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The physiological and pathophysiological roles of neuronal histamine: An insight from human positron emission tomography studies

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Abstract

Histamine neurons are exclusively located in the posterior hypothalamus, and project their fibers to almost all regions of the human brain. Although a significant amount of research has been done to clarify the functions of the histaminergic neuron system in animals, a few studies have been reported on the roles of this system in the human brain. In past studies, we have been able to clarify some of the functions of histamine neurons using different methods, such as histamine-related gene knockout mice or human positron emission tomography (PET). The histaminergic neuron system is known to modulate wakefulness, the sleep–wake cycle, appetite control, learning, memory and emotion. Accordingly we have proposed that histamine neurons have a dual effect on the CNS, with both stimulatory and suppressive actions. As a stimulator, neuronal histamine is one of the most important systems that stimulate and maintain wakefulness. Brain histamine also functions as a suppressor in bioprotection against various noxious and unfavorable stimuli of convulsion, drug sensitization, denervation supersensitivity, ischemic lesions and stress susceptibility.

This review summarizes our works on the functions of histamine neurons using human PET studies, including the development of radiolabeled tracers for histamine H1 receptors (H1R: ^{11}C-doxepin and ^{11}C-pyrilamine), PET measurements of H1R in depression, schizophrenia, and Alzheimer's disease (AD), and studies on the sedative effects of antihistamines using $H_2^{15}O$ and H1R occupancy in the human brain. These molecular and functional PET studies in humans are useful for drug development in this millennium.

Keywords: Histamine; Histamine neurons; Antihistamines; Brain; PET; Depression; Schizophrenia; Alzheimer's disease

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1. Histaminergic neuron system

Central histaminergic neurons, which originate in the tuberomammillary nucleus (TM) of the posterior hypothalamus, are extensively projected to areas such as the whole cerebral cortex, thalamus, basal ganglia, and amygdala (Panula et al., 1984; Watanabe et al., 1984). The presence of histaminergic neuron system (Fig. 1) was first reported by Watanabe et al. in 1983 (Watanabe et al., 1983), and its precise location and distribution was demonstrated in the rat brain using an antibody to L-histidine decarboxylase (HDC, a histamine-forming enzyme) in 1984 (Watanabe et al., 1984). Interestingly, in the same year, Panula et al. (1984) independently demonstrated the presence of the histaminergic neuron system using an antibody against histamine itself. Neuronal histamine works as a neurotransmitter that acts through 4 subtypes of histamine receptors denoted as H_1, H_2, H_3 and H_4. Histamine H1 receptors (H1R) modulate a variety of physiological functions such as wakefulness, sleep–wake cycle, appetite control, learning and memory, stress, seizure, and emotion (Table 1) (Brown et al., 2001; Watanabe & Yanai, 2001; Haas & Panula, 2003). Histamine H2 receptors (H2R) are thought to have almost similar functional properties to H1R in the brain. On the other hand, histamine H3 receptors (H3R), which were initially found as autoreceptors in the presynaptic sites of the histaminergic neuron system, are known to be also located on other neurons as heteroreceptors for regulating the release of other neurotransmitters such as 5-hydroxytryptamine, norepinephrine, acetylcholine, dopamine, and GABA. Therefore, H3R antagonists are expected to be useful in the treatment of psychiatric disorders such as sleep disorders and cognitive impairment (Cowart et al., 2004; Hancock & Fox, 2004; Passani et al., 2004; Stark et al., 2004; Hancock & Brune, 2005; Leurs et al., 2005).

Various functions of the histaminergic neuron system have been demonstrated using mainly pharmacological tools including an HDC inhibitor of (*S*)-α-fluoro-methyl histidine (FMH),

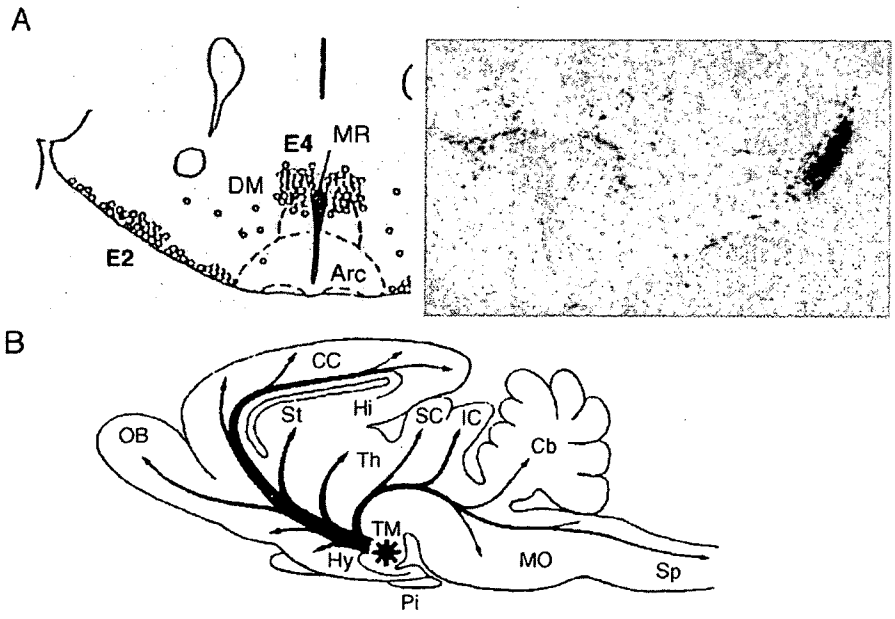

Fig. 1. Localization of histamine neurons in the rat hypothalamus. (**A**) Histamine neurons are visualized by an antibody raised against rat HDC. Neurons expressing HDC are exclusively located in the hypothalamus, and divided into E1 to E5 groups. The picture shows E2 and E4 subgroups. DM, dorsomedial hypothalamic nucleus; Arc, arcuate hypothalamic nucleus. (**B**) Distribution of histaminergic nerve fibers in the rat brain (sagittal section). Arrows indicate projections of neural fibers from neurons in the TM (asterisk). Abbreviations: Cb, cerebellum; CC, cerebral cortex; Hi, hippocampus; Hy, hypothalamus; IC, inferior colliculus; MO, medulla oblongata; OB, olfactory bulb; Pi, pituitary; SC, superior colliculus; Sp, spinal cord; St, striatum; Th, thalamus; TM, tuberomammillary nucleus.

H1R and H2R antagonists and H3R agonists and antagonists (Watanabe et al., 1990). For example, the following pharmacological agents have been used to activate the histaminergic neuron system: L-histidine, a precursor of histamine; HTMT, an H1R agonist; metoprine, an inhibitor of histamine *N*-methyltransferase (HMT, a histamine-inactivating enzyme); thioperamide and clobenpropit, H3R/H4R antagonists.

An alternative approach to clarify the physiological functions of histamine neurons is to manipulate histamine-related genes. Over the past decade, the use of genetically altered mice has become routine in many fields of biomedical research. For investigators that utilize rodents as experimental system, the technical development of production of mice with specific genetic alternations has promised a unique opportunity for a wide variety of sophisticated investigation of mice. As shown in Table 2, there are 6 different knockout mice available for experiments on the histaminergic neuron system (Inoue et al., 1996; Yanai et al., 1998; Kobayashi et al., 2000; Ohtsu et al., 2001; Toyota et al., 2002). Nowadays, many laboratories are using knockout mice of histamine-related genes in combination with classical pharmacological agents in order to clarify the functional roles of the histaminergic neuron system (Table 1).

Although these classical and new modalities are very useful to elucidate the functions of histamine neurons only in animals, it is reasonable to assume the same functions in humans from the animal studies. Autopsied human brain and cerebrospinal fluid (CSF) had been utilized for a long time to directly study human brain chemistry. However, non-invasive brain imaging modalities have nowadays become acceptable alternative approaches to understand human brain chemistry (Volkow et al., 1997; Phelps, 2000; Wong & Pomper, 2003). Imaging techniques enable us to assess the properties of brain tissues and to obtain information of how the brain works across scales from system level to molecular level. Commonly used imaging modalities include computerized axial tomography (CT), magnetic resonance imaging (MRI), electroencephalography (EEG), magnetoencephalography (MEG), magnetic resonance spectroscopy (MRS), functional magnetic resonance imaging (fMRI), single-photon emission tomography (SPECT) and positron emission tomography (PET). Among these techniques, PET is a unique method to non-invasively examine brain chemistry in humans.

