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Preclinical pharmacology of the α4β2 nAChR partial agonist varenicline related to effects on reward, mood and cognition

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ABSTRACT

The pharmacological properties and pharmacokinetic profile of the α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist varenicline provide an advantageous combination of free brain levels and functional potencies at the target receptor that for a large part explain its efficacy as a smoking cessation aid. Since α4β2 and other nAChR subtypes play important roles in mediating central processes that control reward, mood, cognition and attention, there is interest in examining the effects of selective nAChR ligands such as varenicline in preclinical animal models that assess these behaviors. Here we describe results from studies on varenicline's effects in animal models of addiction, depression, cognition and attention and discuss these in the context of recently published preclinical and preliminary clinical studies that collected data on varenicline's effects on mood, cognition and alcohol abuse disorder. Taken together, the preclinical and the limited clinical data show beneficial effects of varenicline, but further clinical studies are needed to evaluate whether the preclinical effects observed in animal models are translatable to the clinic.

1. Introduction

Agonists and partial agonists of various subtypes of nicotinic acetylcholine receptors (nAChRs) are being pursued as potential treatments for a variety of central nervous system disorders, such as addiction, pain, depression, schizophrenia, Parkinson's disease, Alzheimer's disease, ADHD, and alcoholism [1–5]. Progress has been made in the search for a treatment of nicotine dependence with the discovery and approval of the α4β2 nAChR partial agonist varenicline [6], which has been shown to be more effective as a smoking cessation aid than other approved smoking cessation treatments [7]. The first part of this paper summarizes the rationale for α4β2 nAChR partial agonists in nicotine dependence and describes the pharmacological and pharmacokinetic properties of varenicline that provide plausible explanations for improved efficacy compared to other α4β2 nAChR partial agonists that have been studied clinically, cytisine and dianicline [8,9].

Given the interest in nAChR subtypes as targets for novel therapeutics for disorders other than nicotine addiction, recent studies on varenicline have focused on exploring its effects in preclinical models thought to be predictive of effects on alcohol addiction, mood, cognition and attention, among others. Specifically, varenicline has been tested in studies examining its effects on cortical neurotransmitter release, in the behavioral despair test to assess antidepressant-like activity, and in several sensorimotor models that reflect positive modulation of pre-cognitive processes. Results of our studies will be discussed, together with recently published preclinical data on varenicline, in the context of nAChR subtypes that may be involved in mediating these effects. In addition, reference will be made to clinical data on nicotine withdrawal and to emerging results from preliminary studies that collected data on varenicline's effects on alcohol self-administration, mood and cognition.

2. Methods

2.1. Subjects and chemicals

Male C57Bl/6J mice (20–27 g) supplied by the Jackson Laboratory (Bar Harbor, ME) were used in the acoustic startle studies. Male Sprague–Dawley and male Wistar rats (260–320 g)

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supplied by the Charles River Laboratories (Kingston, NY) were used in the microdialysis, electrophysiology and novel object recognition experiments, and in the sustained attention model, respectively. Animals were allowed a minimum of 1 week acclimation prior to experimentation and were housed under standard laboratory conditions under a 12-h light/dark cycle (lights on at 6:00 A.M.) with food and water available *ad libitum*, except for rats used in the sustained attention tasks, which were food restricted. Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals [10] and all procedures were performed with the approval of the Pfizer Institutional Animal Care and Use Committee.

Varenicline tartrate and dianicline HCl were synthesized at Pfizer Global Research and Development (Groton, CT). Cytisine was purchased from Austin Chemical Co. (Buffalo Grove, IL, USA), risperidone from MP Biomedicals LLC (Aurora, OH). Chloral hydrate, D-amphetamine sulfate (−), nicotine bitartrate and all chemicals were obtained from Sigma–Aldrich (St. Louis, MO). Risperidone was dissolved in 1% glacial acetic acid; all other test compounds in saline or water and administered s.c., unless indicated otherwise, at doses expressed as the free base in volumes of 10 ml/kg body weight for mice and 1 ml/kg body weight for rats.

2.2. Neurotransmitter release in rat prefrontal cortex

Microdialysis of dopamine (DA), norepinephrine (NE), acetylcholine (ACh) and histamine in rat prefrontal cortex was performed as previously described [11–13]. Briefly, several days after implantation of a guide cannula under isoflurane anaesthesia, a 4 mm microdialysis probe was inserted into the prefrontal cortex and perfused overnight at 0.6 μl/min with artificial cerebral spinal fluid (NaCl 147 mM; $CaCl_2$ 1.3 mM; KCl 2.7 mM; $MgCl_2$ 1.0 mM, for the ACh assay containing 0.1 μM neostigmine). The next day the perfusion rate was increased to 2 μl/min and every 15 min 30 μl samples were collected on-line and automatically injected onto a reversed phase HPLC system with amperometric detection (for DA, NE and, after post-column enzymatic conversion to H_2O_2, for ACh) or fluorometric detection (for histamine). After basal transmitter output had stabilized (at least six samples), saline or drug was injected s.c. and drug effects were expressed as the percentage of the mean of the last five pre-drug basal levels. For dose–response curves the effect of each dose was calculated as the mean percentage of baseline ± SEM of samples collected over 0–1 h (DA, NE) or 0–3 h (ACh, histamine). Statistical significance was analyzed using one-way ANOVA with post hoc Student–Newman–Keuls tests.

2.3. Auditory gating and neuronal network oscillations in rat hippocampus and entorhinal cortex

Unilateral hippocampal (CA1 or CA3) and entorhinal cortex (EC) field potentials (EEG) and auditory evoked potentials were recorded simultaneously by metal monopolar macroelectrodes from chloral hydrate-anaesthetized rats as described previously [14]. The femoral artery was cannulated for the administration of drugs or additional doses of anaesthetic. Field potentials were amplified, filtered (0.1–100 Hz), displayed and recorded for on-line and off-line analysis (Spike 3). The auditory stimulus consisted of a pair of 10 ms, 5 kHz tone bursts with a 0.5 s delay between the first "conditioning" stimulus and second "test" stimulus. Auditory evoked responses (CA3 and EC) were computed by averaging of responses to 50 pairs of stimuli presented with an interstimulus interval of 10 s. Percentage of gating was determined by the formula: (1 − test amplitude/conditioning amplitude) × 100. Amphetamine (D-amphetamine sulfate, 1 mg/kg, IV) was administered in order to disrupt sensory-gating. Quantitative EEG analysis was performed by means of Fast Fourier Transformation (Spike 3). Drug effects on spontaneous theta oscillations were studied in the hippocampus CA1 and EC; relative theta power was determined using fast Fourier transformation by calculating the percentage of total power that occurred in the theta (3.5–5.5 Hz) frequency band as compared with the 0- to 15-Hz frequency band as described previously [15]. Statistical significance was determined by means of two-tailed paired Student's *t*-test.

2.4. Prepulse inhibition of the acoustic startle response in mice

Effects on prepulse inhibition (PPI) of the acoustic startle response (ASR) were measured in C57Bl/6J mice, which have an inherently low level of PPI. Test compounds were administered 30 min (nicotine) or 60 min (varenicline, risperidone) before testing, which was performed as described in Schmidt et al. [16]. Briefly, individual mice were placed into acoustic startle chambers with loudspeakers that provided background white noise (68 dB) and the acoustic startle stimuli (120 dB). After a 5 min acclimation period with only background noise, a series of 37 acoustic startle stimuli was presented in quasi-random order and with randomly varied inter-trial intervals (10, 15 or 20 s). PPI was calculated as a percentage score for each prepulse trial type with the formula: 100 − [(prepulse + pulse)/pulse] × 100. ASR amplitude was calculated as the average response in milliNewtons ± SEM to the pulse alone trials. Means were statistically compared using ANOVA followed by Dunnett's *t*-tests for post hoc analyses.

