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In vivo pharmacological profile of S 38093, a novel histamine H3 receptor inverse agonist

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ABSTRACT

S 38093, a novel histamine H3 receptor inverse agonist, was tested in a series of neurochemical and behavioral paradigms designed to evaluate its procognitive and arousal properties.

In intracerebral microdialysis studies performed in rats, S 38093 dose-dependently increased histamine extracellular levels in the prefrontal cortex and facilitated cholinergic transmission in the prefrontal cortex and hippocampus of rats after acute and chronic administration (10 mg/kg i.p.).

Acute oral administration of S 38093 at 0.1 mg/kg significantly improved spatial working memory in rats in the Morris water maze test. The compound also displayed cognition enhancing properties in the two-trial object recognition task in rats, in a natural forgetting paradigm at 0.3 and 1 mg/kg p.o. and in a scopolamine-induced memory deficit situation at 3 mg/kg p.o. The property of S 38093 to promote episodic memory was confirmed in a social recognition test in rats at 0.3 and 1 mg/kg i.p.

Arousal properties of S 38093 were assessed in freely moving rats by using electroencephalographic recordings: at 3 and 10 mg/kg i.p., S 38093 significantly reduced slow wave sleep delta power and induced at the highest dose a delay in sleep latency. S 38093 at 10 mg/kg p.o. also decreased the barbital-induced sleeping time in rats.

Taken together these data indicate that S 38093, a novel H3 inverse agonist, displays cognition enhancing at low doses and arousal properties at higher doses in rodents.

1. Introduction

Disorders of learning and memory are characteristic of cerebral aging. As a greater proportion of the population survives beyond the age of 75, an increasing number of individuals will likely suffer from chronic age-related cerebral neurodegenerative diseases such as Alzheimer's disease. Cognitive disorders are thought to be due to a lower efficacy of the neurons to both synthesize and release certain neurotransmitters such as acetylcholine and histamine (Panula et al., 1998). Furthermore, there is a gradual loss of synaptic plasticity and of neuronal processes, cellular loss, especially involving cholinergic neurons, which is accelerated in specific brain regions. Under these conditions, cognitive functions decline as they cannot be maintained by the remaining and deficient neuronal networks.

H3 antagonists/inverse agonists might be useful for treating cognitive dysfunctions (Esbenshade et al., 2008; Passani et al., 2009; Brioni et al., 2011) since it is well established that H3 antagonists can enhance the release of neurotransmitters involved in vigilance, alertness and cognition enhancement including histamine by blockade of H3 autoreceptors (Arrang et al., 1983) as well as the release of acetylcholine, norepinephrine, dopamine, and serotonin via the blockade of H3 heteroreceptors (Blandina et al., 1996; Brown et al., 2001). These findings, in addition to the wide central nervous system projection of histaminergic neurons and localized expression of H3 in cortical and limbic system areas have made this receptor an attractive drug target for treating cognitive decline.

Preclinical behavioral data have highly confirmed that H3 receptor antagonists/inverse agonists are effective in cognition. Indeed, their administration in rats significantly improved performance in diverse cognition paradigms, including passive avoidance, object (Medhurst

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et al., 2007) and social recognition (Prast et al., 1996; Esbenshade et al., 2012). It also increased acquisition in a five-trial inhibitory avoidance test (Fox et al., 2002, 2003; Esbenshade et al., 2012) and spatial learning and reference memory in water maze test (Medhurst et al., 2007; Esbenshade et al., 2012). Moreover, pharmacological blockade of H3 receptors enhances attention as evaluated in the five-choice, serial-reaction time test (Ligneau et al., 1998) and the attentional set shifting paradigm (Medhurst et al., 2007). Procognitive effects of H3 receptor antagonists/inverse agonists have also been observed in animals with both age and scopolamine-induced cognitive impairment (Meguro et al., 1995; Onodera et al., 1998; Giovannini et al., 1999; Ligneau et al., 2007).

S 38093 was identified as a novel H3 antagonist/inverse agonist which possesses a favorable biopharmaceutical profile in rodents and monkeys (for detailed mechanistic characterization and pharmacokinetic properties, see Sors et al., 2017). This report describes in-depth the *in vivo* neurophysiological profile of S 38093 and its cognition-enhancing properties in animal models. From these results, S 38093 emerged as a favorable candidate for the treatment of memory deficits.

2. Materials and methods

2.1. Drugs

Two forms of S 38093 were used either S 38093-1 (oxalate salt) or S 38093-2 (hydrochloride salt with a salt/base ratio of 1.126, its chemical formula is $C_{17}H_{24}N_2O_2$, HCl). S 38093-1 and S 38093-2 was provided by Servier. Thioperamide and scopolamine were purchased from Sigma Chemical Co. (France). Sodium barbital was purchased from Merck (France).

2.2. Animals

Spatial learning, Social recognition, Cortical electroencephalogram recording, Barbital-induced sleep tests and histamine microdialysis studies were performed in rats at the Institut de Recherches Servier (IdRs, Croissy-sur-Seine, France) and protocols were reviewed by the local Ethic Committee of IdRS.

Concerning histamine microdialysis, all procedures conformed to the European Directive 2010/63/EU, and its adaptation to French law (decree 2013/118), on the protection of animals used for scientific purpose. Acetylcholine microdialysis studies were conducted on rats at Pharmaness (Sardinia) according to the guidelines for the care and the use of experimental animals of UE (CEE No. 86/609).