Table 1
Stimulatory and inhibitory CNS functions of neuronal histamine

Functions	Deduced functions
Stimulatory CNS functions	
Sleep–wake cycle	Maintenance of wakefulness
Locomotion	Increased locomotor activity
Cognition	Augmented learning and memory
Energy metabolism	Induction of brain glycogen hydrolysis
Nociception	Increased pain perception
Inhibitory CNS functions	
Feeding	Inhibition of feeding behavior
Convulsion	Inhibition
Stress	Inhibition of stress-induced excitation
Methamphetamine-induced psychosis	Inhibition of kindling formation
Neural plasticity	Inhibition of denervation-induced supersensitivity

Table 2
Available knockout mice of histamine-related genes

Histamine H1 Receptor Gene Knockout Mice (Inoue et al., 1996; Yanai et al., 1998)
Histamine H2 Receptor Gene Knockout Mice (Kobayashi et al., 2000)
Histamine H3 Receptor Gene Knockout Mice (Toyota et al., 2002)
Histamine H4 Receptor Gene Knockout Mice (Hofstra et al., 2003)
Histamine H1 and H2 Receptor Gene Double Knockout Mice (Suzuki et al., 2005b)
Histidine Decarboxylase Gene Knockout Mice (HDC-KO) (Ohtsu et al., 2001)

Although PET is inferior to other techniques in spatial and temporal resolution, its sensitivity is quite high. As brain neurotransmission takes place at very low concentrations, typically in the subpicomolar range, specific neurotransmission in the human brain, such as activity of the histaminergic neuron system, can be detected using PET. We provide here a conceptual and methodological review of our PET studies conducted to clarify the physiological and pathophysiological functions of histamine neurons in humans.

2. Methodological development for positron emission tomography studies of histamine receptors

2.1. Radioligand binding study of histamine H1 receptors

Certain prerequisites for suitable compounds have to be met. First of all, the ligand should possess high affinity and selectivity for the H1R. It should be able to cross blood–brain barrier for high potency in the CNS, and it must at its final step of synthesis contain an atom or functional group suitable for radiolabeling such as $-N^{11}CH_3$, $-S^{11}CH_3$ or $-O^{11}CH_3$, $-^{18}F$. Because of low radiation doses to humans, carbon-11 (half life, 20 min) labeled radiopharmaceuticals are preferable to fluorine-18 (half life, 110 min) labeled ones in early phase PET studies.

For imaging H1R in human brain, [^{11}C]pyrilamine (Yanai et al., 1988) and [^{11}C]doxepin (Iwata et al., 2002) were labeled by *N*-methylation with [^{11}C]methyliodide or [^{11}C]methyl triflate (Fig. 2). However, [^{11}C]methyl triflate has now replaced [^{11}C] methyliodide as a ^{11}C-labeled precursor because of its high reactivity. Pyrilamine is a classical H1R antagonist often used as [^{3}H]pyrilamine in binding experiments. Doxepin, on the other hand, is a tricyclic antidepressant that has an extremely high affinity for the H1R but low affinity for other binding sites (Kanba & Richelson, 1984; Nguyen et al., 2001). Using PET, we have previously compared the binding characteristics of [^{11}C]doxepin and [^{11}C]pyrilamine in the human brain, and found that the distribution patterns of both C-11 tracers are almost identical with a higher signal-to-noise ratio in the case of [^{11}C]doxepin (Villemagne et al., 1991; Yanai et al., 1992b, 1992c). This finding was reconfirmed in another study using H_1R gene knockout (H_1KO) mice, where more than 95% of [^{3}H]doxepin specific binding in the brain was lost in H1R-KO mice, indicating that [^{11}C]doxepin binding observed in the human PET study reflects well the specific binding to H1R (Inoue et al., 1996).

Recently, [^{11}C]doxepin has been frequently used for a variety of human PET studies because of its high affinity (Fig. 3). Using

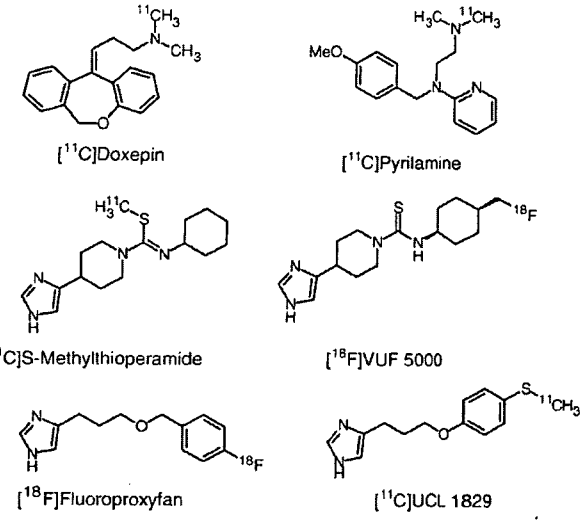

Fig. 2. Chemical structures of PET probes for H1R and H3R. Carbon-11 labeled pyrilamine and doxepin were only used in humans. For H2R, several H2 antagonists are easily labeled with [^{11}C]methyliodide or [^{11}C]methyl triflate, although there are no reports demonstrating specific binding in vivo.

PET and [^{11}C]doxepin, previous studies have shown the age-related decline of H1R binding (Yanai et al., 1992a), and the correlation between H1R binding and cognitive deficits in Alzheimer's disease (AD) (Higuchi et al., 2000), H1R binding and severity of major depression (Kano et al., 2004), and H1R binding and schizophrenia (Iwabuchi et al., 2005). Using the same technique, that is, PET and [^{11}C]doxepin, other studies have demonstrated the interaction between histamine receptor occupancy and cognitive impairment induced by sedative antihistamines (Okamura et al., 2000; Tagawa et al., 2001).

2.2. Attempts to develop selective radioligands for histamine H3 receptors

H3R were initially found in presynaptic sites of the histaminergic neuron system. These presynaptic sites contain autoreceptors that regulate the synthesis and release of histamine in the brain. Several therapeutic applications have been proposed for H3R antagonists such as schizophrenia, attention deficit hyperactivity disorder (ADHD), and AD. In this respect, non-invasive PET imaging techniques are highly beneficial particularly for measurement of receptor occupancy. However, radiolabeled ligands, with high affinity as well as good selectivity for the investigated receptor, are needed for these neuroimaging techniques. Although a large number of H3R ligands have been described, suitable radiolabeled compounds for PET are still lacking (Sasse et al., 2001). We have previously reported the binding characteristics of [^{3}H]*S*-methylthioperamide (SMT) to rat tissues and compared its binding with that of [^{3}H](*R*)α-methylhistamine ((*R*)α-MeHA), a selective H3R agonist (Yanai et al., 1994). The binding of [^{3}H]SMT to the membrane of rat forebrain was found to be stereoselective, saturable, reversible and temperature-dependent. [^{11}C]SMT can easily be synthesized using [^{11}C] methlyiodoide. Other compounds that have been labeled for use with highly advanced imaging techniques of animal PET include, [^{18}F]fluoroproxyfan (Iwata et al., 2000), [^{18}F]VUF 5000 (Windhorst et al., 1999), and [^{11}C]UCL1829 (Fig. 2). However, precise biodistribution studies with these compounds have not been well performed in rodents with animal PET (Phelps 2000). In this respect, H3R gene knockout mice would be useful to demonstrate H3R specific binding in vivo using animal PET. However, further development of new radiotracers is needed for PET imaging of H3R in the human brain.

2.3. Quantification of in vivo H1R binding by positron emission tomography

Quantification of in vivo PET measurement is an important issue because evaluating the binding characteristics B_{max} and K_D is more complicated in single PET scanning. To date, several methods have been described to quantify H1R binding in the human brain. A 4-compartment analysis is ideal for estimating rate constants for both the tracer delivery (k_1 and k_2) and the binding of the tracer to the receptors (k_3, k_4 and BP) (Yanai et al., 1990). However, as shown in Fig. 4A, a 3-compartment analysis is often used on dynamic PET images usually lasting for 90 min (Szabo et al., 1993; Higuchi et al., 2000). This analytical method may not always be always appropriate for generating parametric images from data in large sample sizes due to the long calculation time.

In contrast, graphical analyses are capable of constructing parametric images in a shorter time and are robust even when processing data with sizable noise. Actually, many studies have

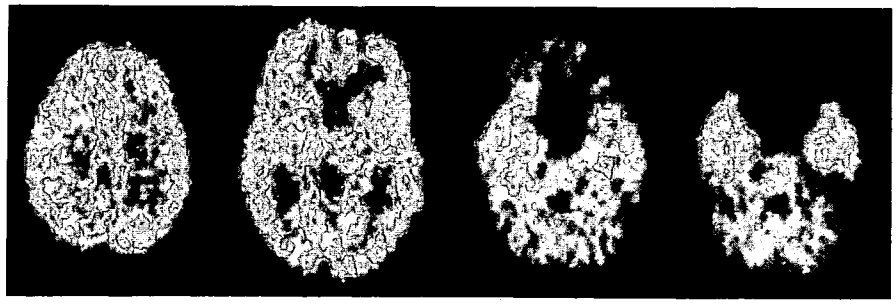

Fig. 3. Distribution of H1R in a young healthy human subject measured using PET and [^{11}C]doxepin. As shown in this figure, the density of H1R is high in the cingulate cortex, fronto-temporal cortex, amygdala and hippocampus. Low density of H1R is observed in the cerebellum. Single oral administration of 2 mg D-chlropheniramine abolished the specific binding of [^{11}C]doxepin in the human brain (data not shown).