2.5. Sustained attention task in rats

The effects of varenicline in the sustained attention task (SAT) were determined in rats performing the standard SAT and a distractor version (dSAT) of the task. This operant procedure consists of a random sequence of signal and non-signal events followed by a variable inter-trial interval (ITI), as described in detail by McGaughy and Sarter [17]. Briefly, following a signal (center panel light illumination for 500, 50, or 25 ms) or a non-signal event (no light illumination), levers were extended into the chambers 2 s later and remained active for 4 s. Correct responses (lever presses) were either hits (signal trial) or correct rejections (non-signal trial) for which animals were rewarded. Incorrect responses were misses (signal trial) or false alarms (non-signal trial) which were unrewarded and triggered an ITI. An error of omission was defined as the failure to operate a lever within 4 s after a trial initiation. To obtain an overall measure of performance that reflects the animals' accuracy in signal and non-signal trials, a 'vigilance index' (VI) was calculated, ranging from −1 (total inaccuracy) to +1 (perfect response accuracy), with 0 indicating random lever selection. The task lasted for 40 min and was divided into five 8-min blocks. In the dSAT, a distractor (chamber houselights flash on/off at 0.5 Hz) was presented during the second and third blocks of dSAT, which reduces the discriminability of the signal and increases the demand on top-down control to maintain performance [18]. The animals were exposed to 'distractor' sessions 2–3 times within a 2-week period prior to drug administration. Varenicline (0.032, 0.1, 1 mg/kg) or saline was administered 30 min before performance in the SAT or dSAT was assessed. The relative number of hits was calculated as [hits/(hits + misses)] for each signal length, the relative number of correct rejections was calculated as [correct rejections/(correct rejections + false alarms)] and errors of omission were recorded. Repeated-measures ANOVA was used to assess the effects of dose, block and signal duration on VI, and hits. For correct rejections and omissions, the factor 'signal duration' was not included. Significant interactions were followed by a dependent *t*-test when appropriate for within-subjects factors to determine the specific source for significant interactions.

2.6. Novel object recognition test in rats

The effects of varenicline in the novel object recognition (NOR) test were measured using a procedure based on that described by Ennaceur and Delacour [19]. Male CD rats were individually placed into a test arena (62 cm × 42 cm × 36 cm) for a 3 min habituation period on day 1. On day 2 rats were placed into the same arena that now contained two identical objects and were allowed to explore them for a 3-min 'sample' period. Rats that accumulated at least 10 s of contact time with one or both of the objects qualified for further testing. On day 3, after a 24-h delay, rats were once again placed into the arena that now contained one familiar and one novel object for a 3 min 'choice' test, and the time spent in contact with either the familiar or novel object was automatically recorded (CleverSys software). Rats were dosed with vehicle or varenicline 60 min prior to placement in the test arena on test days 1–3. Mean exploration times for the novel vs the familiar objects on day 3 were compared statistically using dependent *t*-tests.

3. Efficacy of varenicline and other partial agonists as smoking cessation aids

Nicotine dependence is a chronic relapsing condition that makes smoking extremely difficult to quit because of pronounced withdrawal symptoms that result in a strong urge to relieve craving by smoking and rapidly delivering nicotine to the brain [20]. The addictive properties of nicotine are currently thought to be mediated via interactions with several nAChR subtypes located in different brain areas, of which mesolimbic α4β2 and α7 nAChRs seem to play a crucial role [21–24], but other subtypes have also been implicated [25–27]. The rationale behind the development of partial agonists with high affinity for central α4β2 nAChRs as smoking cessation therapy was that effective treatments would not only provide some level of nicotine-like reinforcement to relieve craving during abstinence, but would also attenuate the rewarding effects of nicotine during smoking. It was hypothesized that this could be achieved with partial agonists that interact with nicotine's target nAChR to reduce withdrawal symptoms in the absence of nicotine, and to prevent nicotine's binding to α4β2 nAChRs and thereby its reinforcing effects, in the presence of nicotine [6,9,28].

The clinical efficacy of varenicline, an α4β2 nAChR partial agonist designed as a smoking cessation aid and for which the discovery strategy, synthesis and pharmacological properties have been described in detail [6,11,28], is consistent with the dual mode of action hypothesis. Patient-reported data from clinical trials support the notion that reduced craving and smoking reward contribute to varenicline's efficacy in smoking cessation [29]. Pooled data from two identical smoking cessation trials showed that varenicline 1 mg BID varenicline reduced craving more than 150 mg BID bupropion ($p < 0.01$) and placebo ($p < 0.001$) during the first week of attempted abstinence. In patients who lapsed, varenicline also reduced ratings of satisfaction and psychological reward after the first cigarette more than bupropion ($p < 0.005$) and placebo ($p < 0.001$).

Two other α4β2 nAChR partial agonists that share the mechanism of action with varenicline, the natural product cytisine [30,8] and SSR591813 or dianicline [9], have been clinically tested. Since abstinence rates are reported to be higher for varenicline than for either dianicline or cytisine [7,8,31,32], their *in vitro* pharmacological and *in vivo* PK properties were compared to explain the clinical differences among these partial agonists [33]. By correlating free human brain concentrations, estimated from therapeutic human plasma levels and rat brain-to-plasma ratios, with binding affinities and functional potencies at α4β2 nAChRs, we found that concentrations equal to predicted human brain concentrations of varenicline (K_i α4β2 = 0.4 nM) can extensively desensitize and to a lesser extent activate α4β2 nAChRs *in vitro* (Fig. 1), properties which are considered a requirement for pharmacological activity [1,5]. This is not the case for dianicline or for cytisine. Dianicline has low *in vitro* affinity (K_i α4β2 = 105 nM) and weak functional potencies at α4β2 nAChRs and free brain levels are predicted to be too low for desensitization and activation of α4β2 nAChRs. Cytisine has high *in vitro* affinity (K_i α4β2 = 2 nM) and potent functional activity at α4β2 nAChRs, but minimal brain penetration, resulting in insufficient free brain levels for an optimal pharmacological effect. A similar comparison of predicted brain levels with the functional potencies of varenicline at α7 nAChRs, at which varenicline is a full agonist (K_i α7 = 125 nM), showed that varenicline concentrations equal to predicted human brain concentrations would cause limited desensitization and minimal activation of α7 nAChRs *in vitro* (Fig. 1).

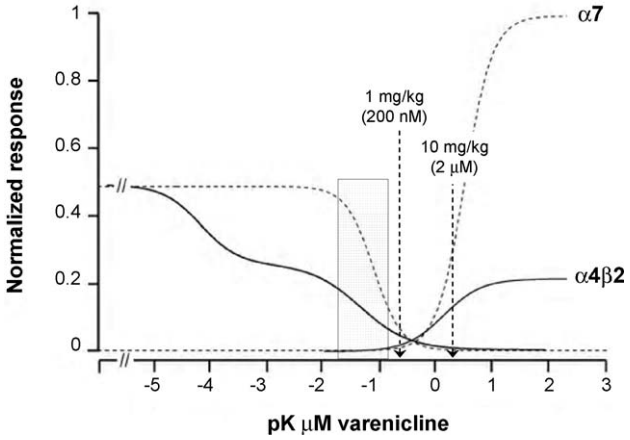

Fig. 1. Plot of the concentration activation and concentration inhibition curves obtained for varenicline at the human α4β2 and α7 nAChR expressed in oocytes measured by voltage clamp. The activation responses are normalized to the maximal response to 100 μM ACh = 1, inhibition responses are normalized to the half maximal response (0.5) obtained with 30 μM ACh. Activation and inhibition curves are the curves of best fit through the data points (not shown). The grey bar represents the predicted steady state unbound brain concentration of varenicline after the recommended therapeutic dose of 1 mg BID. The dashed arrows indicate estimated rat unbound brain levels after 1 or 10 mg/kg varenicline. (Adapted from [33].)

