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RESEARCH PAPER

Galantamine improves apomorphine-induced deficits in prepulse inhibition via muscarinic ACh receptors in mice

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Background and purpose: Galantamine, a weak acetylcholine esterase (AChE) inhibitor and allosteric potentiator of nicotinic ACh receptors (nAChRs), improves apomorphine-induced deficits in prepulse inhibition (PPI), sensory information-processing deficits, via a nAChR-independent mechanism. The present study examined the role of muscarinic ACh receptors (mAChRs) in the effect of galantamine, and studied the mechanism of galantamine-induced increases in prefrontal ACh levels in mice.
Experimental approach: Apomorphine (1 mg kg^{-1}) was administered to male ddY mice (9–10 weeks old) to create a PPI deficit model. Extracellular ACh concentrations in the prefrontal cortex were measured by *in vivo* microdialysis.
Key results: Galantamine- and donepezil-mediated improvements in apomorphine-induced PPI deficits were blocked by the preferential M_1 mAChR antagonist telenzepine. The mAChR agonist oxotremorine also improved apomorphine-induced PPI deficits. Galantamine, like donepezil, increased extracellular ACh concentrations in the prefrontal cortex. Galantamine-induced increases in prefrontal ACh levels were partially blocked by the dopamine D_1 receptor antagonist SCH23390, but not by antagonists of mAChRs (telenzepine) and nAChRs (mecamylamine). Galantamine increased dopamine, but not 5-HT, release in the prefrontal cortex.
Conclusions and implications: Galantamine improves apomorphine-induced PPI deficits by stimulating mAChRs through increasing brain ACh levels via a dopamine D_1 receptor-dependent mechanism and AChE inhibition.
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Keywords: galantamine; donepezil; prepulse inhibition; apomorphine; AChE inhibitor; muscarinic ACh receptor (mAChR); ACh release; dopamine release

Abbreviations: AChE, acetylcholine esterase; ANOVA, analysis of variance; mAChR, muscarinic ACh receptor; nAChR, nicotinic ACh receptor; PPI, prepulse inhibition

Introduction

Central cholinergic dysfunction causes the cognitive symptoms of various neurological diseases (Friedman, 2004). Schizophrenic patients do not show decreased cell density in the nucleus basalis of Meynert, a typical pathological change in Alzheimer's disease, but do show decreased levels of nicotinic ACh receptors (nAChRs) and M_1/M_4 muscarinic ACh receptors (mAChRs) (Friedman, 2004). In addition, single-photon emission computed tomography in living, unmedicated schizophrenic patients showed fewer mAChRs in several brain regions including the frontal cortex (Raedler *et al.*, 2003), and neuropharmacological studies showed that atypical antipsychotic drugs preferentially increase ACh concentrations in the prefrontal cortex (Ichikawa *et al.*, 2002; Shirazi-Southall *et al.*, 2002). These observations indicate that cholinergic alterations may be involved in the sensory gating abnormalities seen in schizophrenia, providing a rationale for pharmacological approaches directed at cholinergic targets to enhance the cognitive abilities of schizophrenic patients.

Prepulse inhibition (PPI) refers to the normal inhibition of the startle response when a weak stimulus (the prepulse) immediately precedes an intense startling stimulus (the pulse) (Graham, 1975). The PPI of startle is an operational measure of the pre-attentive filtering process known as sensorimotor gating, and abnormalities in pre-attentive information processing may be predictive of, or lead to, complex cognitive deficits in schizophrenia (Braff *et al.*, 1999; Geyer *et al.*, 2001; Swerdlow *et al.*, 2006). In addition, PPI performance may be