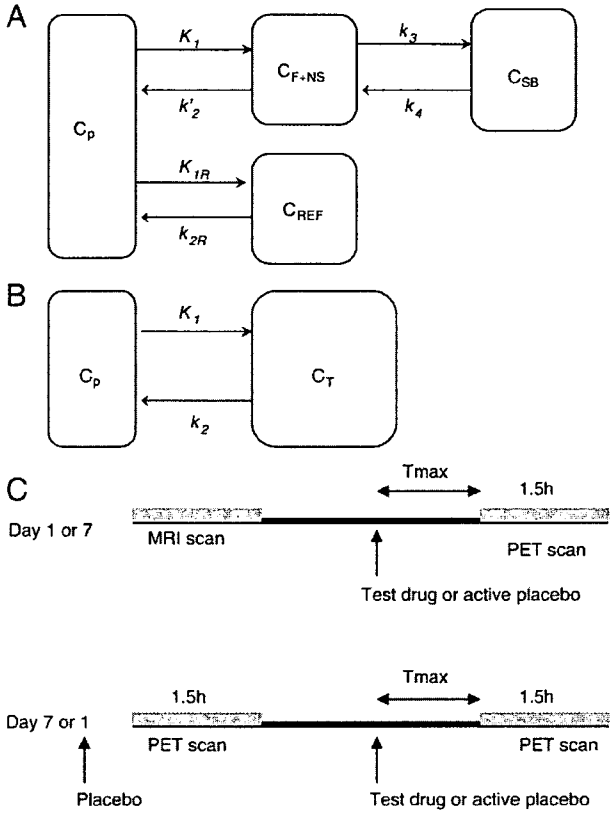

Fig. 4. (A) A 3-compartmental model applied to PET ligands receptor studies. In this model, there are 3 compartments for plasma tracer (Cp), tracer that is free and nonspecifically bound in tissue (CF+NS), and specifically bound in tissue (CSB). At the bottom is a reference tissue model that uses a compartment for plasma tracer (Cp) and a single tissue compartment (CREF) to represent free+nonspecific binding. For H1R, the cerebellum is used as reference tissue because of negligible H1R binding. Binding potential ($BP=k_3/k_4$). (B) 2-compartment model applied to both reference and target tissues (C_T). Distribution volume ($DV=K_1/k_2$). (C) A placebo-controlled design for receptor occupancy studies with carbon-11 labeled ligands. Human subjects undergo 3 PET measurements after single oral administration of test drugs, active placebo, and placebo in a 3-way crossover design, with minimum washout intervals of 7 days between drug treatments. In this study design, 2 PET scans per day are performed on the same subject. Administered doses are adjusted so that maximal radiation exposure to a healthy volunteer does not exceed 5 mSv per 5 years as recommended by our ethics committees. MRI scans are performed in order to examine brain structures. Drugs are administered at the time of plasma peak concentration (T_{max}).

reported the use of graphical methods (Yanai et al., 1992a;1995a, 1995b; Okamura et al., 2000; Tagawa et al., 2001; Kano et al., 2004; Tashiro et al., 2004; Iwabuchi et al., 2005). Recently, Suzuki et al. have confirmed that graphical methods using arterial input function (Logan et al., 1990) and reference tissues (Logan et al., 1996) are both applicable to quantification of H1R binding to [^{11}C]doxepin (Suzuki et al., 2005a).

Mochizuki and Zhou have separately reported that, in the case of H1R binding to [^{11}C]doxepin, a 3-compartment model does not always give reasonable results. Their work demonstrated that H1R binding can be more stably described by a 2-compartment model rather than by a 3-compartment model (Zhou et al., 2003; Mochizuki et al., 2004a, 2004b). Later, Mochizuki et al., developed a simplified static scan protocol for better application in clinical trials. They demonstrated that [^{11}C]doxepin-H1R binding is better described with a 2-compartment rather than a 3-compartment model and proposed the use of distribution volume ($DV=K_1/k_2$) as an index of [^{11}C]doxepin binding (Mochizuki et al., 2004a, 2004b). They also confirmed that their static scan protocol produces reliable DV values with a high correlation coefficient ($r=0.94$) (Mochizuki et al., 2004a, 2004b). Using this protocol, a placebo-controlled crossover study was conducted to compare H1R occupancy by both olopatadine, a novel second-generation antihistamine, and ketotifen (Tashiro et al., 2006).

2.4. Study protocol for positron emission tomography measurement of receptor occupancy

Another important issue in PET measurement is the study design of receptor occupancy. Investigators are able to minimize potential error due to inter-subject variability by using a placebo-controlled design (Fig. 4C). The most important advantage of PET receptor occupancy study is methodological objectivity, which makes interpretation of the results easier and clearer. Placebo-controlled receptor occupancy studies were first performed with PET to investigate the binding of dopamine D_1 and D_2 receptors to second-generation antipsychotics (Karlsson et al., 1995; Nyberg et al., 1999, 2002) and antidepressants (Martinez et al., 2001; Nakayama et al., 2002). In placebo-controlled crossover design, exposure of subjects to radiation could be increased as the same subject is scanned more than twice. However, exposure to radiation should be minimized by the use of a minimum dose and by a 3D data acquisition mode with high sensitivity.

3. Positron emission tomography studies on the sedative effects of histamine H1 receptor antagonists in humans

3.1. Stimulatory effects of brain histamine on cortical activation

Neuronal histamine plays a pivotal role in maintaining wakefulness. It functions as a wake amine and is involved in the circadian rhythm (Monnier et al., 1970). In animal experiments, FMH has been shown to shorten the waking time in the dark period and to prolong the slow-wave-sleep (SWS) time in the light period in cats (Lin et al., 1988). In accordance with this finding, thioperamide has been reported to increase the waking time and (R)αMeHA to decrease SWS time in cats (Lin et al., 1990). These results were confirmed by studies using knockout mice of histamine-related genes. H1KO mice have been shown to move less at night and more during the day (Inoue et al., 1996; Yanai et al., 1998). It is very interesting that the same distorted circadian rhythms were observed in the H2R mutant mice (unpublished data) and H3R gene knockout mice (Toyota et al., 2002) as well as HDC gene knockout mice (Parmentier et al., 2002).

When we consider the involvement of the histaminergic neuron system in the actions of orexin and adenosine, it can be assumed as shown in Fig. 5 that their effects on the sleep–wake

centers are due to activation and inactivation of the histaminergic neuron system, respectively (Scammell et al., 1998; Nishino et al., 2001; Ko et al., 2003). In support of this notion, Huang et al. (2001) have shown a direct involvement of the histaminergic neuron system in the action of the orexinergic system.

3.2. Sedative effects of antihistamines and measurement of H1R occupancy in human brain

H1R antagonists, or antihistamines, are often used for treatment of allergic diseases such as allergic rhinitis and atopic dermatitis (Adelsberg, 1997; Mann et al., 2000). However, antihistamines often induce sedative side effects in addition to their main anti-allergic effects. First-generation antihistamines usually induce sedation and psychomotor retardation among allergic patients. For instance, diphenhydramine, hydroxyzine, promethazine and chlorpheniramine are highly sedative and can create potentially dangerous problems for patients involved in driving or those operating planes or heavy machinery, even at recommended doses (Passalacqua et al., 1993; Adelsberg, 1997). These first-generation antihistamines have been often used as positive controls of sedation (Gengo et al., 1987; Gengo & Gabos, 1987; Ramaekers et al., 1992; Patat et al., 1995; Lee & Maibach, 2001; Vermeeren et al., 2002; Tashiro et al., 2005).

In contrast, second-generation antihistamines, including fexofenadine, loratadine and cetirizine, are regarded as being less impairing and sedating and are, therefore, safer to use in day-to-day activities. However, recent investigation has demonstrated that not all second-generation antihistamines manifest similar 'non-sedative' profiles (Mattila & Paakkari, 1999). Currently available second-generation antihistamines can be further separated into 2 subgroups (Hindmarch et al., 2002). One category includes those drugs that cause little cognitive and psychomotor impairment at low or recommended doses, but cause dose-related impairment at higher doses, such as cetirizine and loratadine (Gengo & Gabos, 1987; Gengo et al., 1990; Ramaekers et al., 1992). The other category consists of those antihistamines that do not cross the blood–brain barrier (BBB), inducing no sedative side-effects even at higher than recommended doses, such as fexofenadine (Hindmarch et al., 1999; Weiler et al., 2000; Hindmarch et al., 2002; Tashiro et al., 2005).

