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Review

Studies on Functional Roles of the Histaminergic Neuron System by Using Pharmacological Agents, Knockout Mice and Positron Emission Tomography

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WATANABE, T. and YANAI, K. *Studies on Functional Roles of the Histaminergic Neuron System by Using Pharmacological Agents, Knockout Mice and Positron Emission Tomography*. Tohoku J. Exp. Med., 2001, **195**(4), 197–217 ── Since one of us, Takehiko Watanabe (TW), elucidated the location and distribution of the histaminergic neuron system in the brain with antibody raised against L-histidine decarboxylase (a histamine-forming enzyme, HDC) as a marker in 1984 and came to Tohoku University School of Medicine in Sendai, we have been collaborating on the functions of this neuron system by using pharmacological agents, knockout mice of the histamine-related genes, and, in some cases, positron emission tomography (PET). Many of our graduate students and colleagues have been actively involved in histamine research since 1985. Our extensive studies have clarified some of the functions of histamine neurons using methods from molecular techniques to non-invasive human PET imaging. Histamine neurons are involved in many brain functions, such as spontaneous locomotion, arousal in wake-sleep cycle, appetite control, seizures, learning and memory, aggressive behavior and emotion. Particularly, the histaminergic neuron system is one of the most important neuron systems to maintain and stimulate wakefulness. Histamine also functions as a bioprotection system against various noxious and unfavorable stimuli (for examples, convulsion, nociception, drug sensitization, ischemic lesions, and stress). Although activators of histamine neurons have not been clinically available until now, we would like to point out that the activation of the histaminergic neuron system is important to maintain

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This review is partly based on the Special Lecture given by TW at the 74th Annual Meeting of the Japanese Pharmacological Society on March 25, 2001 at Yokohama (*Folia Jap. Pharmacol. Soc.*, **118**[3], 159–169 [2001] [in Japanese]), and summarizes the studies carried out in our hands with no aim to cover all the related literature. This review was written to commemorate the retirement of TW on March 31, 2002, and the 3rd anniversary of professorship of KY.

mental health. Here, we summarize the newly-discovered functions of histamine neurons mainly on the basis of results from our research groups. ──── histamine; histidine decarboxylase; pharmacology; brain; knockout mouse; PET
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One of us (TW) came back to Osaka University School of Medicine after a 4-year stay in the USA and transferred to the Department of Pharmacology in 1975. At that time, the histaminergic neuron system had not been identified because the Falck-Hillarp's formalin fluorescence histochemistry could not be successfully applied to histamine in the brain. Therefore, the strategy was to purify L-histidine decarboxylase (a histamine-forming enzyme, HDC), prepare its antibody and identify the histaminergic neuron system immunohistochemically with the anti HDC antibody as a marker. An early report on the study of histaminergic neurons was written on the occasion of the retirement of Prof. Hiroshi Wada (Watanabe 1992). The location and distribution of this neuron system of rat brain was first demonstrated in the evening of December 22, 1982 in the laboratory of Dr. Masaya Tohyama with Drs. Yoshitaka Taguchi and Sadao Shiosaka (Fig. 1, Watanabe et al. 1983, 1984). To our surprise, the histaminergic neuron system was clarified with antibody against histamine itself by Drs. Pertti Panula (Panula et al. 1984) and Harry M.W. Steinbusch (Steinbusch and Mulder 1984) independently in the same year. The fundamental features of the histaminergic neuron system were elucidated within 3 years under the excellent and active supervision of Dr. M. Tohyama (Takeda et al. 1984a, b; Hayashi et al. 1985; Inagaki et al. 1987, 1988a, b), and in laboratories to which the antibody was sent (Senba et al. 1985; Koehler et al. 1986; Steinbusch et al. 1986; Ericson et al. 1987; Wouterlood et al. 1988). Later the general morphology and its relations to various functions were reviewed by TW and others (Watanabe and Wada 1991; Yamatodani et al. 1991; Wada et al. 1991). In 1985, TW was offered a professorship from the Department of Pharmacology at the Tohoku University School of Medicine in Sendai. Thus, our fruitful studies on the functions of the histaminergic neuron system have been initiated since then. The following describes the functions of this

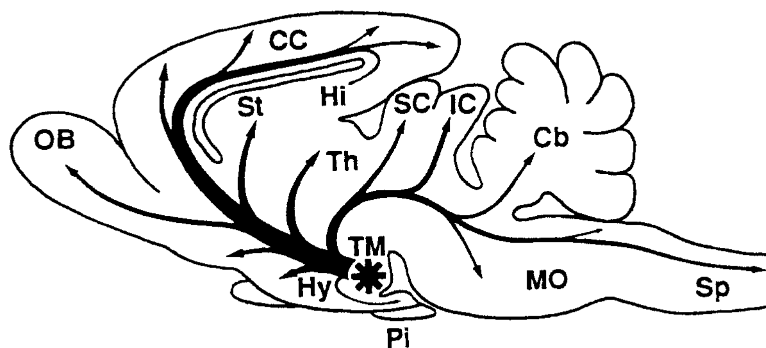

Fig. 1. Location and distribution of the histaminergic neuron system. The cell bodies of the histaminergic neuron system are in the TM of the posterior hypothalamus and send nerve fibers of varicosities to allmost all the regions of the brain.
Cb, cerebellum; CC, cerebral cortex; Hi, hippocampus; Hy, 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.

Table 1. *Functions of histamine neurons in the brain**

Parameter	FMH	H1R-KO	HDC-KO	Deduced function of histamine
Spontaneous locomotion	Decrease	Decrease		Locomotor activation
Circadian rhythm: awakeness	Decrease	Decrease	Decrease	Maintenace of awakeness**
Convulsion	Exacerbated	Exacerbated	Exacerbated	Protection against convulsion**
Nociception		Insensitive	Insensitive	Sensitization to nociception
Behavioral sensitization	Fascilitated		Fascilitated	Inhibition of drug sensitization
Ischemic neuron death	Fascilitated			Protection against ischemic lesion
Learning & memory	Beneficial			Promotion**
Stress	Exacerbated			Anti-stress

FMH, α-fluoromethylhistidine; MAP, methamphetamine; H1R- and HDC-KO, H1 and HDC-receptor- knokout mice.
*Data were restricted to those obtained in our laboratories.
**Confirmed in humans by PET studies.

