A Novel Potent and Selective Histamine H₃ Receptor Antagonist Enerisant: In Vitro Profiles, In Vivo Receptor Occupancy, and Wake-Promoting and Procognitive Effects in Rodents[S]

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ABSTRACT

Histamine H₃ receptor antagonists/inverse agonists are known to enhance the activity of histaminergic neurons in the brain, thereby promoting arousal and cognition. Here, we report the in vitro and in vivo pharmacological profiles for a newly synthesized histamine H₃ receptor antagonist/inverse agonist: [1-(4-{3-[(2R)-2-methylpyrrolidin-1-yl]propoxy}phenyl)-1H-pyrazol-4-yl](morpholin-4-yl)methanone monohydrochloride (enerisant hydrochloride). In vitro assays showed that enerisant was a competitive antagonist/inverse agonist with a high affinity and selectivity for human and rat histamine H₃ receptors. Enerisant showed antagonist activity in vivo, as assessed using R-α-methylhistamine (a histamine H₃ receptor agonist)–induced dipsogenia, and occupied the histamine H₃ receptor in the frontal cortex in a dose-dependent manner. Enerisant also enhanced the extracellular levels of histamine in the posterior hypothalamus and the levels of dopamine and acetylcholine in the medial prefrontal cortex of rats. Enerisant exerted a procognitive effect or reversed scopolamine-induced cognitive impairment in a social recognition test and a novel object recognition test in rats at doses at which less than 50% of the histamine H₃ receptor were occupied (0.03–0.3 mg/kg, p.o.). In contrast, higher doses (3–10 mg/kg, p.o.) at which nearly all the histamine H₃ receptors were occupied were needed to exert wake-promoting effects in rats. These results indicate that enerisant is a potent and selective histamine H₃ receptor antagonist/inverse agonist with the potential to promote arousal and procognition in rats. Moreover, the results also suggest that the histamine H₃ receptor occupancy required to exert a pharmacological effect may vary depending on the domain that is being tested.

SIGNIFICANCE STATEMENT

Enerisant is a novel histamine H₃ receptor antagonist/inverse agonist that exerts wake-promoting and procognitive effects in addition to increasing the release of neurotransmitters related to these pharmacological effects in rodents. Moreover, an in vivo receptor binding study revealed that the in vivo occupancy of the histamine H₃ receptor required to exert the pharmacological effects of enerisant varied, and such variations in required occupancy should be taken into account when performing dose selection in clinical studies.

Introduction

The histaminergic system in the central nervous system is implicated in sleep/wake regulation, attention, cognition, and metabolic homeostasis via four subtypes of histamine receptors (H₁, H₂, H₃, and H₄ receptors), all of which belong to a family of G protein–coupled receptors (Leurs et al., 2011). Of these, the histamine H₃ receptor is highly and widely localized in the brain, particularly in areas involved in arousal and cognitive processes such as the cerebral cortex, hippocampus, basal ganglia, and hypothalamus (Martinez-Mir et al., 1990; Lovenberg et al., 1999; Drutel et al., 2001). In these regions,

the histamine H₃ receptor functions as an autoreceptor and/or a heteroreceptor to modulate the release of several neurotransmitters in a negative manner (Arrang et al., 1983; Esbenshade et al., 2008). Conversely, histamine H₃ receptor blockade reportedly enhances the release of multiple neurotransmitters involved in arousal, vigilance, alertness, and cognition (Bacciottini et al., 2002; Giannoni et al., 2010).

Because of the variety of roles that the histamine H₃ receptor plays in neurotransmission, considerable efforts have been expended to develop histamine H₃ receptor antagonists/inverse agonists as potential therapeutic agents for the treatment of sleep/wake disorders such as narcolepsy as well as attention-deficit hyperactivity disorder, Alzheimer's disease, schizophrenia, and other disorders associated with cognitive impairment (Fox et al., 2003, 2005; Ligneau et al., 2007; Medhurst et al., 2007; Esbenshade et al., 2012; Raddatz

ABBREVIATIONS: BSA, bovine serum albumin; CHO, Chinese hamster ovary; CSF, cerebrospinal fluid; EEG, electroencephalogram; enerisant hydrochloride, [1-(4-{3-[(2R)-2-methylpyrrolidin-1-yl]propoxy}phenyl)-1H-pyrazol-4-yl](morpholin-4-yl)methanone monohydrochloride; GTPγS, guanosine 5′-(γ-thio)triphosphate; mPFC, medial prefrontal cortex; RID, ratio of investigation duration; SD, Sprague-Dawley; TASP0410461, 6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(5-fluoropyridin-3-yl)-3,4-dihydroquinolin-2(1H)-one.

et al., 2012). To date, however, most clinical trials using histamine H$_3$ receptor antagonists/inverse agonists have failed, despite numerous potent compounds having been synthesized and tested preclinically (Ghamari et al., 2019). Pitolisant, however, has been approved for the treatment of narcolepsy and excessive daytime sleepiness (Javaheri and Javaheri, 2020; Lamb, 2020).

Although the efficacies of numerous histamine H$_3$ receptor antagonists/inverse agonists have been tested in many preclinical studies, few reports have thoroughly compared the direct relations between receptor occupancy and several domains of pharmacological actions (Miller et al., 2009; Esbenshade et al., 2012). Antagonists have been shown to exhibit differing relationships between behavioral efficacy and the levels of histamine H$_3$ receptor occupancy when the histamine H$_3$ receptor occupancy was evaluated by using an appropriate radiolabeled ligand for detecting in vivo receptor binding (Miller et al., 2009). Therefore, delineating the appropriate receptor occupancy for different pharmacological effects is important. Moreover, because the determination of histamine H$_3$ receptor occupancy using in vivo receptor binding is analogous to positron emission tomography imaging studies in clinical settings, the results obtained can be applicable and beneficial to clinical development by enabling the optimal doses to be determined and tested for specific disorders.

We recently synthesized a novel, selective and potent histamine H$_3$ receptor antagonist/inverse agonist: [1-(4-{3-[(2R)-2-methylpyrrolidin-1-yl]propoxy}phenyl)-1H-pyrazol-4-yl](morpholin-4-yl)methanone monohydrochloride (enerisant hydrochloride). First, we characterized the in vitro profiles and the pharmacological effects of enerisant, including its wake-promoting and procognitive effects. We also used a radiolabeled ligand ([^{3}H]TASP0410461) that can be used to label the histamine H$_3$ receptor in vivo to determine the histamine H$_3$ receptor occupancy of enerisant, enabling the pharmacological actions of enerisant to be examined in relation to histamine H$_3$ receptor occupancy.

Materials and Methods

Animal. Male Sprague-Dawley (SD) rats (7–13 weeks; Charles River, Yokohama, Japan) were used to evaluate in vivo binding of histamine H$_3$ receptor to investigate R-α-methylhistamine–induced drinking behavior and for the electroencephalogram (EEG) studies and the spontaneous locomotor activity test. Male Wistar rats (9-10 weeks; Charles River) were used for the novel object recognition test. For the social recognition test, adult male SD rats (9 weeks) or juvenile male SD rats (4 weeks) were used. C57BL/6J mice (7 weeks; Charles River) were used to evaluate spontaneous locomotor activity. All the animals were maintained under 12-hour light/dark cycles (lights on at 7:00 AM) in a temperature- and humidity-controlled holding room, with food and water available ad libitum.

All the animal study protocols were approved by the Animal Care and Use Committee of Taisho Pharmaceutical Co., Ltd. and the EEG studies were approved by the Institutional Animal Ethics Committee of NISSEI BILIS Co., Ltd.

