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Regional Differential Effects of the Novel Histamine H_3 Receptor Antagonist 6-[(3-Cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide hydrochloride (GSK189254) on Histamine Release in the Central Nervous System of Freely Moving Rats

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ABSTRACT

After oral administration, the nonimidazole histamine H_3 receptor antagonist, 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide hydrochloride (GSK189254), increased histamine release from the tuberomammillary nucleus, where all histaminergic somata are localized, and from where their axons project to the entire brain. To further understand functional histaminergic circuitry in the brain, dual-probe microdialysis was used to pharmacologically block H_3 receptors in the tuberomammillary nucleus, and monitor histamine release in projection areas. Perfusion of the tuberomammillary nucleus with GSK189254 increased histamine release from the tuberomammillary nucleus, nucleus basalis magnocellularis, and cortex, but not from the striatum or nucleus accumbens. Cortical acetylcholine (ACh) release was also increased, but striatal dopamine release was not affected. When administered locally, GSK189254 increased histamine release from the nucleus basalis magnocellularis, but not from the striatum. Thus, defined by their sensitivity to GSK189254, histaminergic neurons establish distinct pathways according to their terminal projections, and can differentially modulate neurotransmitter release in a brain region-specific manner. Consistent with its effects on cortical ACh release, systemic administration of GSK189254 antagonized the amnesic effects of scopolamine in the rat object recognition test, a cognition paradigm with important cortical components.

The discovery of the histamine H_3 receptor (H_3R) back in 1983 was a major scientific breakthrough that provided key new perspectives in histamine research (Arrang et al., 1983). Originally detected on varicosities of histaminergic axons as an autoreceptor that restricts histamine synthesis and release (Arrang et al., 1987), the H_3R is also located on histaminergic somata where it provides a tonic inhibition. Indeed, application of the H_3R antagonist thioperamide enhanced the firing of histaminergic neurons (Haas and Panula, 2003). These neurons arise solely from the tuberomammillary nucleus (TMN) of the posterior hypothalamus, from where they send diffuse projections throughout the entire central nervous system (Inagaki et al., 1988; Panula et al., 1989). Consistent with this wide-ranging output, histamine is directly and indirectly involved in a variety of basic homeostatic and higher brain functions, such as the control of the sleep-wake cycle, appetite, nociception, cognition, and emotion (Haas and Panula, 2003; Passani et al., 2004), and H_3R antagonists have been shown to increase wakefulness, improve cognitive performance, and reduce body weight in animal mod-

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ABBREVIATIONS: H_3R, histamine H_3 receptor; ACh, acetylcholine; H_4R, histamine H_4 receptor; NBM, nucleus basalis magnocellularis; TMN, tuberomammillary nuclei; GSK189254, 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide hydrochloride; ANOVA, analysis of variance; HPLC, high-performance liquid chromatography.

els (Haas et al., 2008). Such findings suggest the potential use of these compounds for the treatment of Alzheimer's disease and other dementias, attention-deficit hyperactivity disorder, cognitive deficits in schizophrenia, obesity, and sleep disorders (Esbenshade et al., 2008). In this regard, network analyses of the brain and its dysfunction suggest that agents with multiple and complementary modes of action are more likely to show broad-based efficacy against core and comorbid symptoms. Because H_3Rs also act as heteroreceptors moderating the in vivo release of several neurotransmitters, including acetylcholine (ACh; Blandina et al., 1996), dopamine, noradrenaline, and serotonin (Blandina et al., 1998), H_3R antagonists are good candidates for simultaneous modulation of multiple neuronal systems. Therefore, it is not surprising that much effort is focused on the development of clinically suitable H_3R ligands by academic and industrial laboratories (Esbenshade et al., 2008).

All histaminergic neurons are believed to express H_3Rs, and a response to H_3R ligands is a criterion for their identification in vitro. Contrary to this general assumption, we have recently reported that histamine neurons projecting to the striatum and nucleus accumbens are insensitive to thioperamide (Giannoni et al., 2009), suggesting that histamine neurons are functionally more heterogeneous than described previously (Wada et al., 1991). However, thioperamide is also known to display appreciable histamine H_4 receptor (H_4R) antagonist activity, making these observations somewhat difficult to interpret (Hough, 2001; Gbahou et al., 2006). Moreover, thioperamide belongs to the first-generation imidazole-based H_3 antagonists that proved to be unsuitable for clinical application in humans, because of a number of liabilities, including drug-drug interactions, cytochrome P450 inhibition, and lack of selectivity (Celanire et al., 2005). As a result, a large number of potent nonimidazole H_3R compounds have recently been developed, and some of these are currently being evaluated in clinical trials (Celanire et al., 2005; Sander et al., 2008).

The present study addresses an interesting question about whether histaminergic neurons organized into functionally distinct circuits impinging on different brain regions show differential sensitivity to GSK189254, a potent and selective nonimidazole H_3R antagonist, that displays efficacy in a number of rodent cognition paradigm H_3Rs (Medhurst et al., 2007). GSK189254 has entered phase II clinical trials for the treatment of neuropathic pain (Sander et al., 2008). Although previous studies have shown that GSK189254 can increase the release of ACh, noradrenaline, and dopamine (Medhurst et al., 2007), its effects on histamine release have not yet been described.

In the current study, we used the dual-probe microdialysis technique to pharmacologically block H_3Rs in the TMN and monitor modification of histamine, ACh, or dopamine releases in histaminergic projection areas. Single-probe microdialysis experiments were also performed to investigate whether blockade of local H_3Rs in the histaminergic projection areas influenced histamine release within these brain regions. The present study also evaluated the capacity of GSK189254 to influence scopolamine-induced amnesia in rats, measured by object recognition, a short-term memory paradigm.

Materials and Methods

Male Sprague-Dawley rats (225–275g body weight; Harlan, Milan, Italy) were housed in groups of three in a temperature-controlled room (20–24°C), allowed free access to food and water, and kept on a 12-h light/dark cycle (light starts at 7:00 AM). All the experiments were performed in strict compliance with the European Economic Community recommendations for the care and use of laboratory animals (86/609/CEE), and were approved by the Animal Care Committee of the "Dipartimento di Farmacologia Preclinica e Clinica - Universitá di Firenze."

Surgical Procedures. Rats anesthetized with chloral hydrate (400 mg/kg i.p.) and positioned in a stereotaxic frame (Stellar; Stoelting Co., Wood Dale, IL), were implanted with one or two guide cannulae (Metalant AB, Stockholm, Sweden). In the dual-probe experiments, one probe was placed in the TMN of every rat, with a second probe placed in one of four locations: nucleus basalis magnocellularis (NBM), dorsal striatum, nucleus accumbens, or prefrontal cortex. In the single-probe experiments each rat was implanted with a single probe in the NBM or the dorsal striatum. Coordinates from bregma (Paxinos and Watson, 1998) were: TMN, AP = −4.3, L = −1.1, DV = −7.2; prefrontal cortex, AP = +3.2; L = −1.0; DV = +2.8; NBM, AP = −0.8; L = −2.8; DV = +6.5; dorsal striatum, AP = 0, L = −4, DV = +4; nucleus accumbens, AP = +1,7, L = −1.4, DV = +6.3 (Fig. 1). A surgical screw served as an anchor, and the cannulae were fixed to the skull with acrylic dental cement.

Histology. The placement of microdialysis membranes was verified post mortem. Rats were overdosed with chloral hydrate, and the brains removed and stored in 10% formalin for 10 days. Forty-micrometer sections were then sliced on a cryostat, mounted on gelatin-coated slides, and stained with cresyl violet for light microscopic observation. Data from rats in which the membranes were not correctly positioned were discarded (less than 10%).

