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A study of the nicotinic agonist SIB-1553A on locomotion, and attention as measured by the five-choice serial reaction time task

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Abstract

SIB-1553A is a novel ligand with reputed agonist selectivity at nicotinic receptors containing the β_4 subunit. As such, it represents an interesting pharmacological tool with which to probe the function of nicotine receptor subtypes. In the present studies, we compared SIB-1553A with nicotine in its ability to stimulate locomotion and to enhance attention in rats as assessed using the five-choice serial reaction time task (5-CSRTT). In nicotine-naive rats, SIB-1553A (10–40 mg/kg) induced a comparable increase in locomotion to nicotine (0.4 mg/kg), whereas in nicotine-sensitised rats, an enhanced locomotor response was seen to nicotine (0.4 mg/kg) but not to SIB-1553A (10–80 mg/kg). Similarly, chronic treatment with either SIB-1553A or nicotine did not lead to a cross-sensitised locomotor response. Unlike nicotine, SIB-1553A-induced locomotion was insensitive to antagonism by either mecamylamine (1 mg/kg) or DHβE (3 mg/kg), suggesting a non-nicotinic mechanism. In young and aged rats, nicotine (0.4 mg/kg) enhanced attention as demonstrated by an increase in response accuracy and speed. SIB-1553A (3–10 mg/kg) did not mimic any of these changes and at the highest dose tended to disrupt performance. These results lend further support to the involvement of a high affinity site, possibly $\alpha_4\beta_2$, in the locomotor and attentional-enhancing properties of nicotine.

Keywords: Nicotine; SIB-1553A; Five-choice serial reaction time task (5-CSRTT); Locomotor activity; $\alpha_4\beta_2$; β_4; Rat

1. Introduction

Nicotine administration produces highly diverse effects, encompassing changes in body temperature, locomotor activity, cardiovascular and gastrointestinal function, cortical blood flow and nociception. Included in this range of effects are those which indicate potential therapeutic benefit in certain patient populations. Specifically, nicotine appears to be particularly beneficial in treating disorders, which are characterised by some form of cognitive impairment, such as attentional deficit hyperactivity disorder (Conners et al., 1996; Levin et al., 1996a), schizophrenia (Levin et al., 1996b) and Alzheimer's disease (AD) (Sahakian et al., 1989; White and Levin 1999).

Neuronal nicotinic receptors are thought to comprise both $\alpha_{(2-9)}$ and $\beta_{(2-4)}$ subunits, which arrange to form pentameric receptors. Principal CNS forms appear to be the $\alpha_4\beta_2$ and a homomeric α_7 subtype, although other combinations certainly exist (Lena and Changeux 1997; Lukas et al., 1999). Functionally, nicotinic receptors can be subdivided according to their affinity for nicotine, with receptors containing the β_4 subunit displaying a 10–100-fold lower affinity for nicotine compared to those containing the β_2 subunit (Luetje and Patrick 1991).

The recent development of a number of subtype selective ligands has provided tools with which to probe the function of particular nicotinic receptor subtypes. Interest in subtype selective ligands has arisen from the belief that selectively activating specific receptor subtypes may enable the retention of nicotine's therapeutic potential whilst minimising other, undesirable effects. One such ligand is SIB-1553A (4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]-phenol hydrochloride), which has been described as a novel selective agonist of human nicotinic receptors containing the β_4 subunit whilst having little or no agonist activity at sites containing either β_2 or α_7 subunits (Reid et al., 1997; Vernier et al., 1999). Thus, in HEK cells stably transfected with various human nicotine acetylcholine (ACh) receptors, SIB-1553A showed considerably greater efficacy to increase intracellular Ca^{2+} levels in cells expressing $\alpha_2\beta_4$,

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$\alpha_3\beta_4$ and $\alpha_4\beta_4$ compared to $\alpha_4\beta_2$ subunit complexes (Vernier et al, 1999). In vivo microdialysis studies further demonstrated that SIB-1553A stimulated striatal DA and hippocampal acetylcholine release to a magnitude approximately 6- and 10-fold, respectively, greater than nicotine itself (Reid et al., 1997; Vernier et al., 1999). SIB-1553A has also shown efficacy in ameliorating both pharmacological, lesion and age-induced cognitive deficits in rodent and nonhuman primate species (Bontempi et al., 1997; Menzaghi et al., 1997). Based on this preclinical profile, SIB-1553A is currently in phase 2 clinical trials for the symptomatic treatment of AD.

The present study assessed the behavioural effects of SIB-1553A for two reasons. Firstly, SIB-1553A represents a useful probe to investigate nicotine receptor subtype function, as it is presently one of the few β_4 subunit selective ligands described in the literature. Secondly, since SIB-1553A is currently undergoing clinical evaluation as a symptomatic treatment for AD, further preclinical characterisation may provide insight into its potential clinical utility.