Drugs. [1-(4-{3-[(2R)-2-Methylpyrrolidin-1-yl]propoxy}phenyl)-1H-pyrazol-4-yl](morpholin-4-yl)methanone monohydrochloride (enerisant hydrochloride) (Fig. 1) was synthesized at Taisho Pharmaceutical Co., Ltd., and dissolved in dimethyl sulfoxide for the in vitro studies and in saline or distilled water for the in vivo studies. [^{3}H]6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(5-fluoropyridin-3-yl)-3,4-dihydroquinolin-2(1H)-one (TASP0410461) (specific radioactivity: 524 GBq/mmol) was synthesized at Sekisui Medical Co., Ltd. (Tokyo,

Japan). [^{3}H]N-α-methylhistamine (specific radioactivity: 3156 GBq/mmol), [^{35}S]guanosine 5′-(γ-thio)triphosphate (GTPγS) (specific radioactivity: 46.25 TBq/mmol), and Chinese hamster ovary (CHO)-K1 cells expressing human H$_3$ receptor were purchased from PerkinElmer Inc. (Waltham, MA). R-α-methylhistamine dihydrobromide was purchased from Tocris Bioscience (Bristol, UK). Thioperamide maleate, GTPγS, R-α-methylhistamine dihydrochloride, and scopolamine hydrochloride were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). Donepezil hydrochloride was purchased from Eisai Co., Ltd. (Tokyo, Japan).

Radioligand Receptor Binding Assays for Histamine H$_3$ Receptor. The affinity of enerisant for the histamine H$_3$ receptor was determined using [^{3}H]N-α-methylhistamine binding to the membranes of CHO-K1 cells expressing human recombinant H$_3$ receptor (for human histamine H$_3$ receptor) or the membranes of rat cerebral cortex (for rat histamine H$_3$ receptor). Membrane preparations in 50 mmol/l Tris-HCl buffer (pH 7.4) containing 5 mmol/l EDTA and 0.1% (w/v) bovine serum albumin (BSA) were incubated with [^{3}H]N-α-methylhistamine (1 nmol/l for CHO-K1 cells; 0.75 nmol/l for rat cerebral cortex) in the presence or absence of increasing concentrations (0.001–10,000 nmol/l) of enerisant at 25°C for 1 hour. Nonspecific binding was determined in the presence of R-α-methylhistamine (10 μmol/l) for the CHO-K1 cells or thioperamide (10 μmol/l) for the rat cerebral cortex. We used R-α-methylhistamine to determine nonspecific binding for human histamine H$_3$ receptor according to the manufacturer's technical data sheet (PerkinElmer Inc.). The reaction mixtures were filtered by rapid filtration under a vacuum through a UniFilter GF/C microplate (PerkinElmer Inc.) presoaked with 0.3% (w/v) polyethyleneimine, after which the filters were washed with 50 mmol/l Tris-HCl buffer (pH 7.4) containing 5 mmol/l EDTA using a Cell harvester. The filter-bound radioactivity was counted using a TopCount NXT (PerkinElmer Inc.). The experiments were performed in duplicate. Specific binding was determined by subtracting the nonspecific binding from the total binding. The K$_i$ value was calculated using the equation K$_i$ = IC$_{50}$/(1 + (L/K$_d$)), where L is the concentration of [^{3}H]N-α-methylhistamine and K$_d$ was determined using various concentrations of the radioligand. When the binding mode of enerisant to the histamine H$_3$ receptor was investigated, [^{3}H]N-α-methylhistamine binding was conducted using various concentrations of [^{3}H]N-α-methylhistamine (0.25, 0.5, 1, 2, 4, 8, 16 nmol/l) in the absence or presence of enerisant (3 and 10 nmol/l), and the K$_d$ and B$_{max}$ values were determined using a nonlinear least-squares curve-fitting procedure.

Additional Receptor Bindings and Enzyme Assays. The affinity of enerisant to other histamine receptor subtypes (H$_1$, H$_2$, and H$_4$ receptors) was evaluated using respective binding assays for each of the human recombinant receptors (Sekisui Medical Co., Ltd., Ibaraki, Japan). Enerisant was run against a panel of 66 receptor binding assays and 45 enzyme assays (Cerep, Le Bois l'Evêque, France) according to previously reported methods. Enerisant was tested in duplicate at 1 and 10 μmol/l.

[^{35}S]GTPγS Binding Assay for Histamine H$_3$ Receptor. The antagonist and inverse agonist activities of enerisant were determined by examining [^{35}S]GTPγS binding to the membranes of CHO-K1 cells expressing human recombinant histamine H$_3$ receptor (for human histamine H$_3$ receptor) or the membranes of rat frontal cortex (for rat
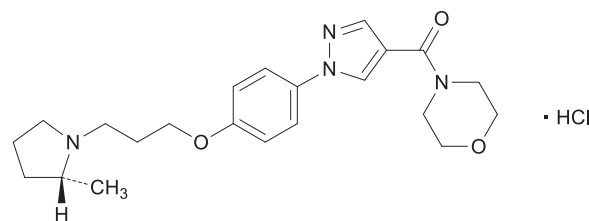

Fig. 1. Chemical structure of enerisant hydrochloride.

histamine H_3 receptor). For human histamine H_3 receptor, membrane preparations in 20 mmol/l HEPES buffer (pH 7.4) containing 100 mmol/l NaCl, 3 mmol/l $MgCl_2$, 10 μg/ml saponin, 0.1% (w/v) BSA, and 3 μmol/l GDP were incubated with increasing concentrations (0.001–10,000 nmol/l) of enerisant in the presence or absence of R-α-methylhistamine (30 nmol/l) at 30°C for 20 minutes, after which the reaction mixtures were further incubated with [^{35}S]GTPγS (0.2 nmol/l) at 30°C for 30 minutes. Nonspecific binding was determined in the presence of GTPγS (10 μmol/l) instead of R-α-methylhistamine. For the rat histamine H_3 receptor, membrane preparations in 20 mmol/l HEPES buffer (pH 7.4) containing 100 mmol/l NaCl, 10 mmol/l $MgCl_2$, 30 μg/ml saponin, 0.1% (w/v) BSA, 300 μmol/l GDP and 1 U/ml adenosine deaminase were incubated with increasing concentrations (0.001–10,000 nmol/l) of enerisant in the presence or absence of R-α-methylhistamine (295 nmol/l) at 30°C for 20 minutes, after which the reaction mixtures were further incubated with [^{35}S]GTPγS (0.3 nmol/l) at 30°C for 90 minutes. Nonspecific binding was determined in the presence of GTPγS (10 μmol/l) instead of R-α-methylhistamine. The reaction was terminated as described for [^{3}H]N-α-methylhistamine binding. The inverse agonist activity of enerisant was determined using the same experimental conditions except for the absence of R-α-methylhistamine in the reaction mixtures.

R-α-Methylhistamine–Induced Dipsogenia in Rats. Male SD rats were placed in a five-compartment stainless-steel cage and were habituated with free access to food and water before the test. Water was provided through a stainless-steel sipper connected to a silicon-based tube with a 10 ml syringe. Enerisant hydrochloride was orally administered 1 hour before the intraperitoneal injection of R-α-methylhistamine dihydrochloride (3 mg/kg). Access to food was stopped, and the amount of water that was subsequently consumed was recorded for 1 hour immediately after R-α-methylhistamine dihydrochloride injection.

Histamine H_3 Receptor In Vivo Binding. The histamine H_3 receptor occupancy of enerisant was determined by the in vivo binding of [^{3}H]TASP0410461, a histamine H_3 receptor antagonist/inverse agonist, which is a radioligand suitable for labeling the histamine H_3 receptor in vivo (Supplemental Fig. 1). Male SD rats were administered [^{3}H]TASP0410461 (740 kBq/ml, 1 ml/kg) through the tail vein at 1 hour after the oral administration of enerisant hydrochloride (0.1, 0.3, 1, and 3 mg/kg). Thirty minutes after the injection of [^{3}H]TASP0410461, the rats were sacrificed, and the frontal cortex was dissected. In addition, the cerebellum, which is a region with low levels of histamine H_3 receptor, was dissected from the vehicle-treated rats and was used to determine nonspecific binding. The tissues were then homogenized in ice-cold 50 mmol/l Tris-HCl buffer (pH 7.4) containing 5 mmol/l EDTA, and the homogenates were filtered over GF/B glass microfiber filters presoaked with 0.3% (w/v) polyethyleneimine solution, after which the filters were washed with the buffer. The percentage receptor occupancy of enerisant was determined by its ability to compete for the in vivo binding of [^{3}H]TASP0410461 in the frontal cortex and was calculated using the following formula:

$$\text{Receptor occupancy of enerisant } (\%) = (1\text{-A/B}) \times 100,$$

where A is the [^{3}H]TASP0410461-specific binding after each dose of enerisant hydrochloride administration, and B is the [^{3}H]TASP0410461-specific binding after vehicle administration.