Novel object recognition test in rats was conducted at Biotrial (Rennes, France) in accordance with Good Laboratory Practice.

2.3. Microdialysis

2.3.1. Histamine levels in prefrontal cortex

Studies were performed on male Wistar rats (225–300 g) supplied by Charles River (L'Arbresle, France). Animals were adapted to laboratory conditions with free access to chow and water for at least 5 days prior to testing under a 12 h/12 h light/dark cycle with lights on at 7 or 7:30 A.M. Laboratory temperature and humidity were 21 ± 1 °C and 60 ± 5%, respectively.

Rats were anaesthetized with pentobarbital (60 mg/kg, i.p.) and xylocaine (5%) was locally applied for analgesia. Animals were further stereotaxically implanted with a guide-cannula into the prefrontal cortex at the following coordinates: AP: +2.2, L: ± 0.6 and DV: −0.2 according to Paxinos and Watson (1997). Five days later, the microdialysis probe (cuprophane CMA11, ∅ 0.23 mm, length 4 mm, Carnegie Medicin, Phymep, France) was lowered into the guide-cannula, connected to a perfusion pump and perfused at a flow rate of 1 µl/min with artificial cerebrospinal fluid containing NaCl (147.2 mM); KCl (4 mM) and $CaCl_2$ (2.3 mM) at pH, 7.3. After a 2-h stabilization period, samples collection started. Microdialysates were collected over a 4-h period every 30 min. Three samples were collected in basal conditions and six after vehicle or S 38093 administration (3 and 10 mg/kg ip).

Histamine was analysed using HPLC (high performance liquid chromatography) coupled to fluorimetric detection. Histamine was post-column derivatized using OPA as a fluorophore. The derivatives were fluorimetrically detected (RF20, Shimadzu, Champs/Marne, France) (Ex: 340 nm; Em: 440 nm) after HPLC separation using a reverse-phase column (Hypersil C18, 250×2.1 mm, particle size, 5 µm, Sigma, St Quentin Fallavier, France) maintained at 38 °C with a flow rate of 0.4 ml/min. Mobile phase was KH_2P0_4, 100 mM, pH=5.7; decane sulfonate, 1.8 mM and methanol 23%. Assay limit of sensitivity was 3 fmol per 30 µl sample.

All data are expressed as means ± S.E.M. Neurotransmitter levels were expressed as a function of mean basal pre-injection values (=100%).

2.3.2. Acetylcholine extracellular levels in prefrontal cortex and hippocampus

Male Sprague Dawley rats, weighing about 200–250 g, were used for the experiment. At their arrival at facility, rats were housed in humidity and temperature controlled room on a 12 h light/dark cycle (light on at 7:00 h a.m.) with water and food available ad libitum. About 24 h prior microdialysis session, rats were anaesthetized with Equitesin (5 ml/kg) and stereotaxically implanted with vertical probes in the medial prefrontal cortex (AP +3.3; L ± 0.8 and V −6, from bregma) and the hippocampus (AP −4.3; L ± 2.6 and V −3.2, from bregma) according to the Paxinos and Watson (1997) atlas coordinates. Rats were housed individually to allow recovery from the surgery. Animals were randomly assigned to experimental groups (5–8 rats per group). The day after surgery, an artificial cerebrospinal fluid (147 mM NaCl, 4 mM KCl, 2 mM CaCl2, pH 6–6.5) was pumped through the dialysis probe at a constant flow rate of 2.0 µl/min via a microinjection pump. To prevent metabolism of acetylcholine, 10^{-7} M of the acetylcholine esterase inhibitor neostigmine, was added to the artificial cerebrospinal fluid. After stabilization of neurotransmitter basal levels, rats were injected with S 38093-2 (10 mg/kg, i.p., 1 ml/kg) or vehicle (0.9% saline, 1 ml/kg). For chronic experiment, rats were administered once a day for 13 days before the last administration on the day of microdialysis experiment. Collection of samples was performed every 20 min, from 100 min before until 180 min after drug administration.

Acetylcholine extracellular concentrations were determined by an HPLC system equipped with a ODS Hypersil 3×100 cm, 5 µm (Thermo Fisher Scientific Inc., USA) and an electrochemical detector (LC-4B, BAS, IN, USA) in conjunction with an enzyme reactor. The mobile phase passes directly through the enzyme reactor containing acetylcholine esterase (ED 3.1.1.7; type VI-S, Sigma, MO, USA) and choline oxidase (EC 1.1.3.17; Sigma, MO, USA) covalently bound to glutaraldehyde-activated Lichrosorb 10-NH2; acetylcholine is quantitatively converted into hydrogen peroxide which is detected electrochemically at a platinum working electrode set at 560 mV versus an Ag/AgCl reference electrode (LC-4B, BAS, IN, USA). The mobile phase consists of 190 mM K2HPO4, 1 mM tetramethyl ammonium hydroxide, pH 8, pumped at 0.9 ml/min.

Values are expressed as means ± S.E.M. relative to baseline (means of the three stable baseline samples before treatment defined as 100%).

2.4. Spatial learning

Male Wistar rats (CERJ, 400–450 g, 3 months of age) were daily handled over 7 days before testing in order to get used to the experimenter. The test was conducted in a circular tank filled with opaque water and containing in the middle part of one of four imaginary quadrants (north, east, south and west), an escape platform positioned 1 cm underneath the surface of the water. Rats were