Microdialysis Experiments. Microdialysis was performed 48 h after surgery during which rats, housed one per cage, recovered from surgery. The stylet was removed from the guide cannulae, and the microdialysis probes (molecular mass cutoff = 6000 Da; Metalant

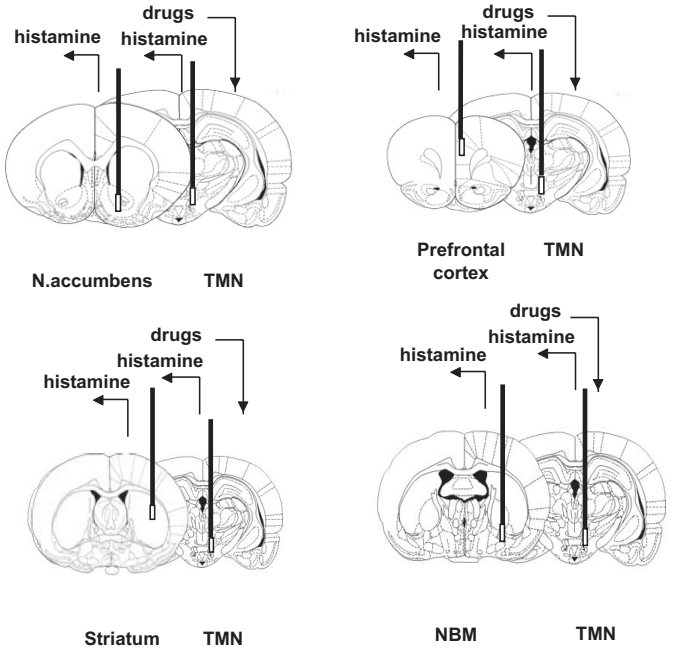

Fig. 1. Schematic diagram showing the position of the microdialysis probes. Rats were implanted with one probe in the TMN to deliver drugs locally and measure neurotransmitter release, and with another probe in the prefrontal cortex, the NBM, the nucleus accumbens, or the dorsal striatum to measure histamine release.

AB) were inserted; the dialyzing membrane protruded 2 mm from the tip of the cannula. Probes were perfused with Ringer's solution (147 mM NaCl, 1.2 mM $CaCl_2$, and 4.0 mM KCl, at pH 7.0) at a flow rate of 2 μl/min by use of a microperfusion pump (Mod CMA/100; Carnegie Medicine, Stockholm, Sweden). Two hours after insertion of the microdialysis probes, when neurotransmitter release became stable, collection of 15-min fractions was started. Spontaneous release was defined as the average value of the first four 15-min fractions collected during 60 min of perfusion with Ringer's solution before drug treatment. All subsequent fractions were expressed as percentage increase of this value. In the dual-probe experiments, both probes were perfused with control medium in the first four 15-min fractions to measure histamine spontaneous release; GSK189254 was then added only to the TMN-perfusing medium, dissolved in the Ringer's solution. GSK189254 structure is shown in Fig. 2. Drug addition did not modify the pH of the medium. In the single-probe experiments the brain region of interest was perfused with control medium in the first four fractions, and GSK189254 was then added to the perfusing medium. In the experiments aimed at measuring ACh release from the prefrontal cortex, neostigmine bromide (0.1 μM), a cholinesterase inhibitor, was added to the medium perfusing the prefrontal cortex to recover detectable ACh concentrations in the dialysate.

Determination of Histamine. To prevent degradation of histamine, 1.5 μl of 5 mM HCl was added to each sample. The dialysates were kept at −80°C until analysis. Histamine contents in the dialysates were determined by HPLC-fluorometry (Cenni et al., 2006). In brief, the column (Hypersil ODS, 3 μm, 2.1 × 100 mm; Thermo Fisher Scientific, Waltham, MA) was eluted with 0.25 M potassium dihydrogen phosphate containing 5% octanesulfonic acid (Sigma-Aldrich, St. Louis, MO) at a flow rate of 0.4 ml/min. The eluate from the column was mixed first with 0.1% *o*-phthalaldehyde solution at a flow rate of 0.1 ml/min and then to a solution containing 4 M sodium hydroxide and 0.2 M boric acid (flow rate, 0.137 ml/min) to adjust the reaction mixture to pH 12.5. The reaction took place at 45°C. Then 17% orthophosphoric acid was added to the solution (flow rate, 0.137 ml/min) to reach a final reaction mixture at pH 3. The fluorescent intensity was measured with a spectrofluorometer (series 1100; Agilent, Waldbronn, Germany) at 450 nm with excitation at 360 nm. The sensitivity limit was 10 fmol and the signal/noise ratio was higher than 3. Histamine levels in the dialysate samples were calculated as femtomoles every 15 min.

Determination of ACh. To prevent degradation of ACh, 5 μl of 0.5 mM HCl was added to each sample. ACh was assayed in the dialysate by HPLC with electrochemical detection using an ACh/choline assay chromatographic kit (BAS Bioanalytical Systems, West Lafayette, IN) consisting of an ACh analytical column (BAS MF-6150) and an ACh/choline immobilized enzyme reactor (BAS MF-6151). The mobile phase was 50 mM Tris/$NaClO_4$ containing 0.05% ProClin (BAS CF-2150; Bio Analytical Systems), a broad-spectrum antimicrobial suited as a preservative for enzymes, pH 8.5, at 1 ml/min flow rate. ACh, separated in the analytical column, was hydrolyzed in the immobilized enzyme reactor by acetylcholinesterase to acetate and choline that was oxidized by choline oxidase to produce betaine and hydrogen peroxide. Hydrogen peroxide was electrochemically detected by a platinum-working electrode at +500 mV with a Ag/AgCl reference electrode. The sensitivity limit was 125 fmol, and the signal/noise ratio was higher than 3. To evaluate the amounts of ACh in the samples, a linear regression curve was made with ACh standards, and the peak areas of this compound in the samples were compared with those of the standards by means of an integrator (P.E. Nelson model 1020; PerkinElmer Life and Analytical Sciences, Waltham, MA). ACh levels in the dialysate samples were calculated as femtomoles every 15 min.

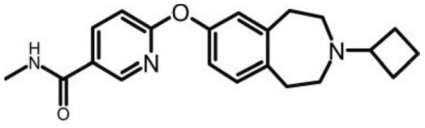

Fig. 2. Chemical structure of GSK189254.

Determination of Dopamine. To prevent degradation of dopamine, 1.5 μl of 5 mM HCl was added to each sample. Dopamine contents in the dialysates were determined by HPLC with coulometric detection. The mobile phase was 75 mM $NaH_2PO_4 \cdot H_2O$, 3 mM 1-octanesulfonic acid sodium salt, 1.2 mM EDTA, 8% acetonitrile, pH 3.4, with phosphoric acid, at 0.8 ml/min flow rate. Samples were injected into a HPLC apparatus equipped with a Macherey-Nagel 125/3 NUCLEOSIL 100-5 C18 AB column with Waters Bondapak 10 μm 125A C18 Precolumn, a LC-10AD Shimadzu Pump, a SIL-10ADvp Shimadzu autoinjector (Shimadzu Italia, Milan, Italy), 3 ESA model 6210 cells (12 electrodes, POTENTIAL E1 −250 mV, E2 −200 mV, E3 −200 mV, E4 −80 mV, E5 −80 mV, E6 0 mV, E7 100 mV, E8 200 mV, E9 300 mV, E10 350 mV, E11 400 mV, E12 400 mV), and ESA CoulArray model 5600A detector (ESA Analytical, Ltd., Dorton, UK). Chromatograms were processed using the CoulArrayWin MFC Application software. The sensitivity limit was <1 nmol. Dopamine levels in the dialysate samples were calculated as femtomoles every 15 min.