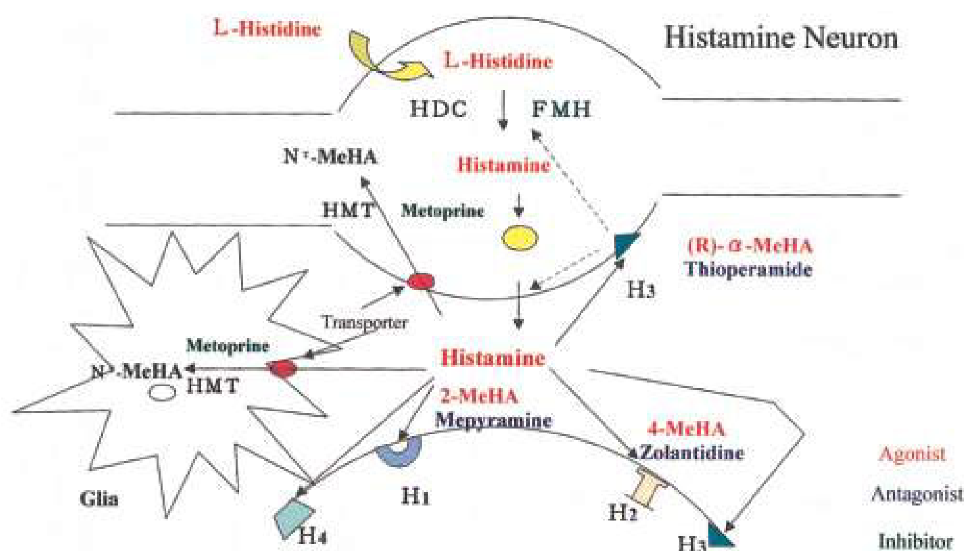

Fig. 2. Pharmacological agents used for studies on functions of the histaminergic neuron system. Stimulating, ——►; Inhibitory, ------►.
H1, H2, H3 and H4, histamine H1, H2, H3 and H4 receptors, respectively; FMH, α-fluoromethylhistidine; N$^{\tau}$-MeHA, N$^{\tau}$-methylhistamine; HMT, histamine *N*-methyltransferase; 2-, 4-MeHA, 2-, 4-methylhistamine.

neuron system as shown in Table 1 after brief explanation of the methods used.

Methods used for studies on functions of the histaminergic neuron system

Pharmacological agents used for studies on functions of the histaminergic neuron system. First, we studied the functions of the histaminergic neuron system pharmacologically by using various agents described in Fig. 2 in collaboration with Drs. Kazutaka Maeyama and Kenji Onodera and many capable graduate students. To inactivate the histaminergic neuron system, we used an HDC inhibitor, (S)-α-fluoromethylhistidine (FMH), H1 and H2 antagonists such as pyrilamine (mepyramine) and zolantidine, respectively, and an H3 agonist, (R)-α-methylhistamine (RαMeHA). FMH was the most useful, particularly in earlier stages of our studies (Kollonitsch et al. 1972; Watanabe et

al. 1990). For its activation, we used L-histidine, a precursor amino acid of histamine, metoprine, an inhibitor of histamine *N*-methyltransferase (HMT) (a histamine-inactivating enzyme), 2-thiazolylethylamine, an H1 agonist, and thioperamide and clobenpropit, H3 antagonists. The results obtained by these studies are summarized in Table 1 (Maeyama and Watanabe 1998; Onodera et al. 1994, 1998; Watanabe 2001). Recently two monographs have been published (Watanabe et al. 2001; Hasenoehrl and Huston 2001).

Imaging of histamine H1 receptors in the human brain by PET. Autopsied human brains and cerebrospinal fluid (CSF) have been utilized for a long time in human brain chemistry. Alternative approaches to human brain chemistry are non-invasive brain imaging modalities. Imaging techniques enabled us to assess the properties of brain tissues and to obtain information of how the brain works across scales from the system level to the molecular level. In addition, neuroimaging is a powerful and innovative tool for studying the pathology of psychiatric and neurological diseases and the drugs used in their treatment. In 1988, another of us, Kazuhiko Yanai (KY), who had been working in the Department of Pediatrics and Cyclotron RI Center and just came back from Prof. Henry N. Wagner, Jr.'s laboratory in Johns Hopkins University, became a faculty member of the Department of Pharmacology and did a lot of work on the distribution of histamine H1 receptors in the living human brain by using positron emission tomography (PET). We were very interested in the clinical studies on non-invasive human brain imaging because there were no clear studies on the pathophysiology of the histamine neurons in the human brain. The first PET imaging of H1 receptors in the human brain with TW as a volunteer was obtained on April 24, 1990. The development of this technique was the second breakthrough in our research on histamine if the morphological elucidation of the histaminergic neuron system is assumed to be the first one.

The distribution of H1 receptors in a living human brain was measured with [^{11}C]-mepyramine (or pyrilamine) or doxepin as a radiotracer by PET (Yanai et al. 1992a). The densities of H1 receptors were most prominent in the frontal, temporal and parietal cortices, the anterior cingulate, thalamus, hippocampus, less prominent in the striatum and occipital cortex, and least prominent in the pons-medulla oblongata and cerebellum. These densities were mostly blocked by the premedication of *d*-chlorpheniramine before the PET scan except those in the thalamus and stiatum, indicating that most bindings are specific but a few non-specific binding sites exist in these two latter regions. The further validity of the use of doxepin as a radiotracer for H1 receptor imaging was later obtained by the in vitro doxepin binding experiments of histamine H1 receptor gene knockout (H1R-KO) mice; [^{3}H]-doxepin did not bind specifically to membrane fractions prepared from brains of H1R-KO mice though a specific binding was detected with wild-type mice (Inoue et al. 1996).

Knockout mice of histamine related genes. In 1994, TW was asked to evaluate the phenotypes of brain functions of histamine H1 receptor (H1R)-gene knockout (KO) mice by Prof. Takeshi Watanabe,* Kyushu University. Isao Inoue, his graduate student, flew to Sendai with many mice from Fukuoka and our study on KO mice commenced (Inoue et al. 1996). Prof. Takeshi Watanabe had also produced H2R-KO mice (Kobayashi et al. 2000), and we so asked him to prepare HDC-KO mice. But he did not agree with our proposal, because the responses of peripheral tissues to histamine are very weak in mice except acid secretion in the

*Our initials are the same, T. Watanabe, when abbreviated and so the editor wrote to us in a cover letter at the time of its galley proof that one of the credited authors, T. Watanabe, of a paper submitted to *Proc. Nat. Acad. Sci. USA* (Inoue et al. 1996) might be duplicated.

stomach and thus the mice were not of great use to his studies on immunology at that time. However, later he obtained interesting results in B and T cell systems (Banu and Watanabe 1999; Jutel et al. 2001). Therefore, we decided to produce HDC-KO mice on our own.

Prior to that decision, in 1993, Dr. K. Maeyama was promoted to Prof. of Department of Pharmacology, Ehime University School of Medicine and Dr. Hiroshi Ohtsu joined us from The First Department of Internal Medicine of our Medical School. He was interested in molecular biology and had been collaborating with us on cloning human HDC cDNA (Mamune-Sato et al. 1992) and gene (Yatsunami et al. 1994), and finally produced HDC-KO mice, which will soon be the third breakthrough (Ohtsu et al. 2001).