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related to cognitive processes in healthy males (Bitsios *et al.*, 2006). The acetylcholine esterase (AChE) inhibitors, galantamine and donepezil, improve apomorphine-induced PPI deficits in rats (Hohnadel *et al.*, 2007) and mice (Koda *et al.*, 2008), but the mechanism of this improvement is unclear. Galantamine is a weaker AChE inhibitor than donepezil (Woodruff-Pak *et al.*, 2002), but has additional allosteric potentiating effects at nAChRs (Maelicke *et al.*, 2001; Santos *et al.*, 2002; Dajas-Bailador *et al.*, 2003; Samochocki *et al.*, 2003). In addition, nicotine administration enhances PPI in both human and animals (Acri *et al.*, 1994; Kumari *et al.*, 1996; 1997; Adler *et al.*, 1998) and reverses apomorphine-induced PPI deficits in rats via nicotinic α_7 receptors (Suemaru *et al.*, 2004). However, the beneficial effects of AChE inhibitors are not blocked by the nAChR antagonists, mecamylamine and methyllycaconitine (Koda *et al.*, 2008), and mAChR agonists reverse apomorphine-induced PPI deficits in rats (Stanhope *et al.*, 2001; Jones *et al.*, 2005). Therefore, we examined the role of mAChRs in the improvement of apomorphine-induced PPI deficits by AChE inhibitors in mice using the preferential M_1 mAChR antagonist, telenzepine (Eltze *et al.*, 1985; Doods *et al.*, 1987; Bymaster *et al.*, 1993). In this relation, we compared the effects of galantamine and donepezil on extracellular ACh levels in the prefrontal cortex, since the prefrontal cortex plays a key role in the regulation of PPI of acoustic startle in animal models (Bubser and Koch, 1994; Swerdlow *et al.*, 1995; de Jong and van den Buuse, 2006). In addition, we examined the effect of galantamine on prefrontal dopamine and 5-HT levels to study the mechanism(s) underlying the effect of galantamine on extracellular ACh levels, since prefrontal ACh release is regulated by dopamine (Imperato *et al.*, 1993; Acquas *et al.*, 1994; Hersi *et al.*, 1995; Di Cara *et al.*, 2007), and 5-HT (Consolo *et al.*, 1996; Somboonthum *et al.*, 1997).

Methods

Animals

Procedures involving animals and their care were conducted according to Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. Male ddY mice (8 weeks old) were housed in groups of 5–6 per cage ($24 \times 17 \times 12$ cm^3) under controlled environmental conditions (22 ± 1°C; 12–12 h light–dark cycle, lights on at 08:00 h; food and water *ad libitum*) for at least 1 week before being used in the experiment. We used a total of 336 mice in all the experiments; different mice were used in each experiment.

Measurement of startle response and PPI

All PPI testing took place within startle chambers acquired from San Diego Instruments (San Diego, CA, USA) as previously reported (Sakaue *et al.*, 2003; Koda *et al.*, 2008). Each startle chamber consists of a 5.1 cm (outside diameter) Plexiglas cylinder mounted on a platform (20.4 cm length × 12.7 cm width × 0.4 cm thick) with a piezoelectric accelerometer unit attached below the Plexiglas cylinder. The piezoelectric unit transduces vibrations into signals that are rectified and stored by a microcomputer interface. The Plexiglas cylinder and platform are located in a sound-attenuated chamber (San Diego Instruments) with a loudspeaker (28 cm above the cylinder), and house light. Calibration procedures using a vibrating standardization unit (San Diego Instruments) were performed between experiments to ensure equivalent sensitivities across the chambers. The sound levels for background noise and various stimuli in each chamber were calibrated with a digital sound-level meter.

Each test session began by placing a mouse in the Plexiglas cylinder where it was left undisturbed. After a background noise of 65 dB had been presented for the 5 min acclimation period, each mouse was exposed to four consecutive blocks with a total of 100 trials over the approximately 30 min test session. One block consisted of 25 trials including five different trial types: pulse-alone trials in which a 40 ms broadband 120 dB burst was presented; three different prepulse-pulse trials in which the onset of a 20 ms broadband noise preceded the onset of the 120 dB startle pulse by 100 ms (prepulse intensities 3, 6 and 9 dB above the 65 dB background noise were used); and no-stimulation trials in which only the background noise was presented. Trials were presented in a pseudo-random order separated by an average of 15 s (range 7–23 s). The startle response was recorded for 100 ms (measuring the response every 1 ms) starting at the onset of each startle stimulus. The maximum startle amplitude recorded during the 100 ms sampling window was used as the dependent variable.

Surgery and microdialysis procedures

Mice were anesthetized with sodium pentobarbital (40 mg kg^{-1}, i.p.) and stereotaxically implanted with a guide cannula (one site per animal) for a dialysis probe (Eicom, Kyoto, Japan) in the prefrontal cortex (A + 1.9 mm, L – 0.5 mm, V – 3.8 mm, from the bregma and skull) (Franklin and Paxinos, 1997). The cannula was cemented in place with dental acrylic, and the animal was kept warm and allowed to recover from anaesthesia. Postoperative analgesia was provided with a single injection of buprenorphine (0.1 mg kg^{-1}, i.p.) (Ago *et al.*, 2006a; 2007). The active probe membranes were 3 mm long in the prefrontal cortex of mice. On the day after surgery, the probe was perfused with Ringer's solution (147.2 mmol·L^{-1} NaCl, 4.0 mmol·L^{-1} KCl and 2.2 mmol·L^{-1} $CaCl_2$; Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) at a constant flow rate of 2 µL min^{-1} for the dopamine and 5-HT simultaneous assay or 1 µL min^{-1} for the ACh assay. A stabilization period of 3 h was established before the onset of the experiments. Microdialysis samples (20 µL) were collected every 10 min for the dopamine and 5-HT simultaneous assay or 20 min for the ACh assay, and injected immediately onto a high-performance liquid chromatography column for detection of dopamine and 5-HT (Ago *et al.*, 2002; 2003; 2006a; 2007) and ACh (Ago *et al.*, 2006b; Sato *et al.*, 2007) as previously reported. After the experiments, Evans Blue dye was microinjected through the cannula to histologically verify the position of the probe.

