The acetylcholinesterase inhibitor galantamine inhibits d-amphetamine-induced psychotic-like behavior in Cebus monkeys

Maibritt B. Andersen, Thomas Werge, Anders Fink-Jensen

Research Institute of Biological Psychiatry, Sct. Hans Hospital, Roskilde, Denmark (M.B.A., T.W.), Laboratory of Neuropsychiatry and Department of Psychiatry, Rigshospitalet University Hospital, Copenhagen, Denmark (A.F.-J.).
Galantamine inhibits psychotic-like behavior in monkeys

Corresponding author: Anders Fink-Jensen, email: a.fink-jensen@dadlnet.dk, Telephone: +45 22755843, Fax: +45 3545 6238, Address: Laboratory of Neuropsychiatry and Department of Psychiatry, Rigshospitalet University Hospital, Blegdamsvej 9, DK 2100 Copenhagen, Denmark

Number of text pages: 15
Number of tables: 2
Number of figures: 4
Number of references: 33
Number of words in the abstract: 187
Number of words in the introduction: 506
Number of words in discussion: 575

List of non-standard abbreviations:

Acetylcholinesterase: AChE
Cholinergic receptor: AChR
Abstract

Cholinergic receptors (AChRs) are reported altered in brains from schizophrenic patients and a growing body of evidence suggests that muscarinic receptor agonists exhibit antipsychotic potential. Centrally acting selective muscarinic receptor agonists are currently not available in the clinic, but acetylcholinesterase inhibitors, which indirectly stimulate AChRs by blocking the breakdown of acetylcholine by acetylcholinesterase (AChE) are widely used in the clinic against Alzheimer’s disease. AChE inhibitors have been reported to exhibit antipsychotic efficacy in Alzheimer’s disease patients and these compounds have also been investigated as adjunctive treatment to antipsychotic medication in schizophrenic patients with varying results. However, monotherapy with AChE inhibitors in schizophrenic patients has not been evaluated. We wanted to investigate the antipsychotic potential of the AChE inhibitor galantamine, which also allosterically potentiate nicotinic receptor stimulation. To this end we investigated its ability to antagonize d-amphetamine-induced psychotic-like behavior in EPS-primed Cebus monkeys. Galantamine inhibited d-amphetamine-induced unrest, arousal and stereotypy. Side effects such as emesis, sedation and EPS were minor or not existing. The results indicate, that AChE inhibitors have anti-psychotic potentials and suggest that clinical trials investigating antipsychotic effects of AChE inhibitors as monotherapy would be of interest.
Introduction

AChR consist of muscarinic G protein coupled receptors and nicotinic ionotropic receptors, and both receptor subtypes have been shown to be altered in schizophrenic patients. Decreased density of cholinergic muscarinic (M1 and/or M4) receptors in postmortem frontal cortex (Crook, et al., 2001), hippocampus (Crook, et al., 2001) and caudate putamen (Dean, et al., 1996) and reduced number of nicotinic receptors in the hippocampus (Freedman, et al., 1995), cortex and thalamus (Breese, et al., 1997) from schizophrenic patients has been reported. In the cited study by Freedman et al., 1995 post-mortem hippocampal sections were labeled with [125I]-α-bungarotoxin, where the α-bungarotoxin-sensitive nicotinic receptor are thought to function as homooligomers and possibly oligomers composed of α7, α8, or α9 subunits (Court et al., 1999). Muscarinic receptor agonists induce antipsychotic-like behavior in rodents (Bymaster, et al., 1998; Fink-Jensen, 2000; Shannon, et al., 2000), in monkeys (Andersen, et al., 2003) and in humans (Bodick, et al., 1997). Selective, centrally acting muscarinic receptor agonists are currently not available for clinical use, but centrally active AChE inhibitors such as donepezil, rivastigmine and galantamine are widely used in the clinic against Alzheimer’s disease. These three compounds all inhibit the breakdown of acetylcholine by the enzyme acetylcholinesterase, by which they indirectly stimulate muscarinic and nicotinic receptors. Galantamine, in addition to this effect, also potentiate nicotinic receptor stimulation by an allosteric mode of action (Samochocki, et al., 2003).