A number of studies, utilising both pharmacological and genetic approaches, have begun to elucidate the receptor subtypes contributing to nicotine's behavioural effects. The locomotor stimulant and reinforcing properties of nicotine are widely thought to be mediated through increased DA activity within the mesocorticolimbic system (Corrigall et al., 1992, 1994; Reavill and Stolerman, 1990; Louis and Clarke, 1998). Both of these actions are potently blocked by the high-affinity competitive antagonist DHβE injected either systemically (Stolerman et al., 1997; Watkins et al., 1999; Grottick et al., 2000a) or centrally into dopamine-containing nuclei (Corrigall et al., 1994). Mice lacking the β_2 subunit neither self-administer nicotine nor show increased accumbens DA release following acute nicotine administration (Picciotto et al., 1998), and the $\alpha_4\beta_2$ preferring agonist SIB-1765F induces similar levels of activity to nicotine in both nontolerant and nicotine-sensitised rats (Menzaghi et al., 1997; Grottick et al., 2000a). Finally, cross-sensitisation develops between the hyperactivity produced by SIB-1765F and nicotine (Grottick et al. 2000b).

Recent studies utilising the five-choice serial reaction time task (5-CSRTT; Carli et al., 1983) have indicated that as with normal humans (Levin et al., 1998; Wesnes and Warburton, 1984), nicotine can improve aspects of attentional function in rats (Mirza and Stolerman, 1998; Stolerman et al., 2000; Grottick and Higgins, 2000). The role of nicotine receptor subtypes in this enhancement is less well characterised, although we have recently shown that nicotine-induced changes in performance can be mimicked by SIB-1765F (Grottick and Higgins, 2000). Converging evidence therefore suggests that the $\alpha_4\beta_2$ receptor complex is a likely substrate both for nicotine-induced locomotion and attentional enhancement. This does not however exclude the possibility of additional and/or alternative mediation by other nicotine subunit-containing receptors.

In the present studies, SIB-1553A was compared directly with nicotine in its ability to increase locomotion in both nontolerant and sensitised rats and to alter performance of the 5-CSRTT. Where effects of SIB-1553A were observed, attempts were made to block these changes with nicotinic antagonists.

2. Methods

All studies were conducted at F. Hoffmann-La Roche (Basel, Switzerland) and complied with local Cantonal and Swiss federal law regulating animal experimentation.

2.1. Locomotor activity studies

Male, Sprague–Dawley rats (RCC, Fullinsdorf, Switzerland) were used throughout. The animals were housed four per cage in a light- and temperature-controlled environment (lights on at 06:00–18:00h) with food available ad libitum. All testing was conducted during the animals' light phase.

2.1.1. Experiment 1: SIB-1553A dose–responses in nontolerant and sensitised rats

To study the effect of SIB-1553A on locomotor activity, two approaches were taken—a study in nicotine-nontolerant rats (Experiment 1A) and a second study in nicotine-sensitised rats (Experiment 1B). A repeated-measures design was used for both studies, with the rats ($n=8$ rats per group) habituated to the test apparatus ($36 \times 24 \times 19$ cm, Benwick Electronics, UK) for three $\times$ daily 2-h sessions before formal activity testing commenced. In the nicotine-nontolerant studies (Experiment 1A), rats received (−)-nicotine or SIB-1553A (0–80 mg/kg sc) as a single injection prior to test, which was of 90-min duration. A 30-min acclimation period to the test apparatus preceded testing and a washout period of 2–3 days intervened between each treatment cycle. An identical protocol was used for the nicotine-sensitised rats (Experiment 1B), except when they received 10 daily injections of nicotine (0.4 mg/kg sc) before drug testing began. The animals continued to receive nicotine injections on the days between treatment cycles. In the sensitised animals, except for test days, the daily nicotine injections were administered noncontingently with exposure to the test apparatus. A dose of 0.4-mg/kg nicotine was included in each study as a positive control.

2.1.2. Experiment 2: interactions between SIB-1553A and nicotinic antagonists

Experiment 2 investigated interactions between nicotine (0.4 mg/kg) and mecamylamine (1 mg/kg) (Experiment 2A), between SIB-1553A (15 mg/kg) and mecamylamine (1 mg/kg) (Experiment 2B) and between SIB-1553A (15 mg/kg) and DHβE (3 mg/kg) (Experiment 2C). All other aspects of the experiments were identical to those described above for the nicotine-nontolerant rats.

2.1.3. Experiment 3: investigating cross-sensitisation between nicotine and SIB-1553A

A final experiment investigating cross-sensitisation between the locomotor stimulant properties of nicotine and SIB-1553A was also conducted (Experiment 3). This study utilised a similar design to that used in the nicotine-sensitised study, except that groups of rats ($n = 8$ per group) received 10 daily injections of either vehicle, nicotine (0.4 mg/kg sc) or SIB-1553A (15 mg/kg sc) for 10 days prior to test. Testing consisted of acute challenge with either vehicle, nicotine (0.4 mg/kg) or SIB-1553A (15 mg/kg). Habituation and testing proceeded in an identical manner to that described above.