These data demonstrate that combining key translational *in vitro* and *in vivo* parameters with predicted therapeutic human brain concentrations is extremely valuable for the elucidation of the role of receptor subtypes in clinical drug effects. In this respect it is important to note that human varenicline exposures are limited by nausea and, at high doses, vomiting [34]. In contrast, unbound brain levels in species that can not vomit, such as rats and mice, can be several fold above maximal human levels and at these high concentrations varenicline can functionally interact with several other nAChR subtypes (Table 1), as illustrated in Fig. 1 for doses of 1 and 10 mg/kg varenicline. This is not possible for human exposures and has to be taken into account for the interpretation of animal data on the contribution of receptor subtypes to a pharmacological effect.

Table 1
K_i values (nM) of varenicline and nicotine at human nAChR subtypes expressed in HEK293 cells (α4β2, α3β4, α6/4β4), IMR32 cells (α7) or Torpedo electroplax membrane (α1βγδ). Data from Refs. [11,33].

nAChR	α4β2	α7	α3β4	α6/α3β4	α1βγδ
Varenicline	0.4	125	86	111	8200
Nicotine	6.0	2110	518	268	1480

4. Effects of varenicline on alcohol intake

Soon after the approval of varenicline as a smoking cessation aid, preclinical, and subsequently clinical studies were initiated to investigate its potential as a novel pharmacotherapy for alcohol use disorders (AUD). The rationale to explore an α4β2 nAChR partial agonist as an AUD treatment was based on the fact that nicotine dependence and AUD are highly co-morbid disorders and that nAChRs are implicated in alcohol reward circuitry, since ethanol can either directly or indirectly activate nAChRs [35]. In addition, there were anecdotal reports that patients treated with varenicline also reduced their alcohol consumption. It has not yet been established which nAChR subtype(s) mediate ethanol's rewarding effect, but based on effects of selective antagonists and on studies in transgenic animals, the α4β2, α3β2 and/or α7 subtypes are thought to be involved [36]. Therefore, varenicline's α4β2 selectivity and demonstrated human safety prompted studies on its potential as treatments for AUD.

Steensland et al. [37] were the first to demonstrate that varenicline selectively reduces alcohol consumption and alcohol seeking, but not sucrose seeking or water consumption, in high-alcohol consuming rats. In addition, chronic varenicline administration decreased ethanol consumption without a rebound increase in ethanol intake after cessation of treatment. In another preclinical animal study, it was found that low varenicline doses improved ethanol-associated disruptions in cognitive processes in mice, which may contribute to its efficacy [38]. More recently, Ericson et al. [39] provided neurochemical evidence that varenicline can modulate the effects of ethanol and nicotine on brain reward systems. They showed that acute varenicline inhibited acute ethanol-induced dopamine release and that after 5 days of treatment, additional varenicline administration inhibited dopamine release in rat nucleus accumbens produced by the co-administration of nicotine and ethanol.

The preclinical data show varenicline's potential to reduce alcohol intake, and provide insight into which nAChR subtypes could mediate this effect. The minimal effective dose of 1 mg/kg for ethanol intake in rats [37] and of 0.1 mg/kg for cognitive deficits in mice [38] correspond to unbound varenicline brain concentrations of approximately 200 nM and 38 nM, respectively. A comparison of these concentrations with functional potencies of varenicline at α4β2 and α7 nAChRs, suggests that effects on cognition might involve desensitization and minimal activation of α4β2 nAChRs, while α7 nAChRs may not play an important role in this effect (Fig. 1). In contrast, concentrations required for reducing ethanol intake in rats are at the high end of varenicline's therapeutic concentration range and can desensitize and activate both α4β2 and α7 nAChRs (Fig. 1). It is thus likely that desensitization of α7 nAChRs plays a more important role than full activation in varenicline's effect on maintaining alcohol abstinence. Obviously other nAChR subtypes could play an important role in the mechanism of action of varenicline in reducing ethanol intake, such as α3β2, β3* or α6* nAChRs [36]. Binding affinities of varenicline for some of these other subtypes are orders of magnitudes lower than for α4β2 AChRs [11], but functional potency data at these nAChRs are not yet available for varenicline.

Clinical data from a recent double-blind, placebo-controlled human laboratory alcohol self-administration study in 20 non-alcohol-dependent heavy drinking smokers, are consistent with these preclinical data. McKee et al. [40] reported that 1 week of varenicline pretreatment significantly reduced alcohol consumption, attenuated alcohol craving and increased the likelihood of remaining completely abstinent during an *ad libitum* self-administration period. Although this was a small study of short duration, the data provide support for the several clinical trials that are planned to study the effects of varenicline on drinking behavior in smokers, non-smokers and patients with mental disorders (see: http://clinicaltrials.gov/ct2/results?term=varenicline+OR+Chantix&pg=5).

5. Effects of varenicline and other nAChR ligands in depression models

There is mounting evidence that over-activation of nAChRs by endogenous ACh can contribute to the development and exacerbation of depressive symptoms. The cholinergic–adrenergic theory of depression [41] was originally based on physostigmine-induced worsening of mood and has been supported by several recent datasets [42], such as the anti-cholinergic properties of SSRIs and the efficacy of nAChR antagonists and partial agonists in animal depression models and in clinical studies in depressed patients. All classical antidepressants have been shown to have weak nAChR antagonist properties that are thought to contribute to their antidepressant effects [43,44]. In addition, studies with the non-selective nAChR antagonist mecamylamine have shown that blockade of nAChRs results in antidepressant-like activity *per se* and in potentiation of the activity of classical antidepressants. This was demonstrated both in animal depression models [45–47] and in clinical trials that examined the effects of adding mecamylamine to antidepressants as an augmentation strategy for treatment-resistant patients [48,49].

While nAChR antagonists reduce over-activation of nAChRs by receptor blockade, agonists and partial agonists can reduce nAChR signaling by reduced activation or desensitization of nAChRs. Electrophysiological studies have demonstrated that (partial) agonists are several orders of magnitude more potent in desensitizing, i.e. inactivating, than in activating nAChRs [1,5,33,50]. Since this is also the case for nicotine, nAChR desensitization by nicotine could account for antidepressant-like activity in animal models and for the reported antidepressant effect of transdermal nicotine, e.g. in depressed non-smokers during a 4-week, double-blind, placebo-controlled trial [51]. Based on experiments in transgenic mice and with receptor-selective compounds that suggested that α4*, β2* and/or α7* are the subtypes most likely involved in the antidepressant-like effects of nAChR ligands, selective reduction of the activity of α4β2 and/or α7 nAChRs would be hypothesized to exert antidepressant activity. Preclinical studies in mouse models of antidepressant efficacy, the behavioral despair (forced swim) and the tail-suspension tests, have indeed demonstrated antidepressant-like activity of α4β2 nAChR partial agonists, such as cytisine [45,52,53], ispronicline [54] and other experimental α4β2 nAChR partial agonists [55,56].

Since varenicline potently desensitizes α4β2 nAChRs (IC_{50} = 3–6 nM [11]) and can also, at higher concentrations, desensitize α7 nAChRs (IC_{50} = 80 nM; Fig. 1 and [33]), the effects of varenicline were examined in the behavioral despair test using C57Bl and CD-1 mice [55–57]. Selected results of the studies in CD-1 mice are summarized in Fig. 2, which shows that low doses of varenicline significantly reduced immobility time in this model, indicating that, like other α4β2 nAChR partial agonists, varenicline has antidepressant-like activity in this test.

