MODULATION OF HIPPOCAMPAL THETA OSCILLATION BY
HISTAMINE H3 RECEPTORS

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List of Abbreviations:
ADHD, attention deficit hyperactive disorder, BPS, Phosphate Buffer Saline; CA, cornu ammonis; EEG, electroencephalograph; EMG, electromyogram; FFT, fast Fourier Transform; GABA, gamma-aminobutyric acid; H3 histamine3; HDC, histidine decarboxylase gene; MS/DB, medial septum/diagonal band of Broca; nPO, nucleus pontis oralis

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Abstract

Preclinical findings demonstrate pro-cognitive actions of histamine3 (H3) receptor antagonists/inverse agonists. Since a prominent role of neuronal network oscillations of the hippocampus, such as theta band oscillation has been recognized in numerous cognitive functions, in the present study the potential involvement of H3 receptors in modulation of hippocampal theta activity has been investigated using various recording paradigms. Systemic administration of the selective H3 receptor antagonists/inverse agonists, thioperamide and ciproxifan (0.1 mg/kg to 1 mg/kg, i.v.) dose-dependently increased hippocampal theta power, similarly to methylphenidate (0.1 mg/kg to 1 mg/kg, i.v.), in chloral hydrate anaesthetized rats. When hippocampal theta oscillation was elicited by electrical brainstem (nucleus pontis oralis) stimulation, ciproxifan (1 mg/kg, i.v.) augmented the power of stimulation-induced theta. In contrast, systemic administration of methylphenidate (1 mg/kg, i.v.) did not modify elicited theta. In order to analyze the role of H3 receptors on stage and behavior dependent hippocampal theta activity, polysomnographic recordings were carried out together with field potential recordings at the hippocampal fissure, in freely moving rats for 8 h during the light phase of the circadian cycle. Systemic administration of ciproxifan (3.0 mg/kg, i.p.) promoted wakefulness with a concomitant reduction in cortical delta power, and augmented novelty-induced hippocampal theta activity. These findings provide evidence that H3 receptors play an important role in regulation of hippocampal theta oscillation, representing one of the probable mechanisms involved in histamine-induced modulation of higher brain functions, such as attention and learning.
Introduction

Pharmacological and biochemical studies have revealed the existence of presynaptic autoreceptors on histamine neurons that mediate the inhibitory effects of histamine on its own neuronal synthesis and release (Arang et al., 1983). Subsequent cloning of these receptors, named histamine3 (H3) receptors, led to their classification as G-protein-coupled receptors that are broadly distributed in the central nervous system (Lovenberg et al., 1999; Haas and Panula, 2003). Histamine-containing tuberomammillary neurons innervate the vast majority of the forebrain, and recent electrophysiological findings on neurochemically identified histamine neurons demonstrate that their activity is closely associated with the sleep-wake cycle (Takahashi et al., 2006). These findings are in line with earlier observations that enhanced endogenous histamine neurotransmission or exogenous histamine1 (H1) receptor agonists lead to wakefulness and arousal (Lin, 2000). Studies in knock-out mice lacking the histidine decarboxylase gene (HDC/-) further support the wake-promoting effects of histamine (Parmentier et al., 2002). Antagonists of H3 receptors also display wake-promoting effects (Passani et al., 2004), presumably via enhanced histamine release since their wakening effect is absent in H1 receptor knock-out mice (Parmentier et al., 2007).

Activity of histamine neurons has been linked not only to the sleep-wake cycle but also to different stages of wakefulness and attention. For example, HDC/- mice are inferior in maintaining wakefulness in conditions requiring higher levels of vigilance, such as during exposure to novel environment (Parmentier et al., 2002). The hypothesis that histaminergic neurons play a role in regulating waking states is also supported by
electrophysiological observations in behaving mice: activity of histaminergic neurons varies in the different waking states, being lowest during quiet waking, moderate during active waking, and highest during attentive waking state (Takahashi et al., 2006). The morphology of the histaminergic system is also in line with its presumed function, since histamine neurons have broad projections, interacting with all wake-promoting neuronal systems such as the cholinergic, noradrenergic, serotonergic and orexinergic neurons and innervating brain areas associated with attention such as the hippocampal circuitry (Haas and Panula, 2003).