2.2. The 5-CSRTT

2.2.1. Subjects

Thirty-eight, male, Lister–Hooded rats (Harlan, Netherlands) weighing 300–400 g were used. Rats were housed in groups of four in holding rooms at controlled temperature (20–22 °C) with a 12-h light/dark cycle (lights on at 06:00 h). Access to food was restricted so as to maintain 85% of free-feeding body weight. Except for during testing, water was available ad libitum at all times.

2.2.2. Apparatus

Five-choice operant chambers (Med Associates, St. Albans, VT) housed in sound-insulated and ventilated enclosures were used for all experiments. Each chamber consisted of an aluminum enclosure (25×30 cm), containing on one wall a food hopper and house light and on the opposite wall an array of five square niches ($2.5 \times 2.5 \times 2.5$ cm) arranged on a curved panel and raised 2.5 cm from the grid floor. An LED (standard conditions: 150 lux) was positioned at the rear of each niche. All apertures in the chamber including the food hopper were controlled by a photocell placed across the entrance. Operant chambers were controlled by the Kestrel Control System (Conclusive Solutions, Harlow, UK).

2.2.3. Training procedure

Rats were initially given access to a handful of pellets (45-mg Noyes Formula P Food Pellets) in their home cage for 2 consecutive days. Training commenced with two daily 30-min sessions in which subjects were placed in the operant chambers, and both the food hopper and five light niches were filled with approximately five pellets each. On subsequent days, no food was placed in the chambers before sessions began. Training on the five-choice task began with the illumination of the house light and delivery of a food pellet. A nose poke into the magazine tray started the first trial, which consisted of an intertrial interval (ITI, 5 s) followed by the random illumination of one of the five lights for a fixed interval (stimulus duration, SD). If a nose poke was registered in the illuminated niche before the end of either the SD or a fixed interval after this period (limited hold, LH), a further pellet was dispensed and a Correct Trial registered. An incorrect nose poke (Incorrect Trial) or failure to respond within the allotted time (Missed Trial) resulted in a Time Out (TO) period in which the house light was extinguished for 5 s. Responding into one of the five niches during the ITI (premature response), or after a correct trial was registered (perseverative response), resulted in a further TO. Finally, if a rat responded into a niche during a TO, the TO was restarted.

Each training session ran for either 100 trials or 60 min, whichever was shorter. Initially, stimulus parameters were such that SD was set at 60 s, and ITI, TO and LH were 5 s. For all subjects, the SD was progressively reduced until a criterion duration of 0.5 s was achieved. All other parameters remained at their initial levels throughout training and test. Training continued under the target stimulus parameters until subjects had achieved consistent performance above a threshold of 75% correct ([correct/(correct + incorrect)] * 100) and < 20% omissions for at least a 2-week period.

2.2.3.1. Experiment 4: effects of nicotine and SIB-1553A in nontolerant and sensitised rats on five-choice performance. Following training, 26 subjects were divided into two groups and administered with daily injections of either nicotine (0.2 mg/kg sc) or vehicle for at least 20 days prior to the start of experimentation. Mean performance for the two groups prior to experimentation: vehicle group 78.5 ± 0.4 (percent correct), 9 ± 1 (omissions); nicotine group 79.4 ± 0.4 (percent correct), 9 ± 1 (omissions). Two separate dose–responses were derived in these rats: Nicotine (0.1–0.4 mg/kg sc) and SIB-1553A (1–10 mg/kg sc), both of which followed a fully repeated-measures design, with doses assigned pseudorandomly across test days. Between test days, subjects continued to receive injections of either vehicle or nicotine after daily sessions in the five-choice apparatus.

2.2.3.2. Experiment 5: effects of nicotine and SIB-1553A over extended sessions of five-choice performance in aged rats. Twelve rats were trained to criterion performance on the 5-CSRTT as described above. These subjects continued to be run in the five-choice task two to three times per week until they had reached approximately 2 years of age. All subjects were then run 5 days/week until performance was again stable (percent correct, 82.7 ± 1.1; omissions, 14 ± 1). A series of studies was then initiated to assess the effects of various nicotinic ligands on performance in these subjects. Therefore, by the time the present studies were performed, rats had some previous experience with nicotine in the five-choice task. The effects of both nicotine (0.4 mg/kg) and SIB-1553A (3–10 mg/kg) was assessed. On test days, the number of trials per session was increased from 100 to 250, and subjects were administered doses of either SIB-1553A or nicotine prior to five-choice testing. All subjects received each dose of test compound, and between test days, subjects continued to be run under

standard conditions (100 trials per session). Subjects who did not complete sessions within the allotted time were omitted from subsequent analysis. Final group sizes were $n = 10$ (nicotine) and $n = 11$ (SIB-1553A).