Additionally, we were very fortunate on two other accounts. First, in 1994, TW was invited to Budapest and gave a special lecture at the European Histamine Research Society Meeting. While staying there, he met a smart young scientist, Prof. Andras Falus, Semmelweis Medical University, who was very much interested in the production of HDC-KO mice and introduced his close friend, Dr. Andras Nagy in the Samuel Lunenfeld Research Institute in Toronto. Dr. Nagy is a distinguished molecular biologist who invented the aggregation method for introduction of genes into an egg in place of microinjection (Nagy et al. 1993). The partial cloning of mouse HDC genes was in hands of Prof. Atsushi Ichikawa, Kyoto Univ., and he agreed to supply gene to us. We later reported on the complete mouse HDC gene (Ishigaki-Suzuki et al. 2000). The other stroke of good luck was that we obtained a three-year Grant-in-Aid for International Collaboration Study from the Ministry of Education, Science and Culture of the Japanese Government, which funded 5 trips to Toronto. In the beginning of 1998, the HDC-KO mice were finally obtained.

HDC-KO mice were prepared by a routine method of homologous recombination method except for use of aggregation technique for gene transfer. The vector was prepared by replacement of the exons 6-9, which contains a concensus sequence of pyridoxal 5′-phosphate binding site (Yamamoto et al. 1990),** with neomycin resistance gene in a reverse direction. The mice were normal in macroscopic observation, the number of siblings, the ratio of births of male to female, fertility and growth rates. The defects of the gene and mRNA were confirmed by Southern and Northern blot analyses, respectively, and the HDC activity was undetectable among the KO mice (Ohtsu et al. 2001) (Table 2). The histamine contents of various organs of HDC-KO mice were very low when compared with those of wild-type mice. Interestingly, however, they did not reach zero, but a small amount of histamine was always found in tissues of HDC-KO mice.

There may be three possible explanations for the origin of histamine in HDC-KO mice; (1) Aromatic amino acid decarboxylase forms histamine from L-histidine, though the Km value for L-histidine is about 10 mM. (2) A new synthetic pathway of histamine is present, i.e., possibly from acetylhistamine or carcinine (a decarboxylated product of carnosine, a dipeptide of β-alanylhistidine) (Flancbaum et al. 1990). (3) Histamine is absorbed from the gut. Of these possibilities, the third one may be most plausible, though the other two cannot be neglected completely. The pK value for the primary amine is higher than 10, and so there is practically little histamine in a non-charged form at physiological pH. However, if there is

**Though there is a consensus amino acid sequence for the presence of PLP binding site, i.e., –Thr Phe Asn Pro Ser Lys Trp– in the primary amino acid sequence from 300 to 306 of mouse HDC, there has been no direct evidence for the presence of PLP bound to HDC. The spectral study of HDC purified from expressed *E. coli* showed the presence of PLP as evidenced by the specific absorption bands at 340 and 415 nm, being similar to that of aromatic amino acid decarboxylase (H. Hayashi, personal communication).

Table 2. *The histamine (HA) content and L-histidine decarboxylase (HDC) activity of organs of wild (+/+), heterozygous (+/−) and HDC-KO(−/−) mice fed with low- and high-histamine diets*

Diet		Low-histamine (L) diet*			(L+HA)diet*
Genotype		+/+	+/−	−/−	−/−
Brain	HA**	58.7±19.8	31.6±10.3	28.8±8.6	83.3±65.4
	HDC***	0.24±0.07	0.12±0.01	0.00±0.00	0.00±0.00
Stomach	HA	4,360±107	2,759±426	99.3±89.0	4,918±1,800
	HDC	1.98±0.44	1.23±0.64	0.03±0.04	0.06±0.03
Skin	HA	21.2±8.5	19.2±1.6	0.26±0.12	10.5±1.3
	HDC	2.12±1.11	0.60±0.16	0.04±0.02	0.00±0.00

*The histamine content of L diet was 0.6 nmol/g diet and 80 μmol of histamine was added to L diet ([L+HA] diet).
**The unit of histamine (HA) level in the brain and stomach was pmol/g tissue and that in the skin was nmol/g tissue. The values are means±S.D., $n=4$.
***The HDC activity was given as pmol histamine formed/min/mg protein. The values are means±S.D., $n=4$. (From Ohtsu et al. 2001)

a large amount of histamine, at neutral pH a certain amount of histamine (1/10 000 of the total histamine when pK of histamine is assumed to be 11) should be present in a non-charged form, which may be absorbed through cell membranes in the gut. This is highly likely, because there are two well-known examples of histamine absorption from the gut; (1) urticaria is often seen with a histamine-rich diet; (2) eating spoiled fish causes diarrhea and even an anaphylactic shock-like reaction (Becker et al. 2001). Fish muscles contain a lot of histidine, which produces histamine by the action of HDC in contaminated bacteria. Dried fish muscle is a major source of protein in conventional mouse diets available from Japanese animal diet companies. Table 3 summarizes the effect of histamine contents in the diets on the histamine levels in the mouse tissues. Thus, we prepared the low-histamine diet and examined the effect of histamine addition to this diet. As shown in the last column of Table 2, the addition of histamine to the low-histamine diet increased the histamine levels, which may be used for rescue experiments of some phenotypes found in HDC-KO mice***.

Table 3. *Effect of histamine content in diets on histamine levels in mouse tissues*

Diet	Histamine in diet (μmol/g)	Histamine content	
		Brain (pmol/g)	Skin (nmol/g)
A	7.28	170	214
B	3.88	341.6	34.9
C	1.21	33	9.52
D	0.27	31.6	0.13

Data were obtained from separate experiments carried out during 1.5 years. From Sakurai and Ohtsu, unpublished.

Functions of histaminergic neuron system

Spontaneous locomotor activity. Our first graduate student, Naruhiko Sakai, measured the spontaneous locomotor activity after administrations of several agents listed in Fig. 2. The locomotor activity of mice decreased signifi-

***The most significant feature of HDC-KO mice is that the number, the size and the density of granules of the mast cells are few, small and less dense (Ohtsu et al. 2001). By using these mice, Dr. Ohtsu and collaborators have obtained many interesting results which have been or will be published. But in this review we omitted most of the results except those concerned with the brain.