The ED_{50} value of each test substance was determined from each dose-response curve by nonlinear least-squares curve-fitting procedure.

Microdialysis Experiments. Male SD rats were secured to a brain stereotaxic apparatus under anesthesia with sodium pentobarbital, and a guide cannula (AG-12 for histamine or AG-4 for dopamine and acetylcholine; Eicom Corporation, Kyoto, Japan) was inserted into the posterior hypothalamus (for histamine, AP: −4.3 mm, L: 1.3 mm, V: 7.2 mm from the bregma) or the medial prefrontal cortex (for dopamine and acetylcholine, AP: +3.2 mm, L: 0.8 mm, V: 1 mm) according to the brain atlas of Paxinos and Watson (1997); the

cannula was fixed in place using the cap nut. The rats were allowed to recover for at least 2 days and were habituated to the sound-proof test cage from the day before the test. A microdialysis probe (A-I-12-02 membrane length 2 mm for histamine, A-I-4-03 membrane length 3 mm for dopamine and acetylcholine; Eicom Corporation) was inserted into the guide cannula and was fixed in place using cap nut under isoflurane (histamine) or ether (dopamine and acetylcholine) anesthesia. On the test day, Ringer's solution (flow rate 2 μl/min for histamine and dopamine, 1 μl/min for acetylcholine) was perfused, and dialysate samples were continuously collected at 12-minute (for histamine), 10-minute (for dopamine), or 20-minute (for acetylcholine) intervals. The dialysate samples were automatically and directly injected into the high-performance liquid chromatography–fluorometry (for histamine) or high-performance liquid chromatography–electrochemical detector (for dopamine and acetylcholine) via an autoinjector. The mean value of histamine levels of five dialysate samples (for histamine) or three dialysate samples (for dopamine and acetylcholine) before the administration of enerisant hydrochloride was defined as the basal level. Dialysate sampling was continued for 180 minutes after the administration of enerisant hydrochloride. The levels in the dialysate samples were determined according to Eicom's technical documents.

EEG Sleep/Wake Study. The EEG studies were conducted at NISSEI BILIS Co., Ltd., Shiga Laboratory (Koka, Japan). Male SD rats chronically implanted with EEG electrodes were kept in a measurement cage placed in a soundproof shielded room (light period: 7:00–19:00), and EEG measurements were recorded 24 hours prior to drug administration. Enerisant hydrochloride (1, 3, and 10 mg/kg) was orally administered at around 11:00, and EEG measurements were recorded until 7 hours after the administration of enerisant hydrochloride. Spontaneous EEG and electromyogram signals were extracted from the electroencephalographs and entered into a frequency analysis program (MTS50061A; Nihon Santeku Co., Ltd, Osaka, Japan) running on a personal computer, and the EEG image signal was inputted into the EEG video system (VY301B; Nihon Kohden Corporation Co., Ltd., Tokyo, Japan). Based on the EEG produced by the EEG frequency analysis program, the sleep/wake cycle was visually analyzed in 20-second epochs for each sleep stage [awake, rest, slow-wave light sleep, slow-wave deep sleep, and fast-wave sleep (rapid eye movement sleep)]. The total duration of each stage every hour was then determined. Locomotor activity was measured using a passive infrared sensor locomotor activity measurement system (Supermex; Muromachi Kikai Co., Ltd., Tokyo, Japan).

Novel Object Recognition Test. The novel object recognition test was performed according to a previously reported method with modifications (Karasawa et al., 2008). The experiments were performed using a transparent open field box measuring 45 × 27 × 30 cm. Male Wistar rats were placed in a test box in which two objects had been placed in two corners. The time spent exploring each object was recorded during the subsequent 5-minute period (defined as the training session). After 2 hours, the rats were placed in the test box once again, and one of the familiar objects used in the previous training session was replaced with a novel object. The time spent exploring each object was then recorded during a 5-minute period (defined as the test session). The time spent exploring each object was measured manually by experimenters who were unaware of the treatments. A preference index, consisting of the ratio of the amount of time spent exploring any one of the two objects (during the training session) or the replaced novel one (during the test session) relative to the total time spent exploring both objects, was used to measure memory preference. Twenty minutes before the training session, scopolamine hydrochloride (0.3 mg/kg, i.p.) was administered, and enerisant hydrochloride (0.03, 0.1 and 0.3 mg/kg) or donepezil hydrochloride (1 mg/kg) was administered orally at 1 hour before the training session.

Social Recognition Test. The social recognition test was performed according to a previously reported method (Shimazaki et al., 2010). The experiments were performed using an open-topped box

$(45 \times 27 \times 30$ cm). An adult rat and an unfamiliar juvenile rat were placed in the test cage, and the length of time during which the adult rat exhibited exploratory behavior (sniffing, grooming, and close following) toward the juvenile rat was recorded (first investigation duration). After 85 minutes, the adult was placed in the same test cage and habituated for 30 minutes, after which the same juvenile (familiar) was placed in the test cage once again for a 5-minute test session; the length of time spent by the adult in exploring the juvenile during this test session was measured (second investigation duration). The time spent exploring the juvenile rats was measured manually by experimenters who were unaware of the treatments. The social memory for each adult rat was defined by determining the ratio of the duration of the second investigation to that of the first investigation [ratio of investigation duration (RID)]. Enerisant hydrochloride (0.01, 0.03, and 0.1 mg/kg) or donepezil hydrochloride (0.3 mg/kg) was administered orally immediately after the first investigation. When the effect of enerisant on scopolamine-impaired social memory was investigated, adult rats were trained in a social memory test to recall prior exposure to a conspecific juvenile, as described above, but with the following changes: the two 5-minute investigation periods were separated by only 30 minutes; enerisant hydrochloride or donepezil hydrochloride was administered to the adults rats orally 1 hour prior to the first investigation, and scopolamine hydrochloride was administered to the adult rats intraperitoneally 30 minutes prior to the first investigation.

Spontaneous Locomotor Activity of Rats and Mice. Animals were placed individually in transparent open-topped vinyl chloride or acrylic cages (for rats, $47 \times 28 \times 30$ cm; for mice, 30 cm diameter, 30 cm height), and spontaneous locomotor activity was recorded for 1 hour using a SCANET apparatus (Melquest Ltd, Toyama, Japan) placed in a sound-proof box. Enerisant hydrochloride (0.3, 3, and 30 mg/kg) was administered orally 1 hour before the start of the measurement.

Pharmacokinetic Analyses. To evaluate the exposure levels of enerisant in the plasma, brain, and cerebrospinal fluid (CSF) after systemic administration, enerisant hydrochloride (1, 3, and 10 mg/kg) was orally administered to male SD rats. The animals were sacrificed, and blood, CSF, and brain samples were collected at 0.5, 1, 2, 3, and 7 hours after the administration of enerisant hydrochloride. Each sample was extracted using protein precipitation and was subsequently analyzed to determine the enerisant levels using a liquid chromatography/tandem mass spectrometry–qualified research method on an API4000 instrument (SCIEX, Framingham, MA).

