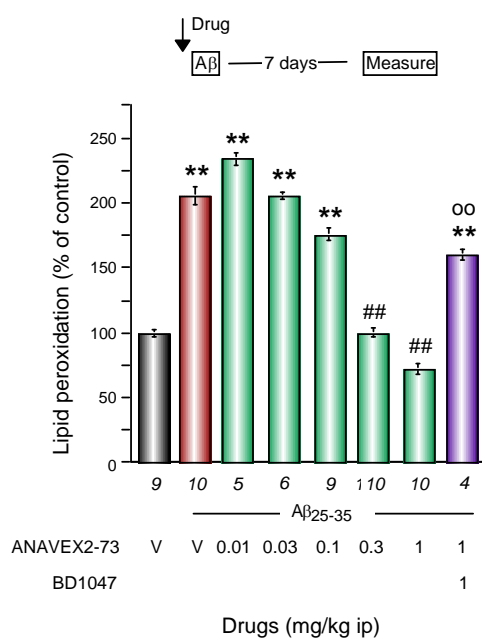
Pre-administration of the σ_1 antagonist BD1047 or the muscarinic antagonist scopolamine blocked the effect of ANAVEX2-73 against $A\beta_{25-35}$ -induced memory deficits



(A) $F(6,81) = 3.13$, $P < 0.01$; (B) $KW = 39.7$, $P < 0.0001$; (C) $F(6,869) = 3.09$, $P < 0.01$; (D) $KW = 28.1$, $P < 0.0001$.

FIGURE 10

ANAVEX2-73 dose-dependently blocked the induction of lipid peroxydation in the hippocampus of $A\beta_{25-35}$ -treated mice



COMMENTS

ANAVEX2-73 also failed to affect learning in control mice, but dose-dependently reversed the deficits induced by $A\beta_{25-35}$ peptide. The compound was effective in both short-and long-term memory tests, with active doses about 300-1000 µg/kg. When injected before the peptide, ANAVEX2-73 protected against the $A\beta_{25-35}$ peptide-induced learning deficits and hippocampal lipid peroxydation. The compound showed a potent neuroprotective activity at 300-1000 µg/kg. ANAVEX2-73 appeared moderately sensitive to the BD1047 pre-treatment in the behavioral tests. The effect was more marked in lipid peroxydation measures. Scopolamine, however, blocked the behavioral effects of ANAVEX2-73 particularly at the 1000 µg/kg dose of compound.

Both ANAVEX1-41 and ANAVEX2-73 are thus promising neuroprotective compounds in this AD animal model. The mechanism of the combined activity at M1/M2/M4 muscarinic receptors and σ_1 receptors must be investigated further.