Object Recognition Paradigm. Object recognition paradigm measures a form of short-term memory based on short and unrepeated experiments without any reinforcement, such as food or electric shocks (Ennaceur and Delacour, 1988). Object recognition is a one-trial task, and does not involve the learning of any rule, being entirely based on the spontaneous exploratory behavior of rats toward objects. In brief, the rats were placed in a white polyvinyl chloride box (70 × 60 × 30 cm) with a grid floor that is easily cleaned, and illuminated by a 75-W lamp suspended 50 cm above the box. The objects to be discriminated were gray polyvinyl chloride shapes: cubes of 8 cm side, pyramids and cylinders of 8 cm height. The day before the test, the rats were allowed to explore the box for 2 min without objects. On the day of the test, two trials, separated by an interval of 60 min, were carried out. In the first trial two identical objects were put in two opposite corners of the box, and the time (t1) required by each rat to complete a cycle of 20 s of object exploration was recorded. Exploration was considered to be directing the nose at a distance less than 2 cm from the object and/or touching it with the nose. During the second trial (T2) one of the objects presented in the first trial was replaced by a new object with a different shape. Rats were left in the box for 5 min. The exploration periods of the familiar (tF) and the new object (tN) were recorded separately, and a discrimination index (D) was calculated according to the formula (tN − tF)/(tN + tF). Care was taken to avoid place preference and olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T2, and cleaning them carefully. Object recognition was carried out in an insulated room to avoid any noise that could impair the performance of the rats.

Statistical Analysis. All values are expressed as means ± S.E.M., and the number of rats used in each experiment is also indicated. The presence of significant treatment effects was first determined by a one-way ANOVA followed by Bonferroni's test. Comparisons using Each Pair Student's *t* test were used to assess differences between groups in the object recognition test. For all statistical tests, $P < 0.05$ was considered significant. For clarity, we reported in figures and figure legends only the significant differences versus the last sample before drug treatment. However, differences were significant versus all baseline samples. Statistical analysis was performed by use of StatView (Abacus Concepts, Berkley, CA) or JMP (SAS Institute, Cary, NC).

Chemicals. GSK189254 was synthesized at GlaxoSmithKline, Harlow, Essex. United Kingdom. The structure is shown in Fig. 2. Scopolamine hydrobromide was purchased from Sigma Chemical (Poole, Dorset, UK). All other reagents and solvents were of HPLC grade or the highest grade available (Sigma Chemical).

Results

After 120 min of equilibration after the insertion of the dialyzing membranes, histamine was released spontaneously at a stable rate from all brain regions investigated. Histological analysis confirmed that the probes were located in the correct areas, with no signs of unusual tissue damage or bleeding (Fig. 1).

Oral Administration of GSK189254 Increased Histamine Release from the TMN. After collection of four, 15-min baseline samples, GSK189254 was administered orally at doses of 1 and 3 mg/kg. The effects on histamine release from the TMN are shown in Fig. 3. GSK189254 (1 mg/kg) significantly increased histamine release approximately 60 min after the administration, up to a peak value of 114 ± 20% (ANOVA, $F_{(14,45)} = 3.139$, $P < 0.0018$). Afterward, histamine release returned to basal levels (Fig. 3A). The administration of a higher dose of GSK189254 (3 mg/kg) increased histamine release significantly (ANOVA, $F_{(14,75)} = 2.823$, $P < 0.002$). The maximal increase was of similar amplitude, 113 ± 28%, but histamine release returned more slowly to basal levels (Fig. 3B).

Histaminergic neurons are more active during wakefulness, their activity being lowest during quiet waking, and highest during attentive waking (Takahashi et al., 2006). In the present study, the experiments were performed during the light phase, and, presumably, histaminergic cell activity was low. Accordingly, most of the rats (90%) were sleeping for the entire period preceding GSK189254 administration that produced clear signs of awakening, but not of agitation or irritability.

Local Perfusion of GSK189254 into the TMN Increased Histamine Release from the TMN and NBM. Using a double-probe microdialysis protocol, histamine release was monitored from both the TMN and NBM. After collection of five, 15-min baseline samples, GSK189254 (1 μM) was infused for 60 min locally into the TMN, where all histaminergic neuronal cell bodies are localized (Inagaki et al., 1988), whereas NBM was always perfused with control medium. As shown in Fig. 4, perfusion with GSK189254 induced a significant increase of histamine release from both the TMN (ANOVA, $F_{(11,48)} = 3.709$, $P < 0.0007$) and the NBM (ANOVA, $F_{(11,48)} = 4.098$, $P < 0.0003$), which returned to baseline values after perfusion with GSK189254 ended. In the TMN the maximal increase was 83 ± 27%. Supposedly, histamine was released by short projections in the posterior hypothalamus, as histaminergic neurons display extensive axonal arborizations within this brain region (Inagaki et al., 1988). When GSK189254 was infused into the TMN, histamine release increased up to a maximum of 92 ± 38% in the NBM.

Local Perfusion of the TMN with GSK189254 Increased the Release of Histamine in Both the TMN and the Prefrontal Cortex, and the Release of ACh in the Prefrontal Cortex. By use of a double-probe microdialysis protocol, histamine release was monitored from both the TMN

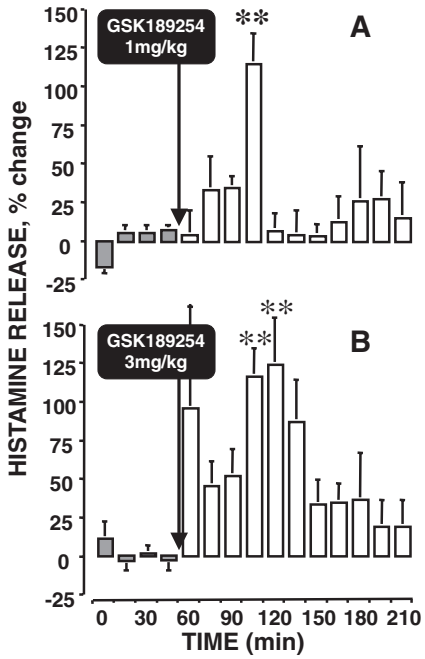

Fig. 3. Time course of histamine release from the TMN of freely moving rats after systemic administration of GSK189254. Histamine release was measured in fractions collected every 15 min. GSK189254 was administered orally at two doses, 1 mg/kg (A) and 3 mg/kg (B). Control values of spontaneous histamine release were calculated for each experiment by averaging the mean of four initially collected 15-min samples. Mean spontaneous release of histamine was 54 ± 4 fmol every 15 min (A), and 74 ± 13 fmol every 15 min (B). Histamine release was expressed as a percentage of spontaneous release. The arrow indicates the time of drug administration. Represented are means ± S.E.M. of 4 (1 mg/kg) and 6 (3 mg/kg) rats. **, $P < 0.01$ versus last sample before drug treatment (ANOVA and Bonferroni's or Dunn's test).

