SSR591813, a Novel Selective and Partial $\alpha_{4}\beta_{2}$ Nicotinic Receptor Agonist with Potential as an Aid to Smoking Cessation


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ABSTRACT

(5aS,8S,10aR)-5a,S,9,10-tetrahydro,7H,11H-8,10a-methanopyrido[2',3':5,6]pyran[2,3-d]azepine (SSR591813) is a novel compound that binds with high affinity to the rat and human $\alpha_{4}\beta_{2}$ nicotinic acetylcholine receptor (nAChR) subtypes ($K_{i}$ = 107 and 36 nM, respectively) and displays selectivity for the $\alpha_{4}\beta_{2}$ nAChR ($K_{i}$ human $\alpha_{4}\beta_{2}$ > 1000, $\alpha_{4}\beta_{3}$ = 116; $\alpha_{4}\beta_{3}$, $\alpha_{4}$ > 6000 nM and rat $\alpha_{4}$ > 6000 nM). Electrophysiological experiments indicate that SSR591813 is a partial agonist at the human $\alpha_{4}\beta_{2}$ nAChR subtype ($EC_{50}$ = 1.3 $\mu$M, IA = 19% compared with the full agonist 1,1-dimethyl-4-phenyl-piperazinium). In vivo findings from microdialysis and drug discrimination studies confirm the partial intrinsic activity of SSR591813. The drug increases dopamine release in the nucleus accumbens shell (30 mg/kg i.p.) and generalizes to nicotine or amphetamine (10–20 mg/kg i.p.) in rats, with an efficacy approximately 2-fold lower than that of nicotine. Pretreatment with SSR591813 (10 mg/kg i.p.) reduces the dopamine-releasing and discriminative effects of nicotine. SSR591813 shows activity in animal models of nicotine dependence at doses devoid of unwanted side effects typically observed with nicotine (hypothermia and cardiovascular effects). The compound (10 mg/kg i.p.) also prevents withdrawal signs precipitated by mecamylamine in nicotine-dependent rats and partially blocks the discriminative cue of an acute precipitated withdrawal. SSR591813 (20 mg/kg i.p.) reduces i.v. nicotine self-administration and antagonizes nicotine-induced behavioral sensitization in rats. The present results confirm important role for $\alpha_{4}\beta_{2}$ nAChRs in mediating nicotine dependence and suggest that SSR591813, a partial agonist at this particular nAChR subtype, may have therapeutic potential in the clinical management of smoking cessation.

Nicotine, the main active substance of tobacco products, is a positive reinforcer in humans and in animals (Goldberg and Henningfield, 1988). It produces interoceptive and discriminative stimuli, and some cross-generalization between nicotine and other addictive drugs has been found (Brioni et al., 1997; Perkins et al., 1999; Cohen et al., 2002). Chronic tobacco use in humans or chronic administration of nicotine in rodents generally results in a state of “physical dependence” characterized by the occurrence of a withdrawal syndrome (Hughes et al., 1994; Malin, 2001). This syndrome consists of somatic (bradycardia, gastrointestinal discomfort, and increased appetite) and affective symptoms (craving, depressed mood, dysphoria, anxiety, irritability, and difficulty concentrating).

Although the molecular mechanisms that contribute to nicotine addiction are poorly understood, neuroadaptations both in the nicotinic cholinergic system and in other neurochemical systems have been found and may be critical for the understanding of nicotine dependence (Di Chiara, 2000; Dani and De Biasi, 2001). The nAChR is a ligand-gated ion channel composed of five subunits (Sargent, 2000). To date, molecular cloning techniques have identified 16 genes encoding ABBREVIATIONS. nAChR, nicotinic acetylcholine receptor; DHjβE, dihydro-β-erythroidine; HEK, human embryonic kidney; DMPP, 1,1-dimethyl-4-phenyl-piperazinium; ANOVA, analysis of variance; FR, fixed ration; IA, intrinsic activity; SSR591813, (5aS,8S,10aR)-5a,S,9,10-tetrahydro,7H,11H-8,10a-methanopyrido[2',3':5,6]pyran[2,3-d]azepine; AHBT 94, (R)-5-(2-azetidinylmethoxy)-2-chloropyridine.
nicotinic acetylcholine receptors (nAChRs) are composed of five subunits: α1, β1, δ, and γ (ε in adult) subunits in skeletal muscle, and α2 to α10 and β2 to β4 subunits in brain, sensory systems, and autonomic ganglia. Most neuronal nAChRs are formed using brain sections from humans and rodents, with the functional properties depending on the subunit composition (Sargent, 2000; Sgard et al., 2002). The distribution of the various subunits in the rat brain has shown distinct expression patterns. The regional distribution of α4 and β2 subunits coincides with high-affinity binding sites for [3H]nicotine, as determined by in situ hybridization and immunoprecipitation (Sargent, 2000).

Several findings suggest that the α4β2 nAChR subtype plays a major role in the reinforcing effects of nicotine. Studies performed using brain sections from humans and rodents have shown that chronic exposure to nicotine increases the density of nicotine binding, mainly to the α4β2 nAChR type (Marks et al., 1992; Perry et al., 1999). Nicotine self-administration is reduced in rats pretreated with the selective α4β2 nAChR antagonist DHβE (Watkins et al., 1999) or in genetically modified mice with functional deletion of the β2 subunit (Picciotto et al., 1998). The β2 subunit is crucial in mediating the dopamine-releasing effects of nicotine as indicated by the absence of striatal dopamine release in β2 subunit knockout mice treated with nicotine. This activity of nicotine on dopamine release, shared by other addictive drugs, is considered as central in the acquisition and maintenance of nicotine addiction (Corrigall and Coen, 1991; Di Chiara, 2000; Cohen et al., 2002).

The current most effective way to stop smoking are those that involve the use of nicotine delivery systems and bupropion, or a combination of both. Bupropion is a dopamine and norepinephrine uptake inhibitor. As such, it increases the concentration of dopamine in the nucleus accumbens and norepinephrine uptake inhibitor. As such, it increases the dysregulation of reinforcement mechanisms that have been hypothesized to lead to compulsive drug taking (Cohen et al., 1999; Dani and De Biasi, 2001). The clinical use of nicotine as a therapeutic agent is severely limited by its cardiovascular and gastrointestinal side effects thought to involve the α4β4 nAChRs (Sargent, 2000). A subtype-selective partial nAChR agonist interacting with the nAChR subtype responsible for the addictive effects of nicotine may be expected to have less abuse potential and less adverse side effects than nicotine and could therefore prove to be of useful therapeutic value in smoking cessation. Herein, we report on the pharmacological properties of the novel nAChR ligand SSR591813 (Fig. 1), which has been selected on the basis of its selective α4β2 partial agonist activity. The focus is on its efficacy in animal models of nicotine addiction.