The hippocampal formation, and hippocampal theta rhythm in particular, has been long implicated in various physiological mechanisms related to orienting exploratory behavior and cognition, such as learning and memory formation (Buzsáki, 2002). Correlation between various cognitive processes and hippocampal theta has been demonstrated in experimental animals (Buzsáki, 2002; Jones and Wilson, 2005) and humans (Caplan et al., 2003; Sammer et al., 2007). In addition, pharmacological studies revealed that drugs shown to have pro-cognitive action enhance hippocampal theta power, and drugs disrupting hippocampal theta oscillations impair hippocampal-dependent memory function (Kinney et al., 1999, Siok et al., 2006, Robbe et al., 2006; McNaughton et al., 2007). It is well established that modulations of activity of the brain histamine system or interaction with various histamine receptors have a profound effect on cognitive processes (Haas and Panula, 2003). Accordingly, a large body of evidence demonstrates that activity of H3 receptors effectively influence cognitive functions: H3 receptor agonists disrupt, whereas antagonists improve cognitive performance in animal models,
such as novel object recognition and a passive avoidance (Giovannini et al., 1999; for review see Wijtmans et al., 2007). Furthermore, H3 receptor antagonists have been shown to prevent scopolamine-induced amnesia, and recent reports on novel H3 receptors antagonists have described improvement in social memory, water maze and attentional set shift in rats (Fox et al., 2003; Passani et al., 2004; Celanire et al., 2005; Esbenshade et al., 2006; Medhurst et al., 2007). Since behavioral studies have established a role of H3 receptors in learning and attention, in the present study, we evaluated the action of histamine H3 receptor antagonists on hippocampal oscillatory activity in various recording paradigms. Effects of ciproxifan and thioperamide, selective antagonists/inverse agonists of H3 receptors, were evaluated on spontaneous hippocampal EEG in anaesthetized rats, and ciproxifan was tested on brainstem stimulation-induced theta activity. Effects of ciproxifan were also studied on hippocampal EEG activity in non-anesthetized rats either at their home cage or during their exposure to novel environment. Since H3 receptor antagonists have been considered as potential treatment for attention deficit hyperactive disorder (ADHD), methylphenidate, a clinically used ADHD drug has been also tested in some of our models (Celanire et al., 2005).
Materials and Methods

Animals and Surgical Procedures. All experiments were performed on male, Sprague-Dawley rats (260-420 g) and were conducted under the approved animal-use protocols of each institution and in compliance with the Animal Welfare Act Regulations (9 CFR parts 1, 2 and 3) and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health guidelines.

EEG studies in chloral hydrate anesthetized rats. Rats weighing 260-320 g were anesthetized with 400 mg/kg intraperitoneal (i.p.) chloral hydrate and a cannula was inserted into the femoral vein for intravenous (i.v.) administration of drugs and anesthesia boosters. The animals were placed in a stereotaxic frame for the duration of the experiment and kept warm (37-38°C) using a thermally regulated heating pad (Harvard Apparatus, Holliston, MA, USA). The EEG was recorded using stainless steel, monopolar electrodes (Rhodes Medical, Woodland Hills, CA, USA) placed in the CA1 region of the dorsal hippocampus (AP = -3.0mm from bregma; lateral = 2.0 mm; DV = 3.8 mm from surface of the skull). The ear-bar of the stereotaxic frame served as the ground and reference. All drugs were administered via the i.v. cannula in a cumulative dosing paradigm at a volume of 1 ml/kg. Field potentials (EEG) were recorded using a Grass P55 AC differential amplifier with the positive input grounded and filters set between 0.3 Hz - 0.5 KHz. The signals were digitized at a rate of 1000 Hz and stored off-line for subsequent analysis using Spike2 (version 4) software. Data analysis was performed using the fast Fourier Transform (FFT) by Spike2 at a spectral resolution of 0.24 Hz. Due to the short acting nature of the anesthetic, each animal was allowed to
stabilize for a period of only 5-10 minutes after electrode placement, prior to the start of each experiment. To begin, 5-minutes of baseline EEG were recorded prior to administration of drug, followed by three, i.v. bolus injections of a single drug, each spaced 5 minutes apart, in a cumulative fashion resulting in final doses of 0.1, 0.3 and 1.0 mg/kg. The 5-minute baseline period, as well as the 5-minute period following each successive drug injection, were recorded and analyzed by calculating the percent of total power which occurred in the theta (3.0-5.5 Hz) frequency band as compared to the 0-15 Hz frequency band. Paired Student’s t test was used for statistical analysis and group values were expressed as mean ± S.E.M.

