Adjunctive galantamine, but not donepezil, enhances the antipsychotic-like effect of raclopride in rats

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Abstract

Acetylcholine (ACh) esterase inhibitors like galantamine and donepezil have been tested as adjunct treatment in schizophrenia. Although ACh esterase inhibition might confer some antipsychotic activity, the role of allosteric potentiation of nicotinic ACh receptors (nAChRs), which is an additional mechanism of galantamine, remains elusive. Therefore, the potential antipsychotic-like effects of galantamine and donepezil, respectively, alone, and in combination with the dopamine D₂/D₃ receptor antagonist, raclopride, were tested in the conditioned avoidance response (CAR) test and extrapyramidal side-effect liability was assessed with the catalepsy test. Neither galantamine nor donepezil alone suppressed CAR selectively. Galantamine, but not donepezil, enhanced the raclopride-induced suppression of CAR, predicting augmentation of antipsychotic activity. In contrast to donepezil, galantamine did not increase catalepsy, alone or combined with raclopride. These data suggest that allosteric potentiation of nAChRs may mediate the antipsychotic-like effect of adjunctive galantamine and provide support for the development of α₇ nAChR-selective allosteric potentiators for schizophrenia.

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Introduction

Pharmacological enhancement of central cholinergic function in Alzheimer’s disease in order to improve cognition, e.g. by acetylcholine (ACh) esterase (AChE) inhibitors, has been found to have some effect also on psychotic symptoms in this disease (Cummings et al., 2006). Although the typical neurodegeneration of cholinergic systems as seen in Alzheimer’s disease has not been observed in schizophrenia, several lines of evidence suggest that aberrant cholinergic neurotransmission may still contribute to its symptomatology. For example, impaired auditory gating, which has been postulated to contribute to cognitive impairment and even hallucinations in schizophrenia (Adler et al., 1998), has been linked to malfunctioning α₇ nicotinic ACh receptors (nAChRs) (Freedman et al., 1997). Accordingly, AChE inhibitors have also been tested as cognitive enhancers in schizophrenia. Both case reports and several small-sized clinical studies indicate that adjunctive use of galantamine and donepezil may improve not only cognitive impairment in schizophrenia, but also negative and positive symptoms (Allen and McEvoy, 2002; Erickson et al., 2005; Norén et al., 2006; Risch et al., 2007; Schubert et al., 2006). Although other studies have failed to confirm these observations (Freudenreich et al., 2005; Keefe et al., 2007; Lee et al., 2007) the above-mentioned studies indicate that AChE inhibitors may confer some antipsychotic activity. Overall, the clinical results obtained with adjunctive galantamine in schizophrenia appear somewhat more robust than those using donepezil, which might reflect the fact that galantamine, in contrast to donepezil, is also an allosteric
potentiator of nAChRs (Maelicke et al., 2000; Schrattenholz et al., 1996). Moreover, our recent experimental data indicate that galantamine, in contrast to donepezil, affects dopaminergic cell firing in the brain by a mechanism that probably involves potentiation of presynaptic α7 nAChRs, leading to increased glutamate release and enhanced dopamine efflux in the prefrontal cortex (Schilstro¨m et al., 2007). The present experimental study was undertaken to specifically analyse the potential antipsychotic effect of galantamine and donepezil, respectively, using the conditioned avoidance response (CAR) paradigm, a preclinical test of antipsychotic activity with high predictive validity. This methodology was subsequently used to explore the possibility of enhancing the suppressant effect on CAR by the selective dopamine D2/D3 receptor antagonist raclopride, using adjunctive treatment with either galantamine or donepezil. As anticholinergic drugs are frequently used to ameliorate parkinsonism and other extrapyramidal side-effects of neuroleptics, the catalepsy test was used to investigate whether the extrapyramidal side-effect (EPS) liability of raclopride would be influenced by adjunctive galantamine or donepezil.

Methods

Animals

Adult male BK1:WR (Wistar) rats weighing 294–522 g (CAR) or 283–357 g (catalepsy) were used in all experiments. Animals arrived at least 5 d prior to experimental use and were housed [four per cage (Makrolon IV)] in the animal facility under standard laboratory conditions with a 12 h reversed light/dark cycle (lights on 18:00 hours). All experiments were performed between 07:00 and 17:00 hours. Food and water was available ad libitum. All experiments were approved by, and conducted in accordance with, the local Animal Ethics Committee (Stockholms Norra och Södra Försöksdjursetiska Kommittéer) (permit no. N93/05).