**Materials and Methods**

**Animals**

Unless indicated otherwise, adult male Sprague-Dawley (microdialysis, self-administration, and behavioral sensitization tests) or Wistar (receptor binding, drug discrimination, and withdrawal signs) rats and OF1 mice (Iffa Credo, L’Arbresle and Charles River, Saint-Aubin-les-Elbeuf, France) were used. Housing was in individual cages (except for the behavioral sensitization tests, eight rats per cage; and rectal temperature measures, five mice per cage) under standard laboratory conditions with lights on between 7:00 AM and 7:00 PM. Food and water were given ad libitum except in the drug discrimination and self-administration studies where animals were restricted to a daily ration of 15 to 20 g of standard laboratory food given at the end of each day.

**Materials and Drugs**

Human embryonic kidney 293 cells stably expressing the human α4β2 nAChR subtype (hα4β2-HEK293) were generated by Sanofi-Synthelabo Recherche (Rueil-Malmaison, France). HEK293 cells stably expressing the human α4β2 nAChR subtype (hα4β2-HEK293) were supplied by Dr. J. Lindstrom (University of Pennsylvania, Philadelphia, PA). SH-SY5Y and IMR-32 neuroblastoma cells, which have been shown to express spontaneously the human α4β2 nAChR subtype (Wang et al., 1996; Nelson et al., 2001), and human medulloblastoma cells (TE671), which express spontaneously the human α4β2 nAChR subtype (Schoepfer et al., 1988), were purchased from American Type Culture Collection (Biovalley, Marne La Vallée, France). Cell lines were maintained as described by suppliers with some modifications: culture medium for hα4β2-HEK293 was supplemented with geneticin (0.5 mg/ml), camaylamine (20 μM), and methotrexate (100 nM), and 1 day before collection, cells were placed in camaylamine-free medium culture; nicotine (100 μM) was added to the culture medium for hα4β2-HEK293 24 to 48 h before use to up-regulate α4β2 nAChR expression (Wang et al., 1998); nicotine (1 mM) was added to the SH-SY5Y and TE671 culture medium 24 h before harvesting.

Radioligands were obtained from the following sources: [3H]cytisine (PerkinElmer Life Sciences, Paris, France); and [3H]α-bungarotoxin and [3H]epibatidine (Amer sham Biosciences Inc., Orsay, France).

SSR591813, ABT 594 hydrochloride, mecamylamine hydrochloride, amisulpride, and bupropion hydrochloride were synthesized by the Medical Chemistry Department of Sanofi-Synthelabo. d-Amphetamine sulfate was purchased from Laboratoire Boyer (Paris, France). (+)-Epibatidine dihydrochloride, R(+)-SKF81297 hydrobromide, (+)-quinpirole hydrochloride, a-xylatecholine, DMPP, a-nicotinoyl ditartrate, DHβE hydrobromide, methyllycaconitine citrate, and R(+)-SCH23390 hydrochloride were obtained from Sigma/RBI (Saint Quentin Fallavier, France). All other compounds were obtained from Invitrogen (Cergy, France) and Sigma/RBI.

During in vivo experiments, drugs were prepared in physiological saline or distilled water containing Tween 80 (0.1%) except for nicotine in the drug discrimination and self-administration studies, which was administered as a solution (pH 7) in saline containing 0.05 N NaOH (10%). Drugs were administered i.p., p.o., or s.c. (1 or 5 m/kg). All doses are expressed as the free bases.

**Membrane Preparations**

For [3H]cytisine binding, rats were killed by decapitation and whole brain except cerebellum was removed and placed on ice. Tis-
sues were homogenized at 4°C in 15 volumes of Tris-HCl buffer (50 mM, pH 7.4) and centrifuged twice for 10 min at 4°C (40,000g). For [3H]-bungarotoxin binding, rat brains were homogenized at 4°C in 15 volumes of 0.32 M sucrose buffer and centrifuged for 10 min (1,000g). Supernatants were centrifuged for 20 min (20,000g). Pellets were subsequently homogenized in 15 volumes of double distilled cold water and centrifuged at 40,000g for 20 min. hαβ2–HEK293 cell pellets were homogenized in ice-cold Tris binding buffer (composition 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, and 1 mM MgCl₂, pH 7.4 at 4°C) and centrifuged twice for 15 min (40,000g). hαβ2–HEK293 cells were homogenized in HEPES binding buffer (composition 140 mM NaCl, 1.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1 mM p-aminobenzamidine dihydrochloride, and 25 mM HEPES, pH 7.5) and washed twice by centrifugation (45,000g for 15 min). The membrane preparation was then incubated for 15 min at 25°C with fresh buffer and centrifuged (45,000g for 15 min). A protocol similar to that used for hαβ2–HEK293 cells was followed for SH-SYSY cells. TE671 cell pellets were homogenized in HEPES binding buffer (composition 118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 1 mM p-aminobenzamidine dihydrochloride, and 20 mM HEPES, pH 7.4 at 4°C) and then centrifuged twice for 15 min at 4°C (40,000g). The pellet was suspended in p-aminobenzamidine-free HEPES buffer, incubated at room temperature for 15 min, and centrifuged again for 15 min at 4°C (40,000g).

**Receptor Binding**

**Rat and Human αβ2 nAChRs.** [3H]Cytisine binding was performed as described previously (Anghel and Arneric, 1994) with slight modifications. Samples containing either hαβ2–HEK293 cell membranes (40–60 μg of protein) or rat brain membranes (200–250 μg of protein), the test compound and 0.5 nM or 2.5 nM [3H]cytisine (32 Ci/mmol) for HEK293 cells and for rat brain, respectively, were incubated in a final volume of 200 μl of Tris-HCl buffer for 2 h at 4°C. Nonspecific binding was determined in the presence of 10 μM nicotine.

**Human αβ2 nAChRs.** [3H] Epibatidine binding was performed as described previously (Marks et al., 1999) and Dineley and Patrick (2000) with slight modifications. Samples containing rat brain membranes (300–350 μg of protein), the test compound and 1 nM [3H]-epibatidine (60 Ci/mmol) were incubated in a final volume of 250 μl at 1 h at 37°C. Nonspecific binding was determined in the presence of 1 μM α-bungarotoxin.

**Human αββ2 nAChRs.** [3H] Epibatidine binding on SH-SYSY cell membranes was performed as described for αβ2 nACr binding except for the radioligand concentration: 0.3 nM [3H]epibatidine.

**Human αββ7 γδ nAChRs.** Samples containing TE671 cell membranes (100–150 μg of protein), 1 nM [3H]-α-β-γ-δ-bungarotoxin (60 Ci/mmol), and the test compound were incubated in a final volume of 250 μl at 2 h at 37°C. Nonspecific binding was determined in the presence of 1 μM α-bungarotoxin.

**Receptor Selectivity.** Interaction of SSR591813 with more than 70 binding sites, including all major classes of neurotransmitter receptor, uptake systems, ion channels, and enzymes was examined. SSR591813 was first tested in all assays at 10 μM. In assays where it caused more than 50% inhibition at this concentration, it was further tested at 10 concentrations to obtain full inhibition curves. Each determination was made in duplicate.

