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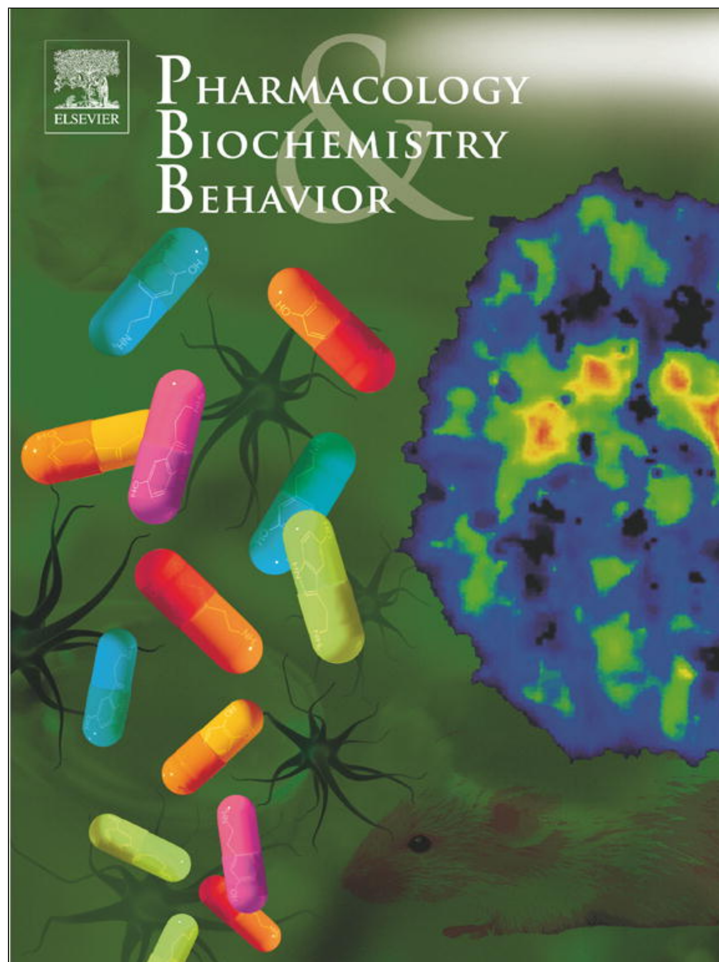

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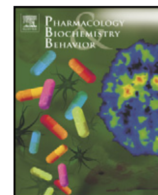

SAR110894, a potent histamine H_3-receptor antagonist, displays procognitive effects in rodents

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A B S T R A C T

SAR110894 is a novel histamine H_3-R ligand, displaying high and selective affinity for human, rat or mouse H_3-Rs. SAR110894 is a potent H_3-R antagonist at native receptors, reversing R-α-methylhistamine-induced inhibition of electrical field stimulation contraction in the guinea-pig ileum. Additionally, SAR110894 inhibited constitutive GTPγS binding at human H_3-Rs demonstrating inverse agonist properties. In behavioral models addressing certain aspects of cognitive impairment associated with schizophrenia (CIAS) and attention deficit/hyperactivity disorder (ADHD), SAR110894 improved memory performances in several variants of the object recognition task in mice (0.3–3 mg/kg, p.o.) or rats (0.3–1 mg/kg, p.o.). Moreover, SAR110894 (1 mg/kg, p.o.) reversed a deficit in working memory in the Y-maze test, following an acute low dose of phencyclidine (PCP) (0.5 mg/kg, i.p.) in mice sensitized by repeated treatment with a high dose of PCP (10 mg/kg, i.p.). In the latent inhibition (LI) model, SAR110894 potentiated LI in saline-treated rats (1 and 3 mg/kg, i.p.) and reversed abnormally persistent LI induced by neonatal nitric oxide synthase (NOS) inhibition in rodents (0.3–3 mg/kg, i.p.). In a social novelty discrimination task in rats, SAR110894 attenuated selective attention deficit induced by neonatal PCP treatment (3 and 10 mg/kg, p.o.) or a parametric modification of the procedure (3 and 10 mg/kg, p.o.). SAR110894 showed efficacy in several animal models related to the cognitive deficits in Alzheimer's disease (AD). It prevented the occurrence of episodic memory deficit induced by scopolamine in rats (0.01–10 mg/kg, p.o.) or by the central infusion of the toxic amyloid fragment β_{25-35} in the object recognition test in mice (1 and 3 mg/kg, p.o.). Altogether, these findings suggest that SAR110894 may be of therapeutic interest for the treatment of the cognitive symptoms of AD, schizophrenia and certain aspects of ADHD.

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1. Introduction

Among the receptors that mediate the action of histamine, the H_3-receptor (H_3-R) has attracted most attention as a possible target for cognitive-enhancing drugs. It exists as a presynaptic autoreceptor that is expressed in high densities in brain regions associated with the control of cognitive functions, such as the hippocampus and the frontal cortex (Arrang et al., 2007; Pillot et al., 2002). There is compelling evidence that H_3-Rs influence learning and memory by modulating the release of acetylcholine (ACh) (Blandina and Passani, 2006; Passani et al., 2000, 2004). For example, mutant mice lacking the H_3-R were insensitive to the amnesic effect of the muscarinic receptor antagonist scopolamine in a passive avoidance paradigm (Toyota et al., 2002), and administration of the H_3-R agonists R-α-methylhistamine or imetit reduced cortical ACh release in freely moving rats at the same doses that impaired rat performance in the object recognition and passive avoidance tests (Blandina et al., 1996). H_3-Rs also behave as heteroreceptors and modulate the release of transmitters other than histamine and ACh, such as dopamine, glutamate, noradrenaline and 5-HT, neurotransmitters that have been shown to affect performance in several cognitive tests (Passani et al., 2000).

Based on the high interest level generated by these findings, it was suggested that inhibition of central H_3-Rs may represent an attractive

Abbreviations: ACh, acetylcholine; ADHD, attention deficit/hyperactivity disorder; AD, Alzheimer's disease; CIAS, cognitive impairment associated with schizophrenia; LI, latent inhibition; NOS, nitric oxide synthase; PCP, phencyclidine.

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target for the potential enhancement of cognitive processes in a number of CNS illnesses, an idea which led to the identification of selective H_3-R antagonists with a rich structural diversity. Among the most investigated compounds are pitolisant, PF-03654746, GSK189254, JNJ-17216498 and ABT-0288 (for recent reviews, see Brioni et al., 2011; Chazot, 2010; Raddatz et al., 2010; Stocking and Letavic, 2008). These drugs improved different domains of cognition in preclinical models using normal rodents, such as attention, working memory, spatial memory, and short- and long-term memory (Brioni et al., 2011). In addition to these facilitatory effects on cognitive processes, H_3-R antagonists attenuated scopolamine-induced deficits in cognitive tests in rodents and those observed naturally in the APP_{Tg2576} mouse model of Alzheimer's disease (AD) (Bardgett et al., 2011; Brioni et al., 2011). Finally, H_3-R antagonists alleviated learning and memory deficits induced by the NMDA receptor blocker, MK-801, suggesting that these drugs may also be effective against cognitive impairment associated with schizophrenia (CIAS) (Bardgett et al., 2009, 2010).

In the current series of experiments, we describe the in vitro and in vivo pharmacological properties of SAR110894, a novel, highly potent, and selective H_3-R antagonist, which has an acceptable oral bioavailability and shows large distribution in the rat brain (personal communication). More specifically, the compound was profiled in a variety of cognitive tests that address various aspects of learning and memory deficits observed in AD, attention deficit/hyperactivity disorder (ADHD) and schizophrenia. SAR110894 was evaluated (1) for its potential to antagonize scopolamine, MK-801- or PCP-induced deficits of working and episodic memory (object recognition in mice and rats and Y-maze in mice); (2) for its activity against a deficit of selective attention in a social recognition test in adult rats treated at the neonatal stage with high doses of PCP or subjected to an overload of information (Terranova et al., 2005); (3) for its ability to attenuate the deficit of episodic memory following the central infusion of a synthetic portion (25–35) of the β-amyloid peptide, which has been shown to represent a valid tool to mimic certain aspects of AD in rodents; and (4) for its potential to alleviate abnormally persistent latent inhibition (LI), using 2 variants of the model: one using the administration of MK-801 to adult rats, and the second using the administration of the nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine (L-NoArg), in the neonatal period to mimic the neurodevelopmental aspect of schizophrenia assessed at the adult stage. LI is a cross-species selective attention phenomenon that is disrupted in patients with schizophrenia (Weiner, 1990).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley, Wistar or female Wistar Han rats (Iffa Credo, L'Arbresle; Charles River, Saint-Aubin-lès-Elbeuf, France; and Tel-Aviv University Medical School, Israel), weighing between 150 and 350 g (6 to 10-week-old) at the time of testing, were used in the selective attention deficit model, novelty discrimination, object recognition (ORT) and LI. Rats were housed in groups of 2 to 8 per cage (see below for further details). Moreover, ten-week-old male CD1 mice (Iffa Credo) weighing 17 to 32 g (6 to 10-week-old) at the time of testing were used in the Y-maze and ORT. Mice were housed in groups of 5 or 6. For in vitro experiments, male Wistar rats, CD1 mice and Dunkin Hartley guinea-pigs (Harlan Ibérica, Spain) were used. All animals were kept under standard laboratory conditions of controlled relative humidity and temperature (21 ± 2 °C) with a 12-hr light/dark cycle and light onset at 7:00 AM. Food and water were freely available. The behavioral experiments were performed between 8:00 AM and 15:00 PM. All experimental procedures described herein were approved by the Animal Care and Use Committee of Sanofi or the Institutional Animal Care and Use Committee of Tel Aviv University, Israel. Our animal facilities and animal care and use programs are in accordance with French legislation which implemented the European directive 86/609/EEC.