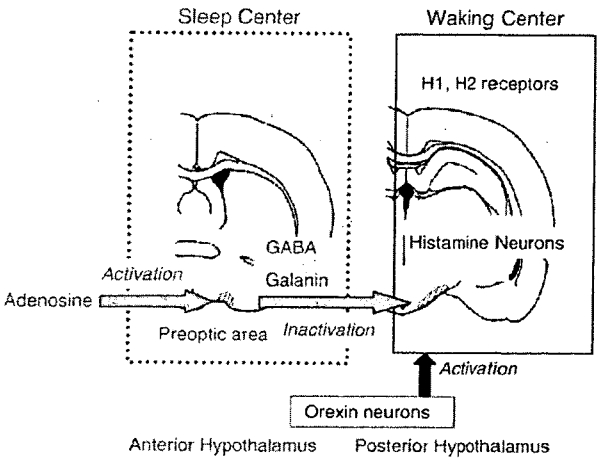

Fig. 5. Hypothetical roles of histamine neurons in sleep–wake mechanism. Histamine neurons function as a center of wakefulness. Anterior hypothalamus and orexin neurons can inhibit and stimulate the activity of histamine neurons, respectively. Adenosine activates the sleep center of the anterior hypothalamus, and caffeine can inhibit the action of adenosine.

Numerous studies have reported the results of various performance tests that compare the sedative profiles between first-generation antihistamines and placebo, or between first- and second-generation antihistamines (Gengo et al., 1987; Gengo & Manning, 1990; Ramaekers et al., 1992; Gandon & Allain, 2002; Hindmarch et al., 2002; Tashiro et al., 2005). However, due to limited sensitivity in psychomotor tests, it has been difficult to detect statistically significant differences in the comparison between 2 different second-generation antihistamines. A recent meta-analysis has demonstrated that proportional impairment ratios (PIR) are useful for comparison of antihistamines sedative properties, especially among second-generation antihistamines (Shamsi & Hindmarch, 2000). Calculation of PIR, however, is not easy because all previous reports on sedation due to antihistamines should be reviewed (Shamsi & Hindmarch, 2000).

Animal studies suggest that sedation is caused by blockade of H1R in histaminergic neurotransmission. Sedative antihistamines that easily penetrate the BBB occupy a large proportion of post-synaptic H1R, and variations in cerebral H1R occupancy of antihistamines may be a result of their different BBB permeability. We first reported that H1R occupancy in humans can be measured using PET with [^{11}C]doxepin (Yanai et al., 1995a, 1995b, 1999; Okamura et al., 2000; Ramaekers & Vermeeren, 2000; Tagawa et al., 2001; Tashiro et al., 2004). This technique has proved to be a reliable method for evaluating the sedative properties of antihistamines in humans (Fig. 6).

Those antihistamines that penetrate the BBB block H1R binding, whereas non-sedative antihistamines do not affect the imaging patterns (Yanai et al., 1995a, 1995b, 1999; Tagawa et al., 2001). We have previously demonstrated a significant correlation between cognitive decline and brain H1R occupancy induced by D-chlorpheniramine. In addition, we have shown that both cognitive decline and brain H1R occupancy significantly correlated with plasma concentration of D-chlorpheniramine (Okamura et al., 2000; Tagawa et al., 2001). The high incidence of sedation caused by ketotifen is in good agreement with H1R occupancy values of 70–80% (Yanai et al., 1999). Single oral administration of D-chlorpheniramine (2 mg) achieved approximately 50–60% of H1R occupancy (Yanai et al., 1995a, 1995b; Tagawa et al., 2001). As a whole, PET studies demonstrated that first-generation antihistamines occupied more than 50% of available H1R. More than 50% of H1R occupancy is associated with high prevalence of sleepiness and cognitive decline (Yanai et al., 1995a, 1995b; Tagawa et al., 2001).

As for antihistamines with little or mild sedation, previous studies have demonstrated that second-generation antihistamines significantly varied in H1R occupancy. After administration of single oral dose, the values of H1R occupancy (%) of

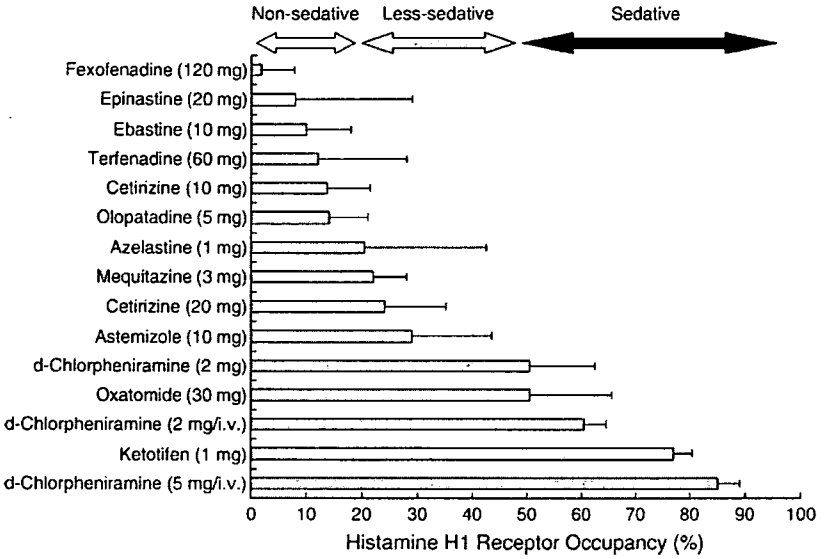

Fig. 6. H1R occupancy caused by various antihistamines in the human brain. Values of H1R occupancy from published and unpublished data (N=3–12) are summarized for the frontal cortex. Sedative, less-sedative and non-sedative antihistamines can be classified according to their H1R occupancy of 50–100%, 20–50% and 0–20%, respectively.

some second-generation antihistamines are as follows (Fig. 5): fexofenadine 120 mg (2%) (Tashiro et al., 2004), cetirizine 20 mg (24%) (Tashiro et al., 2004), epinastine 20 mg (10%) (Yanai et al., 1995a, 1999), terfenadine 60 mg (12%) (Yanai et al., 1995b, 1999), astemizole 10 mg (29%), azelastine 1 mg (21%), mequitazine 3 mg (22%) (Yanai et al., 1999) and ebastine 10 mg (10%) (Tagawa et al., 2001). As a whole, second-generation antihistamines would occupy between 0 to 30% of brain H1R (Yanai et al., 1999). H1R occupancy of several second-generation antihistamines, including fexofenadine and ebastine has no correlation with their plasma drug concentrations, suggesting that even high doses of these antihistamines do not penetrate the BBB. Interestingly, ceterizine dose-dependently occupies H1R in the human brain. All these data suggest that the sedative properties of antihistamines can be divided into 3 categories: sedative, less-sedative and non-sedative.

3.3. Development of new antihistamines and Consensus guideline on new generation antihistamines

The Consensus Group on New Generation Antihistamines (CONGA) was convened in 2002 under the auspices of the British Society for Allergy and Clinical Immunology (BSACI). The proposal from CONGA summarizes the current situation concerning evaluation of the sedative profiles of antihistamines (Holgate et al., 2003). It states that all of the following 3 measurements should be undertaken to classify a specific antihistamine as non-sedating: (1) incidence of subjective sleepiness; (2) objective cognitive and psychomotor functions; and (3) PET measurement of H1R occupancy. For evaluation of the incidence of subjective sleepiness, double-blind, placebo-controlled studies should be performed in a relatively large population sample and no statistically significant difference should be observed between placebo and the target drug. Similarly, cognitive studies should be conducted in a large population sample, and no significant differences should be observed between placebo and the active treatment in at least 2 different tasks. The target agent should also be examined for H1R occupancy in order to confirm its non-sedative profile. The recommended criterion for H1R occupancy is a maximum of around 20% at the highest recommended dose (Holgate et al., 2003). Following these recommendations, if any impairment of CNS function is demonstrated for the target agent, it should be classified as a "relatively non-sedative" or "less sedative" drug, not as a "non-sedative" drug. A lack of effect on the CNS should be recognized as an important "pre-requisite" for new generation antihistamines and "non-sedative" properties should be certified only when the above-mentioned criteria have been fulfilled (Holgate et al., 2003). As a result, the role of PET in drug development will become more important in the future.

3.4. Functional neuroanatomy of sedation due to antihistamines studied with [^{15}O]H_2O-positron emission tomography

In addition to PET application to measurement of receptor occupancy, another important application is to examine the neural mechanism of CNS pharmacological effects. During the past 5 years, functional MRI and PET have been used increasingly to map the modulatory effects of psychopharmacological agents on cognitive activation of networks in the human brain (Honey & Bullmore, 2004). Such pharmacological imaging studies can be informative about the pharmacodynamics and specific neurotransmitter mechanisms that underlie adaptation of the neurocognitive system to variation in task performance. In particular, PET and functional MRI are powerful tools for understanding subjective affective feelings as shown in our study on the modulating system of histamine-

induced itching sensation in the human brain (Mochizuki et al., 2003). For these purposes, regional cerebral blood flow and blood oxygen level dependent (BOLD) signal are measured using $[^{15}O]H_2O$-PET and functional MRI, respectively.