Statistical Analysis. For the in vivo study, data were generally analyzed by applying the F-test or Bartlett's test to examine the homogeneity in variance, followed by Student's t test (for comparisons between two groups) and Dunnett's test or Steel's test (for multiple comparisons). For the novel object recognition test and the social recognition test, data were analyzed using Student's t test for comparisons between the two groups and a one-way analysis variance for multiple comparisons followed by the Dunnett's test. All the statistical analyses were performed using SAS system version 8.2 (SAS Institute Japan, Tokyo, Japan). A value of $P < 0.05$ was regarded as significant.

Results

In Vitro Profiles. The affinity of enerisant for the histamine H_3 receptor was determined by evaluating [^{3}H]N-α-methylhistamine binding to the membranes of CHO-K1 cells expressing human histamine H_3 receptor or the membranes of rat cerebral cortex. Enerisant had a high affinity for the histamine H_3 receptor, with IC_{50} values of 2.89 (2.57–3.26) nmol/l for human histamine H_3 receptor and 14.5 (10.1–20.8) nmol/l for rat histamine H_3 receptor (Table 1). K_i values of enerisant computed were 1.65 ± 0.05 mmol/l (for human histamine H_3 receptor) and 7.87 ± 0.65 nmol/l (for rat

histamine H_3 receptor), respectively. Enerisant, at concentrations of 3 and 10 nmol/l, increased the K_d value without changing the B_{max} value of [^{3}H]N-α-methylhistamine binding to rat histamine H_3 receptor (Fig. 2), showing that enerisant competitively inhibits [^{3}H]N-α-methylhistamine binding. Enerisant inhibited R-α-methylhistamine–stimulated [^{35}S] GTPγS binding to human histamine H_3 receptor and rat histamine H_3 receptor with IC_{50} values of 1.06 (0.166–6.78) and 10.5 (4.79–22.8) nmol/l, respectively, indicating that enerisant acts as an antagonist at the histamine H_3 receptor (Table 1). Moreover, enerisant inhibited basal [^{35}S]GTPγS binding to human histamine H_3 receptor with an EC_{50} value of 0.357 (0.196–0.650) nmol/l, indicating that enerisant acts as an inverse agonist at the histamine H_3 receptor (Table 1). In contrast, enerisant had negligible effects on binding to human histamine H_1, H_2, and H_4 receptor subtypes (Table 1). Moreover, enerisant displayed negligible affinities for 66 other receptors, transporters, and ion channels at concentrations of 1 and 10 μmol/l, whereas it inhibited approximately 50% of receptor bindings for some receptors at 10 μmol/l (Supplemental Table 1). Enerisant displayed negligible effects on 45 enzymes at concentrations of 1 and 10 μmol/l (Supplemental Table 2). Therefore, enerisant is a potent and selective antagonist/ inverse agonist of histamine H_3 receptor.

Rat Dipsogenia Model. The histamine H_3 receptor agonist R-α-methylhistamine increases water intake, and this effect has been reported to be mediated through the central histamine H_3 receptor (Lecklin et al., 1998; Fox et al., 2002). Thus, this model was used to evaluate the in vivo antagonist activity of enerisant. In this study, R-α-methylhistamine dihydrochloride (3 mg/kg, i.p.) produced a dipsogenia response in rats ($P < 0.0001$), and the oral administration of enerisant hydrochloride attenuated the dipsogenia response, reaching statistical significance at doses of 0.3 mg/kg ($P = 0.0002$) and 1 mg/kg ($P = 0.0001$) (Fig. 3).

In Vivo Histamine H_3 Receptor Occupancy in the Frontal Cortex of Rats. The histamine H_3 receptor occupancy of enerisant was determined by evaluating the in vivo receptor binding of [^{3}H]TASP0410461 in the frontal cortex, a brain region where the histamine H_3 receptor is highly expressed. The oral administration of enerisant hydrochloride resulted in the occupancy of the histamine H_3 receptor in a dose-dependent manner (Fig. 4). A dose eliciting a half-maximal receptor occupancy of enerisant hydrochloride was calculated to be 0.78 mg/kg.

In Vivo Neurotransmitter Release. The effects of enerisant on the extracellular levels of histamine in the posterior hypothalamus, of dopamine in the medial prefrontal cortex (mPFC), and of acetylcholine in the mPFC were evaluated using microdialysis in freely moving rats. The subcutaneous administration of enerisant hydrochloride increased the total extracellular histamine levels in the posterior hypothalamus, and an analysis of the total percent of change at each time point after enerisant hydrochloride administration also indicated significant differences between the enerisant hydrochloride–treated (1 mg/kg, s.c.) and vehicle-treated groups ($P = 0.0083$, Fig. 5A). The intraperitoneal administration of enerisant hydrochloride increased the total extracellular dopamine levels in the mPFC, and an analysis of the total percent of change at each time point also indicated significant differences between the enerisant hydrochloride–treated (3 mg/kg, i.p.) and the vehicle-treated groups ($P = 0.005$, Fig. 5B).

TABLE 1

In vitro activity of enerisant at histamine receptor subtypes

Data represent means [95% confidence interval] obtained from three separate experiments (H_3) or a single experiment (H_1, H_2, and H_4).

	IC$_{50}$ or EC$_{50}$ (nmol/l)				
	Human H_1	Human H_2	Human H_3	Human H_4	Rat H_3
Affinity	>10,000	>10,000	2.89 [2.57–3.26]	>10,000	14.5 [10.1–20.8]
Antagonist activity			1.06 [0.166–6.78]		10.5 [4.79–22.8]
Inverse agonist activity			0.357 [0.196–0.650]		

Moreover, the intraperitoneal administration of enerisant hydrochloride increased the total extracellular acetylcholine levels in the mPFC, and an analysis of the total percent of change at each time point also indicated significant differences between the enerisant hydrochloride–treated (1 mg/kg, i.p.) and the vehicle-treated groups ($P = 0.0017$, Fig. 5C).
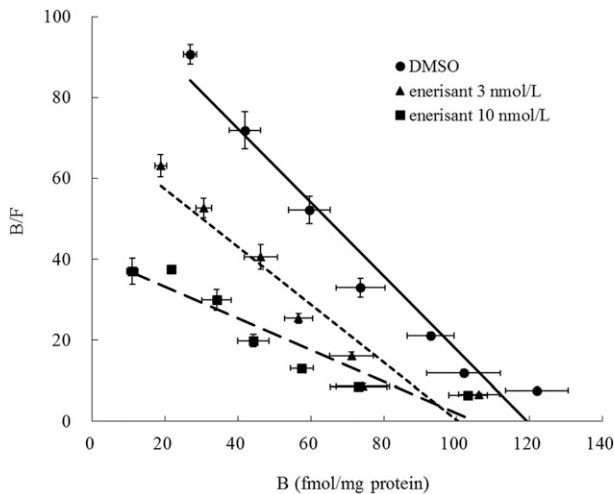
Rat EEG Sleep/Wake Activity. Compared with vehicle treatment, the oral administration of enerisant hydrochloride significantly increased wakefulness at doses of 3 mg/kg ($P < 0.01$) and 10 mg/kg ($P < 0.01$) and decreased slow-wave light sleep at doses of 3 mg/kg ($P < 0.01$) and 10 mg/kg ($P < 0.01$) for 2 hours after administration, whereas enerisant hydrochloride significantly decreased slow-wave deep sleep at doses of 1 mg/kg ($P < 0.01$), 3 mg/kg ($P < 0.05$) and 10 mg/kg ($P < 0.01$) (Fig. 6). Enerisant hydrochloride (1, 3 and 10 mg/kg, p.o.) did not affect the accumulated locomotor activity time at up to 7 hours after administration (data not shown).

Novel Object Recognition Test in Rats. Scopolamine hydrochloride–treated rats spent significantly less time exploring a novel object, compared with vehicle-treated rats ($P < 0.0001$) (Fig. 7A), indicating that scopolamine impaired object memory in rats. Treatment with donepezil hydrochloride (1 mg/kg, p.o.) significantly inhibited the scopolamine-induced impairment of object memory ($P = 0.0005$) (Fig. 7A).

