Methyllycaconitine and scopolamine induced cognitive dysfunction: differential reversal effect of cognitive-enhancing drugs

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Introduction

It is now widely accepted that α7 nAchR plays a central role in cognitive deficits associated with neurodegenerative and cognitive disorders such as **Alzheimer's disease, Parkinson's disease** and **schizophrenia**[1,2].
In support of this concept, changes in the brain expression of α7 nAchR have been reported in patients with neurodegenerative diseases[3,9]. Furthermore, epidemiological studies have reported that nicotine decreases the risk for Parkinson's and Alzheimer's disease[10]. Selective α7 nAchR agonists have been reported to improve the cognitive performance of rodents in various assays[11,13]. Finally, the neuroprotective effect of galanthamine and donepezil has been demonstrated to be mediated by the stimulation of α7 nAchR in rat primary neurons[14,15].
To the best of our knowledge, there is not yet a well established and specific *in-vivo* pharmacological model for studying cognitive deficit associated with the dysfunction of a7 nAchR.

Animal testing and measure of cognitive deficit

Male CD-1 mice are assessed for their spontaneous alternation in the T-maze. **Spontaneous alternation** is the innate tendency of rodents to alternate free choices in a **T-maze** over a series of successive runs. This sequential procedure relies on working memory and is sensitive to various pharmacological manipulations affecting memory processes.
The T-maze apparatus is made of gray Plexiglas with a main stem (55 cm long × 10 cm wide × 20 cm high) and two arms (30 cm long × 10 cm wide × 20 cm high) positioned at 90 degree angle relative to the main stem. A start box (15 cm long × 10 cm wide) is separated from the main stem by a guillotine door. Horizontal doors are also provided to close specific arms during the force choice alternation task.
The percentage of alternation over the **14 free-choice trials** is determined for each mouse and is used as an index of working memory performance.
An alternation is defined as a succession of 2 different arms over consecutive choices (e.g., the sequence right-left-right represents 2 alternations).

Objectives

The aim of the present study is threefold:

- to assess whether specific inhibition of α7 nAchR induces cognitive deficit in mice; the effect of MLA, a reported specific antagonist of α7 nAchR, is investigated.
- to assess whether the cognitive deficit resulting from the inhibition of α7 nAchR could be reversed by clinically approved cognitive-enhancing drugs such as donepezil, memantine and galanthamine
- to undertake a face to face comparison with traditional model involving muscarinic receptor antagonism, i.e., scopolamine-induced cognitive deficit

Results

The degree of cognitive dysfunction is comparable in MLA and scopolamine-based assays but the MLA-deficit shows higher sensitivity and responsiveness to the current cognitive-enhancing drugs than scopolamine-deficit

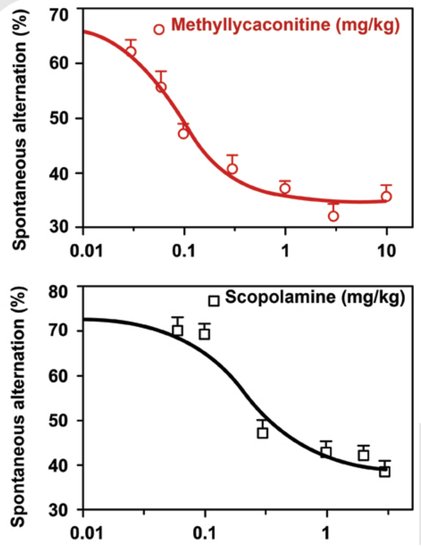

Figure 1 :
Scopolamine and MLA induced cognitive deficit in mice

Scopolamine and MLA elicit a dose-dependent decrease in spontaneous alternation of mice in the T-maze. This suggests that scopolamine and MLA treatment causes cognitive deficit in the mice during the T-maze alternation task.
The ID_{50} for each drug are 0.03 and 0.09 mg/kg, respectively. Maximal effect is comparable (about 30% decrease in the alternation of naïve mice).

- Pre-treatment time : 40 min prior to the T-maze trial
- Drug administration route : intraperitoneal
- Graphs show mean ± sem of n = 10

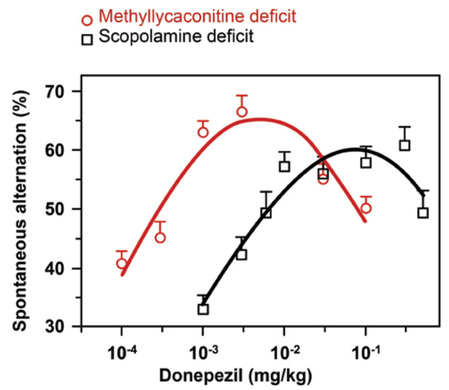

Figure 2 :
Reversal effect of Donepezil

Donepezil produces a dose-dependent increase in spontaneous alternation of both scopolamine- and MLA-treated mice, suggesting a reversion of the cognitive deficit.