Data analysis

All data are expressed as the mean ± SEM. For the acoustic startle response profile, the amount of PPI was calculated

as a percentage score for each prepulse trial type. The following formula was used: %PPI = 100 – {[(startle response to prepulse-pulse trial)/(startle response to pulse-alone trial)] × 100}. Startle amplitude was calculated as the average response to all of the pulse-alone trials and analysed using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test. Data for PPI were analysed using two-way ANOVA for treatment as the intersubject factor and repeated measures with prepulse intensity as the intrasubject factor. The *post hoc* individual comparisons were performed with the Tukey–Kramer test. Data from the 'no stim' trials are not included in the results because the values were negligible, relative to values on trials containing startle stimuli. For *in vivo* microdialysis studies, all data were calculated as per cent change from the dialysate basal concentrations, with 100% defined as the average of three fractions before administration. Analyses were made using two-way ANOVA for treatment as the intersubject factor and repeated measures with time as the intrasubject factor. Statistical analyses were made using a software package Statview 5.0 J for Apple Macintosh computer (SAS Institute Inc., Cary, NC, USA). A value of $P < 0.05$ was considered statistically significant.

Drugs

The following drugs were used: galantamine (Janssen Pharmaceutical K.K., Tokyo, Japan); donepezil (Mitsubishi Tanabe Pharma Co., Yokohama, Japan); apomorphine, SCH23390, oxotremorine, mecamylamine and telenzepine (Sigma, St Louis, MO, USA). All other commercially available chemicals used in the experiments were of superfine quality. Galantamine, donepezil, SCH23390, oxotremorine, mecamylamine and telenzepine were dissolved in saline (0.9% solution of NaCl). Apomorphine was dissolved in saline containing 0.1% w/v ascorbic acid. Drugs were administered at 10 mL kg^{-1} intraperitoneally (galantamine, donepezil, SCH23390, oxotremorine, mecamylamine) or subcutaneously (apomorphine, telenzepine).

Results

Effect of telenzepine, a preferential M_1 mAChR antagonist, on galantamine- and donepezil-induced reversal of PPI deficits in apomorphine-treated mice

Apomorphine (1 mg kg^{-1}, s.c.) caused a marked reduction of PPI of the acoustic startle response in mice. Both galantamine (3 mg kg^{-1}, i.p.) and donepezil (3 mg kg^{-1}, i.p.) reversed apomorphine-induced PPI deficits, as previously reported (Koda *et al.*, 2008). These improvements were significantly antagonized by telenzepine (3 or 10 mg kg^{-1}, s.c.) (Fig. 1), whereas telenzepine alone did not affect PPI or the startle response of naïve mice (data not shown). Galantamine, donepezil and telenzepine did not affect the startle response of apomorphine-treated mice (Table 1).

Effect of oxotremorine, a non-selective mAChR agonist, on apomorphine-induced deficits in PPI of the acoustic startle response

Apomorphine (1 mg kg^{-1}, s.c.) induced deficits in PPI of the acoustic startle response in mice. The mAChR agonist, oxotremorine (0.01–0.1 mg kg^{-1}, i.p.), dose-dependently reversed apomorphine-induced PPI deficits in mice (Fig. 2), whereas oxotremorine alone did not affect PPI or the startle response of naïve mice (data not shown). Oxotremorine did not affect the startle response of apomorphine-treated mice (Table 1).

Effects of galantamine and donepezil on extracellular ACh concentrations in the prefrontal cortex

Basal extracellular levels (means ± SEM) of ACh (not corrected for *in vitro* probe recovery) in the absence ($n = 64$) and presence ($n = 25$) of neostigmine in the perfusion solution were 30 ± 2 and 249 ± 23 fmol per 20 µL respectively (data are obtained from Figs 3 and 4). Galantamine (1 and

Table 1 Effects of galantamine, donepezil, oxotremorine and telenzepine on the startle response of apomorphine-treated mice

Treatment	*Average startle amplitude*		
	Galantamine	*Donepezil*	*Oxotremorine*
Normal mice			
Vehicle	361 ± 45	327 ± 37	342 ± 56
Apomorphine-treated mice			
Vehicle	291 ± 27	286 ± 38	283 ± 58
0.01 mg kg^{-1}			206 ± 18
0.03 mg kg^{-1}			234 ± 40
0.1 mg kg^{-1}			196 ± 36
AChE inhibitor			
+Vehicle	281 ± 34	305 ± 33	
+Telenzepine 3 mg kg^{-1}	333 ± 51	483 ± 87	
+Telenzepine 10 mg kg^{-1}	406 ± 56	302 ± 44	