**EEG studies in urethane anesthetized rats.** Rats weighing 260-320 g were anesthetized with 1.5-1.6 g/kg (i.p.) urethane and prepared as describe above with the following exceptions. A stainless steel screw was placed in the frontal bone to act as reference and ground, and additional holes were burred into the left parietal bone to accept stimulating electrodes in the nucleus pontis oralis (nPO, AP -7.8, lateral 1.8 and DV -6.0. As above, the animals had drug cannulas implanted in the femoral vein and were kept in the stereotaxic frame on a thermally controlled heating pad (37-38°C) for the duration of the experiment where they were allowed to stabilize for 1-2 hours prior to recording. The recording and stimulating electrodes for each animal were similar and consisted of either a pair of twisted, 125µm stainless steel wires (1 mm vertical tip separation) or stainless steel, bipolar electrodes (NEX-100; Rhodes Medical, Woodland Hills, CA, USA). During each experiment, spontaneous and stimulation induced EEG was continuously monitored. Theta was induced in CA1 every 100 seconds by brief
electrical stimulation of the nPO. The stimulus paradigm consisted of a train of 0.3 ms anodal square pulses delivered over a period of 6-seconds at a rate of 250 Hz. Stimulus intensities were either held constant for time-course experiments, or increased in a stepwise fashion. The magnitude of the stimulating current ranged from 0.02 – 0.2 mA depending upon the experiment. For time-course experiments, a stimulus-response curve was generated prior to the start of each study in order to choose a stimulating current that produced a signal frequency of 5-7 Hz and amplitude that was approximately 60-80% of maximum as described in detail earlier (Siok et al., 2006). FFT analysis was performed on the last 5-seconds of EEG during each 6-second stimulation period. The first second during stimulation was not included to avoid stimulus artifact. Total theta power was determined by summing the power in the 4-8 Hz frequency band then normalized for each animal to the mean of the first nine baseline responses measured prior to drug administration.

For experiments where stimulating current was not held constant, the intensity of the stimulation was stepped from 0.02 to 0.18 mA in 0.01 mA increments using the same stimulating paradigm as outlined above (i.e, 0.3 msec square wave delivered at 250 Hz for 6-seconds every 100 seconds). This generated a stimulus-response relationship for both peak theta frequency and total theta power similar to that previously reported by Kinney et al. (1999). The full series of stimulations were delivered prior to and 30 min following administration of vehicle or drug. In these experiments, rats were prepared for brainstem-stimulation and EEG recording as outlined in the above section. Comparisons were made between vehicle and drug treated groups using an analysis of variance, with calculation of F ratio; group values were expressed as mean ± S.E.M.


**EEG studies in freely moving rats.** Experiments were performed on male Sprague–Dawley rats (220-420g, Charles River Laboratories, MA) treated in accordance with NIH guidelines. All procedures were approved by the Institutional Animal Care and Use Committees of Beth Israel Deaconess Medical Center. Fluorocarbon-coated wire electrodes (330 um, stainless steel) for recording neck muscle electromyogram (EMG), a stainless steel jeweler’s screw for frontal cortical electroencephalogram (EEG), and a pair of 125 um stainless steel wires with 1-1.5 mm tip separation for hippocampal EEG recordings were implanted under Ketamine/Xylasine (35-45 and 5 mg/kg of body weight, respectively) anesthesia and the wires were led to miniature connectors mounted on the skull by dental cement, as described previously (Kocsis et al, 2007).