Radioactivity was quantified using solid or liquid scintillation spectrometry. Competition binding data were analyzed using non-linear regression methods. Kᵣ values were derived from the measured IC₅₀ and Kᵦ values for radioactive ligand using the Cheng–Prussoff equation: $K_r = IC_{50}/(1 + (L/K_I))$ where $L$ is the concentration of radiolabeled ligand and $K_I$ is the equilibrium dissociation constant, previously determined by saturation experiments.

**Electrophysiological Studies**

**Human αββ2 nAChRs Expressed in Oocytes.** Under ice anesthesia, ovaries were taken from female Xenopus laevis (UPRES-A6026, Rennes, France) and the follicle cell layer was removed with collagenase under gentle mechanical agitation. Twenty-four hours later, oocytes at stage V/VI were isolated and their nuclei directly injected with 2 ng of cDNAs encoding for the human α and β subunits. Injected oocytes were then incubated at 18°C and used for voltage-clamp experiments after 48 h. Oocytes were voltage-clamped at −60 mV. The effects of SSR591813 and reference compounds were compared with those of 100 μM DMPP. The standard extracellular solution contained 82 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 10 mM HEPES. pH was set to 7.4 with 1 M Tris-OH. The pipette was filled with 3 M KCl. Drugs were diluted in purified water or dimethyl sulfoxide in concentrations that did not exceed 0.08% in the final solution.

**Human αββ2 and αββ2 nAChRs Expressed in HEK293 and IMR-32 Cells, Respectively.** Currents recordings were obtained by using the whole cell configuration of the patch-clamp technique at room temperature. Cells were held at a holding voltage of −60 mV and the effects of SSR591813 or of reference compounds were compared with 1 mM acetylcholine, a concentration that produced maximal response. The standard extracellular solution contained 147 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES. pH was set to 7.4 with 1 M Tris-OH. The pipette was filled with an intracellular pipette medium, containing 140 mM CsCl, 1 mM MgCl₂, 1 mM CaCl₂, 11 mM EGTA, 4 mM ATP, and 10 mM HEPES. pH was set to 7.2 with 1 M Tris-OH. Drugs were diluted in purified water or dimethyl sulfoxide in concentrations that did not exceed 0.08%.

Electrophysiological responses were quantified by calculating the mean current amplitudes induced by the drugs using ACCESS software (Microsoft, Redmond, WA). Concentration-dependence curve calculations used the least-square fitting routine of the Origin software (Origin LabCorp, Northampton, MA). These curves were fitted by using the single site equation: $y = 100 \cdot \frac{C}{C+IC_{50}}$ for agonist effects and $y = 100 \cdot \frac{\text{Max} \cdot C}{(C+C+IC_{50})}$ for SSR591813 antagonist effects. C is the concentration of the tested compound, n the Hill coefficient, and Max the maximal inhibition. Parameters providing the best fit are given with a 95% confidence interval.

**Brain Microdialysis**

Rats were anesthetized with chloral hydrate (0.4 g/kg i.p.) and then mounted in a stereotaxic frame. The microdialysis probes through a guide cannula were implanted in the shell of nucleus accumbens (coordinates: AP, +1.6 mm; L, +0.8 mm versus bregma and 7.5 mm below the dorsal surface) according to the atlas of Paxinos and Watson (1986). Twenty-four hours later the microdialysis probes (CMA/11, length 2 mm and outer diameter 0.24 mm; Phymep, Châtillon, France) were perfused at a constant flow rate of 2 μl/min with cerebrospinal fluid containing 4 mM KCl, 147 mM NaCl, 1.2 mM CaCl₂, and 1.0 mM MgCl₂. A sampling time of 20 min was adopted and 40-μl samples were collected with 10 μl of 1 N HClO₄. The animals were left for at least 3 h to allow the system to equilibrate. Dopamine levels were assayed by high-pressure liquid chromatography with electrochemical detection as described previously (Curet et al., 1996). Briefly, the mobile phase, perfused at 0.8 ml/min, contained 0.1 sodium dihydrogen phosphate, 1 mM EDTA, and 1.5 mM 1-octanesulfonic acid sodium salt in 8% acetonitrile, pH 3.5. Electrochemical detection was performed at a potential of 0.65 V.
with the current gain at 0.5 nA. Time-course effects of nicotine (s.c.) and SSR591813 (i.p.) were evaluated on dopamine levels in serial perfusates expressed as a percentage of the mean value of the 60-min baseline measurements before drug or vehicle administration. Data were analyzed by a two-way ANOVA with repeated measures on factor time, followed by Dunnett’s test comparisons for each time. When combined with nicotine, SSR591813 was administered 1 h before nicotine. Antagonism of nicotine effects by SSR591813 was evaluated by comparing the area under the curve during the 200 min after the challenge injection of nicotine or vehicle. Statistical analysis was carried out by one-way ANOVA followed by Duncan’s multiple range test.

Nicotine and d-Amphetamine Discriminations

Rats were trained to discriminate nicotine (0.4 mg/kg i.p.) or d-amphetamine (0.5 mg/kg i.p.) from saline using a standard, two-lever fixed ratio 10 (FR10), food-rewarded operant procedure. Thus, rats obtained a food pellet (45 mg, formula P; Noyes, Lancaster, NH) each time they pressed 10 times on the appropriate lever in the two-lever operant test chamber (MED Associates, Georgia, VT). Sessions started with the two levers expanded and the house light on. Responses on one lever were rewarded in sessions that followed drug injection, and responses on the other lever were rewarded during the session after saline injection (see Cohen et al., 1997 for further details on the procedure). Daily sessions lasted 15 min. The training procedure was continued until the following criterion was met for a period of 10 successive days: the total number of responses on both levers before the first reinforcement was less than 15. When animals had acquired the discrimination, substitution and antagonism tests were carried out. Rats chosen for the antagonism tests were those that selected the drug lever after nicotine administration. During these tests, the rat was placed in the test chamber at the appropriate time after injection and was reinforced after the first ratio of 10 responses had been completed on either lever. For the reminder of the session, responding on the lever on which the first 10 responses had occurred continued to be reinforced according to the FR10 schedule, responses on the other lever were not reinforced. Switching the lever did not reset FR requirements. All injections were given i.p. 30 min before the start of the sessions except for nicotine and cocaine (i.p., 15 min), amisulpride (i.p., 60 min), ephedrine (s.c., 10 min), DHβE (s.c., 15 min), and mecamylamine and SCH23390 (s.c., 30 min). Drug doses were administered in a mixed order, with at least two training sessions between doses. Results are expressed as the percentage of rats choosing the drug-associated lever and the rate of responding expressed as a percentage of the rate on the preceding saline sessions. SSR591813 effects on rates of lever pressing were analyzed statistically using Friedman analyses of variance followed by Wilcoxon matched pairs, signed-ranks tests. The ED50 discrimination is the dose at which 50% of the rats responded on the drug-associated lever.