2.2. Drugs

The drugs tested included atomoxetine, SAR110894 (currently in clinical Phase II trial in patients with AD. No information of the structure or patent can be disclosed), donepezil, glycine, methylphenidate, ABT-0239 and thioperamide (all synthesized by Sanofi medicinal chemistry). The challenge drugs included scopolamine HBr, R-α-methylhistamine (RAMH), MK-801, phencyclidine (PCP) and L-NoArg (Sigma-Aldrich, Saint Quentin Fallavier, France, Sigma, Israel). The drugs were prepared daily using saline (0.9% NaCl) for intraperitoneal (i.p.) and subcutaneous (s.c.) administrations, or distilled water containing methylcellulose (0.6%) with a drop of Tween 80 for oral (p.o.) administration, unless specified otherwise. Volume of administration was 10 or 20 ml/kg in mice, 2 or 5 ml/kg in rats and 0.1 ml in rat pups. For in vitro studies, SAR110894 was solubilized in pure DMSO at 10 mM concentration and then diluted to the final studied concentrations. Final percentage of DMSO was always kept at 1%. The β_{25-35} amyloid peptide fraction ($A\beta_{25-35}$, ref. SC489) and scrambled β_{25-35} peptide (Sc β_{25-35}, ref. SC942) were purchased from NeoMPS (Strasbourg, France). Peptides (1 mg, purity 97%) were dissolved in 305 μl of distilled water, aliquoted into 50 μl samples and stored at −20 °C. For further details on the administration procedures, see below.

2.3. Selectivity and in vitro functional activity at H_3-Rs

2.3.1. In vitro binding studies

2.3.1.1. Preparation of recombinant receptor. Recombinant human H_3- and H_4-Rs were cloned by the Genomic Science Department of Sanofi and expressed in CHO cells for membrane production (long form 445aa). Cells were scrapped out in incubation buffer (20 mM Tris–HCl, 0.5 mM EDTA, and pH 7.4), disrupted using Ultra-Turrax at a maximum of 12,000 ×*g* for 30 s and centrifuged at 4500 ×*g* for 25 min at 4 °C. Cell pellets were re-suspended in 1 ml ice-cold incubation buffer and stored at −80 °C until use.

2.3.1.2. Preparation of native receptors. Human brain tissue was obtained from the BTIN (Banco de Tejidos para Investigaciones Neurológicas) located at the Universidad Complutense de Madrid (UCM). The tissue was a consented grant of a healthy and fully anonymous donor and was previously tested for the absence of hepatotropic or VIH viruses. Frozen human brain was thawed and homogenized with an Ultra-Turrax at 12,000 ×*g* during 30 s in 20 volumes (w/v) of incubation buffer. After a first centrifugation at 1000 ×*g* for 10 min at 4 °C, the resultant supernatant was centrifuged at 45,000 ×*g* for 25 min at 4 °C. The pellet was homogenized in 20 volumes of incubation buffer and re-centrifuged at 45,000 ×*g* for 20 min at 4 °C. The last pellet was homogenized in incubation buffer and aliquots were stored at −80 °C until use. For experiments using animal tissues, rats or mice were sacrificed by decapitation, and the brain without cerebellum was dissected out on ice. Tissues were homogenized with an Ultra-Turrax (12,000 ×*g* during 30 s) in 20 volumes (w/v) of ice-cold Na/K phosphate buffer and membrane pellets were collected as described for human brain tissues.

2.3.1.3. Receptor binding assay conditions. Binding assays for the H_3-R were performed in Tris–HCl incubation buffer either with recombinant human, or native human, rat and mouse brain membranes, using 0.5 nM $[^3H]N\alpha$-methylhistamine (NEN, NET-1027) as a radioligand and challenged with a dose–response of SAR110894. Non-specific binding was determined in the presence of 10 μM thioperamide. The assay was stopped by rapid filtration through Whatman GF/C filters and radioactive signals were measured in a μbeta counter in the presence of

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scintillant reactant. Affinity for the recombinant H_4-R was carried out following the same procedure but using [^{3}H]histamine (40 nM) as radioligand and histamine (10 μM) to determine non specific binding. In figures, symbols are the arithmetic mean ± standard error of the mean of independent experiments. IC_{50} values are the geometric mean of individual curve IC_{50} and their respective 95% CI. Individual Ki curve was calculated by using the formula: ($Ki = IC_{50}/(1 + [\text{radioligand concentration}]/Kd)$) and Ki experiment values were the arithmetic mean and standard error of the mean of independent experiments. Affinities for the recombinant human H_1- and H_2-Rs were obtained from CEREP (Poitiers, France) using membranes from HEK-293 and CHO expressing cells, [^{3}H] pyrilamine (3 nM), [^{125}I]APT (0.2 nM) as radioligand and pyrilamine (1 μM) and tiotidine (100 μM) to define non specific binding, respectively.

2.3.2. Selectivity profile

The selectivity profile of SAR110894 was determined using 90 receptors and 18 enzymes (CEREP, profile customized from catalog proposal). IC_{50} were determined in case of significant activity obtained at 10 μM (≥50% inhibition).

2.3.3. GTPS-binding

Assays were performed at 25 °C in HEPES-buffer on membranes prepared, as described above, from CHO cells stably expressing the human H_3-R and using the commercial TRF Kit from Perkin-Elmer. Membranes were incubated in Tris 50 mM, pH 7 buffer supplemented with 10 μM GDP, 100 nM Europium-labeled GTP in the absence or in the presence of 50 nM of N-α-methylhistamine (RBI) as agonist and different concentrations of SAR110894. Non-specific binding was determined by 50 μM of non-labeled GTPS. The incubation was carried out in Acrowell plate (Pall corporation, Port Washington, NY, USA) allowing a direct filtration at the end of the 60 min incubation period. Trapped fluorescence (Ex 340 nm, Em 615 nm) was determined using a Victor 2 (Perkin-Elmer).

2.3.4. Electrical field stimulated contractions in isolated guinea-pig ileum

Animals were euthanized under CO_2 and exsanguinated. Each isolated ileum was cleaned and cut into 1.5–2 cm segments and the last 5 cm of the terminal portion was systematically rejected. The strips were mounted between 2 platinum electrodes into organ baths at 37 °C and bubbled with O_2/CO_2 (95:5) at a resting tension of 1 g. After a 1 hour equilibration period, the strips were maximally stimulated using repeated pulses of 0.1 Hz frequency, 1 ms duration and 15 V submaximal intensity (Cibertec Stimulator). Cumulative concentration–response curve for inhibition of electrically stimulated contractions was determined for RAMH in the presence and absence of increasing concentrations of SAR110894 or thioperamide 3 nM. For each experiment, EC_{50} values were obtained using the 4-parameter logistic model according to Ratkowsky and Reedy and used to estimate a pKB by using the Furchgott's formula ($pKB = -\log10$ [concentration of the antagonist] $+ \log10 (r-1)$), where r is the concentration ratio.

2.4. Efficacy and potency in animal models of CIAS and ADHD

2.4.1. Effects of SAR110894 on long-term visual episodic memory using the ORT in mice and rats

The test apparatus was based on that described by Ennaceur and Delacour (1988) in rats and adapted for use in mice. The apparatus consisted of a uniformly lit (100 lx for rats and 20 lx for mice) wooden (rats) and PVC (mice) enclosure (65 L×45 W×45 H cm) for rats and (52 L×52 W×40 H cm) for mice with a video camera positioned 160 cm above the bench. The observer was located in an adjacent room fitted with a video monitoring system. The experiment consisted of 3 sessions. During the first session (context habituation), the subjects were allowed 2 (rats) or 5 min (mice) to become acquainted with the apparatus. Time in active locomotion was manually recorded with a precision of ±0.1 s. The animals were again placed in the enclosure 24 h thereafter for the second (acquisition) session, during which they were exposed to a pair of identical objects (either 7 L×3 W×8 H cm metal triangles or 9 L×3 W×7 H cm plastic pyramid (rats), and either 5.5 L×2 L×3.3 H cm gray metal triangle or 3 L×3 W×3 H cm plastic pyramid (mouse)) placed 10 cm away from the 2 opposite corners of the back wall.

Animals were left in the enclosure for the amount of time necessary to spend at most 20 (rats) or 10 (mice) seconds exploring these 2 objects within a 5 (rats) or 7 (mice) min timeframe. Animals were removed from the cage once they had reached the 20 (rats) or 10 (mice)-second exploration time. Exploration of an object was defined as the animal having its head within 2 (rats) or 1 (mice) cm of the object while looking at it, sniffing it or touching it. Any animal spending less than 20 (rats) or 10 (mice) seconds exploring the 2 objects within 5 (rats) or 7 (mice) min was eliminated from the study. Two different sets of objects were used to allow for cleaning between 2 consecutive animals in order to minimize olfactory cueing. Combinations of orders of presentation and locations of objects were counterbalanced to reduce potential biases owing to spatial or object preferences.

During the third (recall) session, animals were exposed to the familiar (i.e. presented during the previous acquisition session) and a novel (i.e. never presented before) object for 3 (rats) and 5 (mice) min, and the time spent exploring each object was recorded. Any animal spending less than 3 s exploring both objects was discarded from the study. This third session took place 24 (rats) or 48 (mice) hours following the second session. At this intersession interval, there was no longer significant discrimination observed between the 2 objects in control and test animals, that is, rats or mice spent equivalent times exploring the novel and the familiar objects. As a consequence, this interval was used to assess the ability of SAR110894 to improve spontaneous long-term visual episodic memory.

Animals were treated with a single p.o. administration of vehicle (methylcellulose 0.6% in distilled water) or SAR110894 one hour prior to habituation, acquisition and recall sessions. In the combination experiment, SAR110894 and RAMH were administered once p.o. and i.p., respectively, one hour before the recall session.

Data (time exploring each of the 2 objects, in seconds) were analyzed for habituation and acquisition sessions, using a one-way ANOVA assessed on the variables "locomotion" and "time" necessary to reach 20-second active exploration for 2 identical objects, respectively. This was followed when appropriate, by a Dunnett's test for comparing SAR110894-treated groups to the vehicle-treated group. For the recall test sessions, the time spent in exploring each of the 2 objects (expressed in seconds) was analyzed with a 2-way ANOVA with repeated measurements on "object" (Novel (N) or Familiar (F)), followed, when appropriate, by a Winer analysis for comparing the time spent in exploring the familiar versus the novel object for each treatment. This analysis, and all subsequent ones were performed using the SAS software (SAS Institute In., Cary, NC, USA).