The experiments started 5-7 days after surgery and were carried out in groups of 3-4 rats simultaneously. At 8 AM every day, the animals were moved from the animal housing to the recording room, placed in their own dedicated recording boxes, connected to the recording apparatus, and left to accommodate for 2 hr. The recording boxes were made of white opaque plastic, had relatively small horizontal dimensions of 19.5x29 cm and larger than normal height of 28.5 cm to allow rearing without compromising electrophysiological recordings. The boxes had regular bedding and standard water and food containers. The recording sessions lasted 4-8 hours daily after which the rats were moved back to the animal facility for the night. On the test days, started after at least 1 week of control recordings, intraperitoneal (i.p.) injections of saline or ciproxifan were administered at 10 AM., and undisturbed recordings of 2-6 hours followed the injections.
in 4 rats on at least 2 occasions. Recordings during exploration of a novel environment were performed in 7 rats. After 2 hour recording in their home cages, the rats were injected saline or ciproxifan and 30 min later were placed in a novelty box which was of different size (i.e. larger – 38x30 cm) than the recording box and had no bedding but had a couple of unfamiliar objects thrown on the floor. This procedure was repeated for saline and ciproxifan injections on different days; the order of administration of the two substances alternated between rats.

Electrophysiological recordings were made using monopolar electrodes referenced to an indifferent electrode placed over the cerebellum. The cortical EEG and hippocampal field potentials were filtered between 0.1 and 100 Hz and sampled at a rate of 250 samples/s. EMG was high pass filtered at 100 Hz and sampled simultaneously with EEG signals. The EEG signals were subjected to Fast Fourier Transform in 4 or 16 s segments (i.e. 1000 or 4000 points) from which the theta (5-10 Hz) power of hippocampal EEG and the power of delta waves (1-4 Hz) in cortical EEG were calculated. For sleep-wake scoring, these parameters were evaluated together with simultaneous raw EMG integrated over the same segments. 16 s segments were only used for on-line visual inspection and sleep scoring whereas all statistical calculations were based on spectra made for 4 s windows. For different comparisons, delta and theta powers were averaged either over 1 hr periods or separately for slow wave sleep and active waking episodes, respectively. The signals recorded during exploration of the novel environment were handled in a similar way but the averages were made over the first 5 min of exploration. Two-way analysis of variance
(treatment and time as main factors) and paired Student’s t test were used for statistical analysis and group values were expressed as mean ± S.E.M.

Histology. To verify the placement of electrodes after the experiments the rats were deeply anesthetized and perfused through the aortic arch with 0.9% NaCl followed by a fixative solution containing 4% paraformaldehyde (Sigma-Aldrich, Germany), and 15% (V/V%) saturated picric acid (Sigma-Aldrich, Germany) in 0.1M phosphate buffer (PB, pH 7.4, 0.1M) and their brains removed and stored. Sixty micron sections were taken with a freezing microtome and stained with cresyl violet. Hippocampal electrodes were verified in the hippocampus at the level of the fissure shown to give the largest theta signal.

Drugs. Ciproxifan and methylphenidate were synthesized at Pfizer laboratories, thioperamide obtained from Tocris (Ellisville, MO, USA). Drug solutions were made up based upon their salt weights in phosphate buffered saline (PBS) with concentrations adjusted so that injection volumes equaled 1 ml/kg body weight.
Results

Effects of H3 receptor antagonists and methylphenidate on spontaneous hippocampal theta activity in chloral hydrate anesthetized rats

Both H3 receptor antagonists and methylphenidate significantly increased the percentage of theta power when compared to their respective baseline period (Fig. 1). There was no significant increase in the amount of theta after successive injections of vehicle alone. Methylphenidate was the most potent of the three drugs, significantly increasing theta power at the lowest dose tested of 0.1 mg/kg from an average of 17 ± 5% (mean ± SEM) during the baseline period to 53 ± 14% following injection (p < 0.05). At the next highest dose of 0.3 mg/kg, all three drugs significantly increased the percentage of power in the theta band (p < 0.05). Ciproxifan increased theta from 13 ± 2% to 46 ± 10%, thioperamide from 12 ± 1% to 25 ± 2% and methylphenidate from 17 ± 5% to 61 ± 15%. At 1.0 mg/kg, all three drugs had significantly more power in the theta band compared to their respective baseline periods (Fig. 1).