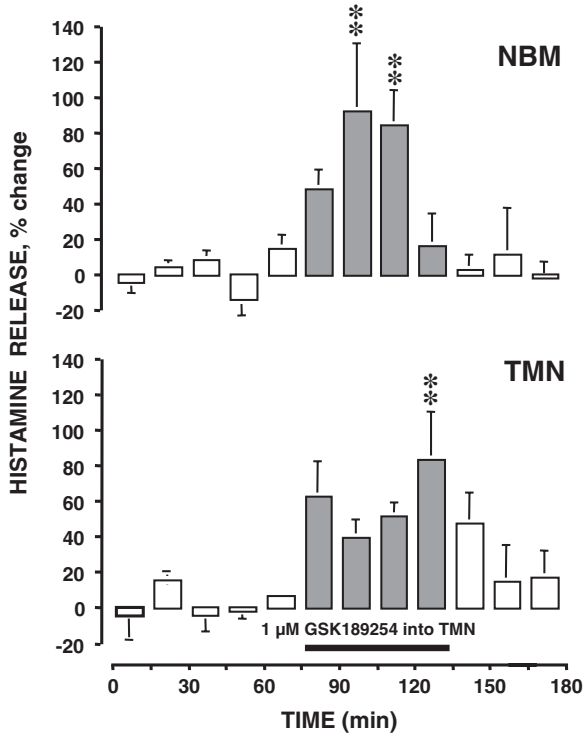

Fig. 4. Influence of GSK189254 administration into the TMN on histamine release from the TMN (bottom) and the NBM (top) of freely moving rats. Histamine was measured in 15-min fractions and expressed as a percentage of spontaneous release. Control values of spontaneous release were calculated for each experiment by averaging the mean of four initially collected 15-min samples. Histamine spontaneous release averaged 94 ± 10 fmol every 15 min in the TMN, and 83 ± 7 fmol every 15 min in the NBM. TMN was perfused with 1 μM GSK189254, and histamine release was measured from the TMN and the NBM. Bar indicates the period of GSK189254 application. Shown are means ± S.E.M. of five experiments. **, $P < 0.01$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

and prefrontal cortex. Infusion of GSK189254 (1 μM) for 60 min locally into the TMN elicited a significant increase of histamine release from both the TMN (ANOVA, $F_{(11,60)} = 3.315$, $P < 0.0013$) and the prefrontal cortex (ANOVA, $F_{(11,60)} = 5.067$, $P < 0.0001$). Histamine release was restored to control levels during subsequent TMN perfusion with control medium (Fig. 5). In the TMN, GSK189254 produced a maximal increase of 91 ± 25%. In the prefrontal cortex, where histamine was released from TMN projections, the maximal increase was 109 ± 19%.

In another set of experiments, releases of histamine from the TMN, and ACh from the prefrontal cortex were monitored (Fig. 6). Neostigmine bromide (0.1 μM), a cholinesterase inhibitor, was added to the medium perfusing the prefrontal cortex to recover detectable ACh concentrations in the dialysate. As shown in Fig. 6, TMN perfusion for 60 min with 1 μM GSK189254 significantly increased releases of histamine from the TMN up to 114 ± 39% (ANOVA, $F_{(11,24)} = 9.222$, $P < 0.0001$), and of ACh from the prefrontal cortex up to 78 ± 6% (ANOVA, $F_{(11,24)} = 14.035$, $P < 0.0001$). Histamine and ACh release was restored to control levels during subsequent TMN perfusion with control medium (Fig. 6).

Local Perfusion of GSK189254 into the TMN Failed to Alter Spontaneous Release of Histamine from the Dorsal Striatum. Perfusion of the TMN with 1 μM GSK189254 increased significantly histamine release from the TMN (ANOVA, $F_{(11,48)} = 10.612$, $P < 0.0001$). Increase of histamine release reached 130 ± 22% of basal levels (Fig. 7). Histamine levels returned to basal values during washout of the compound. Conversely, GSK189254 infused into the

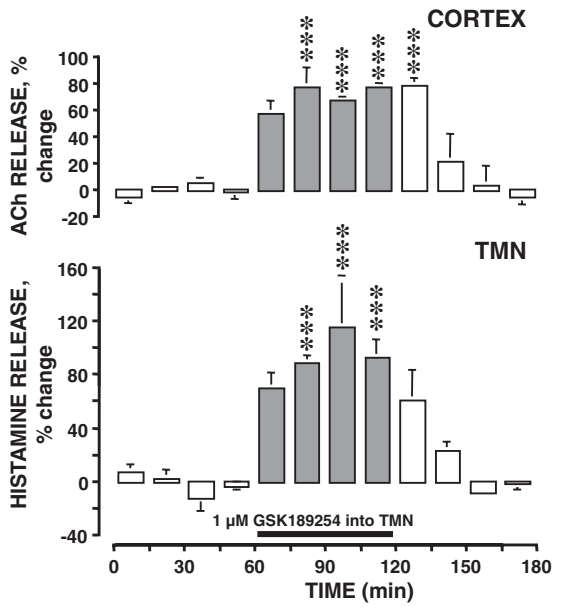

Fig. 6. Influence of GSK189254 administration into the TMN on release of histamine from the TMN (bottom), and of ACh from the prefrontal cortex (top) of freely moving rats. Histamine was measured and calculated as described in Fig. 4. ACh was measured in 15-min fractions and expressed as a percentage of spontaneous release, calculated for each experiment by averaging the mean of four initially collected 15-min samples. Mean spontaneous releases were 54 ± 3 fmol every 15 min (histamine) and 220 ± 80 fmol every 15 min (ACh), respectively. TMN was perfused for 60 min with 1 μM GSK189254, histamine release was measured from the TMN and ACh release from the prefrontal cortex. Bar indicates the period of GSK189254 application. Shown are means ± S.E.M. of three experiments. ***, $P < 0.001$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

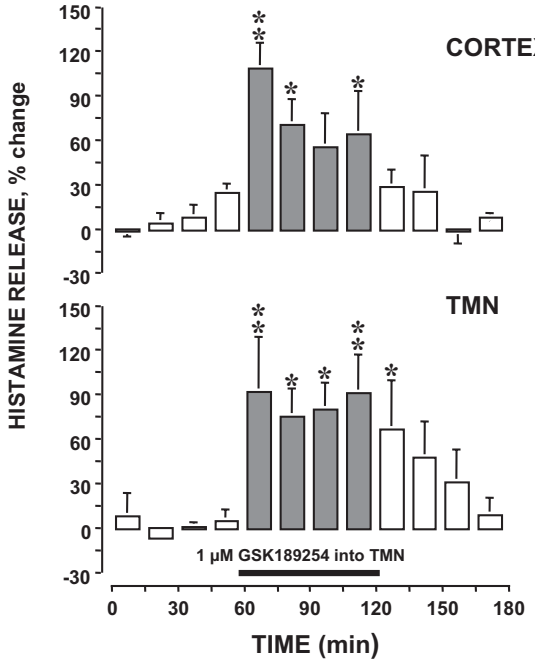

Fig. 5. Influence of GSK189254 administration into the TMN on histamine release from the TMN (bottom) and the prefrontal cortex (top) of freely moving rats. Histamine was measured and calculated as described in Fig. 4. The mean spontaneous release of histamine was 41 ± 4 fmol every 15 min in the TMN, and 36 ± 4 fmol every 15 min in the prefrontal cortex. GSK189254, at a concentration of 1 μM, was infused into the TMN, and histamine release was measured from the TMN and the prefrontal cortex. Bar indicates the period of drug application. Shown are means ± S.E.M. of six experiments. *, $P < 0.05$; **, $P < 0.01$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