CAR

A shuttle-box (530 × 250 × 225 mm), divided into two compartments by a partition, was used. The rats were free to move from one compartment to the other via an opening (75 × 75 mm) in the partition. Upon presentation of the conditioned stimulus (CS), 80 dB white noise (White Noise Generator, Lafayette 1501, Lafayette, IN, USA), the rat had 10 s to avoid the unconditioned stimulus (UCS), an intermittent electric shock in the grid floor of ~0.5 mA (intershock interval 2.5 s, shock duration 0.5 s), by moving into the opposite compartment. The following behavioural variables were recorded: (1) avoidance (response to CS within 10 s); (2) escape (response to CS + UCS); (3) escape failure (if the rat was unable to respond to the shock within 50 s the trial was terminated). The animals were trained for five consecutive days, and were adapted to the shuttle-box 5 min before the training session started. Each training session consisted of about 20 trials randomly distributed over 15 min. Only rats that performed at least 90% avoidance on the last day of training were included in the experiments. All subsequent experimental test sessions were preceded by a pre-test to verify the animals’ continued performance. Pre-tests and experimental tests consisted of about 10 trials randomly distributed over 7.5 min. Drugs were injected within an hour of the pre-test. The same animals were tested repeatedly according to a counterbalanced changeover design serving as their own controls, i.e. all animals received all treatments. Experiments were performed in two sets of 10 animals, each set evaluating the effects of three doses of galantamine and donepezil, respectively, alone or in combination with raclopride. Experimental days were separated by at least 2 d of intermission.

Catalepsy

Animals (n = 112) were placed on an inclined (60°) grid and, excluding the first 30 s, the time the rat remained in the same position was measured for a maximum of 2.5 min. The catalepsy was scored from 0 to 5 according to the time (square root transformation) the animal remained immobile (min): 0 = 0–0.08, 1 = 0.09–0.35, 2 = 0.36–0.80, 3 = 0.81–1.42, 4 = 1.43–2.24, 5 = ≥ 2.25 min, i.e. if the rat remained immobile for ≥ 2.25 min it was scored 5, etc. Each animal was tested once.

Drugs

Raclopride tartrate (Astra Zeneca, Södertälje, Sweden), galantamine and donepezil (Janssen-Cilag AB, Sollentuna, Sweden) were dissolved in saline. All drugs were administered subcutaneously (s.c.) (1.0 ml/kg). The dose of raclopride was chosen based on earlier experiments in which a moderate but significant effect on CAR was observed (data not shown). The doses of galantamine and donepezil were based on previous pharmacokinetic studies (Geerts et al., 2005). Galantamine and donepezil injections were
administered 10 min before the injection of raclopride or saline.

**Statistics**

Statistical evaluation was performed by means of the Friedman two-way analysis of variance (ANOVA), followed by the Wilcoxon matched-pairs signed ranks test for CAR data, or the Kruskal–Wallis one-way ANOVA, followed by the Mann–Whitney U test for catalepsy.

**Results**

**Effects of raclopride on CAR**

Raclopride (0.075 mg/kg s.c.) produced a statistically significant suppression of CAR 20 min after administration, but not at later observation times (90 min and 240 min). No escape failures were recorded with raclopride treatment alone (Figures 1 and 2).

**Effects of galantamine on CAR**

Only the highest dose (5.0 mg/kg s.c.) of galantamine alone significantly suppressed CAR, this effect was only seen 30 min after galantamine administration, but not at later observation times (100 min and 250 min). This dose of galantamine (5.0 mg/kg s.c.) also significantly increased escape failures 30 min after galantamine administration (Fig. 1).

**Effects of the combination of galantamine and raclopride on CAR**

Pretreatment with galantamine (1.0 and 5.0 mg/kg s.c.) significantly enhanced the raclopride (0.075 mg/kg s.c.)-induced suppression of CAR 20 min after raclopride administration compared to animals treated with raclopride alone, this effect was not seen at later observation times (90 min and 240 min). The combination of 1.0 mg/kg s.c. galantamine and 0.75 mg/kg s.c. raclopride induced no escape failures at any time of observation. The combination of 5.0 mg/kg s.c. galantamine and 0.075 mg/kg s.c. raclopride produced a statistically significant increase of escape failures 20 min after raclopride administration, but not at later observation times (90 min and 240 min) (Figure 1).

**Effects of donepezil on CAR**

Only the highest dose (5.0 mg/kg s.c.) of donepezil alone significantly suppressed CAR, this effect was significant 30, 100 and 250 min after donepezil administration (data for 100 min and 250 min not shown). This dose of donepezil (5.0 mg/kg s.c.) also significantly increased escape failures 30 min and
Effects of saline or donepezil (0.1, 1.0 or 5.0 mg/kg s.c.) pre-treatment (10 min) on saline- or raclopride-induced (0.075 mg/kg s.c.) avoidance response in the conditioned avoidance response (CAR) test 20 min after saline or raclopride administration. Each bar represents the median avoidance % (± semi-interquartile range, n = 10 in all groups). Statistical evaluation was performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed ranks test [ANOVA \( \chi^2 \) (d.f. 7) = 55.32, \( p < 0.001 \)].