Effects of ciproxifan and methylphenidate on brainstem stimulation-induced theta activity

Electrical stimulation of the nPO elicited highly regular hippocampal oscillations whose frequency and amplitude increased proportionally to the stimulus intensity, as shown previously (Kinney et al., 1999; Siok et al., 2006; McNaughton et al., 2007). Intensity of the stimulating current necessary to induce hippocampal theta oscillation at 5-7 Hz with an amplitude (i.e., power) between 60-80% of the maximal response was determined. Compared to vehicle treated rats, ciproxifan (1.0 mg/kg, i.v., n=4) caused a significant
increase in theta power (Fig. 2; F[1,84] = 91.52.; p < 0.01). Overall, during the first 15 minutes following injection, ciproxifan increased power by 24% compared to the pre-injection period with the effect lasting for at least 1-hour after administration. By contrast, methylphenidate (1.0 mg/kg, i.v., n=4) had no effect on theta power. Neither ciproxifan nor methylphenidate had any effect on average theta frequency (data not shown). The effects of ciproxifan on current-dependent increases in hippocampal theta power were also evaluated in urethane-anesthetized rats (Fig. 3). At 1.0 mg/kg, i.v. (n=7), ciproxifan again caused a significant increase in power when compared to vehicle treated animals (F[1, 210] = 15.00; p < 0.01) over a range of stimulation intensities. However, current-dependent increases in theta frequency were not affected by ciproxifan.

Effect of ciproxifan on cortical and hippocampal EEG of freely moving rats.
Consistent with earlier reports (Fox et al., 2003), ciproxifan attenuated cortical EEG delta activity. Figure 4A shows the relative delta power calculated for 1 hr segments of the frontal cortical EEG for 2 hours before and 2 hours after ciproxifan (3 mg/kg) and vehicle (saline) injections, in 4 rats. Under control conditions, the spectral power in the delta frequency range (1-4 Hz) constituted ~18 % of the total signal power which did not change after saline injection but dropped to 12-13 % after ciproxifan. The differences between delta power after ciproxifan and saline injections were statistically significant, at corresponding time points (Student’s t-test, p=0.019 and p=0.045 during the 1st and 2nd hour, respectively). The reduction of delta activity was due both to shortening of slow wave sleep and to a decrease in the amplitude of the delta waves. Rats undisturbed after the injection of saline were only awake in 22±5 % of the following 2 hours while after
ciproxifan they spent 42\% ± 7\% of the time awake (Fig. 4B), the difference being significant (p=0.017). In addition, ciproxifan decreased the power of delta waves calculated for episodes of slow wave sleep. The peaks of delta power after ciproxifan were 18\% ± 5\% lower than those before the injection (p=0.026), whereas no significant change was observed after saline injection (-1\% ± 2\%, p=0.258; Fig. 4C, grey bars). At the same time, the prolongation of the periods of wakefulness did not lead to significant changes in average theta activity in the hippocampus (p=0.347). Relative theta power (5-10 Hz) during episodes of high EMG activity of the undisturbed rats in their home cages was similar before and after injections (Fig. 4C, white bars).

Since hippocampal theta activity is closely associated with behavior, the effect of ciproxifan was also tested in the controlled behavior of novelty exploration (Kocsis et al., 2007). After the rats were placed in the novel environment they always engaged in vigorous exploratory activity even when the procedure was repeated in consecutive days. This behavior lasted 5-20 min and was associated with high EMG activity and regular theta waves in the hippocampal EEG. Figure 5A shows the changes in integrated theta power in two rats recorded simultaneously in the novelty box. The rats had been pretreated 30 min earlier by systemic injections of ciproxifan or saline, which alternated in the 2 experiments, in a saline-ciproxifan-saline or in a ciproxifan-saline-ciproxifan sequence, on 3 consecutive days. The traces show an enhancement of theta activity after every ciproxifan administration independent of the order of the injections (see also specimen recordings in Fig. 5B). In a group of 7 rats, the effect of ciproxifan on hippocampal theta power calculated during the first 5 min of active exploration was
found significant ($F[1,14]=19.94$, $p=0.0005$ for treatment; $F[2,14]=1.42$, $p=0.27$ for time, i.e. day 1, 2, and 3; and $f[2,14]=17.43$, $p=0.0002$ for treatment X time interaction). Post-hoc Bonferroni test revealed significant ($p<0.05$) differences between the effects of saline and ciproxifan injections. When the results of the experiments with drug and vehicle injections were pooled together the difference was also found significant (t-test, $p=0.034$, Fig. 5C).
Discussion