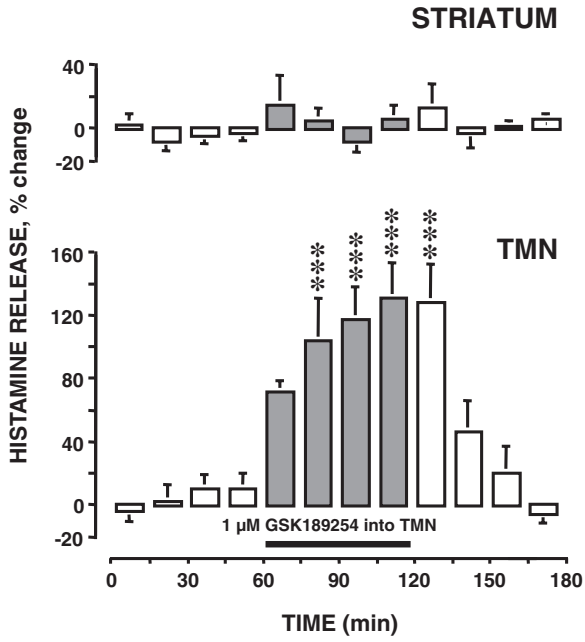

Fig. 7. Influence of GSK189254 administration into the TMN on histamine release from the TMN (bottom) and the dorsal striatum (top) of freely moving rats. Histamine was measured and calculated as described in Fig. 4. The mean of histamine spontaneous release from the TMN was 50 ± 3 fmol every 15 min and 45 ± 4 fmol every 15 min from the dorsal striatum. TMN was perfused with 1 μM GSK189254, and histamine release was measured from the TMN and the dorsal striatum. Bar indicates the period of GSK189254 application. Shown are means ± S.E.M. of five experiments. ***, $P < 0.001$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

TMN did not produce any significant change in the release of histamine from the dorsal striatum, the changes being within variability range (approximately 20%) observed between individual 15-min collection periods during perfusion with control medium (Fig. 7).

Local Perfusion of GSK189254 into the TMN Did Not Alter Spontaneous Release of Either Histamine or Dopamine from the Nucleus Accumbens. When GSK189254 (1 μM) was added to the TMN-perfusing medium for 60 min, the spontaneous release of histamine from the TMN increased significantly by a maximum of 74 ± 12% (ANOVA, $F_{(11,36)} = 6.862$, $P < 0.0001$), but was not significantly changed in the nucleus accumbens, because the changes remained within variability range (approximately 20%) observed between individual 15-min collection periods during perfusion with control medium (Fig. 8).

In another set of experiments, histamine release was monitored from the TMN and dopamine release from the nucleus accumbens. Perfusion of the TMN for 60 min with 1 μM GSK189254 increased significantly histamine release (ANOVA, $F_{(11,24)} = 10.443$, $P < 0.0001$) with a maximal increase of 119 ± 14%, whereas the changes in dopamine release remained within the range of variability (approximately 20%) observed among baseline samples (Fig. 9). Histamine output returned slowly to baseline values after TMN perfusion with GSK189254 ended (Fig. 9).

Single-Probe Experiments: Effects of Local Perfusion with GSK189254 on Histamine Release from the NBM and Striatum. To understand the effects of GSK189254 at axonal domains of histaminergic neurons, each rat was implanted with a single probe in the NBM or the striatum, to locally administer GSK189254 and monitor changes in histamine release. A 45-min application to the NBM of a medium containing 300 nM GSK189254 had no significant effect on spontaneous histamine release, the changes always being within the range of variability (approximately 20%) observed between individual 15-min collection periods during perfusion with control medium (Fig. 10A). Conversely, NBM perfusion with 1 μM GSK189254 for 45 min elicited a significant, transient increase in histamine release (ANOVA, $F_{(10,22)} = 12.538$, $P < 0.0001$) with a maximal value of 117 ± 6% (Fig. 10B). Increased histamine levels persisted during perfusion with GSK189254, after which basal histamine levels were quickly attained. Perfusion of the striatum with 1 μM GSK189254 for 45 min failed to produce any significant change in the spontaneous release of histamine (Fig. 10C).

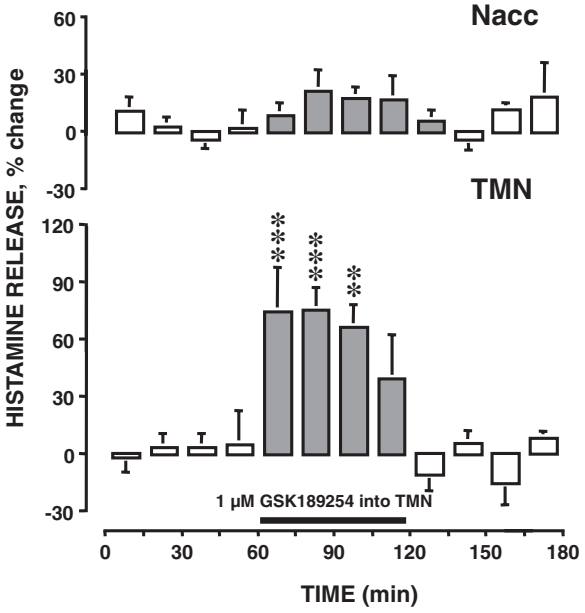

Fig. 8. Influence of GSK189254 administration into the TMN on histamine release from the TMN (lower) and the nucleus accumbens (Nacc; upper) of freely moving rats. Histamine was measured and calculated as described in Fig. 4. The mean spontaneous release of histamine was 55 ± 3 fmol every 15 min in the TMN, and 32 ± 3 fmol every 15 min in the nucleus accumbens. TMN was perfused with 1 μM GSK189254, and histamine release was measured from the TMN and the nucleus accumbens. Bar indicates the period of GSK189254 application. Shown are means ± S.E.M. of four experiments. **, $P < 0.01$; ***, $P < 0.001$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

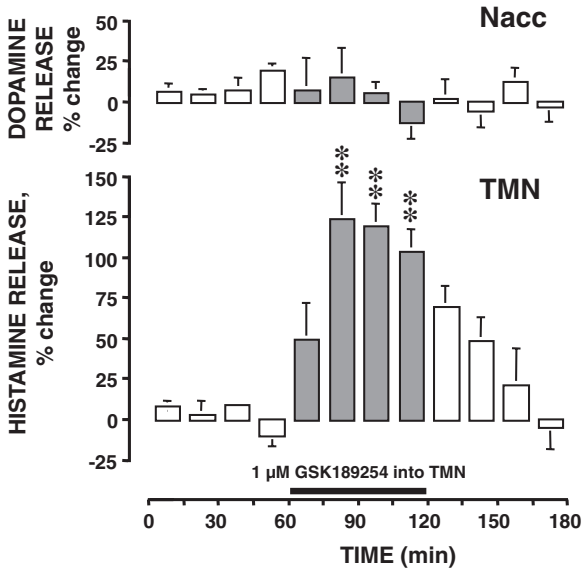

Fig. 9. Influence of GSK189254 administration into the TMN on release of histamine from the TMN (lower), and of dopamine from the nucleus accumbens (Nacc; upper) of freely moving rats. Histamine was measured and calculated as described in Fig. 4. Dopamine was measured in 15-min fractions and expressed as a percentage of spontaneous release, calculated for each experiment by averaging the mean of four initially collected 15-min samples. Mean spontaneous release was 49 ± 18 fmol every 15 min for histamine, and 15.1 ± 1 nmol every 15 min for dopamine. TMN was perfused with 1 μM GSK189254, and histamine release was measured from the TMN simultaneously to dopamine release from the nucleus accumbens. Bar indicates the period of GSK189254 application. Shown are means ± S.E.M. of three experiments. **, $P < 0.01$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