In addition, co-administration of a low dose of varenicline significantly enhanced the effects of a moderately effective dose of sertraline in this model, to the same level as the maximal effect of the tricyclic antidepressant amitriptyline, which is known to consistently yield dose-dependency and near maximal efficacy in this model [52] (Fig. 2). These data suggest that varenicline may have potential as an augmentation strategy for depression by amplifying the SSRI response. The main mechanism of action of varenicline on mood is most likely desensitization of α4β2 nAChRs and possibly to a lesser extent of α7 nAChRs. Given the low doses of varenicline that have activity and produce a robust potentiation of

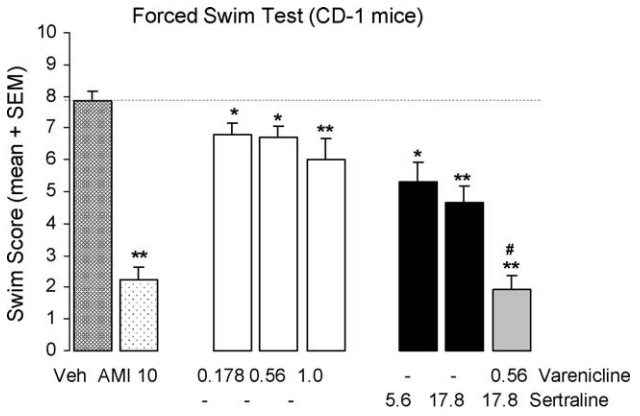

Fig. 2. Effects of amitriptyline (AMI, 10 mg/kg), varenicline alone (0.178, 0.56, 1 mg/kg), sertraline alone (5.6, 17.8 mg/kg) and varenicline + sertraline (0.56 + 17.8 mg/kg) in the behavioral despair (forced swim) test. Data are expressed as total swim score (immobile = 1, active = 0) for 5 min. In separate locomotor activity experiments none of the compounds were found to have stimulant effects, which if present, could have confounded interpretation of the immobility data. $^{*}p < 0.01$, $^{**}p < 0.01$ vs vehicle, $^{\#}p < 0.01$ vs sertraline 17.8 mg/kg (data adapted from Ref. [58]).

the SSRI effect in the mouse behavioral despair test, it is conceivable that antidepressant-like effects of varenicline are mediated via minimal desensitization of α4β2 nAChRs only, which can be achieved with lower doses than those used for smoking cessation (see Fig. 1). A recent study on the antidepressant-like activity of several cytisine analogs in three animal tests also concluded that limited reduction of α4β2 nAChR activity seems to be necessary to result in antidepressant-like effects without affecting other critical pathways [58].

Only limited clinical data are available on the effects of varenicline on mood. A double-blind, placebo-controlled, within-subject crossover study in healthy smokers conducted by Patterson et al. [59] assessed the subjects' mood during a smoking cessation laboratory study. During a mandatory 3 days of smoking abstinence, subjects reported significantly lower levels of negative affect and higher levels of positive affect when taking varenicline than when taking placebo. Another study prospectively examined possible antidepressant effects of varenicline augmentation during an 8-week open-label varenicline study in 18 smokers with treatment-resistant depressive disorders currently on a stable antidepressant or mood stabilizer regimen [60]. Based on the QIDS-SR-16 (16-item Quick Inventory of Depressive Symptomatology-Self-Report) completed by subjects at baseline and every 2 weeks during treatment, there was a statistically significant improvement in mean score beginning at week 2 which was sustained for the full 8 weeks of treatment. Absence of a placebo group makes these data difficult to interpret, but they do encourage further blinded studies.

In conclusion, while larger double-blind clinical studies in depressed patients are necessary to evaluate whether varenicline can improve mood in patients, the available preclinical and clinical data support the notion that selective nAChRs ligands may have antidepressant effects via desensitization of α4β2 and/or α7 nAChRs.

6. Effects of varenicline in cognition models

The rationale for evaluating varenicline's effects on cognitive processes stems from the well-documented findings that cholinergic mechanisms are important for normal cognitive functioning [61,62], that neurodegeneration of the cholinergic system accompanies the cognitive deficits associated with Alzheimer's disease [63], and that administration of drugs that enhance cholinergic signaling, such as donepezil and nicotine, improves cognitive functioning in both animals and humans [64]. Early studies on the effects of nicotine on cognitive performance showed that nicotine abstinence in smokers disrupted cognitive functioning, while both nicotine administration and smoking restored the observed cognitive deficits. More recently, research efforts have focused on the effects of nicotine and nAChR agonists in non-smoking subjects, and there is an abundance of evidence that these agents improve cognitive functioning in non-smoking humans, including healthy volunteers and patients with various neurological and psychiatric disorders, and in a variety of animal models including both normal and impaired rodents and non-human primates (see Ref. [64] for a recent review). Since the α4β2 and α7 nAChR subtypes are generally thought to mediate the cognitive enhancing effects of nicotine, much research has been focused on the design of compounds that have improved selectivity for these receptors compared to nicotine and that have an improved side effect profile [65]. Cognitive enhancing effects have been demonstrated in animal models and in humans for a number of α4β2 ligands, for instance ABT-418 [66–68], ABT-089 [3], and ispronicline [54,69]. There is therefore much interest in the potential cognitive and memory-enhancing effects of a highly potent and selective α4β2 nAChR partial agonist like varenicline, especially given the cognitive deficits associated with smoking cessation for which varenicline is indicated. Below the results of several preclinical studies and recently published data that have begun to evaluate the effects of varenicline on cognitive processes are summarized. Recent emerging clinical data on the effects of varenicline on cognition in normal smokers and in smokers with schizophrenia are also described.

6.1. Effects on neurotransmitter release in rat cortex

Modulation of cortical cholinergic, adrenergic, dopaminergic and/or histaminergic neurotransmitter systems has been shown to play important roles in mediating effects on cognitive processes, including memory and learning [62,70–72]. The effects of varenicline on the rat mesolimbic DA system, where low doses (0.03–1 mg/kg) produce DA increases with a smaller and longer lasting effect than that of nicotine, are well-documented [6,11], and here data on the effects of varenicline on cortical neurotransmitter release are reported.

Interestingly, the results demonstrate that at pharmacologically relevant doses of ≤1 mg/kg, varenicline does not significantly increase extracellular levels of DA or norepinephrine (NE) in rat prefrontal cortex (Fig. 3, left and middle panel). Only after a very high dose of 10 mg/kg, which is associated with plasma levels that are >50-fold higher than levels achieved after recommended human doses, does varenicline produce NE and DA increases with a similar magnitude to those produced by the ADHD drug atomoxetine, a selective NE reuptake inhibitor. Nicotine has comparable effects on cortical DA to varenicline, but appears to be more potent at elevating cortical NE levels (Fig. 3, middle panel).

These results suggest that varenicline-induced increases in cortical DA and NE release are mediated by receptors other than α4β2 nAChRs, but given the high free brain levels of varenicline after 10 mg/kg, estimated to be approximately 2 μM, it is difficult to attribute this effect to a specific nAChR subtype. At these concentrations varenicline can interact with several nAChRs, e.g. with α7 nAChRs (K_i = 125 nM) and cause substantial activation of this nAChR subtype (see Fig. 1), which has been shown to be involved in nicotine-induced cortical DA increase [27].