A few case reports and smaller studies with respect to antipsychotic efficacy of AChE inhibitors in schizophrenic patients have been published. Some studies have demonstrated antipsychotic potential (Allen and McEvoy, 2002; Mendelsohn, et al., 2004; Rosse and Deutsch, 2002), but the majority have not (Bora, et al., 2005; Buchanan, et al., 2003; Erickson, et al., 2005; Freudenreich, et al., 2005; Friedman, et al., 2002; Kumari, et al., 2006; Tugal, et al., 2004), In all studies AChE inhibitors were used as adjunctive treatment in patients already treated with antipsychotics.
Consequently, a ceiling effect may have been obtained by the initial monotherapy with antipsychotics. In addition, several of the antipsychotic compounds used block acetylcholine receptors which may have hampered the effects of the AChE inhibitors.

In conclusion, a possible antipsychotic effect of AChE inhibitors has not been ruled out, as these drugs have not been used as monotherapy in schizophrenic patients. To this end we have tested galantamine in non-human primates. The present study investigated the ability of galantamine to counteract d-amphetamine-induced behavioral effects in EPS-primed Cebus monkeys. The side effect profile of galantamine was also investigated. Several studies from our research laboratory have shown that drugs with antipsychotic activity antagonize d-amphetamine behaviors in Cebus monkeys (Andersen, et al., 2003; Gerlach and Casey, 1990; Brandt-Christensen, et al., 2006). The monkeys are sensitized to extrapyramidal side effects (EPS) by prior long-term treatment with classical DA D2 antagonists and the EPS observed in these monkeys are very similar to EPS induced by antipsychotics in humans and the model is predictive of EPS liability in the clinic (Peacock and Gerlach, 1993). Other potential side effects, e.g. gastrointestinal side effects, were investigated too.
Materials and Methods

Animals

Six male *Cebus* monkeys were used for evaluation of anti-amphetamine effect and side effect profile of galantamine. The monkeys were housed in separate cages in a temperature-regulated environment at a 12-hour light/dark cycle. Visual, olfactory and auditory contact between the monkeys was possible during and between experiments. All experimental procedures carried out in this study were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and with the Danish law regulating experiments on animals.

Compounds and Design

The test drugs were d-amphetamine sulfate and galanthamine hydrobromide (Tocris, Bristol, UK). D-Amphetamine was dissolved in physiological saline and galantamine was dissolved in sterile water. Galantamine was tested in doses of 0.1, 0.3, 0.6 and 1.0 mg/kg alone and in combination with 0.25 mg/kg d-amphetamine. The drugs were injected s.c. at approximately 9 a.m. The monkeys were tested 1-2 times per week throughout the 6-week experiment and the drugs were administered in the same succession to all monkeys. On test days the monkeys did not have access to food or water during the experiment and their toys were removed before the onset of the experiment. All monkeys received all treatments with at least three days between tests serving as their own controls.

Data Evaluation

The monkeys were videotaped in 90-sec. sessions at specific time points throughout the test sessions. The videotapes were rated by an experienced rater for d-amphetamine-induced behaviors and EPS by means of a rating scale previously described (Andersen, et al., 2002; Andersen, et al.,
2003) ranging from 0 (not present) to 6 (extreme presence). The videotapes were rated in random sequence for each monkey. The rated behaviors and symptoms were arousal, unrest, stereotypy, locomotion, sedation, bradykinesia and dystonia (Table 1). Data were evaluated at t = 30, 60, 120 and 180 min after drug administration.