2.2.4. Drugs and injections

(−)-Nicotine hydrogen tartrate and mecamylamine (Sigma), SIB-1553A (synthesised within the Roche CNS Chemistry Department) and DHβE (RBI) were dissolved in 0.9% NaCl solution (saline), and the pH of nicotine was adjusted to ≃7.0 by the addition of sodium hydroxide (Fig. 1). Doses are expressed as that of the base, and drugs were administered at a dose volume of 1 ml/kg. All compounds were administered by the subcutaneous route. Nicotine and SIB-1553A were injected 5 min, mecamylamine 15 min and DHβE 10 min before test.

2.2.5. Statistical analysis

Data from dose–response studies and total session scores from Experiment 4B were analysed using one-factor ANOVA. Drug interaction studies (Experiment 2) and data from the sensitisation study (Experiment 3) were analysed using a two-factor ANOVA. Data from five-choice studies over extended sessions (Experiment 5) also employed a two-factor ANOVA (responses in 50-trial Blocks × Drug treatment). In all analyses, the data were treated as repeated measures, except for the sensitisation study, which utilised one within-subjects factor (challenge compound) and one between-subjects factor (chronic treatment group). Where appropriate, significant main effects were followed by post hoc comparisons using the Newman–Keuls' test.

3. Results

3.1. Experiment 1: SIB-1553A dose–responses in nontolerant and sensitised rats

In nontolerant rats, acute administration of SIB-1553A (10–40 mg/kg) and nicotine (0.4 mg/kg) significantly increased activity [$F(4,28 = 4.3$, $P < .01$; Fig. 2A], with the higher doses of SIB-1553A (20–40 mg/kg) inducing a significant activity above control levels, which did not differ from nicotine. In nicotine-sensitised rats, SIB-1553A (10–80 mg/kg) and nicotine (0.4 mg/kg) again elicited a significant increase in activity [$F(5,35) = 44.4$, $P < .01$; Fig. 2B]. For SIB-1553A, this activity was similar to that seen in nontolerant rats, in that only the highest doses (20–80 mg/kg)

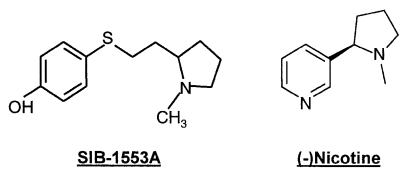

Fig. 1. Chemical structure of SIB-1553A and (−)-nicotine.

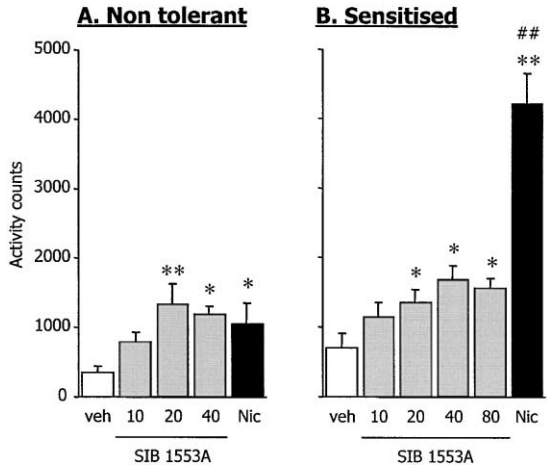

Fig. 2. Effects of SIB-1553A (10–80 mg/kg sc) and nicotine (0.4 mg/kg sc) on locomotor activity in (A) nontolerant and (B) nicotine-sensitised rats. Bars represent means ± S.E.M., $n = 8$ per study. * $P < .05$, ** $P < .01$ vs. vehicle; ## $P < .01$ vs. SIB-1553A treatments.

differed from vehicle controls; however, the nicotine response in this study was approximately fourfold larger than that observed in nontolerant rats, such that post hoc analysis revealed significantly higher activity in nicotine-pretreated rats, compared to SIB-1553A-pretreated rats.

3.2. Experiment 2: interactions between SIB-1553A and nicotinic antagonists

In nontolerant rats, locomotor activity induced by nicotine (0.4 mg/kg) was completely reversed by coadministration of mecamylamine [1 mg/kg; $F(1,7) = 20.0$, $P < .01$; Mecamylamine × Nicotine interaction; Fig. 3], whereas similar levels of activity induced by SIB-1553A were unaffected by coadministration of either mecamylamine [1 mg/kg; $F(1,10) = 2.9$, NS; SIB-1553A × Mecamylamine interaction] or DHβE [3 mg/kg; $F(1,10) = 2.9$, NS; SIB-1553A × DHβE interaction].