Object Recognition. The effects of GSK189254 administration on scopolamine-induced amnesia in the rat object recognition test are depicted in Fig. 11. Rats injected with saline and those receiving GSK189254 in combination with scopolamine spent significantly more time exploring the new object than the familiar one, whereas those treated with scopolamine alone showed no significant difference in the exploration time of the familiar object compared with that of the novel one (Fig. 11). The discrimination index (D) was 0.38 ± 0.08 for saline-injected rats, 0.25 ± 0.07 for those treated with GSK189254 associated with scopolamine, and −0.03 ± 0.07 for the animals that received scopolamine alone. Analysis of variance on the discrimination index confirmed a significant group effect ($F_{(2,30)} = 8.261$, $P = 0.0014$), and Each Pair Student's t post hoc comparisons revealed that the discrimination index of rats receiving scopolamine was

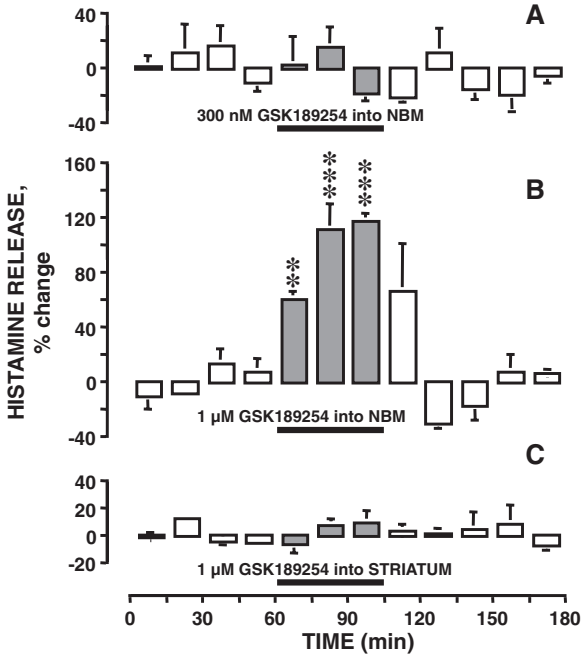

Fig. 10. Effects of local perfusion with GSK189254 on histamine release from the NBM, and the dorsal striatum of freely moving rats: single-probe experiments. Each rat was implanted in the NBM (A and B), or dorsal striatum (C) with a single probe used to simultaneously administer GSK189254 locally and monitor changes in histamine release. Control values of spontaneous release were calculated for each experiment by averaging the mean of four initially collected 15-min samples. The spontaneous release of histamine from the NBM averaged 54 ± 5 fmol every 15 min (A) and 91 ± 6 fmol every 15 min (B), and from the striatum, 38 ± 1 fmol every 15 min (C). The different regions were perfused with 300 nM or 1 μM GSK189254 for 45 min. Bars indicate the period of GSK189254 application. Shown are means ± S.E.M. of four (A), three (B), and three (C) experiments. **, $P < 0.01$; ***, $P < 0.001$ versus last sample before drug treatment (ANOVA and Bonferroni's test).

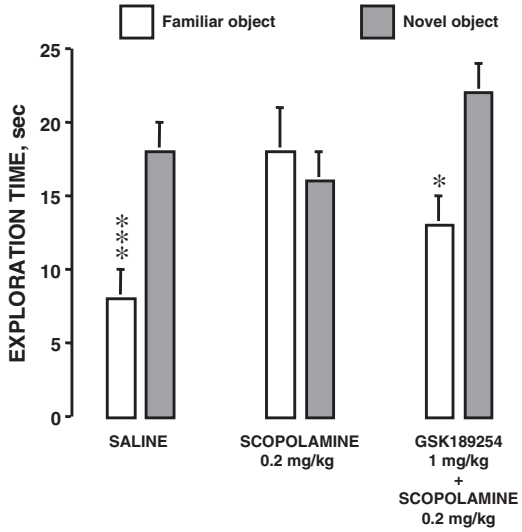

Fig. 11. Effects of pretraining administration of GSK189254 on scopolamine-induced amnesia in the rat object recognition test. Saline (250 μl i.p.), scopolamine (0.2 mg/kg s.c.), and GSK189254 (1 mg/kg i.p.) were dissolved in 0.9% wt/vol NaCl solution to permit the injection of a constant volume (1 ml/kg per rat), and injected 30 min (saline and scopolamine) or 60 min (GSK189254) before the first trial (t1). Shown are means ± S.E.M. of 9 (saline), 12 (scopolamine), and 13 (scopolamine + GSK189254) rats. *, $P < 0.05$; ***, $P < 0.001$ (unpaired t test).

significantly lower from that of rats receiving saline ($P = 0.0005$), and GSK189254 in combination with scopolamine ($P = 0.008$).

Discussion

Cognitive decline characteristic of Alzheimer's disease and related dementias is currently treated with cholinesterase inhibitors, although they provide only modest and transient benefits to some but not all patients (Buccafusco and Terry, 2000; Johnson et al., 2004). Alternative strategies are being actively pursued, and the therapeutic potential of H_3R antagonists/inverse agonists for correcting cognitive deficits is raising great interest (Passani et al., 2004; Esbenshade et al., 2008). Although Bongers et al. (2004) reported that H_3R antagonists/inverse agonists impaired object recognition in mice, numerous other studies have shown that their administration to experimental animals can elicit procognitive effects in several cognitive tasks (Esbenshade et al., 2008). The broad spectrum of efficacy observed across different cognitive domains increases confidence in using H_3R antagonists for the treatment of cognitive deficits in a number of central nervous system disorders. GSK189254 is a novel, selective, brain-penetrant H_3R antagonist/inverse agonist with high affinity for human and rat H_3Rs that improved rat performance in several cognitive paradigms (Medhurst et al., 2007). It is noteworthy that, from a clinical perspective, it has been demonstrated that [^{3}H]GSK189254 can bind to H_3Rs in hippocampal and cortical sections from patients with advanced Alzheimer's disease (Medhurst et al., 2007, 2009), suggesting the persistence of H_3Rs in severe dementia.

GSK189254 was previously shown to modulate ACh, noradrenaline, and dopamine release (Medhurst et al., 2007). In the present study, we investigated the effects of GSK189254 primarily on histamine release after oral dosing and direct administration into different brain regions. Oral administration of GSK189254 significantly enhanced histamine release from the TMN with maximum effect at approximately 60 min postdose, consistent with the T_{max} range from previous pharmacokinetic studies (Medhurst et al., 2007). Doses were similar to those increasing the release of other neurotransmitters, and effective in cognition models (Medhurst et al., 2007). H_3 autoreceptors on histaminergic somata provide a tonic inhibition of firing rate (Haas and Panula, 2003), whereas those on presynaptic terminals restrict histamine synthesis and release (Arrang et al., 1983, 1987). Therefore, blockade of somatic and presynaptic H_3 autoreceptors converge in augmenting histamine levels in the synaptic cleft. Hence, TMN histamine increase may result from blockade of both somatic and presynaptic H_3 autoreceptors. GSK189254 elicited maximal increases of histamine release of similar amplitude at both oral doses (1 and 3 mg/kg), a finding consistent with similar receptor occupancy (80–90%) observed at these doses (Medhurst et al., 2007). Nevertheless, the effect of the higher dose was more prolonged.