Nicotine—Mecamylamine Discrimination

Drug discrimination has been used to study drug dependence. For example, an opioid antagonist has been established as a discrimination stimulus in rats treated 4 h before with a single dose of an opioid agonist (Easterling and Holtzman, 1999). In this paradigm, rats do not discriminate the saline mixture but only opioid withdrawal precipitated by the antagonist. Such acute agonist-induced sensitization to effect of an antagonist has been viewed as evidence of a state of acute dependence. This methodology, validated for opioid and benzodiazepine dependence (Easterling and Holtzman, 1999; McMahon and France, 2003), was applied to nicotine dependence. Thus, rats were trained to discriminate mecamylamine (3 mg/kg s.c., 15 min before testing) after a single administration of nicotine (1 mg/kg s.c., 120 min before testing) further referred as nicotine—mecamylamine, from two injections of saline (saline—saline). Training and testing procedures were identical to those used for nicotine and d-amphetamine discriminations. After training, several doses of mecamylamine (s.c., 15 min before testing) or SSR591813 (i.p., 15 min before testing) were administered in saline- and nicotine-treated rats (1 mg/kg s.c., 120 min before testing). SSR591813 (i.p.) was also injected 30 min before mecamylamine in nicotine-treated rats. Results are expressed as the percentage of rats choosing the nicotine—mecamylamine-associated lever and the rate of responding expressed as a percentage of the rate on the preceding saline sessions. Drug effects on rates of lever pressing were analyzed statistically using Friedman analyses of variance followed by Wilcoxon matched pairs, signed-ranks tests.

Nicotine Withdrawal Signs

The procedure was based on that described previously (Malin, 2001). Rats were anesthetized with a mixture of diazepam (3.3 mg/kg i.p) and ketamine (70 mg/kg ip) and implanted with Alzet osmotic minipumps (model 2001, 7 day; Alza, Palo Alto, CA) placed subcutaneously on the back of the animal parallel to the spine. Pumps were filled with either saline or nicotine in saline resulting in a daily dose of 3 mg/kg. Behavioral observations were performed on day 7 of chronic infusion. The experimenter was unaware of drug treatment. All rats received three injections: SSR591813 (10 mg/kg i.p.) or saline (i.p.), and 30 min later, SSR591813 (10 mg/kg i.p.) or saline (i.p.), immediately followed by nicotine infusion (0.03 mg/kg s.c.). Thus, there were five treatment groups (n = 8): 1) saline-infused rats challenged with saline; 2) nicotine-infused rats challenged with saline; 3) and 4) nicotine-infused rats challenged with either mecamylamine (group 3) or SSR591813 (group 4) immediately before observation; and 5) nicotine-infused rats pretreated with SSR591813 30 min before a mecamylamine challenge. Each rat was placed in a plastic cylindrical container (30 cm in diameter × 30 cm in height) for a 30-min period of observation. The occurrence of the following withdrawal signs was recorded: chews/teeth chatter/yawns, shakes/tremors, abdominal writhe/gasps, ptosis, and genital licks. Ptosis was scored from 0.25 (a quarter-closed eye) to 1 (a closed eye) and was counted once per minute. Data were analyzed statistically by the Kruskal-Wallis test.

Nicotine Self-Administration

Acquisition of self-administration was performed as described previously (Cohen et al., 2002). Rats were selected on their locomotor response to a stimulant dose of nicotine (0.6 mg/kg s.c.; see the “Behavioral Sensitization” for details). Our criterion (150 photocell interruptions during the exploration period and an increase of at least 80 photocell interruptions after nicotine administration) permits a selection of about 50% of the animals. They were trained to press the left lever in standard two-lever operant test chambers on a FR5 schedule of food reinforcement, in 30-min sessions. After acquiring the operant behavior, animals were anesthetized with a mixture of diazepam (3.3 mg/kg i.p) and ketamine (70 mg/kg ip) and implanted with a chronic silastic catheter in the right jugular vein. Catheter patency was maintained by flushing with heparinized saline (30 U/ml) and streptokinase (1000 U/ml) before and after each self-administration session. Five days after surgery, rats were trained in 1-h self-administration sessions. As soon as the rat was put in the experimental cage, the session started and the fan turned on automatically. During sessions, there was no light in the experimental boxes except for a red house light. Responding on the left lever (active lever) was reinforced with nicotine (0.03 mg/kg/infusion) delivered in a volume of 0.018 ml in 1 s (MED Associates pump, model PHM100), whereas responding on the right lever had no consequence. Each infusion was followed by a 19-s time-out period during which responding was counted but not reinforced. No stimuli were associated with infusions and time-out periods were not signaled. Over a period of 2 weeks, the response requirement was progressively increased to a FR5. However, because the FR4 schedule elicited the best discrimination between active and inactive le-
vers, it was chosen for drug testing. Rats were thus retrained on a FR4 schedule. When rats had acquired nicotine self-administration, according to a standard criterion of a minimum of 20 active lever responses, for the majority of days on a FR4, drug testing was started. SSR591813 at doses of 10 mg/kg and 20 mg/kg (n = 15) was injected i.p. 15 min before the session. Doses of SSR591813 were administered in a mixed order. Each dose was administered on three consecutive days with 1 week between the two doses. Data were analyzed statistically by ANOVA for repeated measures for the dose factor and the day factor. Because they did not have a normal distribution, data were transformed using square root transformation.

Behavioral Sensitization

Locomotor activity was assessed in individual photocell activity cages (39 x 39 x 16.5 cm high). Rats were placed in the activity cages for a habituation period of 60 min before drug administration. This procedure reduced basal level of activity to make the test more suitable to demonstrate nicotinic agonist-induced hyperactivity. Doses and time periods in activity cages were chosen on the basis of preliminary experiments that have indicated that nicotine (0.8 mg/kg s.c. and p.o.) slightly increased locomotor activity from 20 to 60 min after injection, in rats habituated to the test apparatus. In the first two experiments, we were interested by the acute effects of SSR591813, a 60-min period was thus used. In the third experiment, the acute effects of nicotine were evaluated, a 20-min period was therefore chosen. SSR591813 was administered p.o. for repeated treatment and s.c. for acute treatment.

Experiment 1. Effect of a Repeated Treatment with SSR591813 on the Locomotor Response to the Drug. Rats were treated with saline or SSR591813 (30 mg/kg p.o., twice a day) for 4 days. Twenty-four hours later, rats were challenged with either saline or SSR591813 (30 mg/kg p.o.), and locomotor activity was measured during 60 min after drug injection.

Experiment 2. Effect of a Repeated Treatment with Nicotine on the Locomotor Response to SSR591813. Rats were treated with saline or nicotine (0.6 mg/kg s.c., once a day) for 4 days. Twenty-four hours later, rats were challenged with either saline or SSR591813 (3, 10, 30 mg/kg s.c.) or nicotine (0.8 mg/kg s.c.), and locomotor activity was measured during 60 min after drug injection.