TABLE 4. *Spontaneous locomotor activity and histamine neurons*

Agents or manipulation	Spontaneous locomotor activity
FMH	*Decrease*
R-α-MeHA	*Decrease*
L-Histidine	*Increase*
Metoprine	*Increase*
Thioperamide	*Increase*
H1R-KO	*Decrease*
H2R-KO	*Decrease*
H3R-KO*	*Decrease*
HDC-KO**	*Decrease*

*Toyota and Lovenberg (2001), submitted.
**Kubota et al. (2001) submitted.

cantly by the administration of FMH, an inhibitor of HDC, and (R)αMeHA, an H3 agonist. On the other hand, the locomotor activity increased by the administration of metoprine, an HMT inhibitor, and thioperamide, an H3 antagonist (Sakai et al. 1991, 1992, 1993) (Table 4). These results indicate that histamine is involved in the activation of ambulation or vigilance. These data were confirmed by experiments using H1R-KO mice (Yanai et al. 1998).

Circadian rhythm of sleep-awakefulness. Since 1970, histamine has been believed to be a wake amine and to be involved in circadian rhythm (Monnier et al. 1970). In our experiments by EEG recording, FMH shortened the waking time in the dark period and prolonged the slow-wave-sleep (SWS) time in the daytime in rats (Kiyono et al. 1985). In accordance with this study, Lin et al. (1990) found that, in cats, thioperamide increased the waking time and (R)αMeHA decreased S2-SWS time. These results were confirmed by studies using KO-mice of histamine-related genes (Lin et al., submitted). The EEG patterns were clearly different between night and day in wild-type mice, but barely different between daytime and nighttime among HDC-KO mice (Lin et al. 2001). The daytime locomotor activity was greater in H1R-KO mice than in wild-type mice, and thus the ratio of ambulation at daytime to that at night time was 0.15 in wild-type but that was 0.55 in H1R-KO mice (Inoue et al. 1996) (Fig. 3). In agreement with this, the ratio was 0.3 in wild type mice and 1.0 among HDC-KO mice. When HDC-KO mice were fed with a high HA diet, the ratio decreased to 0.5, indicating that the partial rescue was successful (Kubota et al., submitted).

The imaging studies of histamine H1 receptors in the living human brain substantiated the histamine-mediated activation theory. The mechanism of the non-sedative nature of the second generation antihistamines has been extensively studied by PET techniques. The drugs that penetrate the blood-brain barrier block the H1 receptor binding, whereas the non-sedative ones do not affect the imaging patterns (Yanai et al. 1995, 1999; Tagawa et al. 2001) (Fig. 4). We demonstrated that cognitive function and brain H1-receptor occupancy by *d*-chlorpheniramine are significantly correlated with the plasma concentration of *d*-chlorpheniramine (Okamura et al. 2000; Tagawa et al. 2001). These data support the conclusion that the impaired cognitive function and subjective sleepiness induced by *d*-chlorpheniramine are caused by H1-recepter occupation, and that H1-recepter occupancy of $\geqq 50\%$ impairs our arousal states. These results clearly suggest that histamine causes wakefulness through H1 receptors in the human brain.

All these data indicate that histamine is important in maintaining wakefulness. When we consider the involvement of the histaminergic neuron system in the actions of orexin and PGD_2 (Scammell et al. 1998; Nambu et al. 1999), their effects might be explained by the activation and inactivation of the histaminergic neuron system, respectively. In support of this notion, Huang et al. (2001) showed the direct involvement of the histaminergic neuron system in the action of the orexinergic system (Nishino et al. 2001). The schematic representation on

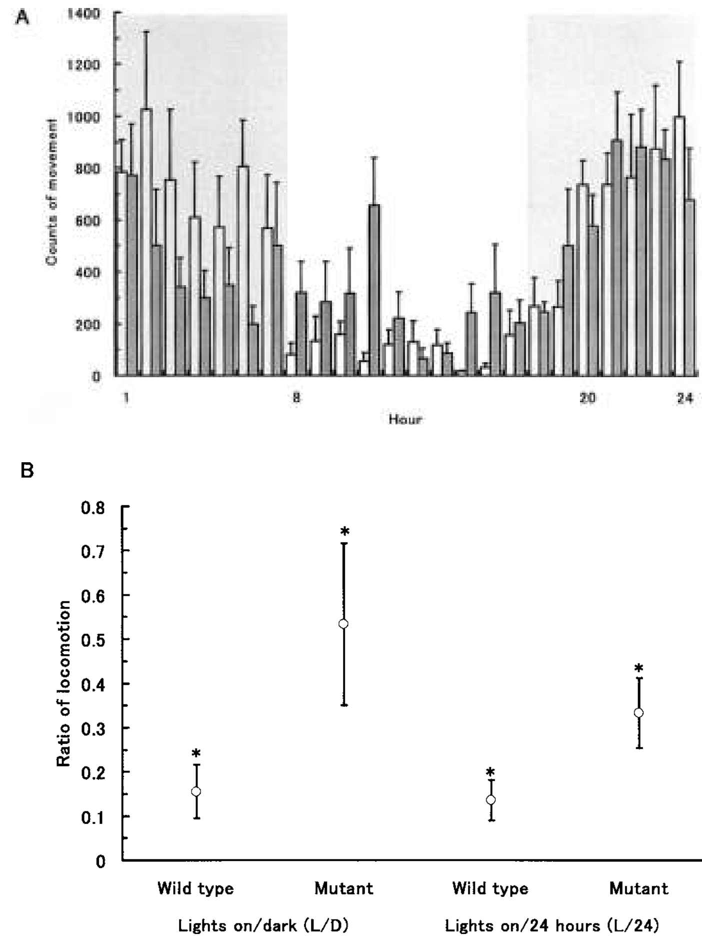

Fig. 3. Sleep-awakefulness circadian rhythm of wild-type and H1R-KO mice (From Inoue et al. 1996). Ciracadian rhythms of spontaneous locomotor activity of H1R-KO and wild-type mice were measured (A). □, +/+(n=8); ▒, −/−(n=8). The ratios of activities at night and daytime were shown in (B), showing the clearer difference in circadian rhythm of both mice.

the role of the histaminergic neuron system in cortical activation is illustrated in Fig. 5 (Modified from Lin et al. 1996; Brown et al. 2001).