2.4.2. Effects of SAR110894 on short-term visual episodic memory deficit following the administration of MK-801 using the ORT in rats

The procedure and statistical analysis were similar to that described for rats in the paragraph above, except for the following 2 points: 1) the third (recall) session was performed one hour after the second session: with this inter-session interval, control animals spend more time exploring the novel than the familiar object, indicating that they are able to discriminate between the 2 objects, an index of a preserved episodic memory capacity; 2) SAR110894 was co-administered (p.o.) with MK-801 (i.p.) one hour prior to the recall session. For the statistical analysis, see previous paragraph.

2.4.3. Effects of SAR110894 on a deficit of spatial working memory induced by PCP sensitization using the Y-maze task in mice

The Y-maze consisted of 3 arms in gray in the shape of a Y. Arms were 28 cm long, 6 cm wide with walls 15 cm high. Movement was tracked

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manually using homemade software by an experimenter located in an adjacent room via a camera mounted directly above the maze. The animal was placed in an arm facing the center (Arm A) for 5 min. A correct alternation occurred when the animal moved to the other 2 arms without retracing its steps (i.e. Arm A to B to C). Movements such as ABA were incorrect. Based on the movement over the entire session, the percentage of correct alternations was calculated (i.e. Total number of alternation × 100)/(Total number of arm entries − 2). Behavioral sensitization to PCP consisted of daily injections of a high dose of PCP (10 mg/kg, i.p.) for 5 consecutive days. After a non-drug period of 4 days, animals were challenged by a low dose of PCP (0.5 mg/kg, i.p.) or vehicle (i.p.), administered 30 min prior to testing. SAR110894 was administered p.o. 60 min before a one session Y-maze testing (i.e. 30 min prior to PCP challenge). For the total number of arm entries, statistics consisted of using a 2-way ANOVA with factors "Experiment" and "Group" performed on all groups to ensure that they were comparable. Percentage of alternation was assessed for PCP sensitization using a 2-way ANOVA with factors "Experiment" and "Group" on "Chronic/challenged saline (i.p.) and vehicle (p.o.)", "Chronic saline (i.p.)/challenged PCP (i.p.) and vehicle (p.o.)" and "Chronic PCP/challenged PCP (i.p.)/vehicle (p.o.)" groups. A post-hoc Dunnett's test was used for "Chronic saline (i.p.)/challenged PCP (i.p.) and vehicle (p.o.)" group. Similarly, another 2-way ANOVA on "Chronic PCP/challenged PCP (i.p.) and vehicle (p.o.) or SAR110894-treated" groups, followed by a global Dunnett's test versus "Chronic saline (i.p.)/challenged PCP (i.p.) and vehicle (p.o.)" group was performed.

2.4.4. Effects of SAR110894 on negative/positive symptoms and cognitive domains of schizophrenia using LI in rats

2.4.4.1. Neonatal treatment. Wistar rats were mated at an age of 3 months. At birth, litters were culled to 10, composed of 5 male and 5 female rats whenever possible. The day of birth was defined as postnatal day 0. On postnatal days 3, 4, and 5 rat pups were given a subcutaneous injection in a volume of 1 ml/kg of either 10 mg/kg L-NoArg, a competitive inhibitor of NOS with selectivity for the neuronal and endothelial isoforms of the enzyme (Furfine et al., 1993), or vehicle. L-NoArg was dissolved in 1 N HCl, diluted with 10 mM phosphate-buffered saline and titrated with 2 M Tris 7.5 pH buffer to a final pH of 5.5. On day 21, the pups were weaned and housed 4 to a cage by sex and litter, and maintained undisturbed till 3 months of age. At adulthood, male rats that were treated neonatally with L-NoArg or vehicle were assigned to eight experimental conditions, pre-exposed-vehicle injected, pre-exposed-drug injected, non-pre-exposed-vehicle injected, and non-pre-exposed-drug injected. In each experimental group there was no more than one rat from the same litter.

2.4.4.2. MK-801 treatment. All drugs were administered i.p. MK-801 was diluted in saline and administered at a dose of 0.05 mg/kg (Gaisler-Salomon and Weiner, 2003), in a volume of 1 ml/kg 30 min before conditioning. SAR110894 and the NMDA co-agonist glycine (which was used as a positive control) were dissolved in saline and administered 30 min in a volume of 3 ml/kg prior to pre-exposure and conditioning stages. No-drug controls received the corresponding vehicle.

2.4.4.3. Apparatus and procedure. LI was measured in a thirst motivated conditioned emotional response (CER) procedure by comparing the suppression of drinking to a tone previously paired with a foot shock in rats that received non-reinforced exposure to the tone prior to conditioning (pre-exposed) and in rats for whom the tone was novel (non-pre-exposed). Parameters that did not produce LI in no-drug controls, 40 pre-exposures and 5 conditioning trials, were used, because persistent LI can be manifested only with such parameters.

Rats were tested in Campden Instruments rodent test chambers with a retractable bottle. When the bottle was not present, the hole was covered by a metal lid. Licks were detected by a Campden Instruments drinkometer. The pre-exposed to-be-conditioned stimulus was a 10 s, 80 dB, 2.8 kHz tone produced by a Sonalert module. Shock was supplied through the floor by a Campden Instruments shock generator and shock scrambler set at 0.5 mA and 1 s duration. Equipment programming and data recording were computer controlled.

Prior to the beginning of each LI experiment, rats were handled for about 2 min daily for 5 days. A 23 h water restriction schedule was initiated simultaneously with handling and continued throughout the experiment. On the next 5 days, rats were trained to drink in the experimental chamber for 20 min/day. Water in the test apparatus was given in addition to the daily ration of 1 h given in the home cages. The LI procedure was conducted on days 11–14 and consisted of the following stages:

Pre-exposure: With the bottle removed, the pre-exposed (PE) rats received 40 tone presentations with an inter-stimulus interval of 50 s. The non-pre-exposed (NPE) rats were confined to the chamber for an identical period of time without receiving the tone.

Conditioning: With the bottle removed, each rat received 5 tone-shock pairings (strong conditioning) given 5 min apart. Shock immediately followed tone termination. The first tone-shock pairing was given 5 min after the start of the session. After the last pairing, rats were left in the experimental chamber for an additional 5 min. Strong conditioning was used in experiments (1 and 2) using MK-801 and neonatal NOS inhibition, because this level of conditioning prevents LI in non-treated controls and thus allows the demonstration of treatment-induced abnormally persistent LI.

Re-baseline: Rats were given a 15 min drinking session as in initial training. Data of rats that failed to complete 600 licks were dropped from the analysis.

Test: Each rat was placed in the chamber and allowed to drink from the bottle. When the rat completed 75 licks the tone was presented for 5 min. The following times were recorded: Time to first lick, time to complete licks 1–50, time to complete licks 51–75 (before tone onset) and time to complete licks 76–100 (after tone onset). Times to complete licks 76–100 were logarithmically transformed to allow parametric ANOVA. Longer log times indicate stronger suppression of drinking. LI is defined as significantly shorter log times to complete licks 76–100 of the pre-exposed (PE) as compared to non-pre-exposed (NPE) rats. In addition, number of licks made during the presentation of the tone, was recorded in 5 blocks of 30 s.

2.4.4.4. Experimental design. Experiment 1 tested the effects of SAR110894 on MK-801-induced persistent LI. The experiment included 6 teen experimental groups in a 2 × 2 × 4 design with main factors of pre-exposure (PE, NPE), treatment (vehicle, MK-801), and pre-treatment (1, 3 and 10 mg/kg SAR110894 or 0.8 g/kg glycine). Experiment 2 tested the effects of SAR110894 on neonatal NOS inhibition-induced persistent LI. The experiment included 16 experimental groups in a 2 × 2 × 4 design with main factors of pre-exposure (PE, NPE), neonatal treatment (vehicle, L-NoArg), and adult treatment (1, 3, 10 mg/kg). As both of these experiments used strong conditioning, the effects of SAR110894 on the non-treated controls allowed the demonstration of SAR110894-induced LI potentiation. Consequently, no separate experiments were conducted to measure this index of antipsychotic activity of SAR110894.

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2.4.4.5. Statistical analyses. Times to complete licks 50–75 and mean log times to complete licks 76–100 were analyzed using 3 way ANOVAs with main factors of PE, neonatal treatment and SAR110894. In cases of significant interactions involving the factor of pre-exposure, LSD post-hoc comparisons were used to assess the difference between the PE and NPE groups within each condition.

2.4.5. Effect of SAR110894 on the impairment of novelty discrimination in adult rats

Adult (160–200 g on arrival) and juvenile (3-week-old, 45–50 g on arrival) male Wistar Han rats were housed individually, or 5 per cage, respectively, in 30 × 40 × 18 cm high cages. Juvenile rats were left 5 per cage for 1 week, and were then used for 1 week in experiments (presentation to adult rats). They were used only once a day, and were chosen at random as first or second juvenile for presentation to the adult. For neonatal PCP treatment experiments, female Wistar Han rats with ten male pups on postnatal day 3 (PN3) were used. Pups were treated on days PN7, PN9 and PN11 with 10 mg/kg of PCP (s.c. administration, 1 ml/100 g body weight) or vehicle (saline). Pups from the same litter received identical treatment. The mother and pups were housed together until weaning at PN21, at which stage pups were housed 5 per cage until 2 weeks before the start of behavioral experiments, when they were housed individually. Pups were not used until they reached the adult stage, when they were used for behavioral experiments (between PN 118 and PN 142). For additional details on this procedure, see Terranova et al. (2005).

2.4.5.1. Experiment 1: effects of PCP treatment at the neonatal stage on novelty discrimination in adult rats. Experiments were performed during the dark phase, under infrared illumination (15 lx). Juvenile rats were isolated 30 min before being placed into the home cage of an adult rat. The cage was placed underneath a video camera, the mesh top removed and replaced by a Plexiglas cover. A first (familiar) juvenile was placed inside the home cage containing one adult rat for a period of 30 min. A second (novel) juvenile was introduced at the end of this period. Durations of investigation behavior (nosing, sniffing, grooming, close chase of the juvenile rat) between the adult rat and each of the 2 juveniles were recorded manually for a period of 5 min following the introduction of the novel juvenile, by an observer located in an adjacent room fitted with a video monitor. SAR110894 or its vehicle (NaCl 0.9%) was administered p.o. to the adult rat 60 min before exposure to the first juvenile. Each adult rat was subjected to 5 treatments: one vehicle and 3 doses of SAR110894 with one or 2 days between each treatment.