Experiment 3. Effect of a Repeated Treatment with SSR591813 Alone and in Combination with Nicotine on the Locomotor Response to Nicotine. Rats were treated with saline or SSR591813 (30 mg/kg p.o.) or nicotine (0.6 mg/kg s.c.) or a combination of nicotine and SSR591813 (10–30 mg/kg p.o.) for 4 days, once a day. Twenty-four hours later, rats were challenged with either saline or nicotine (0.8 mg/kg s.c.), and locomotor activity was measured during 20 min after drug injection. Data were analyzed using one-way or two-way ANOVAs followed by Dunnett’s or Newman-Keuls test.

General Pharmacology

Body Temperature. Potential effects of SSR591813 (3, 10, 30, 100 mg/kg p.o.) on body temperature were studied in mice (n = 8/group). Rectal temperature was determined with a thermistor rectal probe and a digital thermometer Thermalert TH-5 Sensortek (Phymep) just before and 30, 60, 90 and 120 min after drug administration.

Arterial Blood Pressure and Heart Rate in Awake Normotensive Rats. Rats were anesthetized using ketamine (116 mg/kg i.p.). A catheter filled with anticoagulant (povidone) solution was inserted into the femoral artery, passed under the skin, and exteriorized on the first 2 h, and then every 30 min.

Results

Radioligand Binding Studies

SSR591813 displayed specific [3H]cytisine binding to rat brain α4β2 nicotinic receptors with a Kᵢ value of 107 ± 26 nM (n = 3). In contrast, SSR591813 was devoid of affinity for the rat α1 nACHr subtype labeled with [3H]-bungarotoxin (Kᵢ > 6000 nM; n = 2).

The affinity of SSR591813 for the human α4β2 nACHr stably expressed in HEK293 cell line is comparable with that found in rat brain with a Kᵢ value of 36 ± 5 nM (n = 3; Table 1). SSR591813 showed higher affinity for human αβ4 nACHRs than for other human nACHRs: αβ4 (Kᵢ = 116 ± 13 nM; n = 3; 3-fold), αβ3 (Kᵢ = 1791 ± 115 nM; n = 4; 50-fold), and αβ3γδ (Kᵢ > 6000 NM; more than 167-fold).

SSR591813 was devoid of activity (inhibition lower than 50%) at 10 μM in 73 receptor, enzyme, and uptake binding assays (data not shown). Comparatively weak affinities were detected at the muscarinic M1, M2, M3, M4, M5, and serotonin 5HT3 subtypes and these were reexamined to derive Kᵢ values (> 1000 nM).

Functional Characterization

Human α4β2 nACHRs Expressed in Oocytes. In this preparation, SSR591813 behaved as a partial agonist with an intrinsic activity (IA) of 19 ± 3% versus DMPP (100 μM), and an EC50 value of 1.3 ± 0.6 μM (Fig. 2A; Table 2). Data for DMPP were EC50 = 2.4 ± 0.2 μM and IA = 100%; and for epibatidine were EC50 = 0.019 ± 0.0006 μM and IA = 100%. The current induced by 10 μM SSR591813 was fully antagonized by 3 μM DHβE (Fig. 2B). As expected for a compound with high affinity and low efficacy for α4β2 nACHRs, SSR591813 displayed clear antagonistic effects when applied concomitantly with DMPP, with a maximal inhibitory effect of 53 ± 2% and an IC50 value of 0.52 ± 0.08 μM (Fig. 2C).

Human αβ4 nACHRs Expressed in HEK293 Cell Line. SSR591813 at 100 μM (n = 6) only evoked small inward currents, achieving 7 ± 2% of acetylcholine effects (1 mM). DMPP and nicotine were full agonists, and epibatidine behaved as a partial agonist (Fig. 3A; Table 2).

Human αβ3γδ nACHRs Expressed in IMR-32 Cell Line. In comparison with 1 mM acetylcholine, SSR591813 was ineffective up to the highest concentration tested (100 μM; n = 3), epibatidine was a potent but partial agonist, and nicotine and DMPP behaved as full agonists (Fig. 3B; Table 2).

Table 1

<table>
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<tr>
<th>Compound</th>
<th>nACHR subtype</th>
<th>Kᵢ (μM)</th>
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<tr>
<td>SSR591813</td>
<td>α4β2</td>
<td>36 ± 5</td>
</tr>
<tr>
<td></td>
<td>αβ4</td>
<td>116 ± 13</td>
</tr>
<tr>
<td></td>
<td>αβ3γδ</td>
<td>1791 ± 115</td>
</tr>
<tr>
<td>Nicotine</td>
<td>α4β2</td>
<td>&gt;6000</td>
</tr>
<tr>
<td></td>
<td>αβ4</td>
<td>&gt;6000</td>
</tr>
</tbody>
</table>
Increases in extracellular dopamine levels in the shell of the nucleus accumbens induced by SSR591813 and nicotine are shown in Fig. 4A (basal levels in picograms per 40 μl: 2.98 ± 0.14 for vehicle, 3.20 ± 0.10 for nicotine 0.2 mg/kg, 3.71 ± 0.23 and 2.23 ± 0.14 for SSR591813 10 and 30 mg/kg, respectively). ANOVA indicates a significant drug treatment effect [F(3,160) = 8.04, p < 0.01]. A low dose of nicotine (0.2 mg/kg s.c.) increased dopamine levels from 20 to 200 min after administration, with a maximal effect between 40 and 100 min (95%). SSR591813 (10 and 30 mg/kg i.p.) also produced an increase in dopamine levels (40% and 59% at 20 min, respectively). This effect was significant at 20 and 40 min after administration of the highest dose. The magnitude of the SSR591813 effect was approximately 2-fold less than that of nicotine. Pretreatment with SSR591813 (10 mg/kg i.p.) 1 h before nicotine (0.2 mg/kg s.c.) almost completely blocked the effect of nicotine [F(3,16) = 12.13, p < 0.01] (Fig. 4B). The effect of the combination was not different from that of SSR591813 alone.

### Brain Microdialysis

Intracerebral dopamine levels in the shell of the nucleus accumbens induced by SSR591813 and nicotine are shown in Fig. 4A (basal levels in picograms per 40 μl: 2.98 ± 0.14 for vehicle, 3.20 ± 0.10 for nicotine 0.2 mg/kg, 3.71 ± 0.23 and 2.23 ± 0.14 for SSR591813 10 and 30 mg/kg, respectively). ANOVA indicates a significant drug treatment effect [F(3,160) = 8.04, p < 0.01]. A low dose of nicotine (0.2 mg/kg s.c.) increased dopamine levels from 20 to 200 min after administration, with a maximal effect between 40 and 100 min (95%). SSR591813 (10 and 30 mg/kg i.p.) also produced an increase in dopamine levels (40% and 59% at 20 min, respectively). This effect was significant at 20 and 40 min after administration of the highest dose. The magnitude of the SSR591813 effect was approximately 2-fold less than that of nicotine. Pretreatment with SSR591813 (10 mg/kg i.p.) 1 h before nicotine (0.2 mg/kg s.c.) almost completely blocked the effect of nicotine [F(3,16) = 12.13, p < 0.01] (Fig. 4B). The effect of the combination was not different from that of SSR591813 alone.