Convulsion and epilipsy. Hiroyuki Yokoyama, a graduate student from the Department of Pediatrics, extensively examined the functional roles of the histaminergic neuron system in electroconvulsion (Yokoyama et al. 1992, 1993, 1994). In his maximal electroshock seizure experiments, the duration of clonic convulsion was prolonged by FMH administration, but was shortened by metoprine. Thus, an inverse relationship is obtained when the duration of convulsion is plotted *versus* the histamine level in the diencephalon (Fig. 6). Namely, histamine is one of endogenous anticonvulsant. This idea was further supported by experiments in which L-histidine and H3 ligands were used and, more directly, 2-thiazolylethylamine, an H1 agonist, inhibited the clonic convulsion when injected intraventricularly, suggesting that the inhibitory action of histamine is mediated through the H1 receptor system. This idea was also confirmed by the results that H1R-KO mice showed a longer period of electroconvulsion in maximal electroshock model than wild-type mice (Yanai et al. 1998). Accordingly, it was found that HIR-KO mice are more easily susceptible to kindling in pentylene tetrazole-

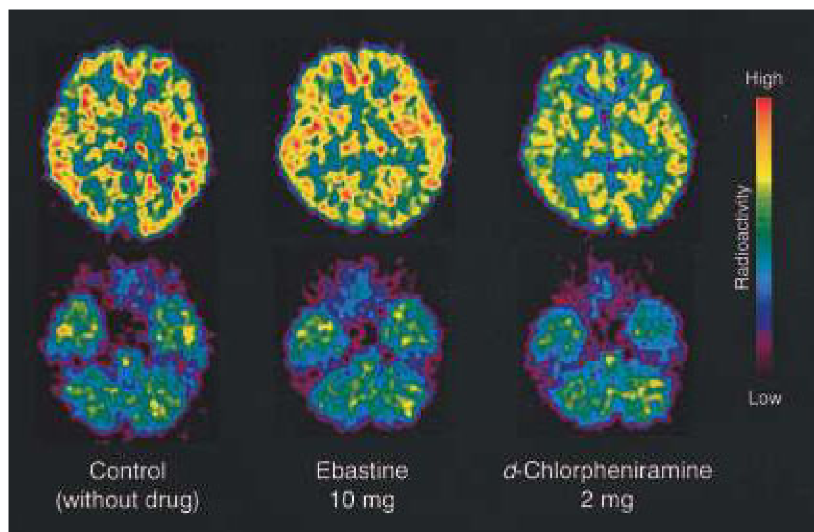

Fig. 4. Distribution of [^{11}C]doxepin binding in human brains with treatment of antihistamines, showing less permeability of the second generation H1 blocker (ebastine) through the blood-brain barrier measured by positron emission tomography (PET) than the first generation (*d*-chlorpheniramine). From Tagawa et al. (2001).

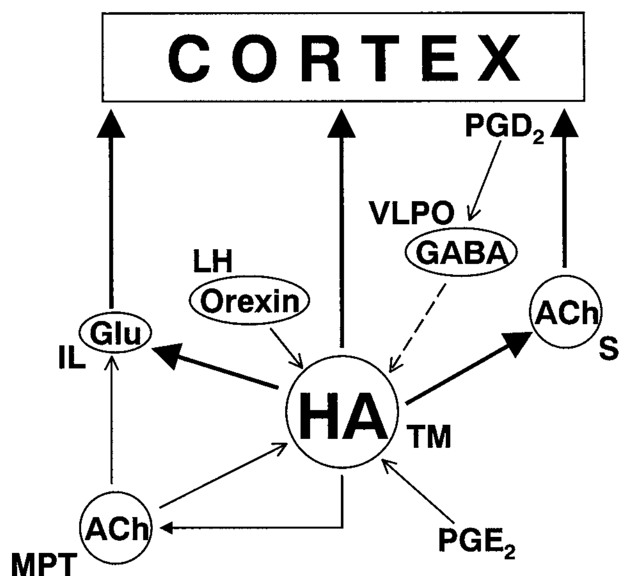

Fig. 5. Mechanism of cortical activation in respect to histaminergice neuron system. The cortical activation through the histaminergic neuron system is shown as ⟶, that through other system as ⟶, the inhibitory action as ⇢. Modified from Lin et al. (1996); Brown et al. (2001). IL, intralaminar nucleus; LH, lateral hypothalamus; MPT, mesopontine tegmentum; SI, substantia innominata; TM, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus.

induced kindling model (Li et al. 1999). In other words, H1R-KO mice reached the kindling state more faster than wild type mice. Similar results were obtained among HDC-KO mice (Li et al., unpublished). The binding capacity to H1 receptors was increased in amygdala kindling model of rats, suggesting that histamine is involved in the suppression of convulsion through H1 receptors (Toyota et al. 1998). In agreement with this finding, the histamine H1

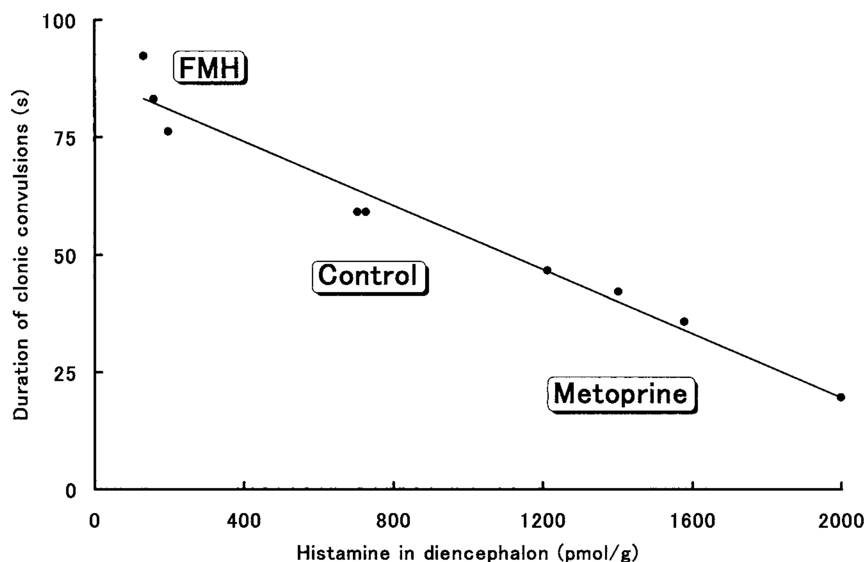

Fig. 6. The inverse relationship between the duration of clonic convulsion of maximal electroshock and the histamine level in the diencephalon in mice (From Yokayama and Iinuma 1996).

TABLE 5. *Convulsion and histamine neurons*

Drug or animal	Action	Duration of electroconvulsion	Formation of kindling
L-Histidine	HA precursor	Reduction	
FMH	Inhibition of HA formation	Prolongation	
Metoprine	Inhibition of HA metabolism	Reduction	
d-Chlorpheniramine	H1 antagonist	Prolongation	Fascilitation
2-Thiazolylethylamine	H1 agonist	Reduction	
Zolantidine	H2 antagonist	No effect	
Thioperamide	H3 antagonist	Reduction	
(R)-α-MeHA	H3 agonist	Prolongation*	
H1R-KO mouse	H1R deficient	Prolongation	Fascilitation
HDC-KO mouse	HA deficient		Fascilitation

*Juvenile mice (3 weeks old).

receptor mRNA level transiently increased by methamphetamine treatment (Kubota et al. 1999). The results are summarized in Table 5.