Although most people taking antihistamines have experienced sedation and impaired performance, the neural correlates of these sedative properties are not well understood in man. In order to assess the sedative effects of antihisatmines in the human brain, reaction time (RT) and accuracy are objectively measured during PET scanning. In this respect, PET is superior to functional MRI because cognitive performance can be accurately measured in PET scanner. For this purpose, regional cerebral blood flow is usually measured using $[^{15}O]H_2O$ as a diffusible tracer. As far as the authors know, Okamura et al. (2000) were the first to visualize the effects of sedative antihistamine-induced sedation using PET and $[^{15}O]H_2O$. They demonstrated that impaired performance in visual discrimination tasks was associated with increased activation in the right prefrontal cortex. This activation in the prefrontal cortex might be induced to compensate for cognitive deterioration caused by antihistamines' suppression of cortical activation. A significant deactivation in the midbrain after treatment with D-chlorpheniramine was also observed, suggesting a suppression of midbrain reticular formation by antihistamines (Okamura et al., 2000). These human PET data are consistent with the concept that neuronal histamine could evoke behavioral arousal through the reticular ascending activating system (Tasaka et al., 1993; Jouvet 1996).

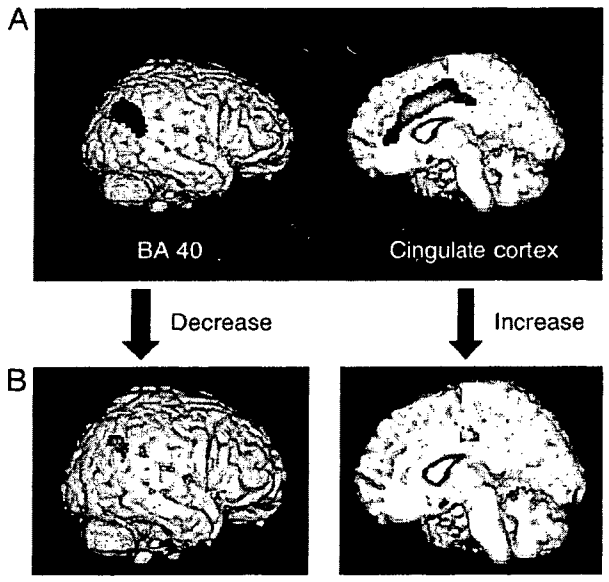

Fig. 7. Visualization of impaired performance by antihistamines in humans using $H_2^{15}O$ and PET. The aim of this study was to visualize the mechanism of brain impairment in spatial cognition (right–left discrimination) after oral administration of D-chlorpheniramine, a first-generation sedative antihistamine. (**A**) Areas where rCBF significantly increased during the spatial discrimination task. (**B**) Areas where rCBF significantly decreased and increased after oral administration of the sedative antihistamine. Normal subjects were randomly assigned to 2 groups (D-chlorpheniramine and placebo) and were asked to perform a spatial discrimination task after oral administration of 6 mg D-chlorpheniramine or a placebo. Administration of D-chlorpheniramine impaired visuomotor spatial discrimination and altered cortical and subcortical activity. Decreased and increased activities were observed in the right parietal cortex (BA 40) which is related to visuomotor spatial cognition and the posterior cingulate cortex which constitutes the attention system of the brain, respectively. These findings clearly suggest that alteration in cortical and subcortical activity contribute to impaired spatial cognition caused by treatment with D-chlorpheniramine.

Antihistamine-induced sedation in humans consists of 2 different aspects; subjective sleepiness and objective impaired performance. As shown in Fig. 7, antihistamine-induced impaired performance is closely related to alteration of task-specific brain activity. These findings clearly suggest that alteration in the cortical and subcortical activity contributes to impaired spatial cognition caused by treatment with sedative antihistamines (Mochizuki et al., 2002). It is generally accepted that histaminergic neurons can activate the cortex either directly by their widespread hypothalamo-cortical projections or indirectly via the thalamo-cortical pathway. Neuronal histamine can also promote cortical activation via the cholinergic system by a dual activation of the substantia innominata and the mesopontine tegmentum (Lin et al., 1996). On the other hand, H1R antagonists are reported to inhibit activation of norepinephrine neurons following central administration of histamine (Fleckenstein et al., 1994). Therefore, task-evoked brain activity might be affected indirectly by modulation of noradrenergic or cholinergic neurons.

4. Changes in histamine H1 receptor density caused by aging and neuropsychiatric disorders

4.1. Effects of aging on the histaminergic neuron system

It has been widely recognized that fundamental functions including attention, learning and memory could decline in aged humans and animals. Several reports have demonstrated that brain histamine is involved in those fundamental functions in both physiological and pathophysiological conditions. Senescence-accelerated mice (SAM) showed a marked age-related deterioration of learning in the passive avoidance test with alteration in the histaminergic neuron system (Meguro et al., 1995). In addition, SAM exhibited a substantial improvement in the latency of passive avoidance response after administration of thioperamide, a potent H3R antagonist (Meguro et al., 1995). Previous studies have shown that both the cholinergic and histaminergic neuron systems are involved in many physiological functions including learning and memory (Bacciottini et al., 2001). H3R are presynaptic modulators of neural transmission in a variety of neuronal circuits relevant to cognition (Blandina et al., 2004). Thus, thioperamide, a prototype H3R antagonist, has been reported to ameliorate cognition in attention and memory tests in non-human experiments and to improve memory impairment induced by scopolamine, MK-801, and abnormal aging, in pentylenetetrazole (PTZ)-kindled mice (Meguro et al., 1995; Watanabe & Yanai, 2001; Jia et al., 2006).

The levels of histamine metabolites are known to increase in human cerebrospinal fluid with age in contrast to other

aminergic transmitters (Prell et al., 1988). Mazurkiewicz-Kwilecki and Nsonwah (1989) have reported that the levels of histamine in the brain normally increase with age. Using PET and [^{11}C]doxepin, we have previously reported an age-related decline in H1R binding in the normal human brain, especially in the prefrontal, temporal, cingulate and parahippocampal regions (Yanai et al., 1992a), which are known to be involved in attention and cognition. It is possible that the increased release of endogenous histamine into synapses in old healthy volunteers results in a decrease in the apparent number (B_{max}) and affinity (K_D) of H1R available for exogenous ligands. Indeed, it has been reported that 20% of H1R was down-regulated in guinea-pig brain by treatment with histamine (Quach et al., 1981).

Histaminergic neurons can activate cortical and subcortical functions through H1R and H2R. Histaminergic neurons in the hypothalamus activate the neocortex and limbic system, particularly the frontal, temporal and cingulate cortices as a neurotransmitter that facilitates cortical arousal (Lin et al., 1996; Okamura et al., 2000). Histamine in the brain also augments hippocampal synaptic currents through the polyamine binding sites of *N*-methyl-D-aspartate (NMDA) receptors (Vorobjev et al., 1993). Based on several animal studies, it has been suggested that histamine plays a modulatory role in the cortical arousal system mainly through H1R (Lin et al., 1988; Inoue et al., 1996; Yanai et al., 1998). Furthermore, other studies revealed that the central histaminergic system could play a modulatory role in learning and memory (Kamei et al., 1990; Kamei & Tasaka, 1993). Thus, it is probable that alterations in histaminergic neurotransmission can disturb arousal and cognitive functions in neuropsychiatric patients as well.

4.2. Alzheimer's disease

In general, it has been believed for a long time that the cholinergic pathway, projecting from the nucleus basalis to the cortical regions, plays a critical role in learning and memory, and that the main pathology of AD is a deficit of cholinergic transmission. Abnormalities have been reported in the brain of AD patients in muscarinic (Higuchi et al., 1999) and nicotinic (Nordberg 1993) acetylcholine receptors, and in acetylcholinesterase (Tanaka et al., 2001). Additional studies have suggested the significant involvement of other neurotransmitter systems in AD. Normal aging has been associated with impairment of cognitive functions including attention and memory, and disturbance in sleep–wake cycle. These abnormalities are more prominent in patients with AD, which is a neurological disorder with progressive deterioration of neurotransmission.

Several studies of autopsied human brains have examined whether the central histaminergic system is affected in patients with AD (Airaksinen et al., 1991). The initial studies did not reveal any change of histamine neurons in AD patients. However, Nakamura et al. (1993) found numerous neurofibrillary tangles in the TM of hypothalamus from AD cases. They also reported the loss of large TM neurons corresponding to histamine neurons in AD. Post mortem measurement of histamine concentration in brains of patients with AD led to contradictory results. Mazurkiewicz-Kwilecki and Nsonwah (1989) showed a significant decrease in histamine concentration in the cortex of AD patients. In contrast, Cacabelos et al. (1989) reported an increase in histamine concentration in the cortex and hypothalamus of AD patients. Recently, Schneider et al. (1997) conducted a more detailed study on the histaminergic system in patients with AD and Down's syndrome. In their report, HDC and choline acetyltransferase activities were similarly reduced in the brains of both AD and Down's syndrome patients versus the controls. In addition, histamine concentration in autopsied brains of AD patients tended to decrease (Panula et al., 1998).