Studies on the effects of varenicline on cortical ACh release are ongoing and preliminary data suggest that varenicline produces only marginal cortical ACh increases in rat prefrontal cortex. After 0.6 and 3.2 mg/kg varenicline, extracellular ACh levels, measured in the presence of an ACh-esterase inhibitor, increased to 138 ± 24% and 150 ± 26% of basal, respectively, which were not significantly

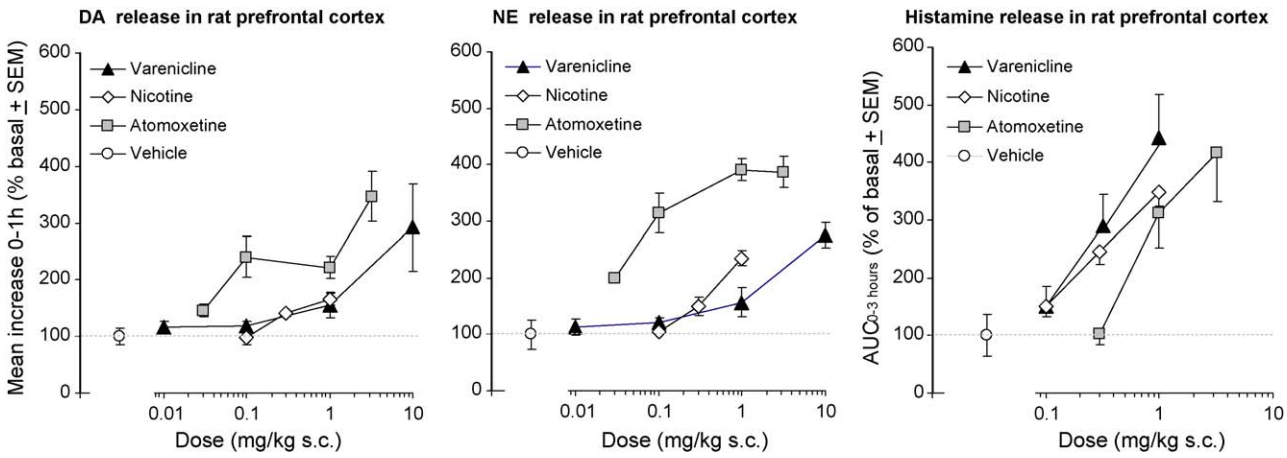

Fig. 3. Effects of varenicline, nicotine and atomoxetine on extracellular levels of dopamine (left panel), norepinephrine (middle panel) and histamine (right panel) in rat prefrontal cortex. Drug effects are calculated as the mean % of baseline ± SEM (n = 3–6) over 0–1 h (DA, NE) and 0–3 h (histamine) after dosing.

different from the effect of vehicle that caused a small transient increase in ACh release. Both 1 mg/kg nicotine and 1 mg/kg atomoxetine produced comparable modest 1.5-fold increases in ACh release.

In view of the lack of effect of pharmacological varenicline doses on cortical NE, DA and ACh release, our finding that low doses of varenicline and nicotine produce pronounced increases in histamine release in rat prefrontal cortex (Fig. 3, right panel) may have relevance for the effects on cognition and attention. Both compounds clearly elevate cortical histamine more readily than cortical DA, NE or ACh, while their potencies to increase *in vivo* histamine release are higher than those of the ADHD drugs atomoxetine and methylphenidate, previously shown to increase histamine release in rat cortex [12]. While underlying receptor mechanisms that mediate varenicline-induced increases in histamine release are not yet known, it is possible that enhanced histamine release, in addition to possible direct effects on α4β2 and other nAChRs, contributes to the cognitive enhancing effects of varenicline.

6.2. Effects on hippocampal auditory gating and theta oscillation in rats

An auditory sensory-gating deficit is present in a number of psychiatric disorders, including schizophrenia, bipolar disorder and Alzheimer's disease [73], and represents a pre-attentional aspect of early sensory processing. Sensory-gating processes that resemble human auditory gating have been demonstrated in experimental animals using clinically equivalent acoustic-stimulation paradigms. Modeling sensory-gating deficits in preclinical studies involves compromising normal physiological gating processes by genetic, behavioral or pharmacological manipulations [74]. Nicotinic receptor agonists and positive allosteric modulators have been shown to be active in these animal models of gating deficits, and auditory gating has been used as a proof-of-mechanism study for an α7 nAChR agonist in schizophrenic patients [75].

A recent study [76] evaluated the effects of varenicline on auditory gating and neuronal network oscillations in the rat hippocampus CA3 and CA1 regions and entorhinal cortex (EC). Administration of varenicline (1 mg/kg, i.v.) or vehicle did not influence auditory gating either in the CA3 or EC in chloral hydrate-anaesthetized rats (Fig. 4A), in contrast to highly potent DA releasers such as amphetamine [74]. Although amphetamine disrupts auditory gating as well as induces hippocampal theta oscillations (see above), dopamine D_2 receptor antagonists reverse only the amphetamine-induced gating deficit, indicating different mechanisms underlying auditory gating and theta oscillations [74]. Varenicline (1 mg/kg, i.v.) significantly reversed an amphetamine-induced gating deficit in CA3, but not in EC (Fig. 4B). These results are in line with recent findings demonstrating that α4β2 nAChRs can modulate auditory gating mechanisms, as shown in the effect of nicotine on auditory gating in DBA/2 mice [77]. In addition, both the nAChR agonist ABT-480 and the selective α4β2 nAChR full agonists 5-I A85380 and TC-2403 improved gating deficits in DBA/2 mice [78]. The finding that varenicline reverses an

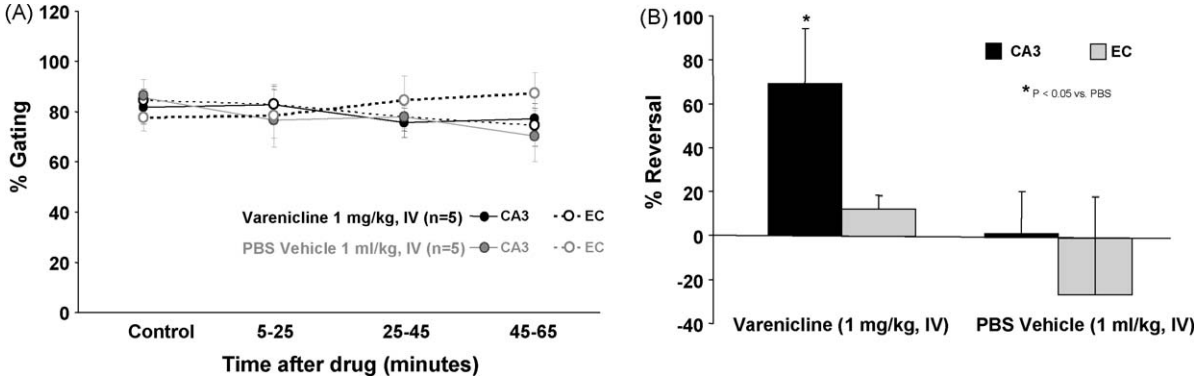

Fig. 4. Effects of varenicline on auditory gating in the hippocampus CA3 region (CA3) and entorhinal cortex (EC). (A) Systemic administration of varenicline (1 mg/kg, i.v., n = 5) or vehicle (n = 5) did not alter physiological auditory gating either in CA3 or EC. (B) Varenicline (1 mg/kg, i.v., n = 5), but not vehicle (n = 5), significantly reversed an amphetamine-induced gating deficit in CA3 but not in (EC). Auditory gating was recorded simultaneously in CA3 and EC from chloral hydrate-anaesthetized rats, physiological gating was disrupted by systemic administration of amphetamine (1 mg/kg, i.v.).

amphetamine-induced gating deficit in rats, together with data showing that varenicline also improves gating deficits in DBA/2 mice [79], suggest that a partial agonist at α4β2 nAChRs is effective as well. It has been reported that an α7 nAChR agonist, PNU-282987, restores gating both in the CA3 and EC in the amphetamine-induced gating deficit model [80]. Since varenicline did not reverse the gating deficit in the EC and varenicline brain levels after 1 mg/kg may be too low to sufficiently activate α7 nAChRs (Fig. 1), this could indicate a potential differential role of nAChR subunits in different brain circuits. Although the role and effectiveness of α7 nAChR agonists in restoring various gating deficits has been unequivocally shown and α7 nAChRs are known to be genetically linked to schizophrenia, non-α7 nAChR agonists may thus also be efficacious in schizophrenia. Forthcoming clinical studies will determine whether α4β2 nAChR agonists can impact auditory gating mechanisms in schizophrenic patients, and subsequently, improve perception and cognitive functioning.