Clinically it has been widely known that H1 blockers cause convulsion as a serious side effect (Churchill and Gammon 1949; Wyngaarden and Seevers 1951; Yokoyama and Iinuma 1996). In fact, PET studies showed that the density of H1 receptors in the brain of patients with complex partial seizure increased in the foci of epileptic discharge (Iinuma et al. 1993). This result may be explained by the up-regulation of H1 receptors, which serves the protection of spreading of abnormal firing. All these basic and clinical data support the idea that histamine is an endogenous anticonvulsant (Kamei 2001).

Behavioral sensitization to methamphetamine. When animals are repeatedly injected with methamphetamine (MAP), the degree of ambulation or catalepsy gradually increases; this lasts even after a long drug-free period. These phenomena are known as "behavioral sensitization" or "reverse tolerance,"

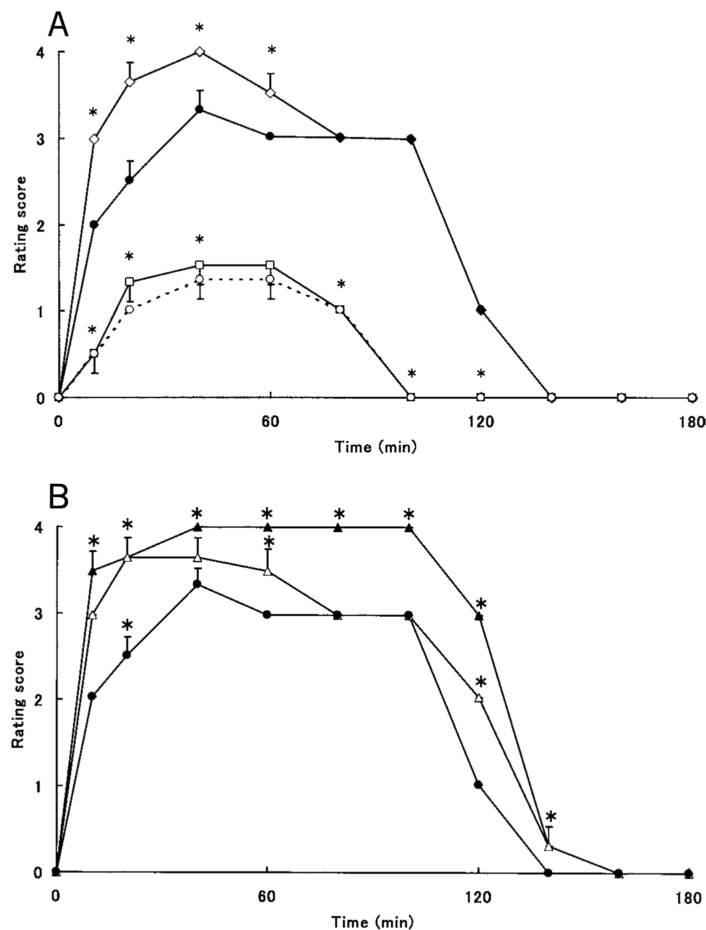

Fig. 7. Effect of histaminergic drugs on catalepsy caused by repeated treatment of rats with methamphetamine. Repeated treatment augments the stereotype scores (Compare ---○--- [saline] with —●— [MAP]). A; MAP plus FMH (—◇—) and L-histidine (—□—), B; MAP plus mepyramine (—△—) and zolantidine (—▲—). From Ito et al. 1997a.

which have served as models of schizophrenia (Sato et al., 1992). The involvement of the histaminergic neuron system in this sensitization was extensively studied by Chihiro Ito, a graduate student from Department of Psychiatry (Ito et al. 1996, 1997a, b). The increase in locomotion by a single dose of MAP was blocked by the treatment with L-histidine and enhanced by FMH. The degree of catalepsy evoked by repeated administration of MAP was also enhanced by FMH but suppressed by L-histidine, a precursor of histamine (Fig. 7a). The H1 and H2 antagonists, pyrilamine (mepyramine) and zolantidine, respectively, facilitated the formation of this phenomenon (Fig. 7b), indicating the involvement of both receptors. Accordingly, the formation of behavioral sensitization was also facilitated in HDC-KO mice (Kubota et al., submitted). All these results indicate that histamine is inhibitory for the formation and maintenance of behavioral sensitization (Table 6).

Responses to nociceptive stimuli. Jalal Izadi Mobarakeh, a graduate student from Iran, examined the effect of nociceptive stimuli in H1R-KO mice in Prof. Sinobu Sakurada's laboratory, Tohoku Pharmaceutical University (Mobarakeh et al. 2000). He used several methods of pain stimuli such as a hot plate, tail pressure, tail pinch, capsaicin and formalin for

Table 6. *Methamphetamine and histamine neurons*

Phase	Agents or manipulation	Effect
Acute	FMH	Facilitated
	H1 or H2 antagonist	Facilitated
	L-Histidine	Suppressed
	HDC-KO	Facilitated
Chronic*	FMH	Facilitated
	H1 or H2 antagonist	Facilitated
	L-Histidine	Suppressed
	HDC-KO	Facilitated

*Sensitization.

thermal, mechanical and chemical stimuli, indicating that histamine enhances the responses to nociceptive stimuli. In all of the examined test paradigms, H1R-KO mice were less sensitive to stimuli than wild type mice. Heterozygotes showed the responses between them in several tests (Fig. 8). Moreover, H1R-KO mice showed a stronger analgesic response to morphine than wild-type mice (Mobarakeh et al. 2002). Similar results were obtained with H2R-KO mice, suggesting that both H1 and H2 receptors are involved in the antinociceptive effect of histamine. Clinically, antihistamines are empirically administered to expect augmented antinociception in preanaesthetic medication. The above findings provide theoretical support for the synergistic antinociceptive effects of morphine by simultaneous administration of H1 or H2 blockers. Similar enhanced effects of morphine were also found when *d*-chloropheniramine was given to ICR mice, but not with the *l*-isomer (Mobarakeh et al. 2001).