The present results demonstrate that systemic administration of thioperamide and ciproxifan, selective H3 receptor antagonists/inverse agonists, as well as the psychostimulant methylphenidate facilitate hippocampal theta oscillation in anaesthetized rats. Furthermore, when hippocampal oscillation is induced by brainstem stimulation, ciproxifan, but not methylphenidate enhances the power of theta activity. In freely-moving rats, ciproxifan promotes wakefulness with a concomitant reduction in cortical delta power, and augments novelty-induced hippocampal theta activity. These findings provide evidence that H3 receptors play a role in regulation of hippocampal theta oscillation, representing one of the probable mechanisms involved in histamine-induced modulation of higher brain functions, such as attention and learning.

Neuronal network oscillations, reflecting neuronal synchronization have been linked to various cognitive processes (Axmacher et al., 2006; Buzsáki and Draguhn, 2004), including hippocampal theta rhythm, one of the most studied network oscillations (Buzsáki, 2002). Neuronal networks generating hippocampal theta oscillation are extensive, covering multiple forebrain and brainstem-diencephalic structures (Bland and Oddie, 2001), and theta-generating circuitries are heavily innervated by various monoaminergic and peptidergic pathways, capable of powerful modulation of their oscillatory activities (Vertes and Kocsis, 1997; Freund 2003). Although histaminergic neurons project to the hippocampus and the septum/diagonal band of Broca (Haas and
Panula, 2003), and presumably innervate both pyramidal and GABAergic interneurons, the role of histamine and various histamine receptors in hippocampal function or hippocampal network oscillations has not been fully investigated. In vivo recordings from the hippocampal CA1 region of freely moving rats have revealed that high-frequency oscillations (200-Hz ripples) are markedly enhanced after injection of the H1-antagonists, indicating a tonic regulation of hippocampal activity by the histaminergic system (Knoche et al., 2003). Furthermore, systemic or intrahippocampal administration of pyrilamine, a selective H1 receptor antagonist, impaired both spatial memory performance and hippocampal theta oscillation during performing memory task in rats (Masuoka and Kamei, 2007).

The present findings show that systemic administration of the H3 receptor antagonists, ciproxifan and thioperamide, enhance the power of spontaneous theta in chloral hydrate anesthetized rats. Methylphenidate elicited a similar response, in line with previous experiments demonstrating that drugs enhancing norepinephrine neurotransmission (such as norepinephrine reuptake inhibitors and psychostimulants), induce or enhance hippocampal theta activity (Berridge and Waterhouse; 2003; Hajós et al., 2003; Krause et al., 2003). Since H3 receptors are located at axon terminals of histamine containing neurons, and function as autoreceptors (Arang et al., 1983), their blockade by ciproxifan and thioperamide could enhance histamine release, and subsequently promote hippocampal theta oscillation. However, H3 receptors are also located on a number of neurochemically heterogeneous axon terminals, and function as heteroreceptors
(Schlicker et al., 1994), therefore ciproxifan and thioperamide could enhance the release of a variety of neurotransmitters, which in turn may modulate hippocampal oscillations.