Apomorphine (1 mg kg^{-1}, s.c.) was administered 10 min before the experiments. Galantamine (3 mg kg^{-1}, i.p.), donepezil (3 mg kg^{-1}, i.p.) or oxotremorine (0.01–0.1 mg kg^{-1}, i.p.) was injected 30 min before the experiments. Telenzepine (3 and 10 mg kg^{-1}, s.c.) was administered 30 min before galantamine or donepezil treatment. Data are expressed as the mean ± SEM from 8–18 mice. One-way ANOVA indicated that galantamine/telenzepine ($F_{4,78} = 1.393$, $P > 0.05$), donepezil/telenzepine ($F_{4,66} = 2.208$, $P > 0.05$) and oxotremorine ($F_{4,64} = 1.695$, $P > 0.05$) did not affect the startle response of apomorphine-treated mice.

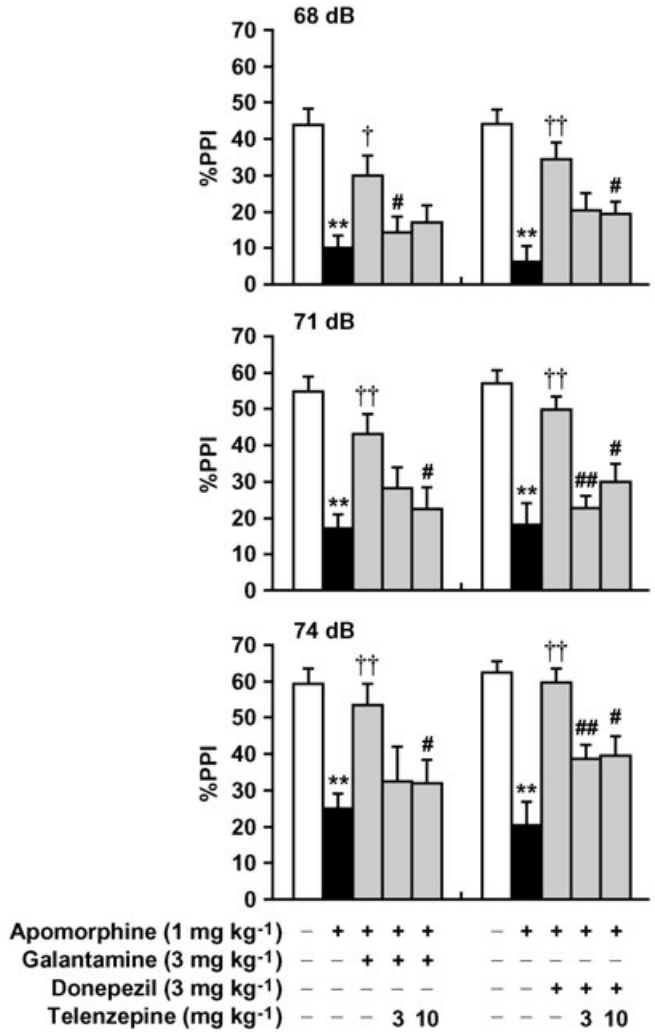

Figure 1 Effect of telenzepine on galantamine- and donepezil-induced reversal of PPI deficits in apomorphine-treated mice. Apomorphine (1 mg kg^{-1}, s.c.) was injected 10 min before the experiments. Galantamine (3 mg kg^{-1}, i.p.) or donepezil (3 mg kg^{-1}, i.p.) was injected 30 min before the experiments. Telenzepine (3 and 10 mg kg^{-1}, s.c.) was administered 30 min before galantamine or donepezil treatment. Data are expressed as the mean ± SEM from 8–18 mice. $^{**}P < 0.01$, compared with vehicle/saline-treated mice; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, compared with vehicle treatment group in apomorphine-treated mice; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with galantamine or donepezil treatment group in apomorphine-treated mice using Tukey–Kramer's *post hoc* test, following repeated measures two-way ANOVA (main effects of prepulse intensity ($F_{2,156} = 60.768$, $P < 0.0001$ and $F_{2,132} = 52.956$, $P < 0.0001$ for galantamine and donepezil respectively) and treatment ($F_{4,78} = 10.032$, $P < 0.0001$ and $F_{4,66} = 16.369$, $P < 0.0001$ for galantamine and donepezil respectively); no significant interaction between treatment and prepulse intensity ($F_{8,156} = 0.906$, $P > 0.05$ and $F_{8,132} = 1.194$, $P > 0.05$ for galantamine and donepezil respectively).