3.3. Experiment 3: investigating cross-sensitisation between nicotine and SIB-1553A

After 10 days of treatment with either nicotine, vehicle or SIB-1553A, two-way ANOVA revealed a significant influence of chronic treatment on the response to acute challenge with nicotine or SIB-1553A [$F(2,20) = 9.2$, $P < .01$], an effect of acute challenge compound [$F(2,40) = 54.7$, $P < .01$] and a significant interaction between the two [$F(4,40) = 3.8$, $P < .01$; Fig. 4]. Response to acute challenge with vehicle [$F(2,20) = 2.8$, NS] and SIB-1553A [$F(2,20) = 3.2$, NS] did not differ between the three chronic groups, whereas activity following acute nicotine challenge was potentiated in the nicotine, as compared to both vehicle- and SIB-1553A-pretreated groups [$F(2,20) = 8.7$, $P < .01$]. In all groups, nicotine and SIB-1553A significantly increased activity above control levels: vehicle group [$F(2,14) = 9.7$,

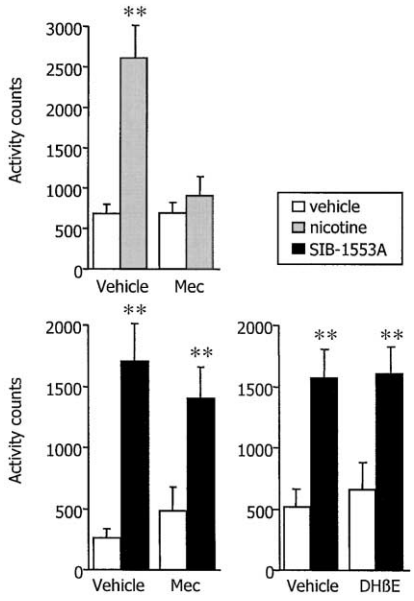

Fig. 3. Effect of the nicotine antagonists mecamylamine (1 mg/kg sc) and DHβE (3 mg/kg sc) on activity induced by nicotine (0.4 mg/kg sc) or SIB-1553A (15 mg/kg sc). Bars represent means ± S.E.M. $n = 8/12$, nicotine and SIB-1553A studies, respectively. ** $P < .01$ compared to vehicle controls. Note that despite the larger response to nicotine than SIB-1553A, nicotine-induced activity was completely blocked by mecamylamine.

$P < .01$], nicotine group [$F(2,14) = 27.2$, $P < .01$] and SIB-1553A group [$F(2,14) = 22.4$, $P < .01$]. Thus, nicotine pretreatment potentiated the subsequent locomotor response to acute challenge with nicotine but not SIB-1553A.

3.4. Experiment 4: effects of nicotine and SIB-1553A in nontolerant and sensitised rats on five-choice performance

As previously reported (Grottick and Higgins 2000), chronic nicotine administration significantly altered the response to acute nicotine challenge (Fig. 5). In subjects who were nicotine naive, nicotine (0.1–0.4 mg/kg) did not alter any parameter of five-choice performance, whereas in nicotine-sensitised rats, nicotine decreased correct latency [$F(3,36) = 3.1$, $P < .05$], increased premature responding [$F(3,36) = 8.3$, $P < .01$] and increased percent correct responses [$F(3,36) = 3.5$, $P < .05$]. Post hoc analysis revealed that the increase in percent correct responses only occurred at the highest (0.4 mg/kg) dose of nicotine, a dose that had no effect on other performance parameters. Vehicle performance for the two groups of subjects did not differ on any measure for the nicotine study.

In contrast to nicotine, SIB-1553A (1–10 mg/kg sc) tended to disrupt performance in both nicotine tolerant and nontolerant rats (Fig. 5), with a higher disruption seen in nicotine-pretreated subjects. Thus, in tolerant rats, SIB-1553A increased correct latency [$F(3,36) = 12.9$, $P < .01$], increased omissions [$F(3,36 = 2.9$, $P < .05$], increased magazine latency [$F(3,36) = 11.0$, $P < .01$] and decreased premature responses [$F(3,36) = 4.7$, $P < .01$]. Only on magazine latency did the disruptive effects of SIB-1553A in nontolerant rats reach statistical significance [$F(3,36) = 3.2$, $P < .05$].

3.5. Experiment 5: effects of nicotine and SIB-1553A over extended sessions of five-choice performance in aged rats

Exposing aged rats to extended sessions of responding in the five-choice task led to a trial-dependent decrease in some performance parameters. Thus, a main effect of trials was obtained for correct latency (nicotine study [$F(4,36) = 5.4$, $P < .01$]; SIB-1553A study [$F(2,36) = 3.9$, $P < .01$]) and omissions (nicotine study [$F(4,36) = 8.4$, $P < .01$]; SIB-1553A study [$F(2,36) = 21.0$, $P < .01$]). Accuracy decreased over trials in the SIB-1553A [$F(2,28) = 2.7$, $P < .05$] but not the nicotine study [$F(2,28) = 1.0$, NS]. Other performance measures did not change over trial blocks, including magazine and incorrect latencies and

Table 1
The effect of various nicotinic agonists on locomotor activity in nicotine-nontolerant and nicotine-sensitised rats and on their ability to cross-sensitise to the psychomotor stimulant effects of nicotine

