The Acetylcholinesterase Inhibitor Galantamine Inhibits d-Amphetamine-Induced Psychotic-Like Behavior in Cebus Monkeys

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ABSTRACT

Cholinergic receptors (AChR) 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 for clinical use, but acetylcholinesterase (AChE) inhibitors, which indirectly stimulate AChR by blocking the breakdown of acetylcholine by 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 potentiates nicotinic receptor stimulation. To this end, we investigated its ability to antagonize d-amphetamine-induced psychotic-like behavior in extrapyramidal side effects (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 antipsychotic potentials and suggest that clinical trials investigating antipsychotic effects of AChE inhibitors as monotherapy would be of interest.

Cholinergic receptors (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 post mortem 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 have been reported. In the cited study by Freedman et al. (1995), post mortem hippocampal sections were labeled with [125I]-bungarotoxin, where the α-bungarotoxin-sensitive nicotinic receptors are thought to function as homo-oligomers and possibly oligomers composed of α₂, α₃, 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), monkeys (Andersen et al., 2003), and humans (Bodick et al., 1997). Selective, centrally acting muscarinic receptor agonists are currently not available for clinical use, but centrally active acetylcholinesterase (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 AChE, by which they indirectly stimulate muscarinic and nicotinic receptors. Galantamine, in addition to this effect, also potentiates 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 shown antipsychotic potential (Allen and McEvoy, 2002; Rosse and Deutsch, 2002; Mendelsohn et al., 2004), but the majority have not (Friedman et al., 2002; Buchanan et al., 2003; Tugal et al., 2004; Bora et al., 2005; Erickson et al., 2005; Freudenreich et al., 2005; Kumari et al., 2006). In all the studies, AChE inhibitors were used as adjunctive treatment in patients already treated with antipsychotic agents. Consequently, a ceiling effect may have been obtained by the initial monotherapy with antipsychotic drugs. 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 because these drugs have not been used as monotherapy in schizophrenic patients. To this end, we have tested galantamine in nonhuman primates. The present study investigated the ability of galantamine to counteract d-amphetamine-induced behavioral effects in extrapyramidal side effects (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 (Gerlach and Casey, 1990; Andersen et al., 2003; Brandt-Christensen et al., 2006). The monkeys are sensitized to EPS by previous long-term treatment with classical dopamine D2 antagonists; the EPS observed in these monkeys are very similar to EPS induced by antipsychotic agents 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 also investigated.

Materials and Methods

Animals. Six male Cebus monkeys were used for evaluation of antipsychotic effect and side effect profile of galantamine. The monkeys were housed in separate cages in a temperature-regulated environment at a 12-h light/dark cycle. Visual, olfactory, and auditory contact between the monkeys was possible during and between experiments. All the experimental procedures carried out in this study were in compliance with the European Communities Council Directive of November 24, 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 Bioscience, 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 AM. The monkeys were tested one or two times per week with at least 3 days between tests. The monkeys did not have access to food or water during the experiment, and their environment at a 12-h light/dark cycle. Visual, olfactory, and auditory contact between the monkeys was possible during and between experiments. All the experimental procedures carried out in this study were in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and with the Danish law regulating experiments on animals.

Data Evaluation. The monkeys were videotaped in 90-s 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 described previously (Andersen et al., 2002, 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 analysis of variance. 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 the tests.

Results

Galantamine and d-Amphetamine. The analysis of variance 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), whereas 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) (Fig. 1). Unrest was significantly affected by drug treatment at all the time points (30 and 60, p < 0.05; 120 and 180, p < 0.01). d-Amphetamine increased unrest at all the time points (30 and 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), whereas 1.0 mg/kg also decreased d-amphetamine-induced unrest at the remaining time points (60, p < 0.01; 120 and 180, p < 0.05) (Fig. 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), whereas 1.0 mg/kg also reduced stereotypy at t = 60 and 120 min (p < 0.05 for both) (Fig. 3). Locomotion was not changed compared with 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 min). Two monkeys vomited within 30 min after administration of d-amphetamine in combination with 1.0 mg/kg galantamine. At the two lower doses, no emetic events were observed (also see 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 to 1.0

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<td><strong>Description of behaviors and rating scales</strong></td>
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mg/kg galantamine produced sedation at $t = 60$ min, whereas 0.6 to 1.0 mg/kg produced sedation at $t = 30$ min as well. Furthermore, 1.0 mg/kg produced sedation at $t = 120$ min (Fig. 4). Locomotion was decreased by 0.6 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 with placebo.

In the present study, $d$-amphetamine induced arousal, unrest, and stereotypy 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 to 1.0 mg/kg when galantamine was administered alone but not when coadministered with $d$-amphetamine (Table 2). The sedation was very mild at 120 and 180 min (Fig. 4). Nevertheless, this side effect was somewhat surprising because 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 have been because of abdominal discomfort or nausea. When tested together with $d$-amphetamine, emesis was only observed in one 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 were 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 antidopaminergic effects of galantamine in primates has not been clarified, but it is likely that the cholinergic muscarinic receptors are involved. Galantamine, through its AChE-inhibiting mode of action, stimulates muscarinic receptors indirectly, and earlier studies have shown functional dopamine antagonism of muscarinic

**Discussion**
M₂/M₄ receptor stimulation in rodents (Bymaster et al., 1998; Fink-Jensen et al., 1998; Fink-Jensen, 2000), 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 are 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, potentiates the effects of nicotinic receptor stimulation. Potentiation of nicotinic receptor stimulation is believed to be advantageous to antipsychotic medical treatment of schizophrenia because activation of nicotinic receptors improves sensory processing deficits in this group of patients (Simosky et al., 2002; Olincy et al., 2006). However, this may not attenuate the effects of amphetamine itself because pretreatment with nicotine increases d-amphetamine-induced hyperactivity in rodents (Birrell and Balfour, 1998). Nicotine receptors are known to desensitize fast, so another possibility for this inhibition would be desensitization of nicotinic receptors. In the present study, it is not possible to determine the specific contribution of nicotinic receptor stimulation to the antiamphetamine effects observed because 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 nonhuman 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 psychiatric disorders.

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References

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