Our current findings also demonstrate that nPO-stimulation induced hippocampal theta can be augmented by systemic administration of the H3 receptor antagonist, ciproxifan. Since nPO stimulation-induced theta is mediated, at least in part, via ascending cholinergic or cholinoceptive neurons (Bland and Oddie, 2001), it has been shown that muscarinic receptor antagonists reduce, whereas acetylcholinesterase inhibitors enhance nPO stimulation-induced hippocampal theta activity (Kinney et al., 1999; Li et al., 2007; Siok et al., 2006; McNaughton et al., 2007). Based on the well-known link between hippocampal theta oscillation and cognitive functions, it has been proposed that modulation of hippocampal theta activity by the cholinergic drugs is a crucial contributor to their cognitive effects. In line with these findings, compounds affecting cognitive functions via other than muscarinic receptors (e.g. n-methyl-aspartate or nicotinic receptors) also have profound effects on nPO stimulation-induced hippocampal theta oscillation (Kinney et al., 1999; Siok et al., 2006; McNaughton et al., 2007). Among the potential pharmacological targets in mnemonic drug discovery the histamine system has been considered: it has been suggested that direct or indirect activation of the histamine pathway, including antagonists or inverse agonists at H3 receptors, would improve cognitive functions (Esbenshade et al., 2006; Medhurst et al., 2007; Passani et al., 2004). Although the precise mechanism via H3 antagonists facilitates brainstem stimulation-induced hippocampal theta activity cannot be currently determined, H3 axon-terminal auto- or heteroreceptors in the hippocampus or its afferents could modulate...
neurotransmission release and enhance stimulation-induced theta activity. Nonetheless, the present findings demonstrate that systemic administration of H3 antagonists enhances the power of nPO-stimulation induced hippocampal theta oscillation, which supports a procognitive action of these compounds. In contrast to ciproxifan, systemic administration of methylphenidate failed to influence nPO-stimulation induced theta activity, in spite of the fact, that it powerfully enhanced spontaneous theta. These findings clearly differentiate H3 receptor antagonists from catecholamine-related stimulants, and indicate that modulation of catecholamine release via H3 heteroreceptors is not the predominant mechanism underlying ciproxifan-augmented theta oscillation. Our findings on methylphenidate also confirm previous observations that drugs enhancing catecholamine neurotransmission, including reboxetine do not augment nPO-stimulation induced hippocampal theta (McNaughton et al., 2007).

Activity of histamine neurons not only correlates with sleep-wake cycle, but enhanced histamine neurotransmission facilitates wakefulness. It has been shown that H3 antagonist ciproxifan, and other nonimidazole H3 receptor antagonists, presumably by augmenting histamine release, enhance wakefulness, and reduce cortical EEG delta power (Fox et al., 2003; Parmentier et al., 2007). Our current findings are in line with these observations: we showed that systemic administration of ciproxifan significantly enhances wakefulness, and reduces cortical delta power in rats. Cortical delta power was significantly reduced even during periods of slow-wave sleep. In contrast, during ciproxifan-induced prolong periods of wakefulness hippocampal theta activity was unaffected. Although ciproxifan did not change power of hippocampal theta oscillation
when rats were kept at their familiar environment, it did significantly enhance novelty-induced theta activity. These findings clearly demonstrate a state-dependent modulation of hippocampal theta activity by the histamine system. In accordance with our previous findings (Kocsis et al., 2007), rats exposed to novel environment showed enhanced hippocampal theta power, most likely reflecting an augmented synchrony between hippocampal neurons. The primary behavioural correlates of theta rhythm in the freely moving rat include exploration as one of the most characteristic theta-behaviors (Vanderwolf, 1969) during which theta is instrumental for the integration of sensory processing with the planning and maintenance of the corresponding motor activity (van Lier et al., 2003; Wyble et al., 2004), as originally proposed by Bland (for review see Bland and Oddie, 2001). This novelty-induced, sensory-related (frequently referred to as Type 2) hippocampal theta oscillation was significantly amplified by ciproxifan, further supporting a close connection between histamine neurotransmission and regulation of degree of active wakefulness and attention.