Likewise, enerisant hydrochloride (0.3 mg/kg, p.o.) significantly attenuated the impairment of object memory induced by scopolamine ($P = 0.0001$) (Fig. 7A).
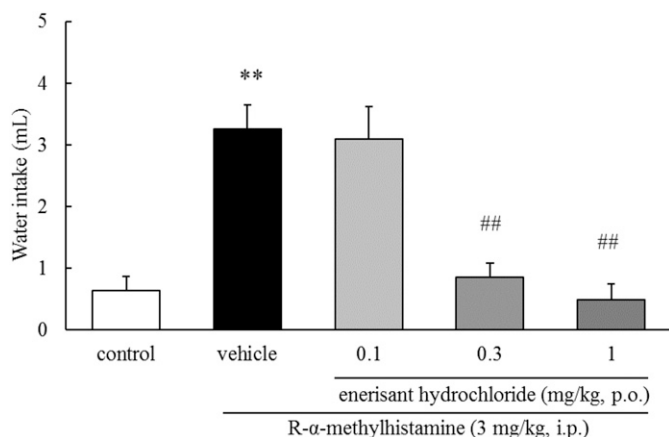
Social Recognition Test in Rats. Rats treated with donepezil hydrochloride (0.3 mg/kg, p.o.) exhibited a significantly shorter RID ($P = 0.004$) (Fig. 7B). Likewise, rats treated with enerisant hydrochloride (0.1 mg/kg, p.o.) exhibited a significantly shorter RID ($P = 0.0093$) (Fig. 7B). When the two 5-minute investigation periods were separated by 30 minutes, the second investigation period of each adult rat toward the same juvenile rat decreased, suggesting that the adult rats retained a social memory of the juvenile rats that were investigated during the first period. The social memory was impaired by treatment with scopolamine hydrochloride ($P = 0.0008$), which was prevented by pretreatment with donepezil hydrochloride (0.3 mg/kg, p.o.) ($P < 0.0001$) (Fig. 7C). Likewise, scopolamine-impaired social memory was significantly attenuated by pretreatment with enerisant hydrochloride ($P = 0.0016$ for 0.03 mg/kg, p.o. and $P = 0.0046$ for 0.1 mg/kg, p.o.) (Fig. 7C).

Spontaneous Locomotor Activity in Rats and Mice. The oral administration of enerisant hydrochloride did not significantly affect spontaneous locomotor activity in rats or mice at doses of 0.3–30 mg/kg (Fig. 8).

	Control	**Enerisant (3 nmol/L)**	**Enerisant (10 nmol/L)**
Kd (nmol/L)	1.28 ± 0.0365	1.64 ± 0.0890	3.78 ± 0.584
Bmax (fmol/mg protein)	123.1 ± 9.615	102.8 ± 6.961	117.3 ± 7.835

Fig. 2. Analysis of the binding mode of enerisant to histamine H_3 receptor using membranes of rat cerebral cortex. (Upper) Linear regression lines in the absence or presence of 3 or 10 nmol/l of enerisant were estimated using the least-squares procedure. Data represent the means ± S.E. of results obtained from three separate experiments. (Lower) Dissociation constant (K_d) and maximum binding (B_{max}) of [^{3}H]N-α-methylhistamine–specific binding to membranes of rat cerebral cortex. Data represent the means ± S.E. of results obtained from three separate experiments.

Fig. 3. Effect of enerisant hydrochloride on R-α-methylhistamine–induced dipsogenia in rats. Water intake was measured for 1 hour immediately after the intraperitoneal administration of R-α-methylhistamine or saline (control). Enerisant hydrochloride or the vehicle was orally administered 1 hour before the intraperitoneal administration of R-α-methylhistamine. Data represent the means ± S.E. (N = 15/group). **$P < 0.01$, compared with response to control group (Student's t test). \#\#$P < 0.01$, compared with response to vehicle-treated group (Steel's test).

Pharmacokinetic Profiles of Enerisant. The plasma, brain, and CSF levels of enerisant after the oral administration of enerisant hydrochloride are summarized in Table 2. The levels of enerisant increased in a greater than dose-proportional manner. Enerisant rapidly reached a maximal level (C_{max}) in plasma within 1 to 2 hours after administration, whereas the levels in the plasma, brain, and CSF were practically equal between 1 and 2 hours after administration at all the doses that were tested. The concentration of enerisant in CSF was 5.17 ng/ml (13.0 nmol/l), which is close to the in vitro affinity (IC_{50} value) for histamine H_3 receptor, at 1 hour after the oral administration of 1 mg/kg of enerisant hydrochloride.

Discussion

In the present study, enerisant was shown to be a potent and selective histamine H_3 receptor antagonist/inverse agonist with wake-promoting and procognitive effects in rodents. Furthermore, enerisant requires different degrees of histamine H_3 receptor occupancy in the frontal cortex to exert these pharmacological effects. In vivo studies of enerisant are summarized in Table 3.

In vitro studies showed that enerisant is a potent, highly selective, and competitive antagonist for the histamine H_3 receptor with a more than 3000-fold selectivity over other histamine receptor subtypes. In addition, enerisant exhibits an inverse agonist activity at the histamine H_3 receptor, as observed with other histamine H_3 receptor antagonists described to date (Esbenshade et al., 2004; Ligneau et al., 2007; Raddatz et al., 2012). It should be noted that enerisant, like other nonimidazole compounds (Esbenshade et al., 2004, 2012; Raddatz et al., 2012), had higher affinity (approximately 5-fold) and more potent antagonist activity (approximately 10-fold) for human histamine H_3 receptor than for rat histamine H_3 receptor, and that this is in contrast with imidazole-containing histamine H_3 receptor antagonists/inverse agonists (Esbenshade et al., 2004), which are less potent at human histamine H_3 receptor than at rat histamine H_3 receptor. It should be additionally mentioned that enerisant

has higher selectivity over histamine receptor subtypes and $\sigma 1$ receptor than pitolisant (Ligneau et al., 2007; Riddy et al., 2019). In vivo functional blockade of the central histamine H_3 receptor by enerisant was demonstrated using a previously characterized R-α-methylhistamine–induced dipsogenia model (Lecklin et al., 1998; Fox et al., 2002). Thus, the oral administration of enerisant hydrochloride inhibited R-α-methylhistamine–induced dipsogenia in rats. In vivo histamine H_3 receptor antagonism was also demonstrated by evaluating histamine release in the posterior hypothalamus. Since the histamine H_3 receptor functions as an autoreceptor on histaminergic somata and presynaptic terminals to provide a tonic inhibition of the firing rate of histamine neurons (Haas and Panula, 2003) and histamine release (Arrang et al., 1983), histamine H_3 receptor antagonists/inverse agonists increase the histamine levels in the synaptic cleft. Enerisant increased the extracellular histamine levels in the posterior hypothalamus, as measured using a microdialysis technique, indicating histamine H_3 receptor antagonism. Collectively, these results show that enerisant is a potent, selective, and competitive antagonist/inverse agonist of the histamine H_3 receptor with excellent in vivo antagonist activity, thereby increasing histaminergic activity.

Histamine H_3 receptor blockade also increases the release of other neurotransmitters in a regionally and functionally specific manner that is dependent on histaminergic innervation and the localization of histamine H_3 receptors. As observed for other histamine H_3 receptor antagonists/inverse agonists, enerisant was shown to increase the releases of acetylcholine and dopamine in the mPFC in the present study. Thus, enerisant increases the extracellular concentrations of neurotransmitters such as histamine, acetylcholine, and dopamine, which are important for vigilance and cognition.