	Nicotine nontolerant		Nicotine sensitised		
Nicotinic agonist	Effect	Antagonism	Effect	Antagonism	Nicotine cross-sensitisation?
Nicotine	↑[a,b]	Blocked by mecamylamine[c] Blocked by DHβE[c]	↑↑[a,b,c]	Blocked by mecamylamine[d] Blocked by DHβE[a,c]	–
SIB-1553A	↑	No effect mecamylamine No effect DHβE	↑	NT NT	No
SIB-1765F	↑[a,b,e]	Blocked by mecamylamine[e] Blocked by DHβE[e]	↑↑[a,b]	NT NT	Yes[b]
AR-R 17779	No effect[a,f]	NT	No effect[a]	NT	No[b]

↑ = significant increase, ↑↑ = larger increase than in nontolerant rats, NT = not tested.

[a] Grottick et al. (2000a).
[b] Grottick et al. (2000b).
[c] Stolerman et al. (1997).
[d] Clarke and Kumar (1983a).
[e] Menzaghi et al. (1997).
[f] Kaiser et al. (1998).

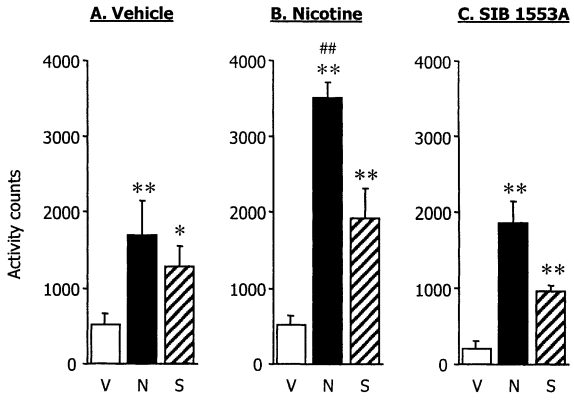

Fig. 4. Locomotor responses to challenge with either vehicle (V), nicotine (0.4 mg/kg sc; N) or SIB-1553A (15 mg/kg sc; S) in rats who had been administered either (A) vehicle, (B) nicotine (0.4 mg/kg) or (C) SIB-1553A (15 mg/kg) for 10 days prior to the start of test. $n=8$ per chronic treatment group. * $P<.05$, ** $P<.01$ compared to respective vehicle controls; ## $P<.01$ nicotine response compared to that in the vehicle group.

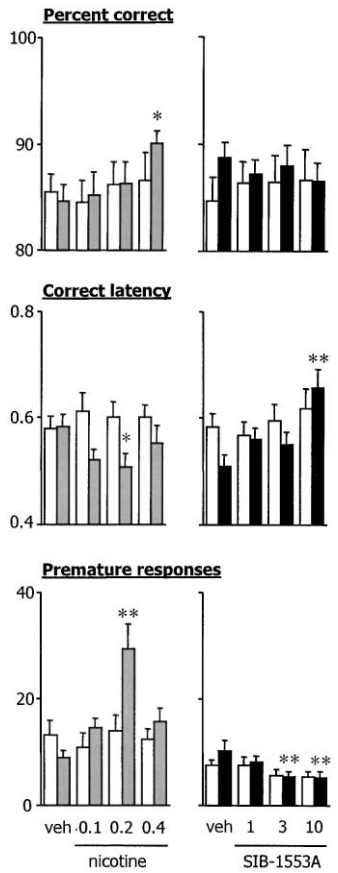

Fig. 5. Effects of nicotine (0.1–0.4 mg/kg sc) and SIB-1553A (1–10 mg/kg sc) on various measures of five-choice performance in nicotine-nontolerant (open bars) and nicotine-sensitised rats (filled bars). Subjects were treated with either vehicle or nicotine (0.2 mg/kg sc) for at least 20 days prior to the start of experimentation. Results are expressed as means ± S.E.M. * $P<.05$, ** $P<.01$ compared to respective vehicle controls. Nicotine data reprinted from Grottick and Higgins (2000), with the kind permission of Elsevier Science.