2.4.5.2. Experiment 2: impairment of novelty discrimination produced by a shortening of the duration of the first presentation and a lengthening of the inter-period interval. The procedure was slightly different from that described for Experiment 1, in the sense that the 2 presentation periods (P1 and P2) were 5 min, and were separated by a 30-min inter-period interval, during which the familiar juvenile was returned to its home cage. This parametric manipulation was implemented to produce an impairment of novelty discrimination by the adult rat during P2. Adult rats were injected p.o. 120 min, i.p. 15 min or i.p. 30 min before the beginning of the test with SAR110894, methylphenidate or atomoxetine respectively.

Data are expressed as the mean of a novelty discrimination index (NDI), which was calculated as the ratio of the time spent investigating the novel juvenile divided by the time spent investigating the familiar juvenile. NDIs were first log-transformed because of the limited number of subjects and the lack of homogeneity of variances between groups. For Experiment 1, statistical analysis was carried out using a 2-way ANOVA, with treatment at the neonatal stage as the between subjects factor, and acute treatment at the adult stage as the within subjects factor, followed by appropriate post-hoc tests. For Experiment 2, statistical analysis used a one-way ANOVA for repeated measures followed by appropriate post-hoc test (Dunnett's test).

2.5. Efficacy and potency in animal models of AD

2.5.1. Effects of SAR110894 on short-term visual episodic memory deficit following the administration of scopolamine using the ORT in rats

The protocol used in this study was identical to that described for MK-801 in the ORT. SAR110894 was administered p.o. 60 min before the acquisition session, while scopolamine (0.3 mg/kg, s.c.) was given 20 min prior to the recall session. For the statistical analysis, see the above ORT experiments paragraph.

2.5.2. Effects of SAR110894 on short-term visual episodic memory deficit following the central infusion of $A\beta_{25-35}$ peptide using the ORT in mice

The peptide solution was incubated at 37 °C for 4 days prior to the administration. 3 μl of the $A\beta_{25-35}$ solution (9 nmol of peptide) was slowly injected into the lateral ventricle of isoflurane-anesthetized mice. Injection was done manually, without the help of a stereotaxic apparatus. Accuracy of the injection was checked in a preliminary experiment using an Indian ink and reached a 95% confidence. The control peptide (scrambled β_{25-35}) consisted of the same sequence of amino acids, but in a random order. It was prepared and administered following the same procedure. Ten days after $A\beta_{25-35}$ administration, animals were tested in the ORT using a slightly different procedure from that described above in mice, in the sense that the acquisition and recall sessions were separated by a 60-min inter-period interval. SAR110894 and donepezil were injected acutely, p.o. or i.p., respectively, 60 or 30 min before the acquisition session.

For the statistical analyses, the scrambled (Sc) β_{25-35} and $A\beta_{25-35}$ groups were compared using a 2-way ANOVA on "Treatment" and "Object" factors with "Object" as repeated factor. The hypothesis of homogeneity of variances being not fulfilled, the analysis was performed on rank data. $A\beta_{25-35}$ control group and treated groups were compared using a 2-way ANOVA on "Treatment" and "Object" factors with "Object" factor as repeated factor. It was followed by a Winer analysis in order to compare the time used to explore the objects according to the treatment. The hypothesis of homogeneity of variances being not fulfilled, the analysis was performed on rank data. When expressed as ratio "new / (familiar + new) × 100", the experiment was validated by comparing the 2 control groups.

3. Results

3.1. Selectivity and in vitro functional activity at H_3-Rs

3.1.1. In vitro binding studies

SAR110894 displayed subnanomolar affinity for recombinant H_3- (Ki = 0.06 nM, Table 1) and native human H_3-Rs (Ki = 0.06 nM) (Fig. 1). In the same conditions, thioperamide and ABT-0239 displayed about 200-fold lower affinity for these receptors. In addition, SAR110894 displays high affinity for rat (Ki = 0.48 nM) and mouse (Ki = 0.4 nM) cortical H_3-Rs. Moreover, SAR110894 is highly selective versus other histamine receptors as evidenced by a minimum of 10,000-fold lower affinity for the other subtypes (H_1-, H_2- and H_4-R). Large panel CEREP receptor profile indicated that SAR110894 is devoid of affinity (Ki > 10 μM) for about 100 classical neurotransmitter receptors, ion channels and enzymes (data not shown). The only interactions were seen with a limited number of off-targets, the most significant ones (i.e. IC_{50} < 0.5 μM) being h5-HT_{2a} and h5-HT_{2c} receptors with a selectivity ratio versus recombinant hH_3-R estimated between 270 and 1500, respectively. However, in functional cellular assay expressions these 2 receptors, SAR110894 behaved as a weak antagonist for (IC_{50} = 4.5 μM and 3.4 μM, respectively) (data not shown).

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Table 1
In vitro binding profile of SAR110894 at human H_1-, H_2-, H_3- and rodent H_3-Rs. Individual Ki curve was calculated by using the formula: (Ki = IC_{50}/(1 + [radioligand concentration]/Kd)) and Ki values were the arithmetic mean and standard error of the mean. Affinities for recombinant H_1- and H_2-Rs were obtained from CEREP.

Receptor (cells, structure, ligand)	Hr-H_1 (HEK-293) [^{3}H]Pyr.	Hr-H_2 (CHO) [^{126}I]-APT.	Hr-H_3 (CHO) [^{3}H]NAMH.	Mouse-H_3 (cortex) [^{3}H]NAMH.	Rat-H_3 (cortex) [^{3}H]NAMH.	Hr-H_4 (CHO) [^{3}H]His.
Ki (nM)						
SAR110894	>10,000	>10,000	0.06	0.48	0.4	>10,000
Ciproxifan	>10,000	>10,000[a]	191.0[b]	1.23[b]	0.79[b]	>10,000
Thioperamide	>10,000	>10,000	19.7	4.2	5.01[b]	11
ABT-0239	>10,000	>1600	14.1	25	11.0[b]	>10,000
GSK189254	>1000[b]	>1000[b]	0.26[b]	3.1[b]	1.29[b]	>1000[a]

[a] Adapted from Esbenshade et al. (2004).
[b] Adapted from Medhurst et al. (2007).

3.1.2. In vitro functional studies

3.1.2.1. GTP-binding. In a functional TRF-GTP-Eu binding model, carried out on membranes of CHO cell stably expressing the human H_3-R receptor, SAR110894 totally and potently reversed RAMH-increased GTP binding (IC_{50} = 1.1 nM) and behaved as an inverse agonist (IC_{50} = 0.065 nM) in basal conditions with a maximal intrinsic activity of 40% reduction of the constitutive signal (Fig. 2).

3.1.2.2. Electrical field stimulated contractions in isolated guinea-pig ileum. SAR110894 antagonism was determined in guinea pig ileum since histamine H_3-Rs were reported to modulate electrical field stimulation (Vollinga et al., 1992). In this model, R-α-methylhistamine was able to block the supramaximal voltage electrical field stimulation contraction with an EC_{50} of 31.52 [24.42–40.68] nM (Fig. 3). The reference H_3-R antagonist thioperamide at 3 nM, reduced RAMH activity with a calculated pKB value of 8.67. Increasing concentrations of SAR110894 produced a competitive right-ward parallel shift in the dose–response curve of R-α-methylhistamine with a pKB of 8.38.

3.2. Efficacy and potency in animal models of CIAS and ADHD

3.2.1. Effects of SAR110894 on long-term visual episodic memory using the ORT in mice: dose–response

Results are presented in Fig. 4a. 2-way ANOVA revealed a significant "object" effect on exploration time of the familiar versus the novel objects during the recall sessions, i.e. 48 h after acquisition [$F(1,72) = 27.82$; $P<0.0001$], a non significant "treatment" effect [$F(5,72) = 1.31$; $P = 0.2699$] and a significant "treatment" × "object" interaction [$F(5,72) = 2.12$; $P = 0.0727$]. Post-hoc analyses (Winer test) on the "object" factor for each level of the "treatment" factor revealed that under control (vehicle) conditions, mice spent an equivalent amount of time investigating the novel and the familiar object, 48 h after the exposure to the familiar object. SAR110894 (at 0.3, 1 and 3 mg/kg) significantly increased exploration of the novel object compared to the familiar one.

3.2.2. Effects of SAR110894 on long-term visual episodic memory using the ORT in mice: antagonism by the H_3-R agonist, RAMH

Results are presented in Fig. 4b. 3-way ANOVA revealed a significant "Object" effect [$F(1,38) = 6.86$; $P = 0.0126$], a non significant "Treatment" effect [$F(1,38) = 0.03$; $P = 0.8616$], and a non significant "RAMH" effect [$F(1,38) = 0.45$; $P = 0.5049$]. "RAMH" × "Treatment" interaction was also found significant at the 10% level [$F(1.38) = 3.83$; $P = 0.0577$] meaning that the treatment effects were different when associated with RAMH. The other between factor interactions were not significant. Post-hoc analyses on the factor "Object" for each level of factors "Treatment" and "RAMH" revealed that under control (saline/vehicle) conditions, the time mice spent at investigating an object was not significantly different for the novel and the familiar object ($P = 0.2156$). Unlike RAMH, which was inactive, SAR110894 (1 mg/kg)

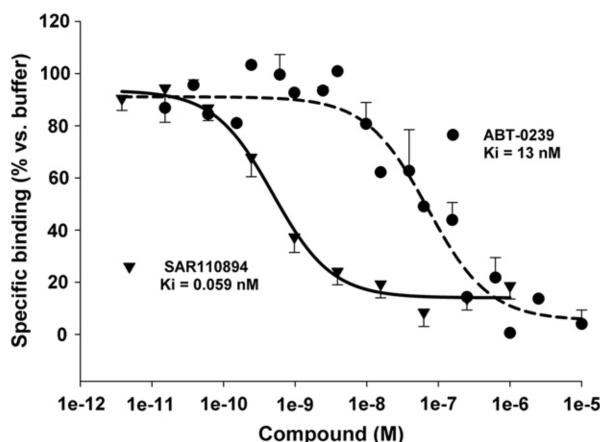

Fig. 1. Dose-dependent displacement of [^{3}H]Nα-methylhistamine (0.5 nM) binding by SAR110894 and ABT-0239 in membranes from parieto-occipital human cortex. Non-specific binding was determined by 10 μM thioperamide. Data are arithmetic means ± standard error of the means of 4 independent experiments. Individual curve Ki was calculated by using the formula: (Ki = IC_{50}/(1 + [radioligand concentration]/Kd)) and Ki experiment values were the arithmetic mean and standard error of mean.