Considering its potential pro-cognitive effects, varenicline was also tested for effects on spontaneous theta oscillations in CA1 and EC in chloral hydrate-anaesthetized rats. The hippocampal formation, and hippocampal theta rhythm in particular, have long been implicated as physiological mechanisms related to orienting exploratory behavior and cognitive processes, such as learning and memory [81]. In addition, pharmacological studies have revealed that drugs shown to have pro-cognitive effects enhance hippocampal theta power, while drugs known to impair hippocampal-dependent memory function, disrupt hippocampal theta oscillations [14,82–85]. Importantly, increased sensitivity to nicotine-induced hippocampal theta rhythm has been reported in knock-in mice carrying hypersensitive α4 nAChRs [86], indicating a potential functional role of α4β2 nAChRs in hippocampal theta oscillation. In line with this hypothesis, our findings demonstrate that varenicline (0.3–1 mg/kg, i.v.) significantly enhanced relative theta power in both CA1 and EC (Fig. 5), representing one of the probable mechanisms involved in the pharmacological effects of varenicline on higher brain functions, such as attention and learning.

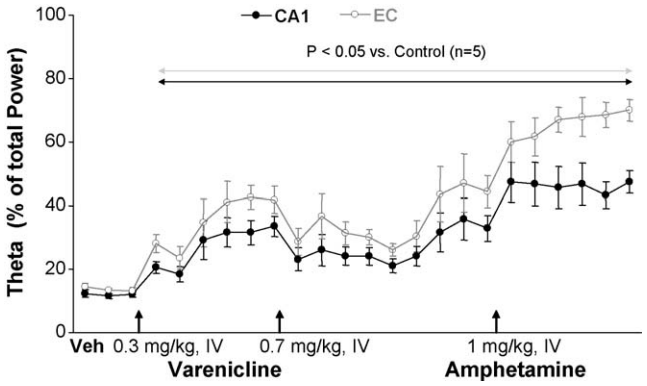

Fig. 5. Effects of varenicline on hippocampal CA1 and entorhinal cortex (EC) theta oscillation. Varenicline (0.3–1 mg/kg iv, cumulative doses, $n = 5$) significantly enhanced relative theta power (expressed as % ± SEM) both in CA1 and EC in chloral hydrate-anaesthetized rats. Subsequent administration of amphetamine (AMP, 1 mg/kg, i.v.) further increased relative theta power. Using fast Fourier transformation, relative theta power was determined by calculating the percentage of total power that occurred in the theta (3.5–5.5 Hz) frequency band as compared with the 0–15-Hz frequency band.

6.3. Effects on prepulse inhibition of the acoustic startle response

The acoustic startle response (ASR) is the reflexive response to an external auditory stimulus (e.g. a loud noise), consisting of the contraction of the skeletal muscles. Prepulse inhibition (PPI) refers to the suppression of the ASR (i.e. reduction in startle amplitude) that occurs when the startling stimulus is immediately preceded by a weak sensory prestimulus or prepulse [87]. PPI is a measure of inhibitory function and information processing and is viewed as an operational measure of 'sensorimotor gating', the process by which excess or trivial stimuli are screened or 'gated out' of awareness, so that attention can be focused on the most salient aspects of the environment [88]. PPI has been shown to be impaired in several neuropsychiatric disorders including schizophrenia and this deficit is hypothesized to reflect abnormal early information processing that may contribute to the cognitive deficits (attentional) and ultimately, the clinical symptoms observed in schizophrenia [88]. In rodents, PPI deficits can be produced by administration of dopaminergic agonists and are blocked by the administration of clinically efficacious antipsychotics. In addition, nicotine administration has been shown to significantly improve PPI in rats (e.g. [89]), in mice (e.g. [90]), and in humans (e.g. [91]). Consequently, many laboratories have used this paradigm to test novel compounds for potential pro-cognitive and/or antipsychotic-like properties.

Effects of varenicline, nicotine and risperidone on ASR and PPI were studied in male C57Bl/6J mice, since they have an inherently low level of PPI [92] and since antipsychotic-induced enhancement of PPI is readily detected in mice naturally exhibiting poor levels of PPI (e.g. [93]). Fig. 6 shows the effects of nicotine and varenicline on ASR and PPI in comparison with the effect of the atypical antipsychotic risperidone, which produced a robust increase in PPI as is characteristic for antipsychotics. Similar to nicotine, varenicline produced a small increase in PPI that was significant

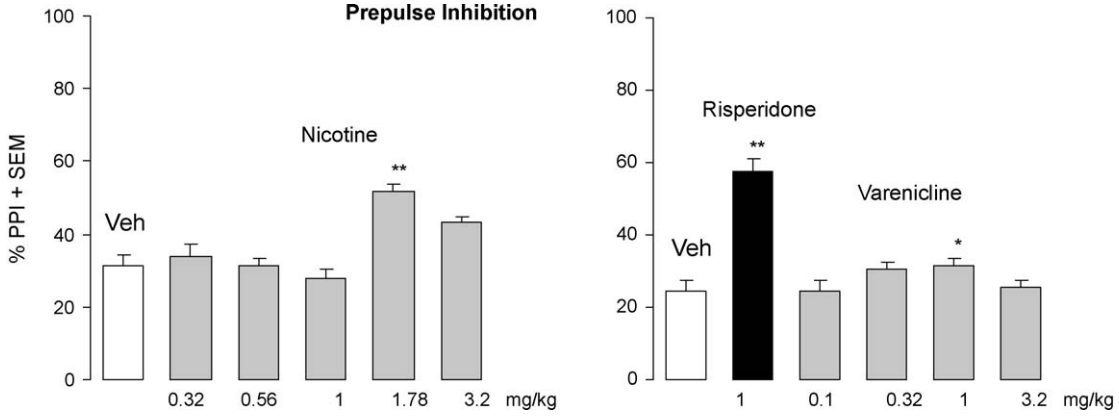

Fig. 6. Effects of nicotine (left panel) and varenicline (right panel) on prepulse inhibition (PPI) of acoustic startle. Data are expressed as mean % PPI + SEM (n = 8–16/group). For comparison the effects of the antipsychotic risperidone (1.0 mg/kg) is shown as well.