In vivo receptor binding, rather than ex vivo receptor binding, has been proposed to be advantageous for determining the in vivo receptor occupancy because the results of ex vivo binding might underestimate the degree of in vivo receptor occupancy as a result of dissociation during ex vivo procedures, as demonstrated for other receptor ligands (Kapur et al., 2001) as well as the histamine H_3 receptor ligand
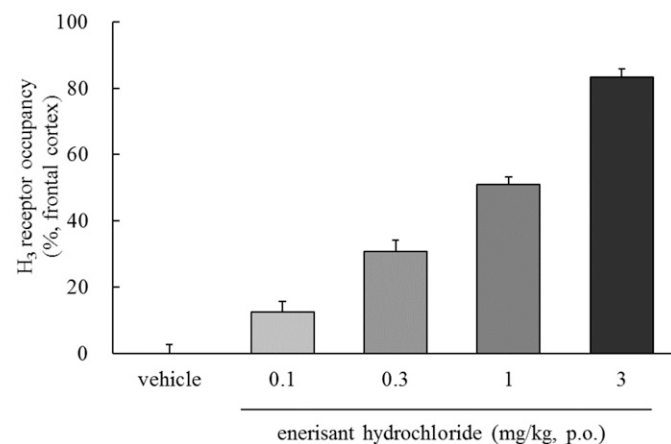
**Fig. 4.** Histamine H_3 receptor occupancy by enerisant in the frontal cortex of rats. Histamine H_3 receptor occupancy (%) in the frontal cortex was measured 30 minutes after the intravenous administration of [^{3}H]TASP0410461. Enerisant hydrochloride or the vehicle was orally administered 1 hour before the injection of [^{3}H]TASP0410461. Data represent the means ± S.E. (N = 5/group).
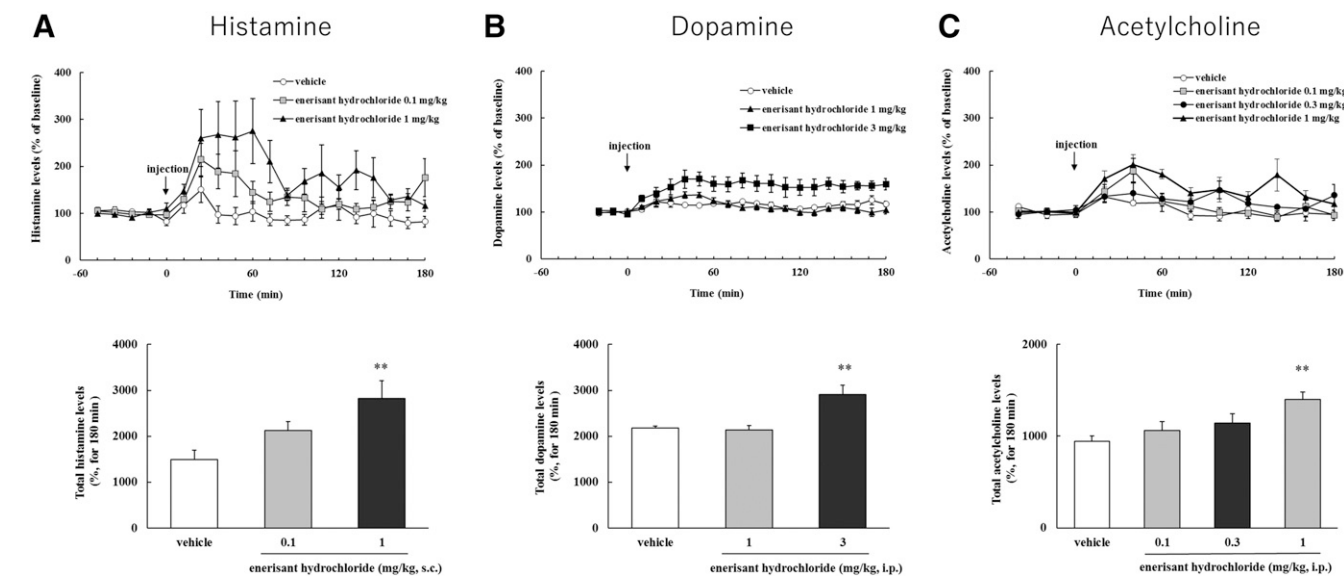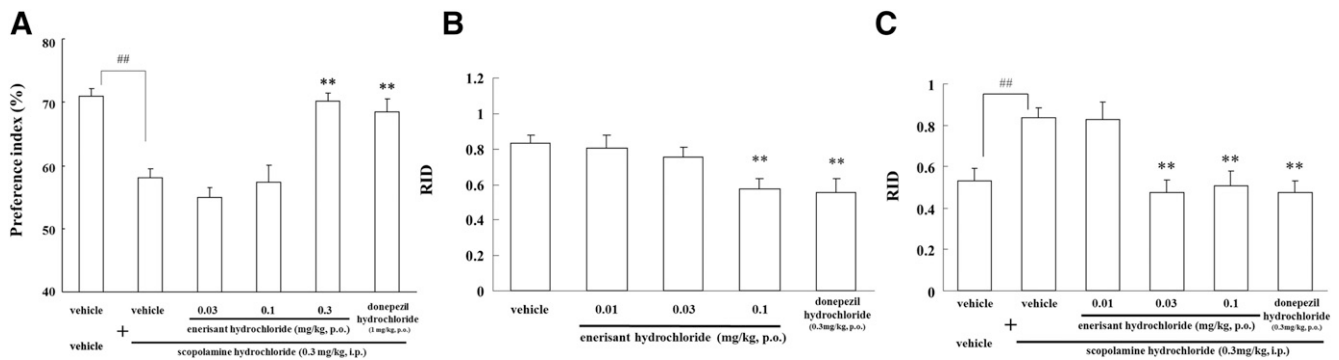
Fig. 5. Effect of enerisant hydrochloride on extracellular levels of histamine, dopamine and acetylcholine in brain regions of rats. (A) Effect of enerisant hydrochloride on extracellular histamine levels in the posterior hypothalamus. (Upper) Time course of histamine levels (%) sampled over 12-minute intervals from 48 minutes before to 180 minutes after the administration of enerisant hydrochloride or the vehicle. The vertical arrow indicates the time of drug administration. (Lower) Total histamine levels were defined as the cumulative percent of the baseline value over a 180-minute period. Data represent the means ± S.E. of results obtained from six rats/group. **$P < 0.01$, compared with the response to the vehicle-treated group (Dunnett's test). (B) Effect of enerisant hydrochloride on extracellular dopamine levels in the medial prefrontal cortex. (Upper) Time course of dopamine levels (%) sampled over 10-minute intervals from 20 minutes before to 180 minutes after the administration of enerisant hydrochloride or the vehicle. The vertical arrow indicates the time of drug administration. (Lower) Total dopamine levels were defined as the cumulative percent of the baseline value over a 180-minute period. Data represent the means ± S.E. of results obtained from five rats/group. **$P < 0.01$, compared with the response to the vehicle-treated group (Dunnett's test). (C) Effect of enerisant hydrochloride on extracellular acetylcholine levels in the medial prefrontal cortex. (Upper) Time course of acetylcholine levels (%) sampled over 20-minute intervals from 40 minutes before to 180 minutes after the administration of enerisant hydrochloride or the vehicle. The vertical arrow indicates the time of drug administration. (Lower) Total acetylcholine levels were defined as the cumulative percent of the baseline value over a 180-minute period. Data represent the means ± S.E. of results obtained from six to eight rats/group. **$P < 0.01$, compared with the response to the vehicle-treated group (Dunnett's test).