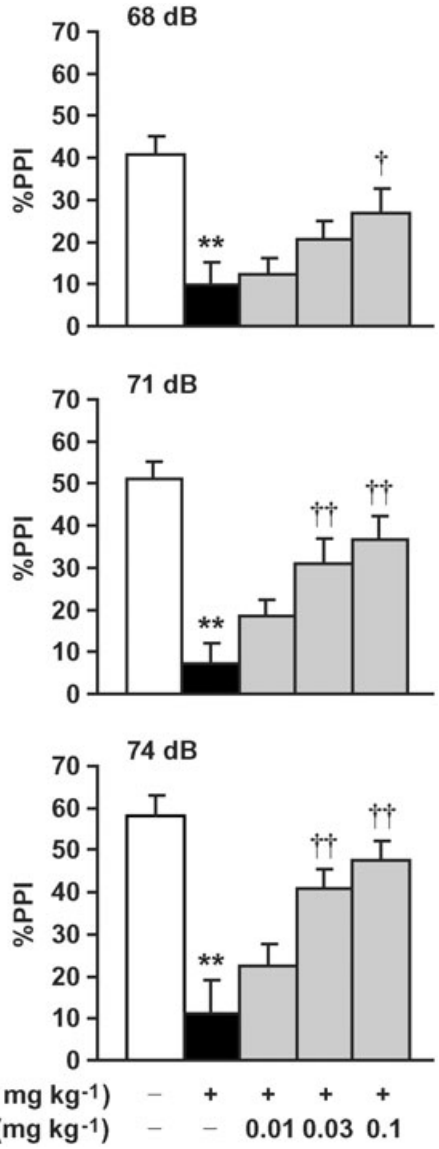

Figure 2 Effect of oxotremorine on apomorphine-induced deficits in PPI of the acoustic startle response in mice. Oxotremorine (0.01–0.1 mg kg^{-1}, i.p.) was injected 30 min before the experiments. Apomorphine (1 mg kg^{-1}, s.c.) was administered 10 min before the experiments. Data are expressed as the mean ± SEM from 10–16 mice. $^{**}P < 0.01$, compared with vehicle/saline-treated mice; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, compared with vehicle treatment group in apomorphine-treated mice using Tukey–Kramer's *post hoc* test, following repeated measures two-way ANOVA (main effects of prepulse intensity ($F_{2,128} = 25.160$, $P < 0.0001$) and treatment ($F_{4,64} = 12.448$, $P < 0.0001$); significant interaction between treatment and prepulse intensity ($F_{8,128} = 2.025$, $P = 0.0484$)).

3 mg kg^{-1}, i.p.) and donepezil (1 and 3 mg kg^{-1}, i.p.) produced a robust increase in extracellular ACh concentrations in the prefrontal cortex in the absence and presence of neostigmine in the perfusion solution (Fig. 3). Inhibition of increase in ACh levels by neostigmine was greater in the effect of donepezil than in that of galantamine.

Effects of SCH23390, a dopamine-D_1 receptor antagonist, mecamylamine, a non-selective nAChR antagonist, and telenzepine on galantamine-induced increase in prefrontal ACh levels

Galantamine (3 mg kg^{-1}, i.p.) caused a robust increase in ACh levels, which could be attenuated by SCH23390 (0.3 mg kg^{-1}, i.p.), but not by mecamylamine (3 and 10 mg kg^{-1}, i.p.) or telenzepine (3 mg kg^{-1}, s.c.) (Fig. 4). SCH23390 alone did not affect basal extracellular ACh levels.