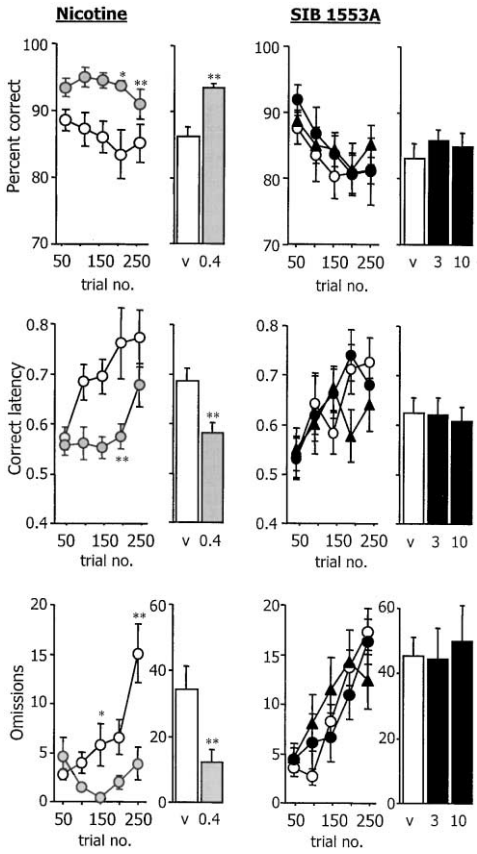

Fig. 6. The effect of nicotine (0.4 mg/kg sc) and SIB-1553A (3–10 mg/kg sc) on the performance of aged rats over extended sessions of responding in the 5-CSRTT. Bars represent session totals, whereas lines represent responses divided into 50 trial bins, ○ = vehicle; ○ = nicotine (0.4 mg/kg); ● = SIB-1553A (3 mg/kg); ▲ = SIB-1553A (10 mg/kg). Results are expressed as means ± S.E.M. * $P<.05$, ** $P<.01$ compared to respective vehicle controls.

perseverative responses. Nicotine (0.4 mg/kg) increased total percent correct [$F(1,9)=14.6$, $P<.01$; Fig. 6], reduced the number of omissions [$F(1,9)=12.0$, $P<.01$] and increased speed to make a correct response [$F(1,9)=18.9$, $P<.01$]. No other measures differed from vehicle controls. A subsequent analysis of the first 100 trials of responding revealed that only percent correct responses were significantly increased by nicotine [$F(1,9)=10.1$, $P<.01$]; both correct latency [$F(1,9)=0.1$, NS] and omissions [$F(1,9)=3.1$, NS] remained unchanged. In contrast to nicotine, SIB-1553A had no effect on any performance parameter.

4. Discussion

The aim of the present studies was to compare nicotine with the novel nicotinic receptor subtype agonist SIB-1553A in tests of locomotion and attention. SIB-1553A produced a behavioural profile, which differed in a number of important respects from nicotine.

Firstly, in both nicotine-sensitised and nontolerant rats, SIB-1553A induced a significant increase in locomotor activity, which did not differ as a function of previous nicotine experience. In accordance with previous observations (Clarke and Kumar, 1983a; Benwell and Balfour 1992), subchronic dosing with nicotine led to a potentiated response to subsequent nicotine but not SIB-1553A challenge. In a further study, we examined this apparent lack of cross-sensitisation by testing whether chronic treatment with either SIB-1553A or nicotine would alter the locomotor response to acute challenge with either drug. Results from this study essentially confirmed that previous nicotine experience does not alter the locomotor response to SIB-1553A and extended this observation to include the inverse—that subchronic dosing with SIB-1553A does not alter the locomotor response to nicotine or SIB-1553A challenge. We have previously demonstrated that the $\alpha_4\beta_2$-preferring agonist SIB-1765F, but not the α_7 agonist AR-R 17779, fully cross-sensitises to the psychostimulant effects of nicotine (Grottick et al. 2000b). The lack of effect with SIB-1553A therefore lends extra support to the suggestion that a high-affinity nicotine site, possibly $\alpha_4\beta_2$, appears pivotal in both the acute and sensitised locomotor responses to nicotine. These findings may also be consistent with single cell RT-PCR and [^{3}H]-nicotine autoradiographic studies in mice lacking various nicotine receptor subtypes. These demonstrate minimal expression of β_4 subunits within the midbrain dopaminergic nuclei but high expression of other subunits, including α_4 and β_2 (Charpantier et al., 1998; Klink et al., 2001; Zoli et al, 1998).

Coadministration of the nicotinic antagonists mecamylamine or DHβE did not antagonise locomotor activity induced by SIB-1553A. This is despite the fact that mecamylamine (Clarke and Kumar, 1983a,b; Reavill and Stolerman, 1990; present study) and DHβE (Stolerman et al., 1997; Watkins et al., 1999; Grottick et al. 2000a) completely attenuate nicotine-induced activity at doses that have no locomotor depressant effects when administered alone. SIB-1553A is described as a preferential agonist of β_4-containing receptors (Vernier et al., 1999). It is however possible that within the behaviourally active dose range, other receptors (including non-nicotinic) are simultaneously activated, thus accounting for the locomotor changes observed. Indeed, it has been reported that SIB-1553A has varying affinity for sigma, muscarinic, adrenergic (α_2), serotonergic (5-HT$_{1/2}$) and histaminergic (H_3) sites (Reid et al., 1997), although specific values were not provided in this abstract. A nicotinic action seems unlikely, given that together mecamylamine and DHβE display reasonable affinity for the majority of known nicotinic sites, including β_4 (Chavez-Noriega et al., 1997). The only major exception to this is the α_7 receptor. However, this seems an unlikely candidate, given that SIB-1553A has negligible affinity for α_7 receptors (Vernier et al., 1999), that various α_7 agonists appear to not induce locomotion (Kaiser et al., 1998; Grottick et al., 2000a,b) and that unlike mecamylamine and DHβE, the selective α_7 antagonist methyllycaconitine does not block nicotine-induced activity (Grottick et al., 2000a). Although this leaves a non-nicotinic action as the most likely explanation for SIB-1553A-induced locomotion, it does not fully negate a β_4 involvement in nicotine-induced activity, as the possibility remains that these other non-nicotinic factors act to simultaneously inhibit the expression of β_4-mediated locomotion. This proposition would be readily testable by investigating the potential inhibitory action of SIB-1553A on nicotine-induced activity. What appears clear from these studies is that the behavioural effects of SIB-1553A include a non-nicotinic component.