Using a kinetic analysis based on a compartment model with PET and [^{11}C]doxepin, Higuchi et al. (2000) compared H1R binding quantitatively between patients with mild, moderate, and severe AD and age-matched controls. They found a decrease in H1R binding that correlated closely with the severity of AD assessed by Mini-mental state examination (MMSE). Though the sample size was rather small, this PET study is the first to describe decreased H1R binding in the living human brain of AD patients. The study suggested a predominant disruption of histaminergic neurotransmission in the neurodegenerative processes in AD patients and concluded that activation of histaminergic neurons could be a potential therapeutic intervention in cognitive impairment.

Histaminergic neurons also have stimulatory effects on cholinergic neurons in the nucleus basalis of Mynert (Khateb et al., 1995). Reduced activity of cholinergic neurons in the nucleus basalis might exacerbate cognitive dysfunction in AD patients. Taken together these findings, including PET results, indicate that deficits in histaminergic neurotransmission may be associated with cognitive decline in AD patients. It is therefore suggested that AD is characterized by multiple deficits in neurotransmitter systems including acetylcholine and histamine neuron systems.

4.3. Depression

Depression is a stress-related psychiatric disorder with disturbance of mood as major symptom, as well as disturbance of sleep, appetite, and physical potential. In general, the pathophysiology of depression has mainly been investigated in relation to dysfunctions of the monoaminergic systems of serotonin, norepinephrine and dopamine. Accordingly, previous human PET studies have focused on alternations in the serotonergic (Meyer et al., 2001a, 2001b, 2003), dopaminergic (Meyer et al., 2001a, 2001b) and noradrenergic (Bremner et al., 2003) neuron systems of depressed patients. Nevertheless, the pathophysiology of depression has remained poorly understood. Interestingly, most symptoms observed in depression, including disturbance of sleep, appetite, and physical potential, are directly associated with the functions of the histaminergic neuron system. Although central histaminergic neurons are known to mediate a variety of these functions, the roles of the histaminergic neuron system in the pathophysiology of depression have been poorly investigated.

As far as the authors know, only 1 PET study measured cerebral H1R binding in depressed patients using PET and

[^{11}C]doxepin (Kano et al., 2004). In that study, the correlation between scores of self-rating depression scale (SDS, Zung et al., 1990) and the regional H1R binding in 10 depressed patients (mean SDS±SD: 53±7.5) and in 10 age-matched healthy subjects (mean SDS±SD: 31.4±4.3) was examined. H1R binding was much lower in the frontal, temporal, occipital cortex and cingulate gyrus of depressed patients than that of controls (Fig. 8). Significant negative correlation was also observed between BP values and SDS scores in the middle frontal, inferior frontal, and in the anterior cingulate gyrus.

There are several possible explanations for the result of this study showing decreased H1R binding in depressed patients. BP may reflect changes in the density of H1R (B_{max}) and/or receptor affinity (K_D), and abnormalities in either or both measures may contribute to difference in BP values between the healthy controls and the depressed patients. Although post-mortem binding assays in the brain of schizophrenic patients have demonstrated low B_{max} and unchanged K_D for H1R (Nakai et al., 1991), no study has so far been reported on these changes in depressed patients. Another possible explanation is that the affinity of [^{11}C]doxepin for H1R may have decreased by increased histamine release from presynaptic sites in the brain of depressed patients. It is, however, still early to conclude which abnormalities in B_{max} and/or in K_D values are more attributable to the decrease in BP observed in depressed patients.

Previous animal studies have reported that neuronal H3R density is decreased with increased release of neuronal histamine in the acute phase of stress response, possibly for adaptation to early-stage stressors (Ghi et al., 1995; Endou et al., 2001). A human brain activation study with PET and [^{15}O]H_2O has demonstrated increased endogenous histamine release under acute stress due to visceral stimulation (Fukudo et al., 2000). In contrast, H1R density in the brain has been shown to decrease in the late stage of food-deprived activity stress (Endou et al., 2001). It has also been reported that chronic stress increases activity of HMT, a histamine-metabolizing enzyme, in the nucleus accumbens and striatum, which might suggest that histamine turnover is associated with vulnerability to stress-induced depression (Ito et al., 1999). These animal and human studies indicate that endogenous histamine is released and/or the rate of histamine turnover is increased under stressful conditions. It is, therefore, suggested that prolonged histamine release and turnover, due to repeated stress, might lead to down-regulation of H1R, that possibly results in decreased binding of [^{11}C]doxepin to H1R observed in PET studies.

A major drawback of the PET study by Kano et al. (2004), would be that 8 of the 10 depressive patients studied were under medication with benzodiazepines and/or fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), though with an extremely weak H1R antagonistic activity (K_D: 6200 nM) (Richelson & Nelson, 1984), and little affinity for H1R (Wong et al., 1983). Though direct influence of these drugs on the results of the study might be very small, reproducibility of the whole result should be confirmed by conducting another PET study with drug-free depressive patients.

4.4. Schizophrenia

Evidence has shown that the histaminergic neuron system is implicated in the pathophysiology of schizophrenia (Usdin et al., 1977; Rauscher et al., 1980). Although several atypical antipsychotics are potent H1R antagonists, the significance of clinical interaction between atypical antipyschotics and the histaminergic neuron system is still unclear. H3R are usually found as presynaptic autoreceptors that control histamine release, and their contribution to the pathophysiology of schizophrenia has been suspected. When animals are repeatedly injected with methamphetamine (MAP), the degree of ambulation or catalepsy gradually increases and tends to stay high even

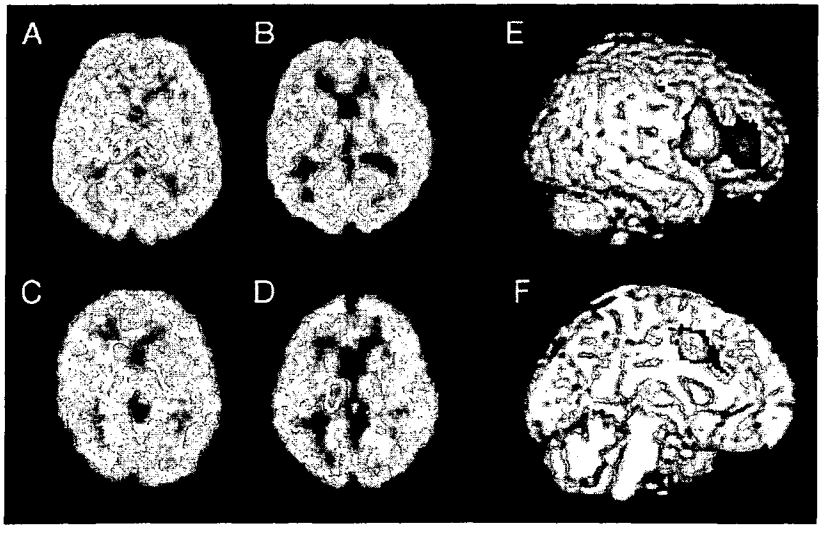

Fig. 8. Comparison of H1R binding between healthy volunteers (**A**, **B**) and depressive patients (**C**, **D**). (**A**) A 26-year-old control subject. (**B**) A 54-year old control subject. (**C**) A 25-year-old depressive patient. (**D**) A 53-year-old depressive patient. Individual PET images of each subject are shown at similar levels. A significant age-dependent decrease in H1R binding was observed in the cortical structures of healthy normal volunteers. (**E**, **F**) Correlation between depression symptoms and H1R binding. The colored areas show brain regions where H1R binding was significantly decreased in proportion to SDS scores.

after a long drug-free period. Such a phenomenon has been known as "behavioral sensitization" or "reverse tolerance", and has served as a model of schizophrenia (Sato et al., 1992). The involvement of the histaminergic neuron system in this sensitization has been initially studied by Ito et al. (1996, 1997a, 1997b). Dai et al. (2005) recently reported that blockade of H1R-mediated neurotransmission attenuates behavioral changes caused by social isolation plus MAP sensitization and that the therapeutic effects of atypical antipsychotics are mediated, at least in part, by interaction with H1R in the brain. In contrast, Pillot et al. (2002) demonstrated that acute administration of ciproxifan, an H3R antagonist/inverse agonist, potentiates the neurochemical and behavioral effects of haloperidol in rats, which resemble the characteristics of atypical antipsychotics.

Neurochemical evidence has shown that the histaminergic neuron system contributes to the pathophysiology of schizophrenia (Usdin et al., 1977; Rauscher et al., 1980). Significant increase in the level of N^{T}-methylhistamine was detected in the cerebrospinal fluid of schizophrenic patients as compared to healthy controls (Prell et al., 1995). In addition, in postmortem binding studies, Nakai et al. (1991) found that the number of H1R in the frontal cortex was reduced in schizophrenic patients. Although previous studies have suggested that schizophrenia is associated with increased activity in brain histamine synthesis or release, brain H1R have not been investigated in schizophrenic patients in vivo except for a study by Iwabuchi et al. (2005). In that study, averaged PET images obtained at 45–90 min after intravenous injection of [^{11}C]doxepin are shown in Fig. 9. Significantly lower radioactivity distribution was observed in the frontal, temporal, occipital and cingulate cortices, striatum and thalamus of schizophrenic patients ($n=10$) than in those of age-matched healthy subjects ($n=10$). H1R density in schizophrenic patients was significantly lower than that in the controls, especially in the frontal, prefrontal and cingulate cortices, all of which are known to be H1R-rich regions. These results are generally consistent with those of H1R binding assays in post-mortem schizophrenic patients (Nakai et al., 1991).