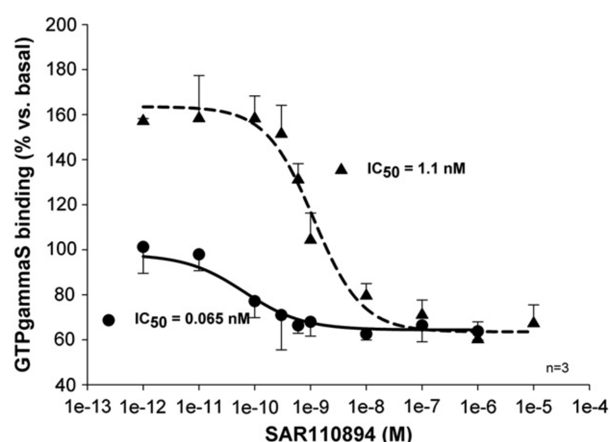

Fig. 2. SAR110894 dose-dependently inhibited basal (circle) and Nα-methylhistamine (50 nM) (triangle)-activated GTP binding on membranes prepared from CHO cells stably expressing the human H_3-R. Hundred nM Europium-labeled GTP was used as a ligand and non-specific binding was determined by 50 μM of non-labeled GTP. Data are arithmetic mean ± standard error of mean of 4 independent experiments. IC_{50} values are the geometric mean and its respective 95% CI.

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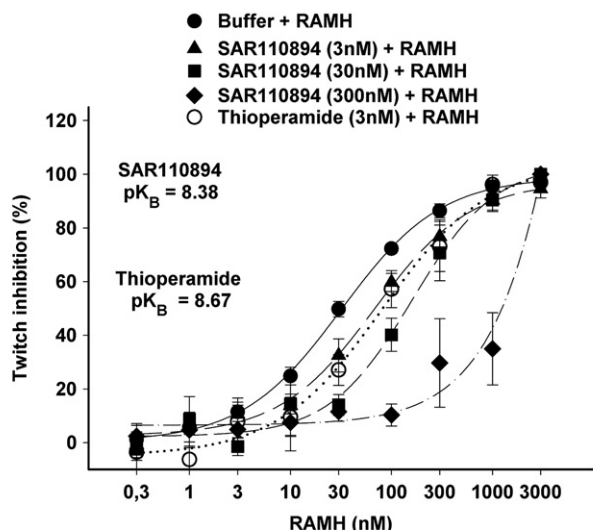

Fig. 3. SAR110894 behaves as a competitive H_3-R antagonist on the R-α-methylhistamine (RAMH)-induced inhibition of electrical-field stimulated guinea pig contractions. Thioperamide was studied as a reference H_3-R antagonist. Data are expressed as mean ± standard error of twitch inhibition determined in 5–6 independent experiments. EC_{50} values were obtained using the 4-parameter logistic model according to Ratkowsky and Reedy and used to estimate a pKB by using the Furchgott's formula (pKB = −log10 [concentration of the antagonist] + log10 (r − 1)), where r is the concentration ratio.

significantly increased the level of exploration of the novel object compared to the familiar one (P = 0.0114). This difference was abolished by RAMH (10 mg/kg) (P = 0.8141).

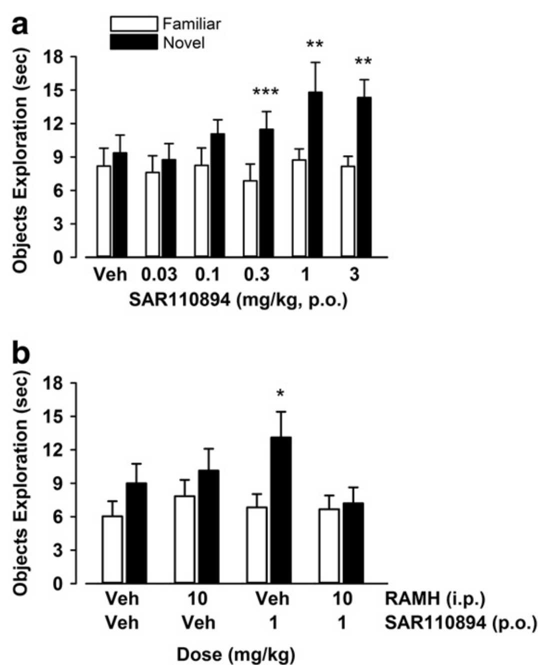

Fig. 4. a) Acute treatment with SAR110894 on long-term visual episodic memory in the ORT in mice. Each bar represents the average exploration time (seconds; means + S.E.M.) of the novel (black columns) and the familiar object (open columns). The interval between the acquisition and the recall session was 48 h. ***P<0.001, **P<0.05 novel versus familiar object for each treatment group. N = 10 to 21 mice per group. b) Antagonism by RAMH of the effects of SAR110894 on long-term episodic memory in the ORT in mice. *P<0.05 novel versus familiar object for each "RAMH" × "Treatment" group. N = 10 to 12 mice per group.

3.2.3. Effects of SAR110894 on long-term visual episodic memory using the ORT in rats

Results are presented in Fig. 5. 2-way ANOVA revealed a significant "object" effect [F(1,42) = 23.00; P<0.0001], a non significant "treatment" effect [F(3,42) = 1.55; P = 0.21] and a significant "treatment" × "object" interaction [F(3,42) = 4.21; P = 0.011]. Post-hoc analyses (Winer test) on the factor "object" for each level of the factor "treatment" revealed that under control (vehicle) conditions, rats spent an equivalent amount of time investigating the novel and the familiar object, 24 h after the exposure to the familiar object. SAR110894 (1 and 3 mg/kg) significantly increased the levels of exploration of the novel object compared to the familiar one.

3.2.4. Effects of SAR110894 on short-term visual episodic memory deficit following the administration of MK-801 using the ORT in rats

Results are presented in Fig. 6. 2-way ANOVA revealed a significant "object" effect [F(1,19) = 14.737; P = 0.0011], a non significant "treatment" effect [F(1,19) = 3.879; P = 0.06] and a significant "treatment" × "object" interaction [F(1,19) = 6.353; P = 0.0208] confirming the validity of the study by showing that control "vehicle + saline"-treated animals significantly discriminated between the new and the familiar objects (P = 0.0002), while "vehicle + MK-801"-treated animals failed to discriminate (P = 0.37). Treatment with SAR110894 (1 mg/kg p.o.) restored the deficit of preferential investigation produced by MK-801 (Fig. 6, third and 4th pairs of bars, from left). The effects of SAR110894 was confirmed by analyses of variance on factor "object" for each level of factor "treatment", which revealed that SAR110894 reversed significantly this deficit at 1 mg/kg, p.o.

3.2.5. Effects of SAR110894 on a deficit of spatial working memory induced by PCP sensitization using the Y-maze task in mice

Results are presented in Fig. 7. Mice subchronically injected with PCP (10 mg/kg, i.p.) and subsequently challenged with a low dose of PCP (0.5 mg/kg, i.p.) displayed a significant reduction of spontaneous alternation compared to control animals and those that received a single challenge dose of PCP {2-way ANOVA [F(3;72) = 3.161, P = 0.0297]}. No significant difference was observed in spontaneous alternation in the group treated by a single challenge dose of PCP "chronic saline/PCP challenge/vehicle-treated" and the "control chronic saline/challenge saline/vehicle-treated" group. Post-hoc Dunnett's *t*-test analysis showed that SAR110894 (at 1 mg/kg, p.o.) reversed the effects of an acute challenge of PCP in PCP-sensitized mice. There was no statistical difference in the total number of spontaneous alternation, which ranged from 34.7 ± 1.4 to 38.2 ± 1.7, depending on the group.

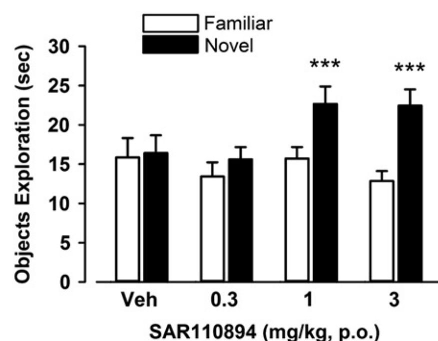

Fig. 5. Effects of SAR110894 on long-term visual episodic memory in the ORT in rats. Each bar represents the average of objects exploration time (seconds; means + S.E.M.) of the novel object (black columns) and of the familiar object (open columns). The interval between the acquisition and the recall session was 24 h. ***P<0.001 novel versus familiar object for each treatment group. N = 8 to 10 rats per group.

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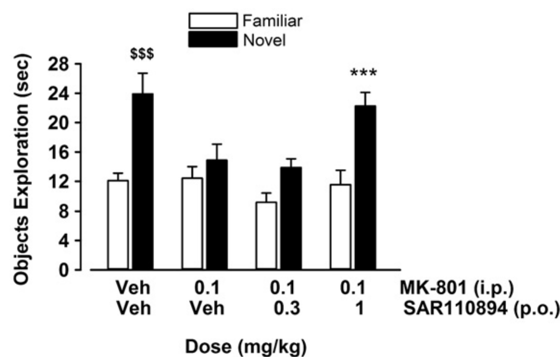

Fig. 6. Reversal by SAR110894 of MK-801-induced deficits on short-term visual episodic memory in the ORT in rats. Data in seconds represent means + S.E.M. of novel and familiar objects exploration. $^{\$\$\$}P<0.001$ novel versus familiar object for each treatment group (Winer analysis following 2-way ANOVA on "vehicle + MK-801" and "vehicle + saline" groups, with repeated measurements on object); $^{***}P<0.001$ novel versus familiar object for each treatment group (Winer analysis following 2-way ANOVA on "vehicle + MK-801" and "SAR110894 + MK-801" groups with repeated measurements on object). N = 9 to 12 animals per group.