### Nicotine Discrimination

In rats trained to discriminate nicotine from saline (Fig. 5A), SSR591813 at 10 mg/kg produced 43% substitution for nicotine. After administration of a higher dose of SSR591813 (20 mg/kg), four of eight rats did not emit enough presses to...
select a lever. Friedman analysis of variance showed a significant SSR591813 effect on response rate ($F_{11.1} = 11.1; p < 0.05$). However, further analyses indicated that none of the doses tested significantly decreased rates of lever pressing compared with control values ($% \text{control value} \pm \text{S.E.M.} : 109 \pm 11, 108 \pm 11, 84 \pm 15, 20 \pm 13$ at 1, 3, 10, and 20 mg/kg, respectively). The nAChR agonists nicotine epibatidine, and ABT 594, produced more than 80% responding on the nicotine-associated lever. Pretreatment with SSR591813 partially antagonized the discriminative effects of the training dose of nicotine (43% inhibition at 10 mg/kg) (Fig. 5B). The effects of SSR591813 at a dose of 20 mg/kg in combination with nicotine could not be evaluated because rats did not emit enough presses ($% \text{control value} \pm \text{S.E.M.} : 113 \pm 11$ after nicotine alone; $117 \pm 12, 65 \pm 15$, and $5 \pm 5$ after nicotine plus SSR591813 at 3, 10, and 20 mg/kg, respectively). Friedman analysis of variance showed a significant drug effect on response rate ($F_{15.6} = 14.8; p < 0.01$). Further analyses indicated that the dose of 20 mg/kg significantly decreased rates of lever pressing compared with control values ($% \text{control value} \pm \text{S.E.M.} : 107 \pm 11$ and $45 \pm 13$ at 10 and 20 mg/kg, respectively). Pretreatment with SSR591813 partially antagonized the substitution of nicotine for $d$-amphetamine (50% inhibition at 10 mg/kg) (Fig. 6B). Friedman analysis of variance showed a significant drug effect on response rate ($F_{10.6} = 10.6; p < 0.05$). However, further analyses indicated that none of SSR591813 doses tested significantly decreased rates of lever pressing compared with control values or nicotine values. Pretreatment with mecamylamine or the selective $\alpha_2 \beta_2$ nAChR antagonist DHβE completely antagonized the nicotine cue, whereas the $\alpha_2$ nAChR antagonist methyllycaconitine produced less than 20% inhibition.

**d-Amphetamine Discrimination**

In rats trained to discriminate between $d$-amphetamine and saline (Fig. 6A), SSR591813 (20 mg/kg) produced 57% substitution for $d$-amphetamine, whereas nicotine, epibatidine, and ABT 594 produced more than 80% substitution. Friedman analysis of variance showed a significant SSR591813 effect on response rate ($F_{14.8} = 14.8; p < 0.01$). Further analyses indicated that the dose of 20 mg/kg significantly decreased rates of lever pressing compared with control values ($% \text{control value} \pm \text{S.E.M.} : 107 \pm 11$ and $45 \pm 13$ at 10 and 20 mg/kg, respectively). Pretreatment with SSR591813 partially antagonized the substitution of nicotine for $d$-amphetamine (50% inhibition at 10 mg/kg) (Fig. 6B). Friedman analysis of variance showed a significant drug effect on response rate ($F_{10.6} = 10.6; p < 0.05$). However, further analyses indicated that none of SSR591813 doses tested significantly decreased rates of lever pressing compared with control values or nicotine values. Pretreatment
with mecamylamine or DHβE fully antagonized the substitution of nicotine for amphetamine, whereas pretreatment with methyllycaconitine produced 60% inhibition.

The role of dopaminergic transmission was investigated in nicotine and d-amphetamine discriminations (Table 3). d-Amphetamine and bupropion fully substituted for d-amphetamine, whereas they partially substituted for nicotine. Quinpirole and SKF81297 fully substituted for d-amphetamine but did not produce more than 45% substitution for nicotine. SCH23390 and amisulpride blocked more potently the stimulus cue in the d-amphetamine discrimination than in the nicotine discrimination.

Nicotine—Mecamylamine Discrimination

Rats successfully learned to discriminate mecamylamine (3 mg/kg s.c. 15 min before testing) after administration of nicotine (1 mg/kg s.c. 120 min before testing) from saline. They reached the discrimination testing criterion in an average of 63 ± 6 training sessions. Administered alone, mecamylamine (1–6 mg/kg s.c. 15 min before testing) substituted for the nicotine—mecamylamine cue at 6 mg/kg, and pretreatment with nicotine (1 mg/kg s.c. 120 min before test-
ing) shifted the dose-response curve to the left (Fig. 7A). Statistical analysis of rates of responding indicated that mecamylamine up to the dose of 3 mg/kg did not significantly decreased rates of lever pressing compared with control values in rats pretreated with saline or with nicotine. The dose of 6 mg/kg significantly decreased response rates in saline pretreated rats (Fr = 11.5; p < 0.05). This dose was not tested in nicotine pretreated rats. Administered alone, SSR591813 (3–20 mg/kg i.p. 15 min before testing) produced 43% substitution at 20 mg/kg, and pretreatment with nicotine did not shift the dose-response curve (Fig. 7B). Statistical analysis of rates of responding indicated that SSR591813 up to the dose of 20 mg/kg did not significantly decreased rates of lever pressing compared with control values in rats pretreated with saline or with nicotine (Fr = 16.8; N.S.). Moreover, SSR591813 (3–20 mg/kg i.p. 30 min before mecamylamine in nicotine-treated rats) decreased the nicotine—mecamylamine discriminative stimulus effects (Fig. 7C). Statistical analysis of rates of responding indicated a significant effect of drug treatment (T = 28.6; p < 0.01). The injection of nicotine followed by mecamylamine nonsignificantly decreased rates of lever pressing compared with control values; SSR591813 at 6, 10, and 20 mg/kg further reduced rates of responding (p < 0.05).

Nicotine Withdrawal Signs

Overall, withdrawal signs in saline and nicotine infused rats after administration of mecamylamine (1 mg/kg), SSR591813 (10 mg/kg), or a combination of both drugs as shown in Fig. 8. Kruskal-Wallis test indicated a significant group effect (T = 9.91; p < 0.05). Nicotine-infused rats challenged with mecamylamine, but not with SSR591813, displayed more withdrawal signs than nicotine-infused rats challenged with saline (p < 0.05). Ptosis, chews, and gasps/writhes were the withdrawal signs the most frequently observed. Acute pretreatment with SSR591813 prevented the occurrence of withdrawal signs precipitated by mecamylamine.