As previously discussed (Grottick and Higgins, 2000) in nicotine-sensitised rats, nicotine induced a small but significant enhancement in attentional performance as evidenced by an increase in accuracy and speed of responding. This effect is unlikely to represent alleviation of a withdrawal state in tolerant rats, as (1) following vehicle administration, tolerant and nontolerant subjects' baseline performance did not differ, (2) administration of the competitive antagonist DHβE to nicotine-tolerant rats did not alter performance (Grottick and Higgins 2000) and (3) nicotine-induced increases in accuracy were above the absolute levels obtained in nontolerant rats. Two explanations could be evoked to explain the selective effect of nicotine in subjects with previous nicotine experience. Firstly, both acute and chronic tolerance develops to the disruptive effects of nicotine (see Stolerman, 1999), which may allow the expression of performance enhancement in the absence of concomitant disruption. Secondly, the selective effects of nicotine in nicotine-sensitised rats may reflect behavioural sensitisation. Sensitisation to the locomotor-activating and DA-releasing effects of nicotine has been established (Clarke and Kumar, 1983a; Benwell and Balfour 1992), and there is some evidence to suggest that chronic dosing with nicotine may lead to progressive enhancement in mnemonic performance (see Levin and Simon 1998; Bernal et al., 1999).

In aged rats, nicotine produced a significant and selective increase in accuracy over the first 100 trials of responding with no concomitant effects on any other measure, thus replicating the effect of nicotine in younger nicotine-sensitised rats. In addition, as the five-choice session progressed, an increase in response speed and a decrease in missed trials became apparent, most likely reflecting reversal of the progressive response disruption seen in vehicle-treated rats. The similarity between nicotine responses in this study and the previous study in which rats had been chronically administered nicotine is intriguing, given that at the time of test, aged subjects had been nicotine free for a period of at least 5–6 weeks. It remains to be established whether this apparent change in the performance-enhancing effects of nicotine reflects a persistence of previous nicotine exposure or an effect of age.

SIB-1553A did not mimic any of these changes in either young or aged rats and at the highest dose (10 mg/kg)

tended to disrupt performance: increasing omissions, decreasing premature responding and increasing the response latency to make a correct response and to collect food. These disruptive effects tended to occur only in nicotine-sensitised rats, although this likely reflects small differences in baseline performance, as in both groups of young rats there was a trend towards disruption, which only reached statistical significance in the nicotine-sensitised group. Thus, higher doses of SIB-1553A were not tested in the five-choice task.

Previous studies with SIB-1553A have indicated enhancements in working or short-term memory tasks (Bontempi et al., 1997). Similarly, in assessing the effects of nicotine, a finding common to many studies is a specific enhancement of short-term rather than reference memory (see Levin and Simon, 1998). This has led to the suggestion that apparent memory improvements may be secondary to enhanced attention, as the most robust effects of nicotine appear in tasks with high attentional load (Warburton and Rusted 1993). The present data therefore appear incompatible with this observation, although it should be remembered that attention is not a unitary concept but rather encompasses a number of distinct processes of which the standard 5-CSRTT likely assesses only a subset. An alternative explanation is that the cognitive effects of nicotine on short-term memory and attention reflect two discrete processes mediated by subsets of receptor types. Except for results from the present study and the finding that the α_7 agonist AR-R 17779 improves radial-arm maze (Levin et al., 1999) but not five-choice performance (Grottick and Higgins 2000), this claim is largely unsubstantiated and awaits further characterisation of subtype-selective nicotinic ligands in attentional and mnemonic tasks.

To summarise, SIB-1553A induced low levels of activity, which did not cross-sensitise to nicotine. This activity was insensitive to antagonism by DHβE and mecamylamine, signifying a non-nicotinic action. This modest effect on locomotor activity may be indicative of reduced dependence liability. Unlike its effects in other cognitive tasks assessing working memory function (Bontempi et al., 1997; Menzaghi et al., 1997), we were unable to detect any beneficial effects of SIB-1553A on the 5-CSRTT. Taken together with previous studies, we can find no evidence to suggest that the nicotine receptor subtypes contributing to locomotor stimulation and attentional enhancement can be dissociated.

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