GSK189254, applied locally through the microdialysis probe into the TMN, significantly increased histamine release from the TMN, the prefrontal cortex, and the NBM, but not from the striatum or nucleus accumbens. The increases in the prefrontal cortex and NBM were probably due to discharge potentiation of histamine neurons sending affer-

ents to these regions, in a way similar to the effects of TMN perfusion with prostaglandin E_2 (Huang et al., 2003), or Orexin-A (Huang et al., 2001). However, during TMN perfusion with GSK189254, histamine levels remained stable in the dorsal striatum and nucleus accumbens, thus indicating that histaminergic neurons projecting to these regions were insensitive to GSK189254. Moreover, dopamine release from the nucleus accumbens did not change during TMN local perfusion with GSK189254. Previous studies showed that, after GSK189254 administration, activation of *c-fos* occurred in cortical areas and the TMN, but not in striatum (Medhurst et al., 2007). According to neuroanatomical studies histaminergic neurons appear as a rather homogeneous cell group with diffuse, overlapping projections throughout the neuraxis (Köhler et al., 1985). Consequently, this system has been described as a regulatory network for whole-brain activity, modulating general states, rather than processing specific functions (Wada et al., 1991). This view is challenged by the current study, which supports the idea that subsets of histaminergic neurons form independent functional units modulated by selective mechanisms according to their respective origin and terminal projections. Much evidence favoring this hypothesis has been reported recently, with findings demonstrating that histamine neurons differ in their sensitivity to glycine (Sergeeva et al., 2001), GABA (Sergeeva et al., 2005; Giannoni et al., 2009), types of stress (Miklos and Kovacs, 2003), bicuculline or thioperamide (Giannoni et al., 2009), and hypercapnic loading (Haxhiu et al., 2001).

Although further studies are required to understand the full implications of such functional heterogeneity of histaminergic neurons, H_3R antagonists may affect only some of those functions. Indeed, the present findings with GSK189254, a highly selective H_3R antagonist without H_4R activity, fully complement the observations with thioperamide (Giannoni et al., 2009), the prototypical H_3R antagonist/inverse agonist that also displays H_4R antagonist activity (Hough, 2001; Connelly et al., 2009). Our conclusion is that H_3R antagonists, as a class of agents, seem to discriminate functionally between distinct histaminergic neurons. Moreover, the present results seem to exclude H_4R participation.

Spatial segregation due to probe localization does not explain why histaminergic neurons projecting to the striatum or nucleus accumbens do not respond to H_3R antagonists. For example, bicuculline administered into the TMN significantly augmented histamine release from the nucleus accumbens (Giannoni et al., 2009), and TMN perfusion with cannabinoid1 receptor agonists increased histamine from the dorsal striatum (Cenni et al., 2006), confirming the existence of histaminergic afferents to these regions. The procedures for implanting the guide cannulae were exactly the same as those used in the present study. Retrograde tracing with dye injections into the striatum or prefrontal cortex labeled most histaminergic somata within the same area, the medial part of the ventral TMN (Köhler et al., 1985). This proximity suggests that the compounds administered through the microdialysis probe affected indiscriminately histaminergic cells projecting to the striatum and prefrontal cortex.

As observed with thioperamide (Giannoni et al., 2009), local perfusion of the striatum with GSK189254 did not alter spontaneous histamine release, and the somatodendritic domain of histaminergic neurons projecting to this region seemed insensitive to GSK189254. It is generally assumed that all histaminergic neurons express H_3Rs, and a response to H_3R ligands is a criterion for their identification. However, several H_3R isoforms have been described, including 6-transmembrane-domain isoforms lacking functional response to H_3R ligands (Bakker et al., 2006). Hence, in vivo insensitivity to GSK189254 and to thioperamide (Giannoni et al., 2009) may depend on high expression of particular isoforms. Alternatively, the magnitude of neuronal responses to extracellular signals might depend on different receptor number at the membrane. In the TMN, some histidine decarboxylase-positive cells display low levels of H_3R immunoreactivity (Giannoni et al., 2009), although no direct evidence demonstrates that these cells are the ones innervating the nucleus accumbens or striatum.

TMN perfusion with GSK189254 significantly increased cortical ACh release. This effect could be a consequence of increased histamine release in the NBM elicited by intra-TMN administration of GSK189254. Indeed, activation of the H_1R depolarizes the cell membrane of NBM cholinergic neurons increasing their tonic firing (Khateb et al., 1995). Moreover, NBM perfusion with either H_3R antagonists or H_1R agonists increases cortical ACh release (Cecchi et al., 2001). A comparable enhancement of cortical ACh release was observed in response to systemic administration of GSK189254 (Medhurst et al., 2007). Reduced availability of ACh in the synaptic cleft is believed to contribute to cognitive deficits. Since GSK189254 was found to increase ACh release in the prefrontal cortex of freely moving rats, one might envisage that H_3R antagonists might exert procognitive effects in paradigms with important cortical cholinergic components, such as object recognition test (Ennaceur and Meliani, 1992). In the current studies, administration of the muscarinic antagonist scopolamine impaired rat performance in object recognition compared with saline-injected animals. Conversely, animals receiving GSK189254 in association with scopolamine did not differ from controls in discriminating between new and familiar objects, consistent with a cognitive improvement after H_3R blockade. GSK189254 has previously been shown to improve cognitive performance in a number of rodent models including scopolamine-induced amnesia in the passive avoidance paradigm and time-delay-induced deficits in the object recognition model (Medhurst et al., 2007). GSK189254 was administered before training, thus supporting a role in acquisition rather than in recall. Previous studies reported that the procognitive effects of H_3R antagonists became evident only when behavioral deficits were pronounced (Blandina et al., 2004). For example, although thioperamide significantly improved the response in a passive avoidance response in senescence-accelerated mice, characterized by a marked age-accelerated deterioration in learning tasks, it was ineffective in mice aging at a normal rate (Meguro et al., 1995). Likewise, administration of thioperamide or clobenpropit, another H_3R antagonist, failed to exhibit any procognitive effects in normal animals measured in object recognition and a passive avoidance response, but prevented scopolamine-induced amnesia in the same tests (Giovannini et al., 1999).

A beneficial effect on a scopolamine-induced deficit is a concomitant observation, but does not prove that cholinergic neurons are involved. Reversal of impairments observed in

this study may be due to the effects of histamine on any number of transmitter systems and/or due to an intrinsic facilitatory role that histaminergic neurons have on cognitive function. Therefore, H_3R antagonists may provide a valid approach to improve cognitive deficits, and the results of ongoing clinical trials in a number of patient populations are eagerly awaited.

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References

Arrang JM, Garbarg M, and Schwartz JC (1983) Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptors. *Nature* **302:**832–837.

Arrang JM, Garbarg M, and Schwartz J (1987) Autoinhibition of histamine synthesis mediated by presynaptic H_3-receptors. *Neuroscience* **23:**149–157.

Bakker RA, Lozada AF, van Marle A, Shenton FC, Drutel G, Karlstedt K, Hoffmann M, Lintunen M, Yamamoto Y, van Rijn RM, et al. (2006) Discovery of naturally occurring splice variants of the rat histamine H3 receptor that act as dominant-negative isoforms. *Mol Pharmacol* **69:**1194–1206.

Blandina P, Bacciottini L, Giovannini MG, and Mannaioni PF (1998) H3-Receptor modulation of the release of neurotransmitters in vivo, in *The Histamine H3 Receptor, a Target for New Drugs* (Leurs R and Timmerman H eds) pp 27–40, Elsevier, Amsterdam.