Nicotine Self-Administration

The effects of SSR591813 on nicotine self-administration on a FR4 schedule of reinforcement are shown in Fig. 9. During control 1-h sessions, rats obtained about seven nicotine injections; the number of lever presses on the active lever was more than 4-fold higher than the number of nicotine injections, indicating that rats emitted responses during time-out periods. SSR591813 at a dose of 20 mg/kg i.p. markedly decreased the number of nicotine infusions [F(3,42) = 3.49; p < 0.05], the number of presses on the active lever [F(3,42) = 3.09; p < 0.05], and the number of presses on the inactive lever [F(3,42) = 5.35; p < 0.01]. Differences from baseline were statistically significant on the 2nd and 3rd days of administration for the number of nicotine infusions and the number of presses on the active lever (p < 0.05), and on the 2nd day for the number of presses on the inactive lever (p < 0.01).

Behavioral Sensitization

Effect of a repeated treatment with SSR591813 on the locomotor response to the drug (Fig. 10A). SSR591813 (30 mg/kg p.o.) increased locomotor activity in saline- and in SSR591813-treated (30 mg/kg p.o. twice daily for 4 days)
Repeated administration of SSR591813 did not produce sensitization to the hyperlocomotor activity of the drug. Effect of a Repeated Treatment with Nicotine on the Locomotor Response to SSR591813 (Fig. 10B) 

Repeated administration of nicotine (0.6 mg/kg s.c., once a day for 4 days) did not change locomotor activity response to SSR591813 (3, 10, and 30 mg/kg s.c.), whereas it produced sensitization to the hyperlocomotor activity of nicotine (0.8 mg/kg s.c.).

Effect of a Repeated Treatment with SSR591813 Alone and in Combination with Nicotine on the Locomotor Response to Nicotine (Fig. 11) 

Repeated administration of nicotine (0.6 mg/kg s.c., once a day for 4 days) produced sensitization to the hyperlocomotor activity of nicotine (0.8 mg/kg s.c.). In contrast, repeated administration of SSR591813 (30 mg/kg p.o. once a day for 4 days) did not produce sensitization to the hyperlocomotor activity of the drug.

TABLE 3
Effects of various drugs affecting dopaminergic transmission in rats trained to discriminate nicotine or \( d \)-amphetamine from saline (\( n = 6–15 \) rats)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Receptor/Site Activity</th>
<th>nicotine Discrimination</th>
<th>( d )-amphetamine Discrimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substitution for the training drug: ED(_{50}) mg/kg (% maximal substitution)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( d )-Amphetamine</td>
<td>DA release</td>
<td>1</td>
<td>0.18</td>
</tr>
<tr>
<td>Buproprion</td>
<td>DA/NE uptake inhibitor</td>
<td>50</td>
<td>3.5</td>
</tr>
<tr>
<td>SKF81297</td>
<td>DA ( D_1 ) agonist</td>
<td>&gt;3</td>
<td>1.6</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>DA ( D_2/D_3 ) agonist</td>
<td>&gt;0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Antagonism of the nicotine substitution: ED(_{50}) mg/kg (% maximal inhibition)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amisulpride</td>
<td>DA ( D_2/D_3 ) antagonist</td>
<td>&gt;30</td>
<td>6.40</td>
</tr>
<tr>
<td>SCH23390</td>
<td>DA ( D_1 ) antagonist</td>
<td>0.02</td>
<td>0.008</td>
</tr>
</tbody>
</table>

DA, dopamine; NE, norepinephrine.
days) did not produce sensitization to the nicotine response. Moreover, SSR591813 (10–30 mg/kg p.o.) coadministered with nicotine (0.6 mg/kg s.c., 120 min) blocked the development of behavioral sensitization to the hyperlocomotor activity of nicotine \( F(7,76) = 15.20; p < 0.01 \).

**General Pharmacology**

**Body Temperature.** SSR591813 up to 100 mg/kg p.o. did not induce hypothermia in mice.

**Cardiovascular Effects in Awake Normotensive Rats.** Neither heart rate nor blood pressure was modified by SSR591813 at any of the doses tested (10–100 mg/kg p.o.) compared with rats receiving vehicle.

**Discussion**

The present study investigated the pharmacological properties of the novel selective and partial \( \alpha_3 \beta_2 \) nAChR agonist SSR591813. The drug displays high affinity for the neuronal rat and human \( \alpha_3 \beta_2 \) nAChR subtype \( (K_i = 107 \text{ and } 36 \text{ nM}, \text{respectively}) \), whereas it has weaker affinity for other nAChR subtypes (selectivity ratio 3–160-fold). In addition, SSR591813 has negligible affinity for more than 70 neurotransmitter receptors, enzymes, and transmitter uptake sites.

Electrophysiological experiments at human \( \alpha_3 \beta_2 \) nAChRs expressed in *Xenopus* oocytes show that SSR591813 is a partial agonist \( (E_{\text{max}} = 19\%) \) with an \( EC_{50} \) value of 1.3 \( \mu \text{M} \). Given the affinity measured in binding experiments (36 nM), one would have expected a lower \( EC_{50} \) value. It is, however, a common observation that the oocyte expression system is at least 1 order of magnitude less sensitive than recombinant mammalian systems. In addition, functional experiments always show a shift in \( EC_{50} \) versus \( K_i \) values, probably because of the desensitization state of the channels in binding experiments (Weber et al., 1975). The partial agonist profile of SSR591813 is further demonstrated by its partial (53%) inhibition of the DMPP response. The absence of functional effects at the other nAChR subtypes tested confirms the selectivity of SSR591813 for the \( \alpha_3 \beta_2 \) nAChR.

nAChRs have a modulatory role on several neurotransmitter systems. An increase in mesolimbic dopaminergic transmission has been implicated in the mechanisms of nicotine addiction (Di Chiara, 2000). SSR591813 increased extracellular dopamine levels in the shell of the nucleus accumbens, but its efficacy was notably lower than that of nicotine. In
addition, it reduced nicotine-induced dopamine release in this brain region. These in vivo results support the partial agonist profile of SSR591813 demonstrated in in vitro experiments. Several studies have implicated the $\alpha_4$, $\alpha_6$, $\alpha_7$, and $\beta_2$ subunits in the nAChR-mediated control of the release of dopamine (Wonnacott et al., 1990). However, the $\beta_2$ subunit seems to play a key role as indicated by the failure of nicotine to elicit dopamine release in $\beta_2$ knockout mice (Picciotto et al., 1998). Both the selective $\alpha_4\beta_2$ antagonist DH$\beta$E and the $\alpha_7$ antagonist methyllycaconitine block nicotine-induced dopamine release (Sacaan et al., 1995; Fu et al., 2000). The effects of SSR591813 on dopamine function could be mediated by $\alpha_4\beta_2$ nAChRs located on dopamine-containing neurons of the ventral tegmental area and on terminal fields of those neurons in the nucleus accumbens (Wonnacott et al., 1990; Fu et al., 2000).