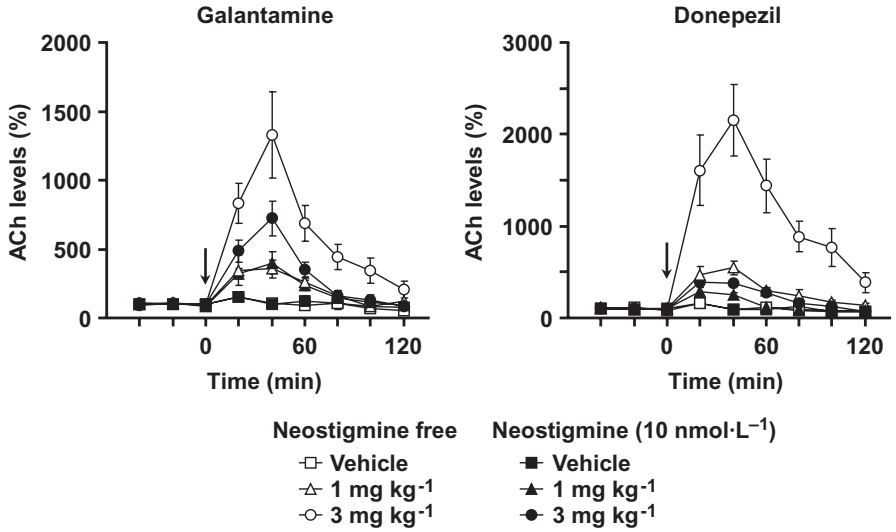

Figure 3 Effects of galantamine and donepezil on extracellular ACh levels in the prefrontal cortex of mice. Galantamine or donepezil at doses of 1 and 3 mg kg^{-1} were injected i.p. at 0 min (arrow). Ringer's solution was perfused with or without neostigmine at 10 nmol·L^{-1} in the probe. Data are expressed as the mean ± SEM from 3–7 mice. Repeated measures two-way ANOVA indicated that galantamine significantly increased prefrontal ACh levels [interaction (treatment × time): $F_{16,96} = 6.245$, $P < 0.0001$ and $F_{16,72} = 8.092$, $P < 0.0001$ for absence and presence of neostigmine respectively]. Donepezil also increased prefrontal ACh levels ($F_{16,88} = 13.444$, $P < 0.0001$ and $F_{16,80} = 9.023$, $P < 0.0001$ for absence and presence of neostigmine respectively).

Effect of galantamine on extracellular levels of dopamine and 5-HT in the prefrontal cortex

Basal extracellular levels (means ± SEM) of dopamine and 5-HT (not corrected for *in vitro* probe recovery) were 3.30 ± 0.54 fmol per 20 µL ($n = 24$) and 6.71 ± 1.40 fmol per 20 µL ($n = 12$) respectively (data are obtained from Figs 5 and 6). Galantamine (1 and 3 mg kg^{-1}) significantly increased extracellular dopamine, but not 5-HT, levels (Fig. 5).

Effect of mecamylamine on galantamine-induced increase in prefrontal dopamine levels

Galantamine (3 mg kg^{-1}, i.p.) caused a robust increase in dopamine levels, but this effect was not inhibited by mecamylamine (3 and 10 mg kg^{-1}, i.p.) (Fig. 6).

Discussion

A disruption of cerebral cholinergic pathways may contribute to the cognitive deficits of schizophrenia, and AChE inhibitors, like ACh receptor agonists, have therapeutic potential for these deficits (Friedman, 2004). We have recently reported in mice that the AChE inhibitors, galantamine and donepezil, improved apomorphine-induced PPI disruption (Koda *et al.*, 2008), as occurs in rats (Hohnadel *et al.*, 2007). Galantamine is a weak AChE inhibitor and potentiates nAChR activity (Dajas-Bailador *et al.*, 2003; Samochocki *et al.*, 2003), although nAChR antagonists (mecamylamine and methyllycaconitine) do not block its ability to modulate PPI deficits (Koda *et al.*, 2008). The present study demonstrated that the preferential M_1 mAChR antagonist, telenzepine (Eltze *et al.*, 1985; Doods *et al.*, 1987; Bymaster *et al.*, 1993), blocked galantamine and donepezil-mediated improvements in apomorphine-induced PPI deficits. This suggests that endogenous ACh preferentially interacts with mAChRs, although both mAChRs and nAChRs are responsible for the improvement of apomorphine-induced PPI deficits (Stanhope *et al.*, 2001; Suemaru *et al.*, 2004; Jones *et al.*, 2005). In addition, the non-selective mAChR agonist, oxotremorine, improved apomorphine-induced PPI deficits in mice. Galantamine, like donepezil, then increased brain ACh levels to improve PPI deficits via mAChRs.

Galantamine at low doses (0.01–0.63 mg kg^{-1} s.c. for the prefrontal cortex and 0.16–0.63 mg kg^{-1} s.c. for the hippocampus) increases brain ACh levels in rats (Di Cara *et al.*, 2007), and donepezil increases extracellular ACh concentrations in the cortex (Rogers *et al.*, 1991; Giacobini *et al.*, 1996), hippocampus (Kawashima *et al.*, 1994; Kosasa *et al.*, 1999; Hatip-Al-Khatib *et al.*, 2004) and striatum (Isomae *et al.*, 2002) in rats. Ours is the first study to show that galantamine and donepezil increase extracellular ACh concentrations in the prefrontal cortex of mice. Galantamine is much more potent in rats than in mice: the effective doses of galantamine in the prefrontal cortex of rats (Di Cara *et al.*, 2007) and mice (in this study) were 0.01–0.63 and 1–3 mg kg^{-1} respectively. Doses of galantamine that increased prefrontal ACh levels (3 mg kg^{-1}) also improved apomorphine-induced PPI deficits in mice. The same dose also improved performance of mice with a nucleus basalis magnocellularis lesion in two different tasks (passive avoidance and swim maze) (Sweeney *et al.*, 1990).