(Le et al., 2008). In the present study, we determined the histamine H_3 receptor occupancy in the brain by utilizing a newly synthesized radiolabeled ligand, [³H]TASP0410461, and obtained results showing that the oral administration of enerisant hydrochloride resulted in a dose-dependent histamine H_3 receptor occupancy in the frontal cortex with an ED_{50} value of 0.78 mg/kg. The in vivo antagonism of enerisant was observed at doses (0.3 and 1 mg/kg) resulting in less than or nearly 50% histamine H_3 receptor occupancy, and this finding is consistent with previous findings for another histamine H_3 receptor antagonist/inverse agonist ABT-239 (Fox et al., 2005; Miller et al., 2009). Moreover, the concentration of enerisant in
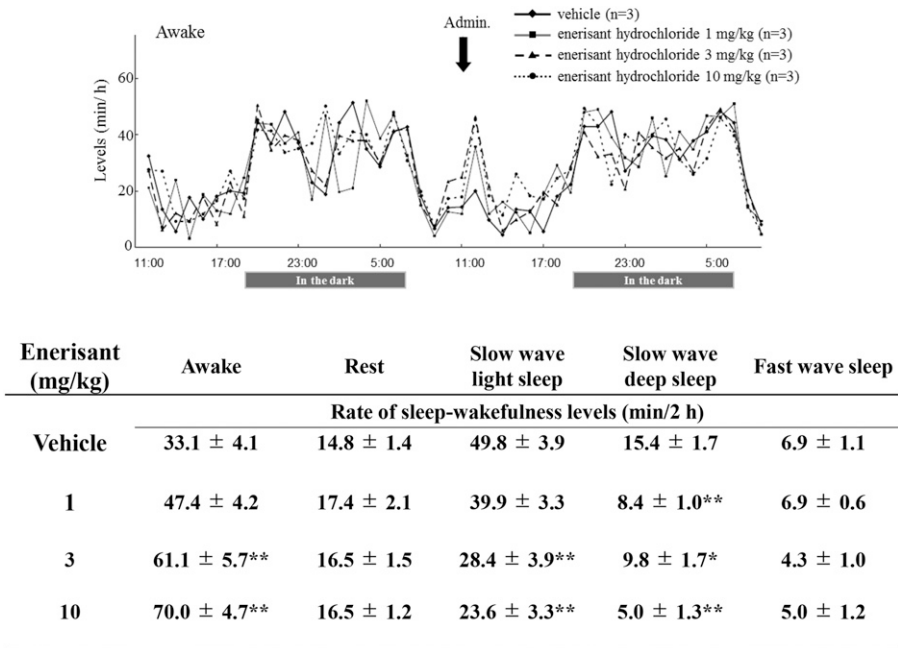
Fig. 6. Effect of enerisant hydrochloride on sleep/wake cycle of rats. (Upper) Effect of enerisant hydrochloride on awake levels. The vertical arrow indicates the time of the oral administration of enerisant hydrochloride or the vehicle. Data represent the means of three rats. (Lower) Effect of enerisant hydrochloride on each parameter (awake, rest, slow-wave light sleep, slow-wave deep sleep, fast-wave sleep) for 2 hours after administration. Data represent the means ± S.E. of results obtained from eight rats/group. *$P < 0.05$; **$P < 0.01$, compared with the response to the vehicle-treated group (Dunnett's test).

| Enerisant (mg/kg)	Awake	Rest	Slow wave light sleep	Slow wave deep sleep	Fast wave sleep
	Rate of sleep-wakefulness levels (min/2 h)				
Vehicle	33.1 ± 4.1	14.8 ± 1.4	49.8 ± 3.9	15.4 ± 1.7	6.9 ± 1.1
1	47.4 ± 4.2	17.4 ± 2.1	39.9 ± 3.3	8.4 ± 1.0**	6.9 ± 0.6
3	61.1 ± 5.7**	16.5 ± 1.5	28.4 ± 3.9**	9.8 ± 1.7*	4.3 ± 1.0
10	70.0 ± 4.7**	16.5 ± 1.2	23.6 ± 3.3**	5.0 ± 1.3**	5.0 ± 1.2

Fig. 7. Effect of enerisant hydrochloride in a novel object recognition test (A) and the social recognition test (B and C) in rats. (A) Effect of enerisant hydrochloride on scopolamine-induced cognitive deficits in the novel object recognition test. Enerisant hydrochloride, donepezil hydrochloride or the vehicle was orally administered, followed 40 minutes later by the intraperitoneal administration of scopolamine hydrochloride. Data represent the means ± S.E. (N = 9-10/group). [##]$P < 0.01$, compared with the response to the vehicle + vehicle-treated group (Student's t test). Enerisant-treated group: [**]$P < 0.01$, compared with the response to the vehicle + scopolamine-treated group (Dunnett's test). Donepezil-treated group: [**]$P < 0.01$, compared with the response to the vehicle + scopolamine–treated group (Student's t test). (B) Effect of enerisant hydrochloride on time-dependent memory impairment in the social recognition test. Enerisant hydrochloride, donepezil hydrochloride or the vehicle was orally administered immediately after the first investigation. Data represent the means ± S.E. (N = 12–15/group). Enerisant-treated group: [**]$P < 0.01$, compared with the response to the vehicle-treated group (Dunnett's t test). Donepezil-treated group: [**]$P < 0.01$, compared with the response to the vehicle-treated group (Student's t test). (C) Effect of enerisant hydrochloride on scopolamine-induced cognitive deficits in the social recognition test. Enerisant hydrochloride, donepezil hydrochloride or the vehicle was orally administered 1 hour prior to the first investigation, followed 30 minutes later by the intraperitoneal administration of scopolamine hydrochloride. Data represent the means ± S.E. (N = 14–15/group). [##]$P < 0.01$, compared with the response to the vehicle + vehicle–treated group (Student's t test). Enerisant-treated group: [**]$P < 0.01$, compared with the response to the vehicle + scopolamine-treated group (Dunnett's test). Donepezil-treated group: [**]$P < 0.01$, compared with the response to the vehicle + scopolamine–treated group (Student's t test).

CSF was 13.0 nmol/l at 1 hour after the oral administration of 1 mg/kg of enerisant hydrochloride, at which dose a 50.9% occupancy was observed. This finding is consistent with the in vitro affinity of enerisant for rat histamine H_3 receptor (IC_{50} = 14.5 nmol/l), indicating that the in vivo receptor binding used in the present study corresponds to the in vitro antagonist activity.

Histamine H_3 receptor antagonists/inverse agonists have been reported to increase wakefulness in several species (Fox et al., 2003, 2005; Barbier et al., 2004; Le et al., 2008; Ligneau et al., 2007; Esbenshade et al., 2012; Raddatz et al., 2012). Likewise, in the present study, we observed that enerisant increased wakefulness and decreased slow-wave light sleep and slow-wave deep sleep in rats. In contrast, the enhanced wakefulness was not accompanied by increased motor activity, which is distinct from that of stimulants such as