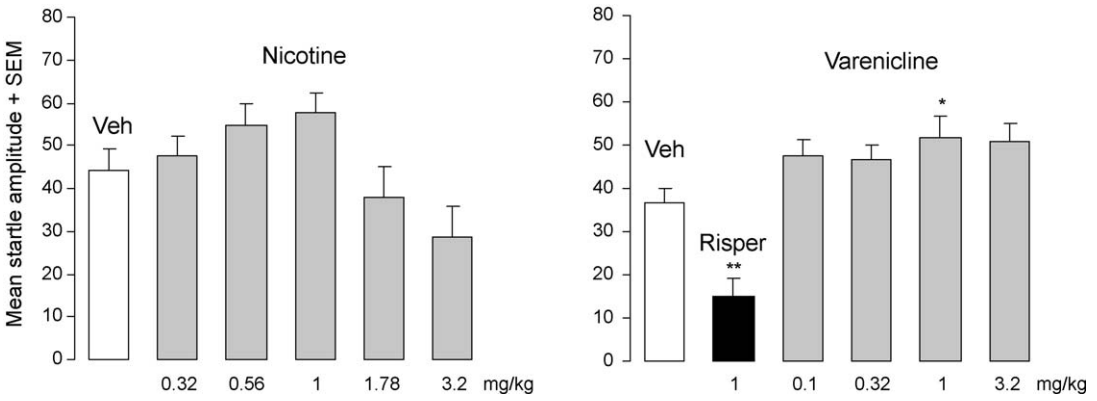

Fig. 7. Effects of nicotine (left panel) and varenicline (right panel) on acoustic startle amplitude. Data are expressed as the mean startle amplitude in milliNewtons + SEM (n = 8–16/group). The effect of the antipsychotic risperidone (1 mg/kg) is shown for comparison.

after 1 mg/kg varenicline. These data in C57Bl/6J mice suggest that varenicline may have weak antipsychotic and/or cognitive enhancing properties.

Risperidone significantly decreased mean startle amplitude (Fig. 7), while nicotine and varenicline failed to show a significant effect, except for a marginal but statistically significant increase with varenicline at a single dose (1 mg/kg). Several additional studies with varenicline, however, failed to show this effect.

6.4. Effects in the sustained attention task

Deficits in top-down control of attention are a prominent feature of neuropsychiatric disorders [94,95], ADHD and age-related cognitive disorders. The sustained attention task (SAT) as such or with a distracter condition (dSAT), is an operant procedure in rats with translational validity in humans [17,97,97]. There is extensive evidence that both SAT and dSAT performance depend on the integrity of the cortical cholinergic input system, specifically at the level of the prefrontal cortex [96,98,99]. While the SAT largely depends on bottom-up attentional processes (signal-driven mechanisms), the distractor mechanisms in rats and humans are thought to be mediated via prefrontal efferent circuitries, which act to optimize receptive field properties and suppress distractor representation in sensory and sensory-associational cortical regions. The distractor reduces the discriminability of the signal, increasing the demand on top-down control to maintain performance [18], and dramatically impairs performance, reflecting exhaustion of the rat's detection capacity in the presence of the distractor [97,100].

Varenicline did not affect performance at 0.032, 0.1 and 1 mg/kg ($p > 0.1$, data not shown) under standard conditions during which responses are driven by bottom-up (in the case of signal events) and associational processes (in the case of non-signal events [94]. In contrast, in the presence of a distracter, when top-down processes are recruited to optimize input processing by filtering out noise, varenicline robustly enhanced the performance during the post-distractor blocks (Fig. 7). Statistical analysis of the effects of varenicline on performance (VI) during the distractor period and the post-distractor period (blocks 2–5), indicated a significant interaction between the effects of dose, session and signal duration ($F_{9,77} = 2.617$; $p = 0.009$). This effect is due to a significant improvement in VI during the first post-distractor blocks, but not during block 5 as the animals' performance recovered. Varenicline attenuated primarily the effect of distractor on the medium signal duration during the first post-distractor block as indicated by dependent t-tests ($t_{(8)} = 3.147$); $p = 0.014$; $t_{(8)} = 3.354$; $p = 0.01$ for 0.1 and 1 mg, respectively (Fig. 1).

The improvement in performance after administration of varenicline was entirely due to an increase in correct responses to signal trials. This is reflected by a significant dose effect ($F_{3,24} = 3.374$; $p = 0.035$) and an overall dose × session × signal duration interaction ($F_{15,123} = 1.853$; $p = 0.033$). Post hoc analysis demonstrated that 1 mg/kg significantly improved the correct response to the longest signal duration (500 ms) during the second post-distractor block ($t_{(8)} = 2.565$; $p = 0.033$) and the medium signal duration during the first post-distractor block ($t_{(8)} = 3.821$; $p = 0.005$; Fig. 8). The relative number of correct rejections remained unaffected (all $p > 0.1$, data not shown). Performance

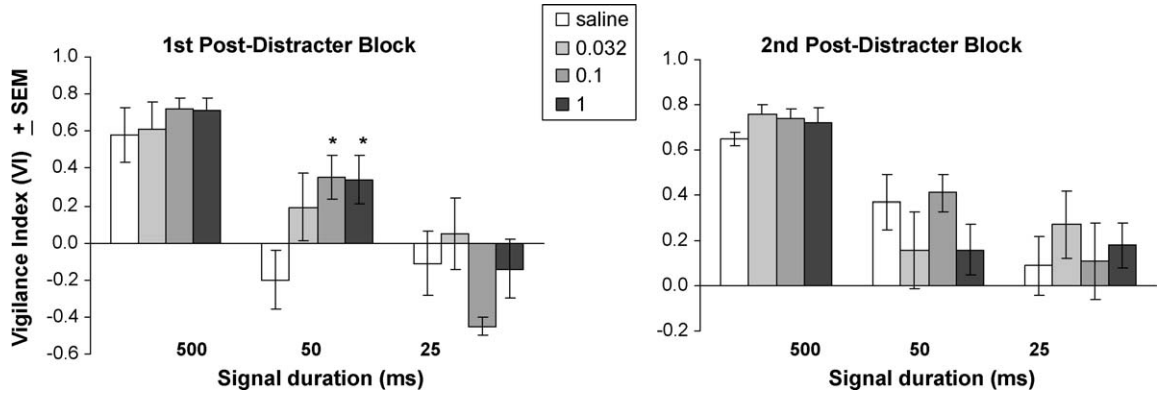

Fig. 8. Effects of varenicline administration on vigilance index (VI) during the first (a) and second (b) post-distractor block for each signal duration as measured by VI (n = 9). There was a significant attenuation of the distractor effect mainly during the first distractor block. Varenicline significantly attenuated this effect at the medium signal duration of 50 ms ($p < 0.01$).

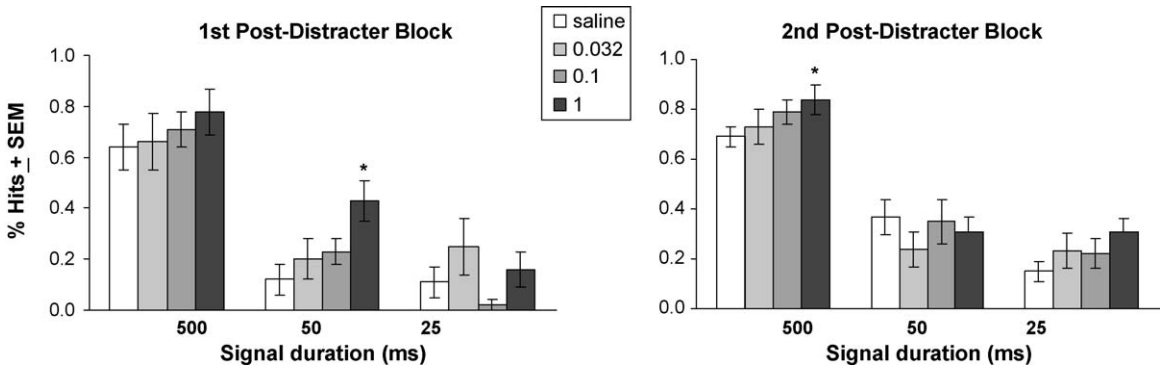

Fig. 9. Effects of varenicline administration on signal detection (hits) during the first (a) and second (b) post-distractor block for each signal duration as measured by hits ($n = 9$). Varenicline significantly improved the detection of the signal and significantly attenuated the distractor effect at the medium signal duration (50 ms) during the first post-distractor block and improved the response to the longest signal duration (500 ms) during the second post-distractor block ($p < 0.05$).

at the shortest signal is considered a "floor effect" and likely prevents the manifestation of distractor effects and drug related improvement in performance. Errors of omission were not robustly affected by the distractor and remained below 10% in all trials (data not shown). The lack of effect on omission errors indicates persistent motivation by the animals to stay on task, which increases their attentional effort to allow a recovery of performance after the distractor condition [100] (Fig. 9).