We found that galantamine and donepezil similarly increase brain ACh levels, although another report (Geerts

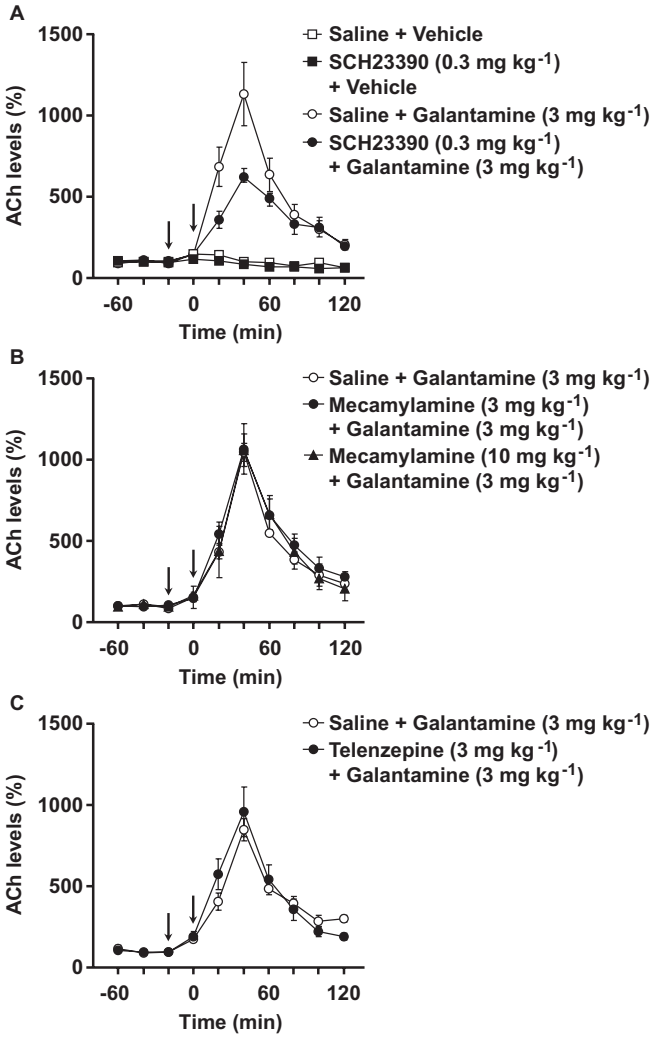

Figure 4 Effects of antagonists of dopamine D_1 receptors (SCH23390), nAChR (mecamylamine) and mAChR (telenzepine) on galantamine-induced increase in prefrontal ACh levels in mice. Ringer's solution was perfused without neostigmine in the probe. Galantamine (3 mg kg^{-1}) was injected i.p. at 0 min (right arrow). SCH23390 (0.3 mg kg^{-1}, i.p.) (A), mecamylamine (3 and 10 mg kg^{-1}, i.p.) (B) and telenzepine (3 mg kg^{-1}, s.c.) (C) were injected 20 min before galantamine treatment (left arrow). Data are expressed as the mean ± SEM from 3–4 mice. Repeated measures two-way ANOVA indicated that SCH23390 attenuated galantamine-induced increase in ACh levels, although SCH23390 itself did not affect basal extracellular ACh levels [interaction (treatment × time): $F_{9,54} = 1.214$, $P > 0.05$ and $F_{9,54} = 5.465$, $P < 0.0001$ for basal and galantamine-induced increase respectively]. On the other hand, neither mecamylamine [interaction (treatment × time): $F_{18,72} = 0.261$, $P > 0.05$] nor telenzepine ($F_{9,54} = 0.864$, $P > 0.05$) affected galantamine-induced increase in ACh levels.

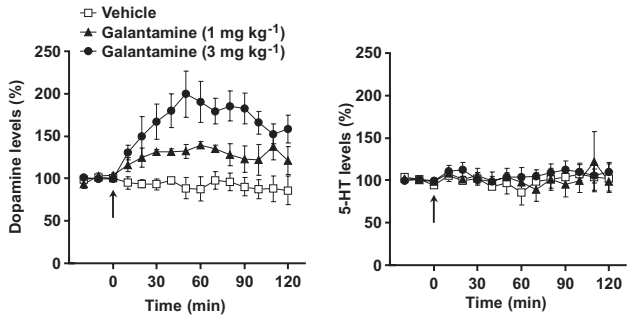

Figure 5 Effects of galantamine on extracellular levels of dopamine and 5-HT in the prefrontal cortex of mice. Galantamine at doses of 1 and 3 mg kg^{-1} were injected i.p. at 0 min (arrow). Data are expressed as the mean ± SEM from four mice. Repeated measures two-way ANOVA indicated that galantamine produced a significant increase in levels of dopamine [interaction (treatment × time): $F_{28,126} = 4.878$, $P < 0.0001$], but not 5-HT ($F_{28,126} = 0.460$, $P > 0.05$).