However, in the scopolamine deficit, the ED_{50} of donepezil was 4 times greater than in the MLA deficit (0.002 mg/kg and 0.0005 mg/kg, respectively).

Furthermore, the reversal effect of donepezil in the scopolamine deficit showed a decline at the dose of 0.5 mg/kg. This decline was also observed in MLA deficit but occurs at doses 15 times lower than in scopolamine deficit.

- Pre-treatment time : 40 min prior to the T-maze trial
- Drug administration route : intraperitoneal (MLA and scopolamine) per os (donepezil)
- Graphs show mean ± sem of n = 10

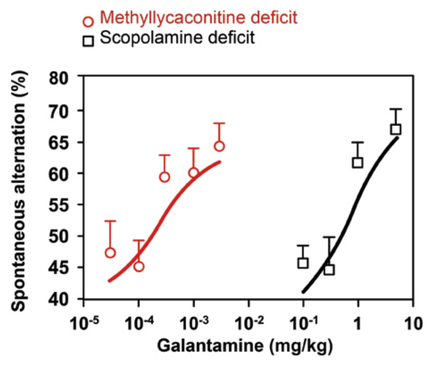

Figure 3 :
Reversal effect of Galanthamine

Galanthamine induces a dose-dependent increase in the spontaneous alternation of both scopolamine- and MLA-treated mice.

The ED_{50} of galanthamine is reduced by 3.5 logarithms in the MLA deficit as compared to that in scopolamine deficit (0.0003 and 0.7 mg/kg, respectively).

- Pre-treatment time : 40 min prior to the T-maze trial
- Drug administration route : intraperitoneal (MLA and Scopolamine); per os (Galanthamine)
- Graphs show mean ± sem of n = 10

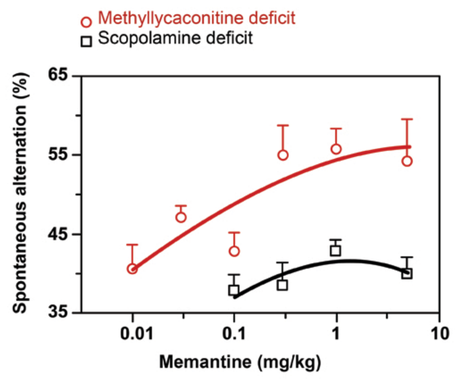

Figure 4 :
Reversal effect of Memantine

Memantine does not produce any significant increase in spontaneous alternation of scopolamine-treated mice.
In contrast, memantine elicits up to 18% increase in spontaneous alternation of MLA-treated mice.

The ED_{50} of memantine in reversing MLA deficit is 0.09 mg/kg.

- Pre-treatment time : 40 min prior to the T-maze trial
- Drug administration route : intraperitoneal
- Graphs show mean ± sem of n = 10

Summary of key findings

- Specific inhibition of α7 nAchR by MLA induces marked cognitive deficit in mice
- MLA-induced cognitive deficit is comparable in intensity to that elicited by scopolamine (inhibition of muscarinic receptor)
- Donepezil and galanthamine show enhanced potency (1 to 3 logarithm magnitude, respectively) in MLA- as compared to scopolamine- induced cognitive deficit
- Memantine reverses MLA-deficit whereas it is ineffective in scopolamine-deficit model

References
1. Neurosci Biobehav Rev. 2008;32(4):693-706
2. Med Hypotheses. 2010 Feb;74(2):281-5
3. Biol Psychiatry. 2001 Feb 1;49(3):175-84
4. Glia. 2003 Jan 15;41(2):207-11
5. Exp Neurol. 2005 Mar;192(1):215-25
6. Arch Neurol. 2007 Dec;64(12):1771-6
7. J Chem Neuroanat. 2000 Dec;20(3-4):299-306
8. Neurobiol Dis. 2000 Dec;7(6 Pt B):666-72
9. Neurotox Res. 2000;2(2-3):157-65
10. Behav Brain Res. 2000 Aug;113(1-2):117-20
11. Neuropsychopharmacology. 2007 Jan;32(1):17-34
12. J.Pharmacol Exp Ther. 2009 May;329(2):459-68
13. Biochem Pharmacol. 2009 Oct 1;78(7):880-8
14. Eur J Pharmacol. 2006 Nov 7;549(1-3):19-26
15. Br J Pharmacol. 2010 Sep;161(1):127-39