Blandina P, Efoudebe M, Cenni G, Mannaioni P, and Passani MB (2004) Acetylcholine, histamine, and cognition: two sides of the same coin. *Learn Mem* **11:**1–8.

Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, Pepeu G, and Giovannini MG (1996) Inhibition of cortical acetylcholine release and cognitive performance by histamine H_3 receptor activation in rats. *Br J Pharmacol* **119:**1656–1664.

Bongers G, Leurs R, Robertson J, and Raber J (2004) Role of H_3-receptor-mediated signaling in anxiety and cognition in wild-type and Apoe−/− mice. *Neuropsychopharmacology* **29:**441–449.

Buccafusco JJ and Terry AV Jr (2000) Multiple central nervous system targets for eliciting beneficial effects on memory and cognition. *J Pharmacol Exp Ther* **295:**438–446.

Cecchi M, Passani MB, Bacciottini L, Mannaioni PF, and Blandina P (2001) Cortical Acetylcholine release elicited by stimulation of histamine H1 receptors in the nucleus basalis magnocellularis: a dual-probe microdialysis study in the freely moving rat. *Eur J Neurosci* **13:**68–78.

Celanire S, Wijtmans M, Talaga P, Leurs R, and de Esch IJ (2005) Keynote review: histamine H3 receptor antagonists reach out for the clinic. *Drug Discov Today* **10:**1613–1627.

Cenni G, Blandina P, Mackie K, Nosi D, Formigli L, Giannoni P, Ballini C, Della Corte L, Mannaioni PF, and Passani MB (2006) Differential effect of cannabinoid agonists and endocannabinoids on histamine release from distinct regions of the rat brain. *Eur J Neurosci* **24:**1633–1644.

Connelly WM, Shenton FC, Lethbridge N, Leurs R, Waldvogel HJ, Faull RL, Lees G, and Chazot PL (2009) The histamine H4 receptor is functionally expressed on neurons in the mammalian CNS. *Br J Pharmacol* **157:**55–63.

Ennaceur A and Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* **31:**47–59.

Ennaceur A and Meliani K (1992) Effects of physostigmine and scopolamine on rats' performances in object-recognition and radial-maze tests. *Psychopharmacology* **109:**321–330.

Esbenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD, and Brioni JD (2008) The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. *Br J Pharmacol* **154:**1166–1181.

Gbahou F, Vincent L, Humbert-Claude M, Tardivel-Lacombe J, Chabret C, and Arrang JM (2006) Compared pharmacology of human histamine H3 and H4 receptors: structure–activity relationships of histamine derivatives. *Br J Pharmacol* **147:**744–754.

Giannoni P, Passani MB, Nosi D, Chazot PL, Shenton FC, Medhurst AD, Munari L, and Blandina P (2009) Heterogeneity of histaminergic neurons in the tuberomammillary nucleus of the rat. *Eur J Neurosci* **29:**2363–2374.

Giovannini MG, Bartolini L, Bacciottini L, Greco L, and Blandina P (1999) Effects of histamine H_3 receptor agonists and antagonists on cognitive performance and scopolamine-induced amnesia. *Behav Brain Res* **104:**147–155.

Haas H and Panula P (2003) The role of histamine and the tuberomammillary nucleus in the nervous system. *Nat Rev Neurosci* **4:**121–130.

Haas HL, Sergeeva OA, and Selbach O (2008) Histamine in the nervous system. *Physiol Rev* **88:**1183–1241.

Haxhiu MA, Tolentino-Silva F, Pete G, Kc P, and Mack SO (2001) Monoaminergic neurons, chemosensation and arousal. *Respir Physiol* **129:**191–209.

Hough LB (2001) Genomics meets histamine receptors: new subtypes, new receptors. *Mol Pharmacol* **59:**415–419.

Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, and Hayaishi O (2001) Arousal effect of orexin A depends on activation of the histaminergic system. *Proc Natl Acad Sci USA* **98:**9965–9970.

Huang ZL, Sato Y, Mochizuki T, Okada T, Qu WM, Yamatodani A, Urade Y, and Hayaishi O (2003) Prostaglandin E2 activates the histaminergic system via the EP4 receptor to induce wakefulness in rats. *J Neurosci* **23:**5975–5983.

Inagaki N, Yamatodani A, Ando-Yamamoto M, Tohyama M, Watanabe T, and Wada H (1988) Organization of histaminergic fibers in the rat brain. *J Comp Neurol* **273:**283–300.

Johnson CN, Roland A, and Upton N (2004) New symptomatic strategies in Alzheimer's disease. *Drug Discov Today* **1:**13–19.

Khateb A, Fort P, Pegna A, Jones BE, and Mühlethaler M (1995) Cholinergic nucleus basalis neurons are excited by histamine in vitro. *Neuroscience* **69:**495–506.

Köhler C, Swanson LW, Haglund L, and Wu JY (1985) The cytoarchitecture, histochemistry and projections of the tuberomammillary nucleus in the rat. *Neuroscience* **16:**85–110.

Medhurst AD, Atkins AR, Beresford IJ, Brackenborough K, Briggs MA, Calver AR, Cilia J, Cluderay JE, Crook B, Davis JB, et al. (2007) GSK189254, a novel H3 receptor antagonist that binds to histamine H3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. *J Pharmacol Exp Ther* **321:**1032–1045.

Medhurst AD, Roberts JC, Lee J, Chen CP, Brown SH, Roman S, and Lai MK (2009) Characterisation of histamine H3 receptors in Alzheimer's Disease brain and amyloid over-expressing TASTPM mice. *Br J Pharmacol* **157:**130–138.

Meguro K, Yanai K, Sakai N, Sakurai E, Maeyama K, Sasaki H, and Watanabe T (1995) Effects of thioperamide, a histamine H3 antagonist, on the step-through passive avoidance response and histidine decarboxylase activity in senescence-accelerated mice. *Pharmacol Biochem Behav* **50:**321–325.

Miklos IH and Kovacs KJ (2003) Functional heterogeneity of the responses of histaminergic neuron subpopulations to various stress challenges. *Eur J Neurosci* **13:**3069–3079.

Panula P, Flügge G, Fuchs E, Pirvola U, Auvinen S, and Airaksinen MS (1989) Histamine-immunoreactive nerve fibers in the mammalian spinal cord. *Brain Res* **484:**234–239.

Passani MB, Lin JS, Hancock A, Crochet S, and Blandina P (2004) The histamine H3 receptor as a novel therapeutic target for cognitive and sleep disorders. *Trends Pharmacol Sci* **25:**618–625.

Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates.* Academic Press, New York.

Sander K, Kottke T, and Stark H (2008) Histamine H3 receptor antagonists go to clinics. *Biol Pharm Bull* **31:**2163–2181.

Sergeeva OA, Andreeva N, Garret M, Scherer A, and Haas HL (2005) Pharmacological properties of GABAA receptors in rat hypothalamic neurons expressing the epsilon-subunit. *J Neurosci* **25:**88–95.

Sergeeva OA, Eriksson KS, and Haas HL (2001) Glycine receptor mediated responses in rat histaminergic neurons. *Neurosci Lett* **300:**5–8.

Takahashi K, Lin JS, and Sakai K (2006) Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. *J Neurosci* **26:**10292–10298.

Wada H, Inagaki N, Yamatodani A, and Watanabe T (1991) Is the histaminergic neuron system a regulatory center for whole-brain activity? *Trends Neurosci* **14:**415–418.

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