The data were analyzed for overall treatment effects at each time point using a one-way repeated measures ANOVA. Student-Newman-Keuls multiple comparison procedure was used to analyze for specific dose effects. The accepted level of significance was p<0.05 for all tests.
Results

**Galantamine and d-amphetamine**

The ANOVA revealed a drug effect on arousal at all four test times (p<0.001 for all). The post hoc analysis revealed that d-amphetamine increased arousal at all four test times (p<0.001 for all). Galantamine at 0.6 mg/kg decreased the d-amphetamine-induced arousal at t = 60, 120 and 180 min (p<0.05 for all) while 1.0 mg/kg decreased d-amphetamine-induced arousal at t = 30 (p<0.05), 60 (p<0.01), 120 (p<0.001) and 180 min (p<0.01) (Figure 1). Unrest was significantly affected by drug treatment at all time points (30 & 60: p<0.05, 120 & 180: p<0.01). D-Amphetamine increased unrest at all time points (30 & 60: p<0.001, 120: p<0.05, 180: p<0.01). At t= 30 min, d-amphetamine-induced unrest was counteracted by 0.6 and 1.0 mg/kg galantamine (p<0.01 for both), while 1.0 mg/kg also decreased d-amphetamine-induced unrest at the remaining time points (60: p<0.01, 120 & 180: p<0.05) (Figure 2). D-Amphetamine produced stereotypy at all four test times (p<0.001 for all). The d-amphetamine-induced stereotypy was significantly reduced by 0.6 and 1.0 mg/kg galantamine at t = 30 min (p<0.05 and 0.01, respectively) while 1.0 mg/kg also reduced stereotypy at t = 60 and 120 min (p<0.05 for both) (Figure 3). Locomotion was not changed compared to placebo treatment. Sedation, dystonia, bradykinesia and oral dyskinesia were not observed when d-amphetamine was administered in combination with galantamine. One monkey vomited following injection of 0.6 mg/kg galantamine and d-amphetamine (within 15 minutes). Two monkeys vomited within 30 minutes after administration of d-amphetamine in combination with 1.0 mg/kg galantamine. At the two lower doses no emetic events were observed. See also Table 2.
**Galantamine**

When administered alone in doses of 0.1, 0.3, 0.6 and 1.0 mg/kg, galantamine produced sedation at t = 30 min (p<0.001), t = 60 min (p<0.001) and t = 120 min (p<0.01). The post hoc analyses showed that 0.3 – 1.0 mg/kg galantamine produced sedation at t = 60 min, while 0.6 – 1.0 mg/kg produced sedation at t = 30 min as well. 1.0 mg/kg also produced sedation at t = 120 min (Figure 4).

Locomotion was decreased by 0.6 mg/kg and 1.0 mg/kg galantamine at t = 30 min (p<0.001 for both) and by 1.0 mg/kg at t = 60 min (p<0.01) compared to placebo. Unrest was decreased at t = 60 min by 0.6 mg/kg (p<0.01) and 1.0 mg/kg galantamine (p<0.05) and at t = 120 min by 1.0 mg/kg (p<0.05) compared to placebo. Arousal was not affected by galantamine. Stereotypy, oral dyskinesia, bradykinesia or dystonia were not observed. At 1.0 mg/kg, three monkeys vomited 1-3 times between 15 and 180 minutes after injection. One monkey vomited within 15 minutes after injection of 0.6 mg/kg galantamine. At the two lower doses no monkeys vomited. See also Table 2.
Discussion