Taken together these results indicate that varenicline significantly improved the animals' capacity to attenuate the impairment of performance under challenging distractor conditions, primarily due to improved signal detection during the post-recovery period. The underlying mechanism for this effect of varenicline is unclear, but recent evidence indicates that cholinergic activity signals in the medial prefrontal cortex selectively mediate the incorporation of signals into ongoing cognitive and behavioral processes [101]. Furthermore, increases in ACh release in the prefrontal cortex are particularly high during performance challenges [99]. The present data suggest that modulation of the prefrontal cholinergic input by varenicline may play a role in the improvement in the control of attention which could underlie its potential beneficial cognitive effects.

6.5. Effects in the novel object recognition test

Declarative memory has been defined as the capacity for the conscious recollection of facts and events [102] and is thought to require relatively few exposures for learning, as compared to non-declarative memory. Object recognition, which is a judgement of prior exposure to an object, is thought to be a critical component of human declarative memory, and is commonly impaired in patients suffering from neurodegenerative diseases and traumatic brain injuries. The novel object recognition (NOR) task in rodents is a relatively simple non-aversive, non-spatial memory test that exploits the subjects' natural drive to explore novel, rather than familiar objects. This test has become the assay of choice for assessing aspects of declarative memory in rodents, since it does not require the use of extensive training procedures or the manipulation of motivational factors (reviewed by [103]). A growing body of research on this test implicates the perirhinal cortex as a critical brain region and supports the use of the test as an experimental tool for studying the effects of drugs on cognitive processes in rodents and non-human primates. Additionally, nicotine [104] and a selective nAChR partial agonist, TC-1734 [105], have been reported to improve performance in the NOR task in rats and mice, respectively, suggesting that increased nicotinic cholinergic neurotransmission can positively affect declarative memory processes in rodents as measured by this task.

The effect of varenicline on declarative memory processes was assessed in a NOR test in rats using a procedure based on Ennaceur and Delacour [18] and results are depicted in Fig. 10. The data show that varenicline significantly enhanced the time spent exploring the novel object suggestive of an improvement in the cognitive processes involved in this test.

6.6. Summary prelinical studies

Preclinical studies in several cognition models have consistently shown positive effects with varenicline treatment at pharmacologically relevant doses. As mentioned above, these results are in agreement with additional published data on the effects of varenicline in a sensorimotor gating model in DBA2 mice [80]. In addition, using a contextual fear conditioning test in mice Gould and co-workers recently reported that varenicline had no effect on its own, but significantly attenuated the deficits associated with nicotine withdrawal [106] and ethanol-induced deficits [38]. An earlier report from this group provided evidence that $\alpha4\beta2$ nAChRs play a critical role in mediating the enhancing effect of nicotine on contextual fear conditioning [107]. According to the preclinical data shown here, varenicline has small to marked effects on cognitive processes, most likely via interactions with $\alpha4\beta2$ and/or $\alpha7$ nAChRs, although it is possible that effects are

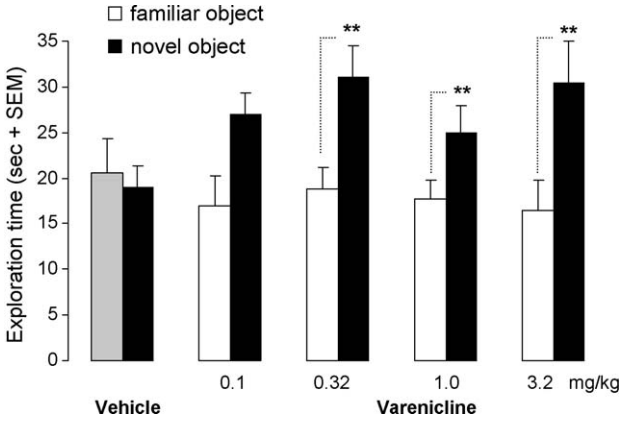

Fig. 10. The effect of varenicline in the novel object recognition test in rats. Bars represent mean (+SEM) exploration time of the familiar object (grey and white bars) and the novel object (black bars). The time spent exploring the novel object by vehicle-treated rats was not significantly different from the time spent exploring the familiar object, indicative of a failure to recognize the novel object. Administration of varenicline (0.1–3.2 mg/kg, s.c.) significantly increased the time spent exploring the novel object, indicative of improved recognition memory in this test. $^{**}p < 0.01$.

mediated via other subtypes for which the functional interaction is not known. In addition, enhanced histamine release could contribute to some of these effects as well. So far, the only clinical data in support of varenicline's cognitive enhancing properties are preliminary reports which are briefly discussed below.

6.7. Clinical studies

Recently published clinical data suggest that varenicline may affect select cognitive functions, although the data are limited and the studied populations and assessment methods are divergent. Varenicline's effects on nicotine withdrawal symptoms, mood, and cognition were assessed in a double-blind, within-subject cross-over study in which 67 normal smokers received varenicline or placebo (in counterbalanced sequence) for 21 days separated by a washout period [59]. Cognitive function was assessed with a battery of computerized tasks at baseline and during a mandatory abstinence period of 3 days during each medication period. Levels of sustained attention (assessed by the Penn Continuous Performance Task) and working memory (assessed by the letter-N-back task) were significantly greater during the varenicline condition than the placebo condition, although the medication effects were small. No significant difference was observed for executive function (assessed by the Conditional Exclusion Test). Furthermore, in a small sample of 12 smokers with schizophrenia or schizoaffective disorder who all received 9 weeks of varenicline treatment in an open-label study [108], some cognitive test scores at the end of treatment were significantly improved compared with baseline. As measured by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), there were significant increases in scores associated with verbal learning and memory, such as list learning, list recall, and language index, but a significant decrease in visual spatial construction index score. Varenicline treatment also did not improve visual spatial performance or memory on the Virtual Water Maze Task, although latency to finding a hidden target was significantly improved among four outpatient subjects. Finally, Evins [109] presented preliminary results of the first 13 week open-label varenicline treatment part of a 40-week double-blind, placebo-controlled trial of varenicline for smoking cessation in adult smokers with schizophrenia, that showed a trend for improved cognitive function associated with schizophrenia.

The small sample sizes and heterogeneity of the studies published thus far make it difficult to assess to what extent these data will generalize to larger populations and obviously additional larger studies will be needed to determine whether the preclinical cognition data translate to humans.

7. Summary and conclusion

The pharmacological properties and the excellent pharmacokinetic profile of the α4β2 nAChR partial agonist varenicline are equally important for its efficacy as a treatment for nicotine dependence and have prompted studies in preclinical models of other disorders in which nAChR subtypes are thought to play key roles. At pharmacologically relevant doses, varenicline has been found to improve cognitive processes and mood in a wide range of animal models. These positive preclinical effects of varenicline are likely mediated via interaction with α4β2 and/or α7 nAChRs, although effects of varenicline at other subtypes not yet studied may play a role. Only limited clinical data are available on varenicline's effects on cognition, mood and alcohol abuse disorder and, while showing some beneficial effects, large double-blind clinical studies are needed to assess whether preclinical effects observed in animal models are translatable to the clinic.

Acknowledgments

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