As described previously, nicotine produced discriminative stimulus effects and some cross-generalization with $d$-amphetamine (Brioni et al., 1997; Stolerman et al., 1997; Cohen et al., 2002). In contrast to the full agonists epibatidine and ABT 594, SSR591813 displayed a partial agonist profile in the nicotine and $d$-amphetamine discriminations, because it partially substituted for both drugs and partially antagonized the nicotine discriminative effects. The finding that the selective $\alpha_4\beta_2$ antagonist DH$\beta$E blocked the effects of nicotine in both discriminations suggests that the $\alpha_4\beta_2$ nAChR subtype mediates the discriminative stimulus effects of nicotine. In contrast, methyllycaconitine reduced the nicotine cue in the $d$-amphetamine discrimination only. Results with drugs affecting dopaminergic transmission indicate that nicotine substitution for $d$-amphetamine is linked to elevated dopaminergic transmission, whereas dopaminergic transmission plays a minor role in the nicotine discrimination (for further discussion, see Gasior et al., 1999; Young and Glennon, 2002). These findings are consistent with previous observations that the dopamine-releasing and -reinforcing effects of nicotine are correlated with its discriminative properties in the $d$-amphetamine but not in the nicotine discrimination (Cohen et al., 2002). The finding that SSR591813 displays partial agonist activity in both discriminations, and particularly in the $d$-amphetamine discrimination, is consistent with its in vitro profile and its in vivo activity on dopamine release.

The self-administration paradigm has been used as a model to predict the therapeutic efficacy of compounds in the treatment of drug addiction and particularly, to assess effects on craving and reinforcing properties. Like other drugs of abuse, nicotine has been shown to sustain i.v. self-administration in animals (Corrigall and Coen, 1989). In the present study, SSR591813 reduced the number of responses on the active lever and the number of nicotine infusions on the 2nd and 3rd days of treatment. It is unlikely that these effects can be attributed to motor impairment induced by SSR591813. The drug inconsistently reduced responses on the inactive lever and, at active doses, SSR591813 did not produce any significant stimulant or depressant behavioral effects that may have interfered with the task. Because SSR591813 consistently reduced rates of responding for food in the drug discrimination tests, the specificity of its effect on nicotine self-administration needs to be assessed further. Investigation of SSR591813 effect on a nicotine dose-response curve would also provide valuable information regarding whether SSR591813 substituted for or antagonized the rewarding effects of nicotine. The present results suggesting that the $\alpha_4\beta_2$ subunit mediates the reinforcing and motivational effects of nicotine are consonant with previous studies showing that nicotine self-administration is not longer seen in mice lacking the $\beta_2$ subunit (Picciotto et al., 1998) and is reduced by a pretreatment with DH$\beta$E (Watkins et al., 1999).

As described previously (Malin, 2001), mecamylamine administered to rats chronically exposed to nicotine precipitates the somatic nicotine withdrawal syndrome. SSR591813 did not precipitate withdrawal signs in nicotine-exposed rats but prevented the occurrence of withdrawal signs precipitated by mecamylamine. These results suggest that $\alpha_4\beta_2$ nAChRs play a major role in the nicotine withdrawal syndrome. This is partly confirmed by the findings that DH$\beta$E, but not methyllycaconitine, precipitates withdrawal (Malin, 2001).
To investigate the affective aspects of nicotine withdrawal, we used a model based on reports of acute dependence in humans (Bickel et al., 1988) and validated in rodents for opioid and benzodiazepine withdrawal (McMahon and France, 2003; Easterling and Holtzman, 1999). In humans, administration of naloxone several hours after an acute injection of morphine produces an opiate withdrawal syndrome and in rats, it produces an aversive state that can serve as a discriminative stimulus (“withdrawal” stimulus). In the present study, rats were trained to discriminate the interoceptive stimulus produced by administration of mecamylamine after an acute injection of nicotine. Increased sensitivity to mecamylamine occurred after administration of nicotine, whereas this was not the case for SSR591813. Moreover, SSR591813 partially blocked the nicotine–mecamylamine cue. One mechanism that has been proposed to account for the effects of an antagonist after acute or prolonged treatment with an agonist is a conversion of receptors to a constitutive active state. In such a system, an antagonist might act as an inverse agonist (for discussion, see Easterling and Holtzman, 1999). The present experiments showing that SSR591813 can prevent the occurrence of a somatic and affective nicotine withdrawal syndrome suggest that its intrinsic activity at $\alpha_4\beta_2$ nAChRs is sufficient to restore normal cholinergic functioning.

Repeated exposure of rats to addictive drugs is well known to cause a long-lasting increase in their psychomotor and motivational effects. This process of behavioral and neurochemical sensitizations has been suggested to play a role in the acquisition and maintenance of addiction (Di Chiara, 2000). In contrast to nicotine, SSR591813 did not produce a behavioral sensitization, there was no cross-sensitization between nicotine and SSR591813, and SSR591813 prevented the development of nicotine sensitization. Dani and De Biasi (2001) have hypothesized that nicotine sensitization is due to
an excess excitability of the nicotinic cholinergic systems that develops with chronic nicotine exposure. This does not seem to apply to SSR591813, possibly because of its low intrinsic activity.

The present results indicate that SSR591813 displays partial $\alpha_4\beta_2$ agonist activity in vitro and in vivo assays. It binds to $\alpha_4\beta_2$ nAChRs yet has submaximal capacity to activate these nAChRs. As a result, SSR591813 exhibits either agonist-like or antagonist-like properties. SSR591813 behaves as an agonist with lower efficacy than nicotine as for its capacity to release dopamine and to substitute for nicotine and for d-amphetamine. SSR591813 behaves as an antagonist in the presence of nicotine; it reduces the dopamine-releasing and discriminative stimulus effects of nicotine, as well as the development of sensitization to nicotine.

How relevant is this partial intrinsic activity to SSR591813’s potential therapeutic effects? It has been hypothesized that smoking behavior is maintained by the positive reinforcing effects of nicotine as well as the avoidance of the withdrawal phase characterized by somatic and affective (craving) symptoms (Malin, 2001). Here, we suggest that SSR591813 could be administered during smoking and tobacco abstinence periods to reduce nicotine intake and to prevent the occurrence of withdrawal signs. Moreover, SSR591813 may prevent dysregulation of reinforcement mechanisms by its interaction with mesolimbic dopaminergic neurotransmission. The lack of behavioral sensitization after repeated administration of SSR591813 and its low efficacy to stimulate dopamine release and to substitute for d-amphetamine, compared with nicotine, suggest that this compound should not display dependence and abuse potential. These aspects need to be further assessed. The lack of affinity of SSR591813 for nAChRs found in the peripheral nervous system, in particular the ganglionic $\alpha_3\beta_4$ nAChR subtype, and its lack of unwanted effects in rodents (particularly cardiovascular) generally associated with full nicotinic receptor agonists suggest that SSR591813 will have a low propensity to induce such side effects in humans.

In conclusion, the present study shows that SSR591813 is a functionally selective partial agonist at the $\alpha_4\beta_2$ nAChR subtype. SSR591813 displays antiaddictive-like activity in animal models in the absence of motor effects. SSR591813 represents a promising alternative to agents currently used for the management of nicotine dependence.

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References