Stress and anxiety. The hypothalamus, which is rich in histaminergic innervation, is highly sensitive to stress. In order to clarify the role of histaminergic neurons in stress responses, we used a model of food-deprived activity stress. This model is defined as the condition in which a rat was forced to run on a wire wheel while food consumption was restricted. This food-deprived activity stress gradually increased locomotion on the running wheel, and actually resulted in decreased body weight and death. The continuous intracerebroventricular injection of histamine reduced

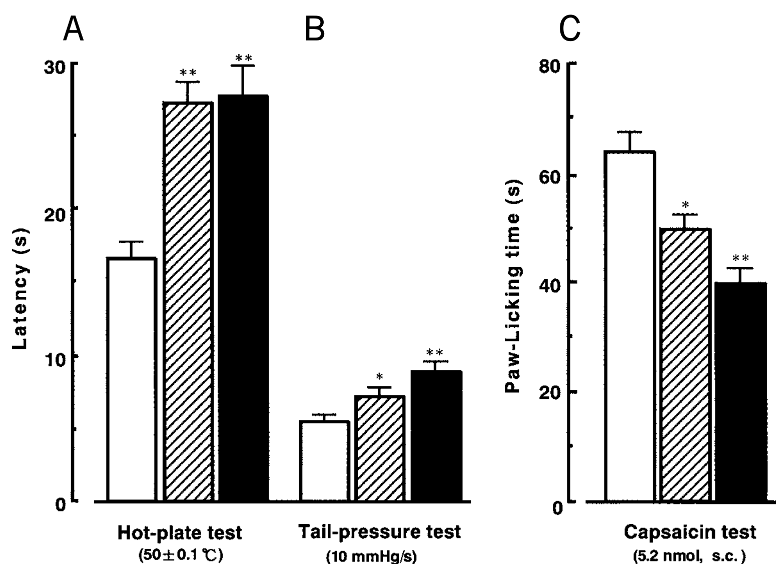

Fig. 8. Nociceptive responses in H1R-KO mice (Mobarakeh et al. 2000). Nociceptive responses of wild (+/+), heterozygous (+/−) and HDC-KO (−/−) mice were measured by using 3 methods (hot plate, A; tail pressure, B; capsaicin C), showing that −/− mice are less sensitive to all the stimuli. □, +/+; ▨, +/−; ■, −/−. (From Mobarakeh et al. 2000)

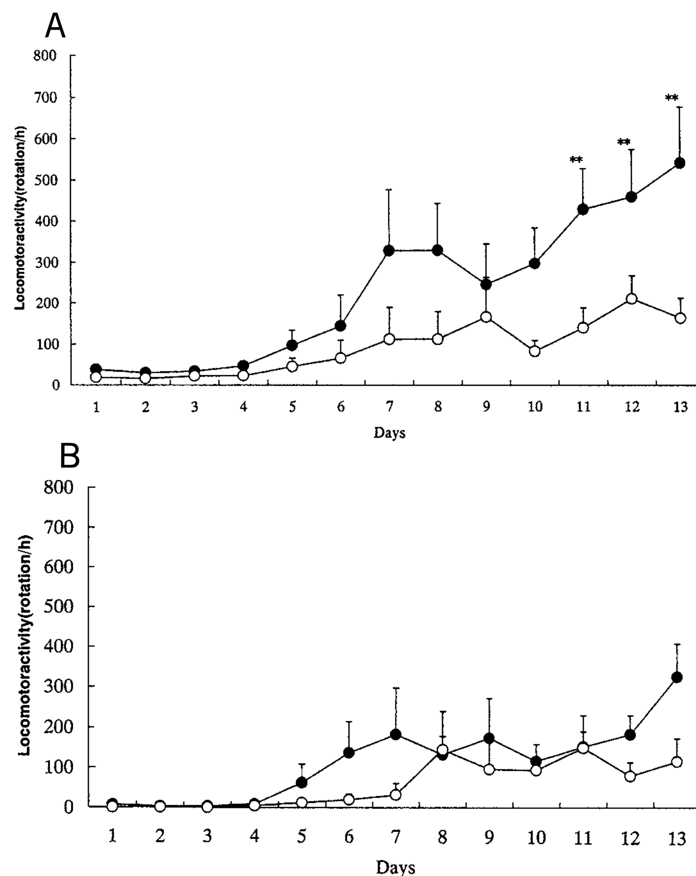

Fig. 9. Effect of continuous i.c.v. injection of histamine on the activity stress in dark (A) and light (B) periods in rats. ●, control; ○, histamine. From Endou et al. (2001).

the hyperactivity caused by food-deprived activity stress in rats, although it did not affect the spontaneous locomotor activity (Endou et al. 2001) (Fig. 9), suggesting that the histaminergic neuron system suppresses the stress-induced hyperactivity. Since this is a model for anorexia nervosa (Avarich et al. 1993; Beneke et al. 1995), neuronal histamine may be related to the pathogenesis of the disease. These data suggest an importance of a diet containing histidine as a food supplement. Chicken extracts contain more than 5% of L-histidine of a total concentration of amino acids, and have been widely used in Chinese communities as a traditional remedy for several diseases caused by stress. In agreement with the high concentration of L-histidine, Yang and Sakurai observed the protective effects of chicken extracts on the hyperexcitation caused by this model of stress (Yang et al. 2001). These findings are quite interesting because the hyperexcitation caused by environments such as stress and drugs such as methamphetamine can be significantly inhibited by the activation of histamine neurons. The contribution of L-histidine or chicken extract to histamine formation in vivo remains to be clarified.

Appetite controls. Neuronal histamine is thought to be involved in the regulation of appetite and energy control (Sakata et al. 1997). Continuous administration of histamine into the hypothalamus suppressed food intakes among rats (Itowi et al. 1988). This was also supported by the increased endogenous histamine through the treatment of metoprine, an HNT inhibitor. In contrast, α-fluoromethylhistidine (α-FHM), a specific HDC inhibitor, increased feeding-associated behaviors (Sakai et al. 1996).

These results suggest that hypothalamic histamine functions as an anorectic transmitter like leptin. Recently, Yamatodani and his coworkers demonstrated with H1 receptor-gene knockout mice that the appetite-suppressive action of leptin is mediated through H1 receptors (Morimoto et al. 1999). These previous findings suggest that clinically available H1 agonists may be useful for the treatment of abnormal obesity (Fukagawa and Sakata, 2001).

Brain ischemia, neural plasticity, learning and memory, and congnition. Many neurotransmitters are thought to be related to delayed neuronal cell death following brain ischemia: some neurotransmitters are neuroprotective, whereas others are neurotoxic. In order to clarify the role of neuronal histamine in brain ischemia, Koreaki Sugimoto, a graduate student from the Department of Neurology, examined the effects of α-FMH on delayed neuronal cell death (Sugimoto et al. 1994). Gerbils were used as a suitable model for studies on brain ischemia because an arterial circle of Willis is absent and the ligation of the carotid artery can easily cause brain ischemia. The results clearly indicated that depletion of brain histamine aggravated neuronal death of hippocampal CA2 neurons after 3-minute ischemia. There are several reports in accordance with his data (Karunushina et al. 1983; Adachi et al. 1993), showing that neuronal histamine functions as a neuroprotective transmitter for ischemic brain damages.