In the present study d-amphetamine induced arousal, unrest and stereotypies in Cebus monkeys. These behaviors were antagonized by galantamine (0.6 – 1.0 mg/kg) at several time points after drug administration. Galantamine did not produce EPS in any of the tested doses. Sedation was observed at doses of 0.3 - 1.0 mg/kg when galantamine was administered alone, but not when co-administered with d-amphetamine (Table 2). The sedation was very mild at 120 min and 160 min. (Figure 4). Nevertheless, this side effect was somewhat surprising, since sedation is normally not observed in patients treated with galantamine. We have no explanation for this discrepancy. When galantamine was given alone, some monkeys vomited at the two high doses. At the highest dose of galantamine, the monkeys, that did not vomit, lay flat on their abdomen at several time points, which could be due to abdominal discomfort or nausea. When tested together with d-amphetamine, emesis was only observed in one out of six animals at the two highest doses of galantamine (see Table 2). In conclusion, galantamine inhibited d-amphetamine induced psychotic-like behavior. However, sedation and emesis was observed and these galantamine-induced side effects may have contributed to its antipsychotic-like effects.

To our knowledge, this is the first study reporting that galantamine inhibits d-amphetamine-induced behavior. The results are in accordance with an earlier study in mice investigating the interaction of galantamine with another indirect dopamine agonist, cocaine: Galantamine was found to inhibit cocaine-induced locomotor sensitization in mice (Hikida, et al., 2003), showing that its functional dopamine antagonism was not confined to d-amphetamine. The mechanism of action behind the anti-dopaminergic effects of galantamine in primates has not been clarified, but it is likely that the cholinergic muscarinic receptors are involved. Galantamine, through its acetylcholinesterase-inhibiting mode of action, stimulates muscarinic receptors indirectly, and earlier studies have shown
functional dopamine antagonism of muscarinic M2/M4 receptor stimulation in rodents (Fink-Jensen, 2000; Fink-Jensen, et al., 1998; Bymaster, et al., 1998), of muscarinic M1/M4 receptor stimulation in rodents (Shannon, et al., 2000; Stanhope, et al., 2001) and of muscarinic M1/M4 receptor stimulation in monkeys (Andersen, et al., 2003). Data from gene targeting techniques is also in concordance with this idea. M1 receptor knockout mice show increased basal locomotor activity and elevated locomotor response to d-amphetamine (Gerber, et al., 2001) and M4 receptor knockout mice show increased locomotor activity response to a dopamine D1 receptor stimulation (Gomez, et al., 1999). Galantamine, in addition to its AChE inhibitory action, potentiate the effects of nicotinic receptor stimulation. Potentiation of nicotinic receptor stimulation is believed to be advantageous to antipsychotic medical treatment of schizophrenia, since activation of nicotinic receptors improves sensory processing deficits in this group of patients (Olincy, et al., 2006; Simosky, et al., 2002). However, this may not attenuate the effects of amphetamine itself, since pretreatment with nicotine inhibits d-amphetamine-induced hyperactivity in rodents (Birrell and Balfour, 1998). Nicotine receptors are known to desensitise fast, so another possibility for this inhibition would be desensitisation of nicotinic receptors. In the present study, it is not possible to determine the specific contribution of nicotinic receptor stimulation to the anti-amphetamine effects observed, since galantamine was not compared with a selective AChE inhibitor such as donepezil.

In conclusion, the present results show that galantamine attenuates d-amphetamine-induced psychotic-like behavior in non-human primates. There is a strong need for new and more efficacious antipsychotic medication and the current study supports the role of AChR as potential novel targets for the medical treatment of schizophrenia and other psychotic disorders.

Acknowledgements
The authors are thankful to Finn Nielsen for technical assistance
References


Crook JM, Tomaskovic-Crook E, Copolov DL and Dean B (2001) Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. Am J Psychiatry 158:918-925.


cross-over trial of adjunctive donepezil for cognitive impairment in schizophrenia. Int J Neuropsychopharmacol 7:117-123.
Footnotes

These studies were partially funded by the Danish National Psychiatric Research Foundation.
Legends for Figures

Figure 1. Effect of galantamine on d-amphetamine-induced arousal (n=6). Arousal was rated on a scale ranging from 0 to 6. AMP = 0.25 mg/kg d-amphetamine. GAL = galantamine. * p<0.05 relative to vehicle, + p<0.05 relative to d-amphetamine. Student-Newman-Keuls test.