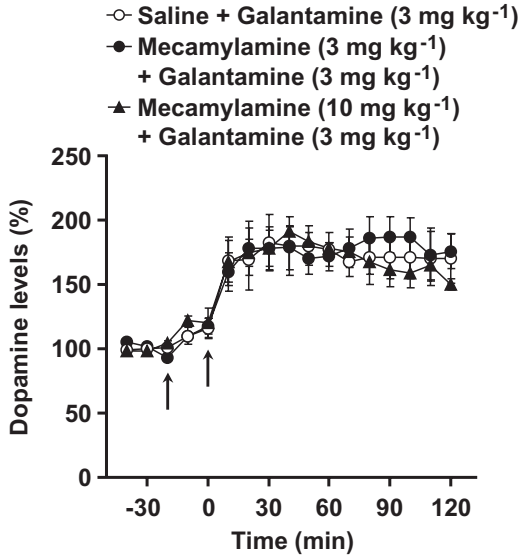

Figure 6 Effect of mecamylamine on galantamine-induced increase in prefrontal dopamine levels. Galantamine (3 mg kg^{-1}) was injected i.p. at 0 min (right arrow). Mecamylamine (3 and 10 mg kg^{-1}, i.p.) was injected 20 min before galantamine treatment (left arrow). Data are expressed as the mean ± SEM from 3–5 mice. Repeated measures two-way ANOVA indicated that mecamylamine did not affect galantamine-induced increase in dopamine levels [interaction (treatment × time): $F_{32,144} = 0.332$, $P > 0.05$].

et al., 2005) indicated that donepezil was 3–15 times more potent than galantamine at inhibiting brain AChE. In addition, the present study showed that inhibition of increase in ACh levels by neostigmine was much greater in the effect of donepezil than in that of galantamine. These observations suggest that mechanisms other than AChE inhibition were involved in the galantamine-induced increases in prefrontal ACh levels. Dopamine D_1 receptors facilitate ACh release in the prefrontal cortex and hippocampus of rats (Imperato *et al.*, 1993; Acquas *et al.*, 1994; Hersi *et al.*, 1995; Di Cara *et al.*, 2007). Galantamine, but not donepezil, enhances dopamine release in the prefrontal cortex in rats, although only at a low dose (0.1 mg kg^{-1}) (Schilström *et al.*, 2007). We found that galantamine increased extracellular dopamine, but not 5-HT, concentrations in the prefrontal cortex, and the effect of galantamine on ACh levels was partially blocked by the dopamine D_1 receptor antagonist, SCH23390. A dopamine D_1 receptor-mediated mechanism may thus contribute to the ability of galantamine to increase prefrontal ACh levels. nAChR-mediated ACh release also occurs in rat brain (Tani

et al., 1998; Reid *et al*., 1999), but nAChR and mAChR antagonists did not affect the ability of galantamine to modify prefrontal ACh levels. We have found in separate experiments that mecamylamine (3 mg kg^{-1}) blocked nicotine-induced hypolocomotion and hypothermia in mice. This suggests that mecamylamine under these conditions does indeed block nAChRs, in agreement with the previous studies (Freeman *et al*., 1987; Damaj *et al*., 1995; Castañé *et al*., 2002). Galantamine may increase prefrontal ACh levels via two different mechanisms, AChE inhibition and through activation of dopamine D_1 receptors by increasing dopamine release. Concerning the mechanism of galantamine-induced increase in dopaminergic neurotransmission, Schilström *et al*. (2007) reported that galantamine-induced increase in dopamine cell firing in the ventral tegmental area was prevented by mecamylamine, but not by the muscarinic receptor antagonist scopolamine in anaesthetized rats. However, the present study showed that galantamine-induced increase in dopamine release in the prefrontal cortex of awake mice was not affected by mecamylamine. Alternatively, Alés *et al*. (2006) have reported that galantamine can potentiate neurotransmitter release by blocking small conductance Ca^{2+}-activated K^+ channels. It remains to be established how galantamine might increase dopamine release in the prefrontal cortex.

In conclusion, we have shown that galantamine and donepezil improved PPI deficits in apomorphine-treated mice in an mAChR-dependent manner. We also showed that galantamine, like donepezil, increases prefrontal ACh levels. Galantamine-induced increases in prefrontal ACh levels required both AChE inhibition and activation of dopamine D_1 receptors.

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Conflict of interest

The authors state no conflict of interest.

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