3.2.6. Effects of SAR110894 on negative/positive symptoms and cognitive domains of schizophrenia using LI in rats

3.2.6.1. Effects of SAR110894 on MK-801-induced persistent LI and LI with strong conditioning. The experiment included 185 rats (*n* per group = 8–10). The experimental groups did not differ in their times to complete licks 51–75 before tone onset (all Ps > 0.05; overall mean A period = 8.23 s). Fig. 8a presents the mean log times to complete licks 76–100 (after tone onset) of the pre-exposed and non-pre-exposed rats in the different experimental conditions. As expected, vehicle-injected rats did not show LI, whereas MK-801-treated rats showed LI in spite of extended conditioning. MK-801-induced abnormally persistent LI was reversed by the NMDA agonist, glycine, but not by 1 to 10 mg/kg SAR110894. In addition, the 2 lower doses of SAR110894 potentiated LI in vehicle-treated rats. 3-way ANOVA with main factors of pre-exposure (0, 40), treatment (vehicle, MK-801) and pre-treatment (1, 3, 10 mg/kg SAR110894), yielded significant main effects of pre-exposure [$F(1,165) = 55.48$, $P<0.0001$], and pre-treatment [$F(4,165) = 5.37$, $P<0.05$], as well as significant interactions of pre-treatment × treatment [$F(4,165) = 2.84$, $P<0.05$]. Post-hoc comparisons revealed a significant difference between the pre-exposed and non-pre-exposed groups in the MK-801-vehicle ($P<0.005$), the MK-801 + 1 mg/kg SAR110894 ($Ps<0.0005$), the MK-801 + 3 mg/kg ($P<0.0001$), the MK-801 + 10 mg/kg ($P<0.0005$), and in the vehicle + 3 mg/kg conditions ($P<0.05$); a close to significant difference in the vehicle + 1 mg/kg condition ($P = 0.069$); and no effect in the 3 remaining conditions.

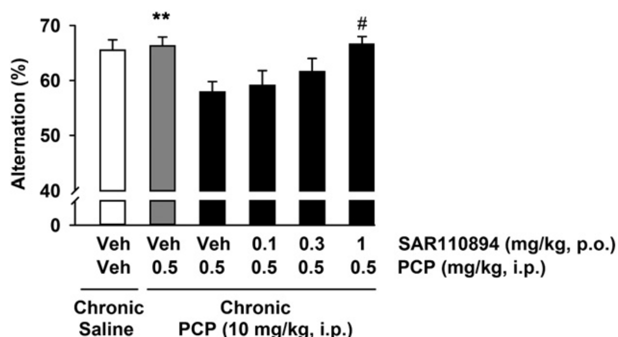

Fig. 7. Effect of a single administration of SAR110894 on spontaneous alternation in the Y-maze test in PCP-sensitized mice. Bars represent number of arm entries (mean + S.E.M.). $^{**}P<0.01$ (versus vehicle control), $^{\#}P<0.01$ (versus acute PCP/PCP-sensitized group). N = 20 mice per group.

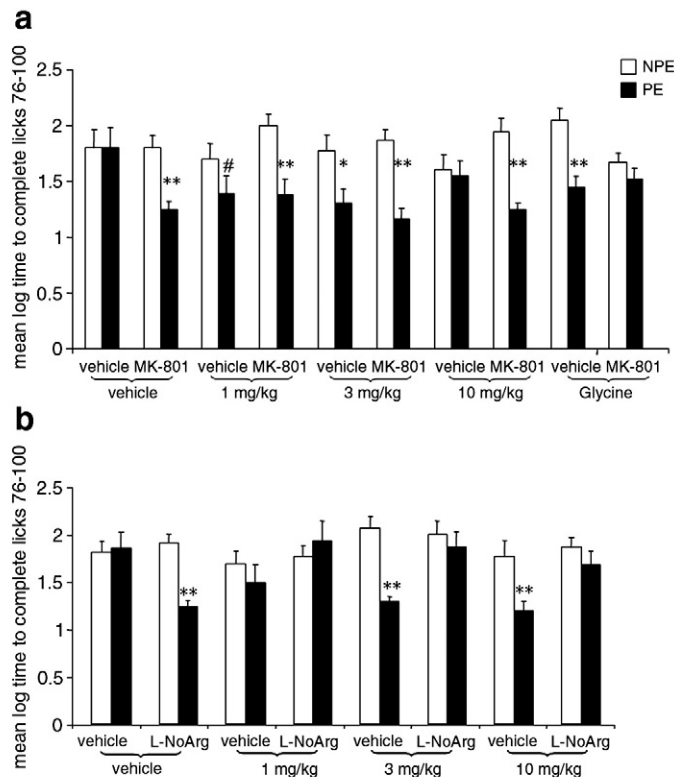

Fig. 8. Effects of SAR110894 on (a) MK-801- or (b) L-NoArg-induced persistent LI. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and non-pre-exposed (NPE) rats treated with MK-801, L-NoArg or vehicle, injected with SAR110894 at doses of 1, 3 and 10 mg/kg, glycine at 0.8 g/kg or vehicle. Forty pre-exposures and 5 conditioning trials were used. SAR110894 was administered i.p. in the pre-exposure and conditioning phases. Asterisks indicate a significant difference between the PE and NPE groups, namely, presence of LI. $^{\#}P = 0.07$, $^{*}P<0.05$ and $^{**}P<0.01$. N = 11 to 12 rats per group.

3.2.6.2. Effects of SAR110894 on neonatal L-NoArg-induced persistent LI. The experiment included 189 rats (run in 4 replications) in 16 groups in a 2 × 2 × 4 design with main factors of pre-exposure (PE, NPE), neonatal treatment (vehicle, L-NoArg), and SAR110894 (vehicle, 1, 3 10 mg/kg). Data from 2 rats were dropped from the analysis. N per group was 11–12. The 16 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all Ps > 0.05; overall mean A period = 8.07 s). Fig. 8b presents the means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed and non-pre-exposed rats neonatally treated with vehicle or L-NoArg and injected with 1, 3 or 10 mg/kg of SAR110894, or vehicle. As can be seen, LI was absent in neonatally vehicle-treated rats whereas neonatally treated L-NoArg rats showed LI. The 3 doses of SAR110894 successfully reversed the abnormally persistent LI in the neonatal L-NoArg-treated rats, so that these rats did not show LI like the neonatal vehicle-treated rats. Noteworthy, SAR110894 at 1 mg/kg, but not at 3 or 10 mg/kg, potentiated LI when administrated to neonatally vehicle-treated rats. 3-way ANOVA with main factors of pre-exposure (PE, NPE), neonatal treatment (Vehicle, L-NoArg) and SAR110894, yielded significant main effects of pre-exposure [$F(1,171) = 6.603$, $P<0.05$] and neonatal treatment [$F(1,171) = 5.19$, $P<0.05$], as well as near significant interactions of pre-exposure × neonatal treatment × SSR110894 [$F(3,171) = 2.181$, $P = 0.092$]. Post-hoc comparisons revealed a significant difference between the pre-exposed and non-pre-exposed groups in the neonatal L-NoArg-rats injected with vehicle ($P<0.05$), and in the neonatal vehicle-rats injected with 1 mg/kg ($P<0.05$), but not in all the other conditions.

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3.2.6.3. Effects of SAR110894 on strong conditioning-induced persistent LI. The experiment included 93 rats (run in 1 replication) in 8 groups in a 2×4 design with main factors of pre-exposure (PE, NPE) and SAR110894 (vehicle, 1, 3, 10 mg/kg). Data of 1 rat were dropped from the analysis. N per group was 11–12. The eight experimental groups did not differ in their times to complete licks 51–75 before tone onset (all Ps > 0.05; overall mean A period = 7.85 s). Fig. 8a (vehicle) presents the means and standard errors of the log times to complete licks 76–100 (after tone onset) of the pre-exposed and non-pre-exposed rats injected with 1, 3 or 10 mg/kg of SAR110894, or vehicle. As can be seen, LI was absent in vehicle-treated rats whereas the 1 and 3 but not 10 mg/kg potentiated LI.2-way ANOVA with main factors of pre-exposure (PE, NPE) and SAR110894 yielded a significant main effect of pre-exposure [$F(1,85) = 6.216$, $P < 0.05$], as well as a significant interaction of pre-exposure × SAR110894 [$F(3,85) = 2.955$, $P < 0.05$]. *Post-hoc* comparisons revealed a significant difference between the pre-exposed and non-pre-exposed groups in rats treated with 1 mg/kg ($P < 0.05$) and 3 mg/kg ($P < 0.05$), but not in the 2 other conditions.

3.2.6.4. Effect of SAR110894 on the impairment of novelty discrimination in adult rats

3.2.6.4.1. Experiment 1: effects of SAR110894 on neonatal PCP-induced novelty discrimination deficits in adult rats. Under control conditions (i.e. acute injection of vehicle), adult rats pretreated with saline at the neonatal stage (foremost left white bar, Fig. 9) spent approximately 3.5 times more time investigating the novel rather than the familiar juvenile (NDI = 3.44 ± 0.80). By contrast, adult rats neonatally pretreated with PCP presented a NDI (0.97 ± 0.09) roughly 3.5 times less time than that of neonatal saline-treated rats (compare the foremost left pair of bars, Fig. 9). This indicates that neonatal PCP-treated rats spent less time exploring the novel juvenile, which can be interpreted as an impairment of selective attention. Treatment with SAR110894 dose-dependently normalized this impairment ($P < 0.01$). The significant differences of novelty discrimination observed between the 2 groups (saline-neonates and PCP-neonates) under acute treatment of vehicle and the lower dose of SAR110894 (1 mg/kg) were abolished when rats were treated with the 2 higher doses of SAR110894 (3 and 10 mg/kg) (the 2 foremost right pairs of bars, Fig. 9). This was supported by post-hoc statistical analysis following a 2-way ANOVA with a significant interaction at the level 0.1 between the global neonatal pre-treatment and acute treatment effects [$F(3,24) = 2.926$, $P = 0.05$]. Note that in the neonatal saline-treated group, SAR110894 had no effect by itself (compare the 4 white bars in Fig. 9).