Effects of galantamine and donepezil on catalepsy

Raclopride (0.075 mg/kg s.c.) or galantamine (0.1, 1.0 or 5.0 mg/kg s.c.), alone or in combination, did not produce catalepsy. Donepezil (5.0 mg/kg s.c.) alone produced slight but significant catalepsy 130 min after its administration (\( p < 0.05 \)). The combination of 5.0 mg/kg s.c. donepezil and 0.075 mg/kg s.c. raclopride produced significant catalepsy [30 min: \( \chi^2(13) = 26.974, p < 0.05 \); 60 min: \( \chi^2(13) = 37.009, p < 0.001 \); 120 min: \( \chi^2(13) = 53.584, p < 0.001 \) (\( p < 0.05 \)) at 30, 60 and 120 min (\( p < 0.05 \), donepezil + raclopride vs. saline + raclopride) after raclopride injection.

Discussion

The main finding of the present study is that a low dose of galantamine (1.0 mg/kg s.c.) can significantly augment the suppressant effect of raclopride in the CAR test without producing any escape failures or catalepsy. These data provide support for the clinical observation that adjunctive galantamine, besides cognitive improvement, may indeed enhance the antipsychotic activity of neuroleptic drugs (Norén et al., 2006). Moreover, our data propose that adding galantamine to a typical \( D_2 \)/\( D_3 \) antagonist does not increase the risk of EPS. In contrast, donepezil could not augment the suppressant effect of raclopride in the CAR test without producing both escape failures and significant catalepsy. These data indicate that donepezil may not confer any specific antipsychotic activity and, moreover, that donepezil when used as...
adjunctive treatment in schizophrenia may increase the risk of EPS and other non-specific behavioural side-effects.

The differences between galantamine and donepezil may be related to their different mechanisms of action. Whereas donepezil is a potent and selective inhibitor of AChE, galantamine, at the doses used, provides only weak inhibition of AChE but acts as a relatively potent allosteric modulator of nAChRs (Geerts et al., 2005; Schrattenholz et al., 1996; Thomsen and Kewitz, 1990). Thus, our data suggest that the antipsychotic-like effect of adjunctive galantamine may, in principle, largely be due to its allosteric modulatory action at nAChRs. This conclusion is supported by the fact that its specific antipsychotic-like effect could not be mimicked by the selective AChE inhibitor donepezil at any dose tested and, moreover, that the antipsychotic-like effect of galantamine was lost at higher dosage. As previously mentioned, galantamine may, through stimulation of α7 nAChRs and a resulting increase in dopaminergic neuronal activity, enhance dopamine outflow in the medial prefrontal cortex (Schilstrom et al., 2007), an effect which is shared by clozapine and other atypical, but not typical, antipsychotic drugs (see e.g. Kuroki et al., 1999). In fact, previous studies directly propose that an enhancement of prefrontal dopamine output per se may serve to enhance the antipsychotic efficacy of typical D2/3 antagonists (El Tayeb et al., 2005; Hertel et al., 1999).

Generally, potentiation of nAChRs, particularly via α7 nAChRs, may improve various aspects of schizophrenia. For example, α7 nAChRs have previously been shown to play an important role in the mechanism of normal auditory gating (Luntz-Leybman et al., 1992), while impaired auditory gating in schizophrenia, which has been postulated to contribute to cognitive impairment as well as hallucinations, (Adler et al., 1998) has been linked to polymorphisms in the α7 nAChR gene (Freedman et al., 1997). Moreover, the partial α7 nicotinic agonist 3-[(2,4-dimethoxy) benzylidene]anabaseine (DMXB-A) has been shown to reduce cognitive deficits in schizophrenia (Olincy et al., 2006). Consequently, allosteric potentiation of α7 nAChRs by galantamine may provide the major explanation of its utility as adjunct treatment in schizophrenia.

With the above interpretation of our data the lack of effect observed with donepezil may seem surprising, since an increase of ACh efflux induced by donepezil is likely to activate nAChRs. However, muscarinic ACh receptors (mAChRs) will also be activated and although some subtypes of mAChRs have been suggested as possible targets for novel antipsychotic drugs (Bymaster et al., 1998), activation of other mAChR subtypes may in fact even counteract antipsychotic activity (Shannon et al., 1999). Interestingly, some preclinical studies indicate that AChE inhibitors may confer antipsychotic activity when given alone (Andersen et al., 2007; Hohnadel et al., 2007) but neither galantamine nor donepezil suppressed CAR selectively, i.e. the significant suppression observed at 5.0 mg/kg of each drug was associated with escape failures. Moreover, since donepezil also produced catalepsy when combined with raclopride, our data indicate that a high degree of AChE inhibition may rather be associated with increased side-effect liability than any specific antipsychotic effect.

In summary, our data imply that galantamine by means of allosteric modulation of nAChRs may enhance the antipsychotic activity when added to typical D2/3 antagonists without increasing the risk of EPS. The present data also propose that AChE inhibition per se, whether alone or in combination with typical antipsychotic drugs, does not generate any antipsychotic-like effect as judged by the CAR test. Our results rather indicate that inhibition of AChE may increase the risk of motor side-effects and suggest that the therapeutic window for galantamine may be limited to a dose range where inhibition of AChE is but modest. Generally, these findings provide indirect support for the development of α7 nAChR-selective allosteric potentiators for adjunct treatment of schizophrenia.

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Statement of Interest

None.

References


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