Figure 2. Effect of galantamine on d-amphetamine-induced unrest (n=6). Unrest was rated on a scale ranging from 0 to 6. See Figure 1 for description of abbreviations. * p<0.05 relative to vehicle, + p<0.05 relative to d-amphetamine. Student-Newman-Keuls test.

Figure 3. Effect of galantamine on d-amphetamine-induced stereotypy (n=6). Stereotypy was rated on a scale ranging from 0 to 6. See Figure 1 for description of abbreviations. * p<0.05 relative to vehicle, + p<0.05 relative to d-amphetamine. Student-Newman-Keuls test.

Figure 4. Sedation after injection of galantamine (n=6). Sedation was rated on a scale ranging from 0 to 6. * p<0.05 relative to vehicle. Student-Newman-Keuls test.
Table 1. Description of behaviors and rating scales.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrest</td>
<td>Restlessness including fidgeting and frequent changes of direction of movement or frequent changes between different behaviors.</td>
<td>0-6</td>
</tr>
<tr>
<td>Stereotypies</td>
<td>Repeated futile movements, abrupt whole body movements and aborted behaviors.</td>
<td>0-6</td>
</tr>
<tr>
<td>Arousal</td>
<td>Degree of vigilance ranging from not awake to extreme vigilance in relation to self or the environment.</td>
<td>0-6</td>
</tr>
<tr>
<td>Locomotion</td>
<td>Horizontal and vertical movement in space</td>
<td>0-6</td>
</tr>
<tr>
<td>Sedation</td>
<td>Degree of drowsiness ranging from fully awake to heavy sleeping (cannot be awakened by gross stimuli).</td>
<td>0-6</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>Slow and/or stiffened movements ranging from normal tempo and flexibility to fixed maintained postures.</td>
<td>0-6</td>
</tr>
<tr>
<td>Dystonia</td>
<td>Clonic movement of head, neck, limbs and trunk. Gaping and grimacing.</td>
<td>0-6</td>
</tr>
</tbody>
</table>
Table 2. Overview of galantamine side effects.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Emetic events (vomiting)</th>
<th>Sedation - at least one time point ($2 \leq X \leq 5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-amphetamine 0.25 mg/kg (amp)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vehicle</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galantamine 0.1 mg/kg</td>
<td>+</td>
<td>1 in 6</td>
</tr>
<tr>
<td>Galantamine 0.1 mg/kg / amp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galantamine 0.3 mg/kg</td>
<td>+</td>
<td>4 in 6</td>
</tr>
<tr>
<td>Galantamine 0.3 mg/kg / amp</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galantamine 0.6 mg/kg</td>
<td>3 in 6</td>
<td>6 in 6</td>
</tr>
<tr>
<td>Galantamine 0.6 mg/kg / amp</td>
<td>1 in 6</td>
<td>+</td>
</tr>
<tr>
<td>Galantamine 1.0 mg/kg</td>
<td>2 in 6</td>
<td>6 in 6</td>
</tr>
<tr>
<td>Galantamine 1.0 mg/kg / amp</td>
<td>2 in 6</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 1

![Bar chart showing arousal levels over time](chart.png)

- **Placebo**
- **Amphetamine**
- **AMP + 0.1 GAL**
- **AMP + 0.3 GAL**
- **AMP + 0.6 GAL**
- **AMP + 1.0 GAL**

**Arousal (mean ± sem)**

- **30 min**
- **60 min**
- **120 min**
- **180 min**

* * * * *+

The chart illustrates the comparison of arousal levels across different groups and time points, with statistical significance indicated by asterisks.
Figure 4

Sedation (mean ± sem)

- 0.0 mg/kg GAL
- 0.1 mg/kg GAL
- 0.3 mg/kg GAL
- 0.6 mg/kg GAL
- 1.0 mg/kg GAL

30 min  60 min  120 min  180 min