3.2.6.4.2. Experiment 2: impairment of novelty discrimination produced by a shortening of the duration of the first presentation and a lengthening of the inter-period interval. Under control conditions (i.e. injection of vehicle), adult rats spent approximately the same time investigating the novel or the familiar juvenile (Fig. 10) (NDI = 1.07 ± 0.07). This indicates that adult rats were not able to discriminate a novel from a familiar nonspecific, which can be interpreted as a disruption of selective attention. Treatment with SAR110894 dose-dependently normalized this impairment. The significant differences of novelty discrimination observed were between vehicle and the 2 higher doses of SAR110894 (3 and 10 mg/kg) (Fig. 10). This was supported by Dunnett's test following a one-way ANOVA for repeated measures with a significant global effect [$F(3,27) = 11.36$, $P < 0.0001$]. Treatment with methylphenidate dose-dependently normalized this impairment. The significant differences of novelty discrimination observed were between vehicle and the dose of 1 mg/kg (Fig. 10). This was supported by Dunnett's test following a one-way ANOVA for repeated measures with a significant global effect [$F(4,36) = 2.68$, $P = 0.05$]. Treatment with atomoxetine significantly normalized this impairment only at the higher dose of 3 mg/kg (the foremost right bar, Fig. 10). This was supported by Dunnett's test following a one-way ANOVA for repeated measures with a significant global effect [$F(2.18) = 5.31$, $P < 0.05$].

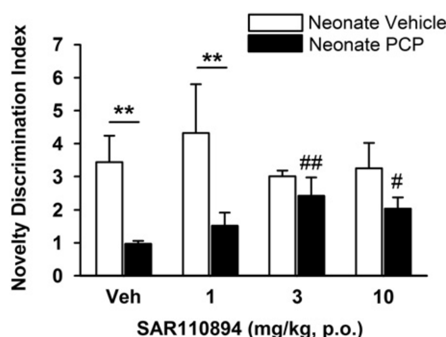

Fig. 9. Antagonism by SAR110894 of the impairment of novelty discrimination in adult rats treated with PCP at the neonatal stage. Each bar represents the mean (+S.E.M.) novelty discrimination index (ratio of the time spent investigating the novel juvenile divided by the time spent investigating the familiar juvenile, in seconds). Post hoc analyses following a 2-way ANOVA: **P<0.01 versus corresponding saline-neonates group; #P<0.05, ##P<0.01 versus acute vehicle PCP-neonates group (Dunnett's test). N = 10 rats per group.

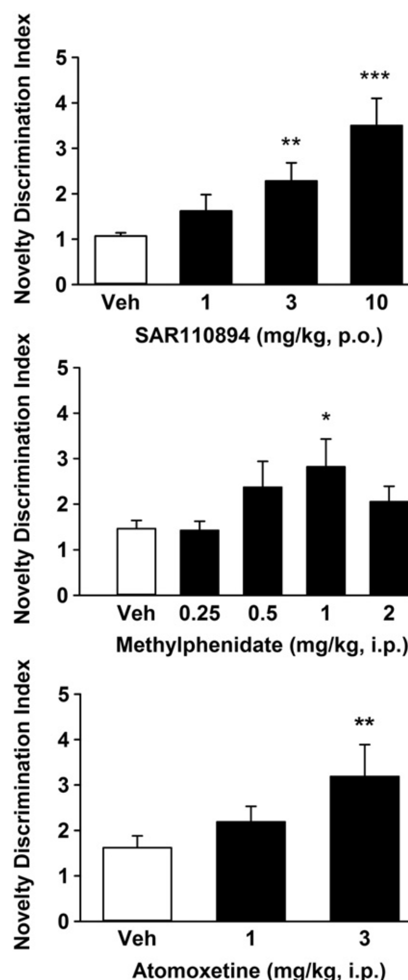

Fig. 10. Effects of SAR110894, atomoxetine and methylphenidate on spontaneous selective attention disruption in adult rats. Bars represent the mean (+S.E.M.) novelty discrimination index. Post hoc analyses (Dunnett's test) following a 2-way ANOVA: *P<0.05, **P<0.01, ***P<0.001 versus vehicle-treated rats. N = 10 rats per group.

3.3. Efficacy and potency in animal models of AD

3.3.1. Effects of SAR110894 on short-term visual episodic memory deficit following the administration of scopolamine using the ORT in rats

The time spent exploring the familiar and novel objects during the recall test session, 1 h after acquisition, is presented in Fig. 11. 2-way

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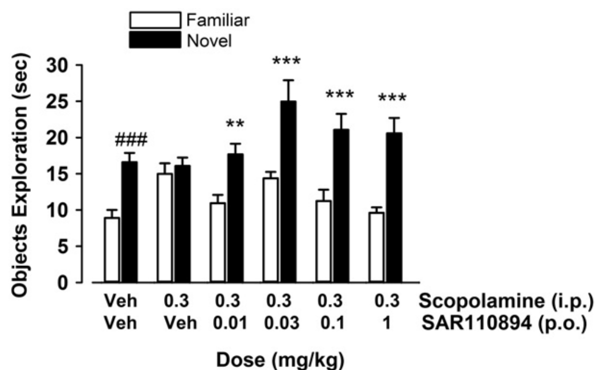
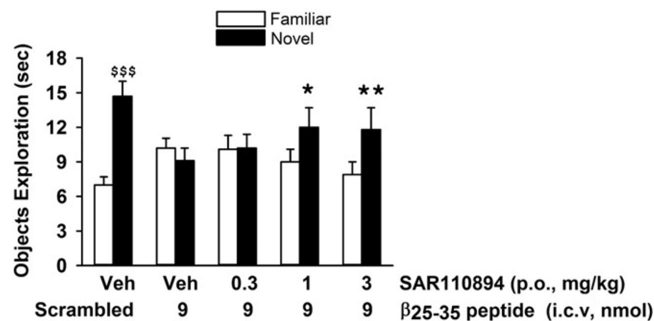


Fig. 11. Effects of SAR110894 on scopolamine-induced visual episodic memory in rats. Data represent exploration time (mean + S.E.M.) of the novel (black columns) and familiar (open columns) object. $^{###}$P<0.001: comparison novel versus familiar object for each treatment groups; Winer analysis following 2-way ANOVA (with "Vehicle + scopolamine" and "Vehicle + saline" groups) with repeated measurements on object. ***P<0.001, **P<0.01: comparison of novel versus familiar object for each treatment group; Winer analysis following 2-way ANOVA (with "Vehicle + scopolamine" and "SAR110894 + scopolamine" groups) with repeated measurements on object. N = 10–11 per group.



Objects Exploration (sec): 18, 15, 12, 9, 6, 3, 0; $$$, *, *, ***; Veh Scrambled; Veh 9; 0.1 9; 0.3 9; 1 9; Donepezil (i.p., mg/kg); β25-35 peptide (i.c.v, nmol)

Fig. 12. Effects of SAR110894 and donepezil on β_{25-35}-induced visual episodic memory in mice. Data represent exploration time (mean + S.E.M.) of the novel (black columns) and familiar (open columns) object, respectively. $^{\$\$\$}$P<0.001: comparison novel versus familiar object for each treatment groups; Winer analysis following 2-way ANOVA (with "Vehicle + β_{25-35}" and "Vehicle + saline" groups) with repeated measurements on object. *P<0.05, **P<0.01, ***P<0.001: comparison novel versus familiar object for each treatment group; Winer analysis following 2-way ANOVA (with "Vehicle + β_{25-35}" and "SAR110894 or donepezil + β_{25-35}" groups) with repeated measurements on object. N = 5–8 per group.

ANOVA revealed a significant "object" effect [F(1,40) = 30; P<0.0001], a non significant "treatment" effect [F(1,40) = 2.98; P = 0.09] and a significant "treatment" × "object" interaction [F(1,40) = 16.85; P<0.001] confirming the validity of the study by showing that control "vehicle + saline"-treated animals significantly discriminated between the new and the familiar object (P = 0.0001), while "vehicle + scopolamine"-treated animals failed to discriminate (P = 0.31). 2-way ANOVA with repeated measures, revealed a significant "object" effect [F(1,64) = 82.96; P<0.0001], a non significant "treatment" effect [F(4,64) = 2.01; P = 0.10] and a significant "treatment" × "object" interaction [F(4,64) = 6.49; P = 0.002]. Post-hoc analysis showed that SAR110894 reversed significantly the deleterious effects of scopolamine from the dose of 0.1 mg/kg, p.o., since animals treated with "SAR110894 + scopolamine" spent more time exploring the new object versus the familiar one (P = 0.002 for 0.01 mg/kg; P<0.0001 for 0.03, 0.1 and 1 mg/kg).

3.3.2. Effects of SAR110894 and donepezil on short-term visual episodic memory deficit following the central infusion of $A\beta_{25-35}$ peptide using the ORT in mice

Analysis of the time spent exploring the 2 identical objects during the acquisition session revealed that no effect of SAR110894, donepezil or the $A\beta_{25-35}$ peptide was observed (P = 0.2139, 0.9451 and 0.9984) on the time necessary to a 15-second total exploration of both objects. The time spent exploring the familiar and the novel object during the recall session, 1 h after acquisition is presented in Fig. 12. 2-way ANOVA confirmed the validity of the study, by showing that $Sc\beta_{25-35}$-treated animals significantly discriminated between the new and the familiar object (SAR110894 and donepezil studies: Ps<0.0001), while $A\beta_{25-35}$-treated animals failed to discriminate. An ANOVA on factor "Object" for each level of factor "Treatment" revealed that treatment with SAR110894 at 1 and 3 mg/kg p.o., or donepezil between 0.1, 0.3 and 1 mg/kg i.p., significantly reversed this deficit (Fig. 12).

4. Discussion

The main objective of this study was to characterize the procognitive profile of the novel H_3-R antagonist, SAR110894, using a variety of tests measuring different facets of cognitive processes in rodents. Results showed that the compound enhanced cognitive performances and attention in normal animals, while attenuating memory and attentional deficits following drug challenge or pathophysiological-related conditions.