A previous study has found a weak independent association between schizophrenia and genetic variants in H1R gene-1536-G/C locus (Mancama et al., 2002). An excess of the H1R-1536-C allele were observed among schizophrenic patients, though statistically the association did not survive a correction for multiple comparisons (Mancama et al., 2002), suggesting that decreased H1R density was not associated with H1R promoter gene polymorphisms. Instead, the above findings suggested increased histamine release from the presynaptic sites of histaminergic neurons in schizophrenics. As in the case of depression, it was suspected that increased presynaptic histamine release resulted in H1R down-regulation. These PET findings are rather preliminary and would need replication because of the relatively small number of subjects and the problem of medication (benzodiazepines and haloperidol).

4.5. Epilepsy

It is believed that brain histamine acts as an endogeneous anti-convulsive agent. The role of histamine in convulsion was examined in several seizure models of electroconvulsion, PTZ-kindling and amygdala kindling (Watanabe and Yanai, 2001). In pharmacological experiments, convulsion duration was prolonged by deletion of histamine by FMH administration, and was shortened by increase of histamine contents by metoprine, an HMT inhibitor or L-histidine. These finding are supported by experiments using H3R ligands. Selective H1 agonists inhibit convulsion in several seizure models, suggesting that the inhibitory action of histamine is mediated through H1R. These pharmacological results were further confirmed by studies using knockout mice with histamine-related genes. H1R gene knockout mice showed longer period of electroconvulsion in maximal electroshock models and were more susceptible to PTZ-induced kindling (Chen et al.,

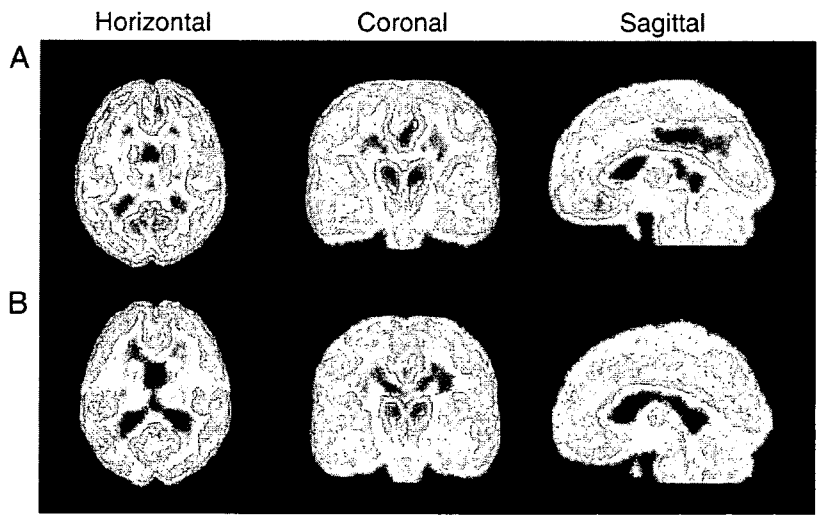

Fig. 9. H1R binding in schizophrenia. The images were obtained 45–90 min after intravenous injection of [^{11}C]doxepin and were averaged in both 10 schizophrenic patients (**B**) and 10 normal controls (**A**). Averaged PET images are shown at the same levels of horizontal, coronal and sagittal brain slices.

2003). Similar results were also obtained with HDC-KO mice. It is well known that brain-penetrating H1R blockers cause convulsion as serious side effects. In addition, our PET studies have shown an increase in H1R in the foci of epileptic patients with complex partial seizures (Iinuma et al., 1993). This result might be explained by up-regulation of H1R, which diminishes the spreading of abnormal firing. In accordance with these human data, the binding potential (B_{max}/K_D) of H1R has been shown to increase in the amygdala kindling model of rats (Toyota et al., 1999).

5. Closing remarks

The histaminergic neuron system with its extensive projections in the brain seems to be involved in many physiological and pathophysiological functions. By combining pharmacological agents, knockout mice of histamine-related genes and PET imaging, it has been shown that the histaminergic neuron system facilitates wakefulness and sensation of pain and itching (Mobarakeh et al., 2000; Yoshida et al., 2005). Brain histamine has also been demonstrated to function as a suppressor in bioprotection against noxious stimuli of convulsion, drug sensitization, denervation, ischemic lesions and stress. These results suggest that neuronal histamine has both stimulatory and inhibitory effects in normal and pathological conditions. Unfortunately at present no effective medication that activates histaminergic neurotransmission is available. In this sense, the recent discovery of H3 and H4 receptors is expected to play an important role in the development of drugs that promote or suppress histaminergic neurotransmission (Hough, 2001). Although the roles of neuronal histamine have been extensively studied in animals, only few human studies are available in the literature. As many pharmaceutical companies are developing new histamine H3 antagonists/agonists as new therapeutics, molecular and functional PET studies in humans would be useful as a new strategy for drug development.

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Autonomic nervous function and localization of cerebral activity during lavender aromatic immersion

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Abstract. Changes in the autonomic nervous activity can be induced by various sensory and emotional stimuli. The authors examined whether the power spectral analysis of heart rate variability (HRV) could detect changes in autonomic tone following a lavender aroma treatment or not. Healthy young women ($n = 10$, 23 ± 3 years old) underwent continuous electrocardiographic (ECG) monitoring before and after (10, 20, 30 minutes) the lavender fragrance stimuli.

HRV was expressed by three indices: low (0.04–0.15 Hz) and high (0.15–0.40 Hz) frequency components (nLF and nHF, respectively) as well as LF/HF ratio. Increases in the parasympathetic tone were observed after the lavender fragrance stimulus as seen as increases in the HF component and decreases in the LF/HF. Additional measurement with positron emission tomography (PET) demonstrated the regional metabolic activation in the orbitofrontal, posterior cingulate gyrus, brainstem, thalamus and cerebellum, as well as the reductions in the pre/post-central gyrus and frontal eye field.

These results suggested that lavender aromatic treatment induced not only relaxation but also increased arousal level in these subjects.

Keywords: Lavender, heart rate variability (HRV), positron emission tomography (PET), relaxation, aroma-therapy

1. Introduction

It is well known that olfactory stimulus has a powerful influence on mammalian behaviors and physiological functions, and the relationship between olfactory sensation and autonomic changes has been studied extensively. Olfaction is mediated by chemoreceptors of olfactory cells located in the nasal mucosae and olfactory neurons in the olfactory bulb, and the olfactory information is further projected to the primary olfactory regions in the brain. Most of these brain regions are strongly connected to or

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are part of the limbic system, the center of autonomic function and emotion. It goes in accordance with the fact that various smells can induce autonomic changes compatible with autonomic relaxation [1,12] or excitation [1,2], as well as changes compatible with basic emotions [19]. Such autonomic changes following treatments with perfumed fragrances (aroma-therapy) can be detected by power spectral analysis (PSA) of heart rate variability (HRV). A few studies have demonstrated usefulness of HRV measurement for evaluation of autonomic changes induced by various odorants such as lavender [8,16].

It has been said that "lavender", one of the most popular flower fragrances used in aroma-therapy, usually does not induce strong emotions but does comfortable feeling and relaxation. The effects of these fragrances on brain functions do not seem to be simple. It is expected that many brain regions, including sensory and limbic regions, are involved in olfaction. It is already recognized that the primary olfactory regions include the anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala, periamygdaloid regions, and entorhinal cortex. The secondary olfactory regions include the main composition of the limbic system: i.e. the hippocampus, ventral striatum and pallidum, thalamus, hypothalamus, orbitofrontal cortex, insular cortex, and cingulate gyrus. So far, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been used to investigate regional brain activation due to various sensory and emotional stimuli. However, functional neuroanatomy of autonomic changes induced by aroma-therapy has not been studied well using PET.

The aim of the present study is to investigate the effects of lavender fragrance on autonomic nervous functions in terms of HRV and to observe brain responses in terms of brain glucose metabolic changes measured by PET.

2. Materials and methods

2.1. Subjects and materials

Ten healthy female volunteers, ranging from 20 to 27 years old (*mean* ± *S.D.*: 23 ± 3.0 years old), were recruited for the present experiment after obtaining their written informed consent. The present study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. The subjects were non-smokers, and none of them had any olfactory problems nor were they on any types of medication at the time of the experiment. Subjects were requested to refrain from foods and drinks that would affect olfactory functions for 24 h (alcohol, coffee, chewing gum, etc.), and to take adequate rest and sleep during the night before the experiment day.