amphetamine (Parmentier et al., 2007). Notably, enerisant increased wakefulness in rats at doses of 3 and 10 mg/kg, at which doses an in vivo receptor binding study showed a histamine H_3 receptor occupancy of more than 80%. The present result was consistent with the previous reports that histamine H_3 receptor antagonists/inverse agonists increased arousal at doses corresponding to nearly full occupancy or more than 80% occupancy of the histamine H_3 receptor (Barbier et al., 2004; Le et al., 2008; Raddatz et al., 2012). Moreover, the present result was consistent with the report that therapeutic dose of pitolisant occupies brain histamine H_3 receptor by more than 80% in humans (Rusjan et al., 2020). In addition, increased wakefulness was observed at a dose at which a significant increase in the extracellular histamine concentration was observed in the posterior hypothalamus, supporting the hypothesis that
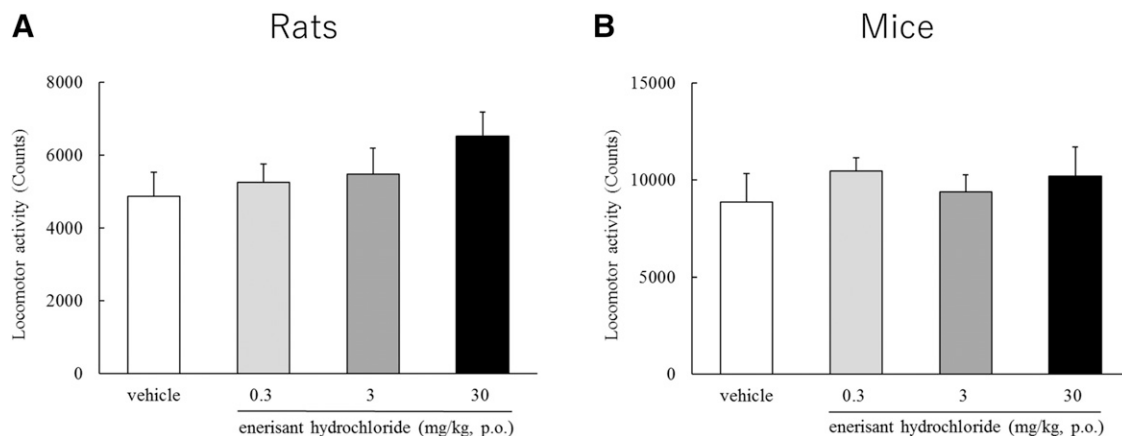
Fig. 8. Effect of enerisant hydrochloride on spontaneous locomotor activity in rats (A) and mice (B). (A) Enerisant hydrochloride (0.3, 3 and 30 mg/kg) or the vehicle was administered orally to rats prior to the measurement of spontaneous locomotor activity for 1 hour. Data represent the means ± S.E. (N = 8/group). (B) Enerisant hydrochloride (0.3, 3 and 30 mg/kg) or the vehicle was administered orally to mice prior to the measurement of spontaneous locomotor activity for 1 hour. Data represent the means ± S.E. (N = 8/group).

TABLE 2

Plasma, brain, and CSF concentrations of enerisant after single oral administration of enerisant hydrochloride in rats

Dose	Time	Plasma	Brain	CSF
mg/kg	h	ng/ml	ng/g	ng/ml
1	0.5	39.2 ± 21.4	10.8 ± 6.80	4.74 ± 3.43
	1	40.2 ± 10.3	13.3 ± 3.01	5.17 ± 1.37
	2	38.9 ± 7.35	13.5 ± 1.78	5.29 ± 1.43
	3	35.6 ± 7.41	12.7 ± 2.94	4.15 ± 0.748
	7	12.4 ± 4.91	5.07 ± 1.50	1.50 ± 0.717
3	0.5	122 ± 49.3	25.5 ± 8.30	13.8 ± 6.00
	1	169 ± 40.4	49.6 ± 14.6	23.6 ± 4.41
	2	176 ± 33.8	48.1 ± 9.10	21.2 ± 4.47
	3	100 ± 29.0	34.5 ± 10.3	13.2 ± 3.51
	7	51.5 ± 15.4	16.2 ± 3.67	5.52 ± 1.76
10	0.5	738 ± 310	179 ± 86.5	110 ± 52.0
	1	802 ± 115	218 ± 50.8	115 ± 28.4
	2	745 ± 219	218 ± 90.4	94.8 ± 28.8
	3	488 ± 78.0	135 ± 21.5	66.9 ± 11.3
	7	208 ± 81.2	56.5 ± 23.0	28.1 ± 15.6

histamine H_3 receptor antagonists/inverse agonists increase wakefulness by increasing histaminergic tones.

Potential cognition-enhancing properties of enerisant were examined in two behavioral tests, a novel object recognition test and a social recognition test, both of which have been used to evaluate the procognitive effects of several agents (de Bruin and Pouzet, 2006; Loiseau et al., 2008; Shimazaki et al., 2010; Chaki et al., 2015). In the present study, enerisant significantly reversed cognitive impairment induced by scopolamine in both a novel object recognition test and the social recognition test. In addition, enerisant significantly enhanced social memory in naïve animals. These results complement previous findings of a procognitive effect of histamine H_3 receptor antagonists/inverse agonists (Fox et al., 2005; Medhurst et al., 2007; Esbenshade et al., 2012; Griebel et al., 2012; Raddatz et al., 2012; Alachkar et al., 2017). Interestingly, although the effective dose varies, these effects occurred at doses corresponding to a histamine H_3 receptor occupancy of less than 30.7% under all the conditions used in this study. In particular, in the social recognition test, the procognitive effects appeared at receptor occupancy levels of 12.6% or less. The present results were consistent with previous findings that histamine H_3 receptor antagonists/inverse agonists exert procognitive effects at doses corresponding to a smaller percentage of occupancy (as low as 10%) and that do not affect wake/sleep EEG (Fox et al., 2003, 2005; Miller et al., 2009;

Esbenshade et al., 2012). The present and previous studies clearly indicate that a much lower receptor occupancy is sufficient for histamine H_3 receptor antagonists/inverse agonists to exert their procognitive effects, at least in rodents. Not only acetylcholine, but also dopaminergic transmission in the frontal cortex is presumably involved in the procognitive effects observed in the above-mentioned models (Millan et al., 2007; Loiseau and Millan, 2009; Pezze et al., 2015), suggesting that the increased release of acetylcholine and dopamine in the mPFC by enerisant may contribute to the procognitive effects in these animal models. Although significant increases in these neurotransmitter levels were seen at doses higher than those efficacious in the novel object recognition test and the social recognition test, it is likely that only subtle, localized changes are required to exert procognitive effects. In this regard, these discrepancies may reflect the limited sensitivity of microdialysis techniques, which do not measure subtle changes in synaptic cleft neurotransmitter levels.

Notably, it has been demonstrated that histaminergic neurons are organized into distinct functional circuits impinging on different brain regions, and that responses to histamine H_3 receptor antagonists differentiate histaminergic neurons according to their projection areas (Blandina et al., 2012). Therefore, it can be conceivable that histaminergic activity in the brain regions relating to arousal and cognition may be

TABLE 3

Summary of the effects of enerisant in animal models

Values represents MED in each model except in vivo H_3 receptor occupancy, which shows ED_{50} value.

Model	Species	MED or ED_{50}
In vivo H_3 receptor occupancy	Rat	0.78 mg/kg (p.o.)
In vivo antagonism		
R-α-methylhistamine–induced dipsogenia	Rat	0.3 mg/kg (p.o.)
Neurotransmitter release		
Histamine release in the posterior hypothalamus	Rat	1 mg/kg (s.c.)
Dopamine release in the mPFC	Rat	3 mg/kg (i.p.)
Acetylcholine release in the mPFC	Rat	1 mg/kg (i.p.)
EEG sleep/wake activity		
Wakefulness	Rat	3 mg/kg (p.o.)
Cognition		
Novel object recognition test (scopolamine)	Rat	0.3 mg/kg (p.o.)
Social recognition test (natural forgetting)	Rat	0.1 mg/kg (p.o.)
Social recognition test (scopolamine)	Rat	0.03 mg/kg (p.o.)
Spontaneous locomotor activity	Mouse, Rat	>30 mg/kg (p.o.)

MED, minimum effective dose.

differentially regulated by enerisant, which may partly account for differential pharmacological actions of enerisant.

In summary, the present study demonstrates potent pharmacological effects after oral dosing with enerisant hydrochloride and suggests that this novel histamine H3 receptor antagonist/inverse agonist may have therapeutic utility in the treatment of sleep and cognitive disorders. We also provide evidence that the histamine H3 receptor occupancy required to exert each effect differs. The present results will provide important information in planning and interpreting clinical studies of enerisant, which will facilitate future mechanism-related studies as well as validations of the effectiveness of this approach in patients exhibiting histamine H3 receptor abnormalities.

Authorship Contributions

Participated in research design: Hino, Chaki.

Conducted experiments: Hino, Marumo, Kotani, Shimazaki, Kaku-Fukumoto, Hikichi, Karasawa, Tomishima, Komiyama, Tatsuda, Chaki.

Contributed new reagents or analytic tools: Nozawa, Nakamura.

Performed data analysis: Hino, Marumo, Hikichi.

Wrote or contributed to the writing of the manuscript: Hino, Chaki.

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