4.1. Selectivity and in vitro functional activity at H_3-Rs

SAR110894 binds to recombinant and native human H_3-Rs with sub-nanomolar affinity, is over 10,000-fold selective versus human H_1, H_2, and H_4 histamine receptors, and displays high selectivity vs a large number of neurotransmitter receptors, ion channels and enzymes. It also potently binds rat and mouse native H_3-Rs. SAR110894 is a potent H_3-R antagonist at native receptors, reversing RAMH-induced inhibition of electrical field stimulation contraction in the guinea-pig ileum. Additionally, SAR110894 behaves as an inverse agonist, inhibiting constitutive GTP binding at human H_3-Rs. Although no head-to-head comparison was performed between SAR110894 and other selective H_3-R antagonists in in vitro assays, depending of the in vitro systems used, the drug appears to be more potent than GSK189254, JNJ-5207852 and ABT-0239 at the human H_3-R, and, functionally, displays a similar mixed H_3-R antagonist/inverse agonist profile as pitolisant, A-331440, ABT-0239 and GSK189254 (Stocking and Letavic, 2008).

4.2. Efficacy and potency in animal models of CIAS and ADHD

Dysfunction in glutamatergic neurotransmission, via NMDA receptors, has been suggested to be involved in the pathophysiology of schizophrenia (for a recent review, see Field et al., 2011). It is well known that noncompetitive NMDA receptor antagonists such as PCP induce schizophrenia-like symptoms, including cognitive deficits, in human volunteers (Goff and Coyle, 2001), observations which are largely substantiated by findings in animals showing that NMDA receptor antagonists impair learning and memory performances (for a recent review, see Neill et al., 2010).

The current results with SAR110894 demonstrate that the drug was able to attenuate spontaneous or NMDA receptor antagonist-induced deficits of several types of memory of relevance to schizophrenia. SAR110894 improved memory performances in several variants of the object recognition task, using either a long-term episodic memory

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procedure in non-treated mice or rats or a short-term episodic memory protocol using animals impaired by an acute dose of MK-801. Moreover, SAR110894 reversed a deficit in working memory in the Y-maze test, following an acute low dose of PCP in mice sensitized by repeated treatment with a high dose of PCP. The latter property fits well with observations in patients with schizophrenia, which were found to exhibit more pronounced negative symptoms than normal volunteers following an acute challenge with the NMDA receptor antagonist ketamine (Lahti et al., 2001), indicating that the former are sensitized to the effects of this PCP analog. Additionally, it is important to note that the positive effects of SAR110894 in the object recognition test in mice using a long-term forgetting delay are mediated by the H_3-R, as they were abolished by the H_3-R agonist RAMH. Although a few studies have examined the effects of earlier, less potent and selective, H_3-R antagonists (i.e., clobenpropit, ciproxifan and thioperamide) on learning and memory deficits produced by NMDA antagonists (Bardgett et al., 2009, 2010; Bernaerts et al., 2004; Huang et al., 2004), there is no similar work using the recent, more selective and potent H_3-R antagonists. However, these studies are generally in agreement with our findings that NMDA receptor antagonist-induced cognitive deficit can be attenuated by H_3-R blockade.

SAR110894 was tested in LI, a cross-species selective attention phenomenon that is disrupted in patients with schizophrenia and rodents treated with psychostimulants, and is sensitive to antipsychotics (Weiner, 2003). To the best of our knowledge, this is the first time that an H_3-R antagonist was tested in this procedure. MK-801-treated rats as well as rats neonatally treated with the NOS inhibitor L-NoArg on postnatal days 4–5, persisted in displaying LI with strong conditioning. SAR110894 potentiated LI in saline-treated rats and reverted the abnormally persistent LI back to control levels in the L-NoArg neurodevelopmental model. However, it failed to reverse MK-801-induced abnormally persistent LI. Potentiation of LI is regarded as a model of the positive symptoms of schizophrenia as it is very sensitive to typical antipsychotics, whereas reversal of MK-801 and L-NoArg is thought to reflect impaired set shifting that is associated with cognitive inflexibility and negative symptoms (Barak et al., 2009; Weiner and Arad, 2009). These findings suggest that SAR110894 may be useful in treating some positive symptoms of schizophrenia in addition to its therapeutic effect on certain aspects of cognition. However, the difference in drug effects between the MK-801 LI and L-NoArg LI models is unclear. Unlike the pharmacological MK-801 LI model, which may mimic the acute neurotransmitter dysfunction at the NMDA receptor believed to play a role in schizophrenia, the L-NoArg LI model may address the neurodevelopmental changes in the brain. Such a model is believed to mimic more closely the widespread disruption of corticomesolimbic circuitries implicated in the pathophysiology of schizophrenia (Lipska and Weinberger, 2000). As the neurodevelopmental model requires no psychomimetic challenge, our demonstration that SAR110894 is active in such a model suggests that H_3-R blockade may be effective at the neuronal circuit level underlying LI, rather than merely interfering with the psychomimetic drug activity. One could speculate that the limbic regions responsible for behavioral flexibility were underactive in animals neonatally treated with L-NoArg, and SAR110894 thus was able to raise the developmentally induced hypoglutamatergic state.

In line with this idea are the findings from the social novelty discrimination task, which showed that SAR110894 was able to reverse selective attention deficit induced by neonatal PCP treatment. Moreover, in this task, in the absence of PCP treatment, but following a parametric modification of the procedure (reduction of time spent in contact with the familiar juvenile during P1), we observed attentional perseveration, and pre-treatment with SAR110894 restored attentional flexibility, as did the anti-ADHD drugs, methylphenidate and atomoxetine. Taken together, the capacity of SAR110894 to reverse pharmacological-, spontaneous- and neurodevelopmental-induced attentional deficit/perseveration provides strong evidence for the potential efficacy of this drug for treating CIAS and ADHD.

4.3. Efficacy and potency in animal models of Alzheimer's disease

There are a number of animal models that may be relevant to aspects of AD. While the disease is associated with deficits in multiple neurotransmitter systems, reversal of scopolamine-induced deficits has been used extensively in a number of cognitive models and may model declining cholinergic function associated with the disease. In this study, SAR110894 prevented the occurrence of episodic memory deficit induced by scopolamine in the object recognition test in rats. Several H_3-R antagonists have been shown to be active in models of scopolamine-induced cognitive deficits, including passive avoidance, object recognition and delayed non-matching to position (DNMTP) tests (Galici et al., 2009; Ligneau et al., 2007; Medhurst et al., 2007; Miyazaki et al., 1995a, 1995b; Molinengo et al., 1999). Interestingly, in the study using DNMTP, the antagonism of scopolamine effects was associated with normalization of ACh neurotransmission in the cortex. Although preliminary findings with SAR110894 indicate that the drug increases cortical ACh levels in rats (Guillot et al., 2008), it is important to note that the cognitive effects in the scopolamine model appeared at doses much lower than those affecting the cholinergic transmission in the cortex (i.e. minimal active dose: 0.01 vs. 30 mg/kg, p.o.), suggesting the possibility that at least some cognitive effects of SAR110894 occur independently of ACh in the cortical area. Moreover, it is worth mentioning that the effects in the scopolamine object recognition test appeared at a dose at least 30 times lower than the minimal active dose in the other memory tests, which ranged from 0.3 to 1 mg/kg.

β-Amyloidogenesis is critical in the pathogenesis of AD (Selkoe, 1994). The 2 major forms of the aggregated β-amyloid peptide comprising 40 or 42 amino acids have been well described as the prominent components of the senile plaques in AD. The core amino acid sequence (25–35) of the amyloid peptide has been shown to mimic the neurotoxic properties of the β-amyloid peptides (1–40) or (1–42) (Yankner et al., 1990). Although it is not clear whether or not the β_{25-35} fragment occurs in the brain of AD patients, it has been demonstrated that the acute injection of the peptide into the cerebral ventricles of rodents produces massive neurotoxic effects similar to those produced by the β-amyloid (1–40) peptide (Kowall et al., 1991; Yamada and Nabeshima, 2000). More specifically, the infusion produces cerebral oxidative and cellular stress, neuroinflammation, and neuroprotective reactions, and modified endogenous amyloid processing. Moreover, β_{25-35}, provokes a rapid glial activation, ACh homeostasis perturbation, and hippocampal morphological alterations (Zussy et al., 2011). Finally, there are reports indicating memory deficits induced by the amyloid fragment when it was injected into the cerebral ventricle or locally into the hippocampus of rodents (Chen et al., 1996; Dornan et al., 1993; Kim et al., 2011; Maurice et al., 1996; Olariu et al., 2001; Zussy et al., 2011). In the current study, the acute intracerebroventricular infusion of the β_{25-35} fragment impaired short-term episodic memory performance in the object recognition task in mice. This deficit can be attributed specifically to β_{25-35}, since the scrambled β_{25-35} peptide was inactive. The administration of SAR110894 one hour before the acquisition session significantly attenuated this deficit, an effect which was comparable to that observed with the prototypical acetylcholinesterase inhibitor, donepezil. Since we haven't assessed the regional effects of β_{25-35} infusion, it can only be speculated on the mechanisms underlying the effects of SAR110894 in this model. The ability of the drug to improve performance following central β_{25-35} infusion may be related to the H_3-R-mediated ACh release in the hippocampus thought to contribute to the cognitive-enhancing effects of H_3-R antagonists. Alternatively, the effects of SAR110894 in the β_{25-35} model may be explained by its ability to counteract Aβ toxicity, an idea based on the observation that the H_3-R antagonist clobenpropit protects cells from injury produced by $A\beta_{42}$ toxicity via a glutamate receptor-mediated mechanism (Fu et al., 2010). Clearly, further studies are warranted to determine more precisely the mechanisms contributing to the cognitive effects of SAR110894 following β_{25-35} infusion.

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4.4. Conclusion

In summary, the present series of experiments showed that SAR110894 is a potent and selective H_3-R antagonist, which has demonstrated consistent efficacy in a broad range of rodent cognition models, addressing different aspects of memory impairment as seen in schizophrenia and AD. It is worth mentioning that the active dose ranges (from 0.01 to 3 mg/kg, p.o., depending on the test) and the magnitude of the effects of SAR110894 varied across tests, which suggests that different mechanisms may have been involved. Collectively, these findings support further the hypothesis that H_3-R antagonists may have a therapeutic utility for diseases where cognitive deficits occur, such as schizophrenia, AD and ADHD.

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