Before starting the study, each subject was interviewed for her preferences to the lavender fragrance, and was also requested to rate her own stress using the Stress Response Scale (SRS-18). The subjects were requested to be seated on a comfortable chair with the eyes open in the PET operation room. Holter ECG recording in the NASA leads (FM-300, Fukuda Denshi, Tokyo, Japan) was performed for evaluation of the HRV. Respiratory rate was also monitored in order to ensure the absence of respiratory changes after lavender administration, but the data were lost because of technical problem. Then, the respiratory data were collected again from other 15 subjects (ranging from 20 to 23 years old; *mean age* ± *S.D.*: 21.0 ± 1.0 years old).

To supply the lavender fragrance, a plaster prepared for aroma-therapy ("Lavender girl", Teikoku Pharmaceuticals, Tokyo, Japan) was attached on the subjects' right shoulder shortly before administration of [^{18}F]labeled fluorodeoxyglucose (FDG) injected through the right cubital vein (37 MBq). After 40-min-long uptake phase of FDG, all the electrodes and the lavender plaster were removed and soon PET scanning procedure was initiated using SET2400W scanner (Shimadzu Inc., Kyoto, Japan). PET

scanning covered each subject's whole brain taking 12 min in total (6 min for emission scan and 6 min for transmission scan). Tissue attenuation of positron annihilation photons was corrected using post-injection transmission data. Measurement was done twice within 3 months period but with an interval of at least 1 week: lavender administration and control conditions. For the control condition, exactly the same experimental procedure was repeated for each subject except for the absence of the lavender plaster. An order of the 2 conditions was balanced for each subject in order to remove an order effect. Five randomly chosen subjects were studied with a "lavender-control" order while the other 5 subjects were studied in the order of "control-lavender".

Additionally, the whole measurements were done within 10 days after the first day of menstruation. The reasons were to match their hormonal environment and to avoid radiation exposures during an early stage of pregnancy. Subjects were requested to rate their own stress using SRS-18 after PET scanning.

2.2. *Frequency-domain analysis of HRV*

Analyzing HRV in the frequency domain is a valuable method to determine quantitatively the sympathetic and parasympathetic modulations of heart rate (HR) [18]. The autonomic modulation of sinus rates has turned out to be a significant prognostic determinant in many cardiovascular diseases [9,18]. Two main spectral components are most commonly distinguished in the HRV spectrum: low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.4 Hz) components, respectively [9,18] (Fig. 1).

For analysis, segments of 10-minute-long records (before and after lavender administration: 10, 20 and 30 min post administration) were used. Beat to beat (R-R) intervals of heart rates were measured by detecting a peak of QRS waves of the ECG, and HR was estimated from the R-R intervals. Power spectral analysis of variability of these cardiovascular parameters was performed with the fast Fourier transformation technique (FFT; 512 points) through a Hamming window. LF and HF components were distinguished by frequency bands [9,18]. For further evaluation, normalized LF and HF (nLF and nHF, respectively) and low-high frequency ratio (LF/HF) were used. nLF and nHF were calculated as follows: $nLF = LF \times 100/(LF + HF)$, $nHF = HF \times 100/[LF + HF])$, respectively, where [LF + HF] in termed as total power (TP) [18].

2.3. *FDG-PET*

PET brain images were analyzed to identify regional changes of glucose metabolic rate using a widely-used software package, statistical parametric mapping (SPM2 [6,7], Functional Imaging Laboratory, London, UK). An FDG brain template distributed by Montreal Neurological Institute, McGill University, Canada, [6] was used for anatomical standardization (spatial normalization) of the PET images by applying Affine and non-linear transformations. Gaussian smoothing of 12 mm full width at half maximum was applied to compensate for errors in spatial normalization. Voxel-wised paired t-statistics was conducted based on the general linear model to find differences in the regional brain activity in the control and lavender fragrance conditions. Because the number of subjects was small, the threshold for the significance was set at $p < 0.001$ without corrections for multiple comparisons.

3. Results

Representative data of HRV measurement taken from a subject are demonstrated in Fig. 1. R-R intervals tended to be longer later in the experiment compared to the initial resting condition. The R-R

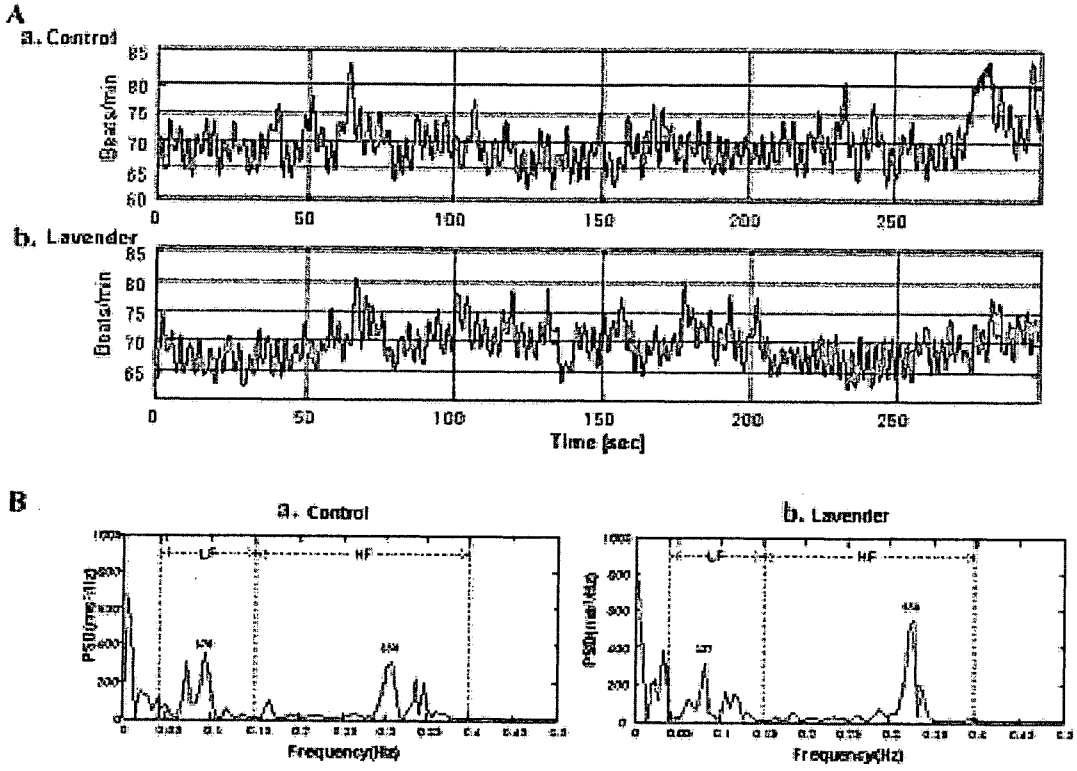

Fig. 1. Representative data of heart rate (HR) and HR variability (HRV) during exposure to control and lavender fragrance (A). Time course of HR changes during control (Aa) and lavender fragrance (Ab) conditions, respectively. Note that the HR gradually decreases and fluctuates more slowly during the lavender fragrance condition (Aa,b). Results of HRV spectral analysis (B) are shown during exposure to the control (Ba) and lavender fragrance (Bb) conditions, respectively. Note that HF component of HRV increased from 350 to 558 $(\text{beats/min})^2/\text{Hz}$ while LF/HF ratio decreased from 1.04 to 0.51, following administration of a lavender fragrance plaster. Abbreviations: LF = low frequency; HF = high frequency.

intervals were somewhat shorter in the lavender administration condition in comparison to the control (Fig. 1A). The fraction of HF was higher and the LF/HF ratio was lower in the lavender condition (Fig. 1B). Similar tendency was observed also in averaged data of 10 subjects as demonstrated in Fig. 2 and Table 1. nHF tended to increase and nLF tended to decrease in the first 10 min following lavender plaster administration, resulting in decreased LF/HF (Fig. 2).

Summary of physiology data obtained from the 10 subjects are shown in Table 2. None of HR, and systolic, diastolic and mean blood pressure (SBP, DBP and MBP, respectively) manifested significant difference between the control and lavender administration conditions. Additional respiration data also indicated no change after lavender administration as follows: 16.9 ± 1.9 (rest), 16.6 ± 1.2 (10 min post-administration of lavender), 16.6 ± 1.3 (20 min), 16.3 ± 1.8 (30 min). However, nHF was significantly higher (*mean* ± *S.D.*: 0.51 ± 0.21 Hz) with lavender use than that in the control condition (0.44 ± 0.11 Hz) ($p < 0.05$). In contrast, changes in LF/HF were significantly lower (1.41 ± 0.67) with lavender use in comparison to the control (1.91 ± 0.82) ($p < 0.05$). SRS-18 scores were significantly lower (14.1 ± 13.2) in the lavender fragrance condition compared to the control (18.8 ± 14.1) ($p < 0.05$). Scores of SRS-18 were also lower in the lavender administration condition (Table 2).