Hippocampal theta generating network is extensive and complex; it includes multiple oscillators in the hippocampus and in brainstem-diencephalic nuclei (Vertes and Kocsis, 1997; Bland and Oddie, 2001; Buzsáki, 2002). H3 receptor antagonists could modify theta activity at various components of this network, presumably in a state- and behavior-dependent manner. Further studies are warranted to explore the precise modulation of the hippocampal activity by the histaminergic system, as well as the site and mode of action of H3 receptors. Nevertheless, facilitation of novelty-induced hippocampal theta by ciproxifan resembles the results observed after systemic administration of the
norepinephrine re-uptake inhibitor reboxetine (Kocsis et al., 2007). Since norepinephrine re-uptake inhibitors are clinically known to improve ADHD symptoms (Biederman et al., 2004), these findings further corroborated the connection between hippocampal theta and attention. Our current results, together with previous observations indicating a particularly important role of histamine in active wakefulness and adequate response to novel environment (Parmentier et al., 2002; Takahashi et al., 2006), suggest that H3 receptor antagonists could affect attention and potentially other domains of cognitive function.
References


Footnotes:

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FIGURE 1. Effects of ciproxifan, thioperamide and methylphenidate on spontaneous EEG in chloral hydrate anesthetized rats. (A) Representative EEG traces and power spectrum from vehicle treated animals and animals treated with 1.0 mg/kg, i.v., of ciproxifan, thioperamide or methylphenidate. (B) Dose response effects of ciproxifan, thioperamide and methylphenidate. Methylphenidate was most potent, significantly increasing the percent of power in the theta band at the lowest dose tested (0.1 mg/kg, i.v.; p < 0.05 compared to pre-drug control period). All three compounds showed significant increases in the percent of power in the theta band at 0.3 and 1.0 mg/kg, i.v. (p < 0.05, paired Student’s t-test).

FIGURE 2. Effects of ciproxifan (1.0 mg/kg, i.v., n=4), methylphenidate (1.0 mg/kg, i.v., n=4) or vehicle (n=4) on brainstem stimulated hippocampal theta activity. Arrows indicate administration of drugs or vehicle. For inter-animal comparisons, total theta power during nPO stimulation was normalized for each rat to the average power measured prior to drug or vehicle administration. Ciproxifan significantly increased power of hippocampal theta oscillation (p<0.01; ANOVA, F=91.5; d.f. 1,84).

FIGURE 3. Effects of systemic administration of ciproxifan (1 mg/kg, i.v., n=7) on stimulation-induced hippocampal theta oscillation in urethane anesthetized rats.
Stimulation intensity was stepped from 0.02 to 0.18 mA in 0.01 mA increments thereby generating a stimulus-response relationship for both theta frequency and power. Ciproxifan significantly increased theta power over a range of stimulation intensities \( (p<0.01; \text{ANOVA } F=15.00; \text{d.f. } 1,210) \), but did not affect theta frequency.

FIGURE 4. Effect of ciproxifan on EEG delta slow waves in freely moving rats. A. Relative delta (1-4 Hz) power averaged over 1 hr segments of frontal cortical EEG before and after i.p. injection of saline and 3 mg/kg ciproxifan \((p<0.05, n=4)\). B. Percent time spent awake during the first 2 hours after saline and ciproxifan injection. C. Percent changes in spectral powers in the delta (1-4 Hz) band of frontal cortical EEG and in the theta band (5-10 Hz) of hippocampal EEG after injection of saline and ciproxifan as compared with controls before injection. Delta and theta were calculated for episodes associated with low and high EMG activity, respectively. Relative power of separate frequency bands were calculated by fast Fourier transform of consecutive 16 s segments and divided by the total power of the EEG signal.

FIGURE 5. Effect of ciproxifan on hippocampal theta activity in a novel environment. A. Integrated theta power in the hippocampus (layer at the fissure between CA1 and dentate gyrus) in two rats in 3 consecutive test days. Large artefacts in each trace mark the transfer of the rats in the novelty box 30 min after systemic injection of saline or ciproxifan (3 mg/kg). Note the sequence of injection of saline-ciproxifan-saline in rat #c5 and reverse order in rat #c1. B. Specimen recordings of hippocampal EEG (hipp) and associated muscle activity (EMG) during exploration of the novel environment (rat #c5).
C. Average theta power in the first 5 min of high theta activity following transfer to novel environment in 7 rats (p<0.05).
Figure 1
Figure 2

Normalized Total Power

Minutes

Ciprofloxacin
Methylphenidate
Vehicle

Baseline
15 Min
30 Min
45 Min
60 Min

Percent change in total theta power compared to baseline period
Figure 3
Figure 4
Figure 5.