We also examined the participation of the histaminergic neuron system in neuronal plasticity of other brain injury models caused chemically or physically (Nakagawa et al. 1994; Ryu et al. 1994, 1995, 1996; Zhao et al. 1996). Jong Hoon Ryu, a Korean graduate student, extensively examined the plasticity of histamine H3 receptors in the rat brain after 6-hydroxydopamine-induced dopaminergic denervation by quantitative autoradiography using tritium-sensitive imaging plates. Our autoradiographic studies demonstrated that H3 receptors were highly up-regulated in the postsynaptic sites of injured neurons in association with denervation supersensitivity. In his studies, tritium-sensitive imaging plates and an imaging analysis system (Fujix Bio-Imaging Analyzer BAS) were used for quantification of tritium-labeled ligand binding studies because of their high sensitivity and accuracy. Our studies are actually the first report to describe the superiority of the Fujix imaging plate system when compared to conventional tritium-sensitive films. Nakagawa et al. (1994) demonstrated the increase in H3 receptor densities in the superior colliculus of rats that were subjected to unilateral enucleation (Fig. 10).

Aging is associated with impairments of attention and memory, and disturbances in wakefulness and cognition are more prominent in patients with Alzheimer's disease which is a devastating neurological disorder with progressive deterioration of neurotransmission in multiple brain areas. There are several reports that brain histamine is involved in the cognition in physiological and pathophysiological conditions. Meguro et al. (1995) reported that thioperamide, a potent H3 antagonist, improved the response latency of passive avoidance response in senescence-accelerated mice (SAM) (Miyamoto et al. 1986). The animals showed a marked age-accerated deterioration of learning tasks in a passive avoidance test. In human studies, Yanai et al. (1992b) reported an age-related decline in histamine H1 recepter binding in the normal human brain by PET. In these studies, the H1 recepter binding was shown to decrease markedly in the prefrontal, temporal, cingulate and parahippocampal regions, which are known to have close associations with attention and cognitive functions. Furthermore, Higuchi et al. (2000) demonstrated a significant decrease of H1 receptors particularly in the frontal and temporal areas of Alzheimer's disease brain compared to the old, normal subjects. The H1 receptor binding correlated closely to the severity of Alzheimer's disease assessed by the

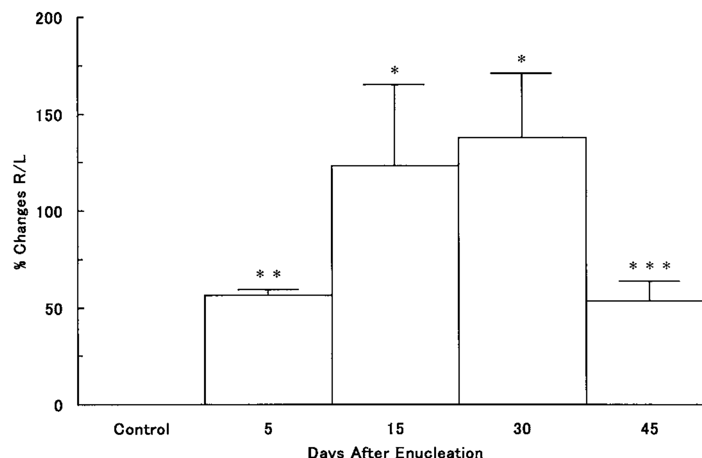

Fig. 10. Effect of unilateral enulceation on H3 receptor desities in the superior colliculus of rats. From Nakagawa et al. (1994).

Mini-Mental State Examination score. Our studies suggested the predominant disruption of the histaminergic neurotransmission in the neurodegenerative processes of Alzheimer's disease and a potential therapy to improve the cognitive impairment by means of the activation of histaminergic neurons.

Concluding remarks

The histaminergic neuron system in the brain has a very wide distribution, suggesting that it is related to many functions. By the combination of studies using pharmacological agents, knockout mice of histamine-related genes and PET, it seems clear that the histaminergic neuron system facilitates spontaneous locomotion and causes wakefulness, and at the same time maintains the homeostasis by suppression of convulsion, behavioral sensitization by drugs, stress and neuronal cell death caused by ischemia, and by sensitization of the sense of nociception. These results suggest that neuronal histamine has both stimulatory and inhibitory effects in normal and pathological conditions.

Brain histamine is indispensable for our mental health. Unfortunately, there are no effective medications to activate histaminergic neurons at present. A next step for the research strategy is the development of new drugs that activate histamine neurons. In this sense, the recent discovery of H3 and H4 receptors is expected to play an important role in this line of research (Hough 2001; Leurs et al. 2001). In summary, the activity of the histaminergic neuron system can maintain wakefulness in normal conditions and protect against hyperexcitation caused by chemical, physical and psychological damages in pathological conditions.

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References

Adachi, N., Oishi, R., Itano, Y., Yamada, Y., Yamada, T., Hirakawa, M. & Saeki, K. (1993) Aggravation of ischemic neuronal damage in rat hippocampus by impairment of histaminergic neurotransmission. *Brain Res.*, **602**, 165-168.

Aravich, P.F., Rieg, T.S., Lauterio, T.J. & Doerries, L.E. (1993) β-Endorphin and dynorphin

abnormalities in rats subjected to exercise and restricted feeding: relationship to anorexia nervosa? *Brain Res*., **622**, 1–8.

Banu, Y. & Watanabe, T. (1999) Augmentation of antigen receptor-mediated responses by histamine H1 receptor signaling. *J. Exp. Med*., **189**, 673–682.

Becker, K., Southwick, K., Reardon, J., Berg, R. & MacCormack, J.J. (2001) Histamine poisoning associated with eating tuna burgers. *JAMA*, **285**, 1327–1330.

Beneke, W.M., Schulte, S.E. & van der Guig, J.G. (1995) An analysis of excessive running in the development of activity anorexia. *Physiol. Behav*., **58**, 451–457.

Brown, R.E., Stevens, D.R. & Haas, H.L. (2001) The physiology of brain histamine. *Prog. Neurobiol*., **63**, 637–672.

Churchill, J.A. & Gammon, G.D. (1949) The effect of antihistaminic drugs on convulsive seizures. *JAMA*, **141**, 18–21.

Endou, M., Yanai, K., Sakurai, E., Fukudo, S., Hongo, M. & Watanabe, T. (2001) Food-deprived activity stress decreased the activity of the histaminergic neuron system in rats. *Brain Res*., **891**, 32–41.

Ericson, H., Watanabe, T. & Koehler, C.H. (1987) A morphological analysis of the tuberomammillary nucleus in the rat brain. Delineation of subgroups using antibody against L-histidine decarboxylase as a marker. *J. Comp. Neurol*., **263**, 1–24.

Flancbaum, L., Brotman, D.N., Fitzpatrick, C.J., Van Es, T., Kasziba, E. & Fisher, H. (1990). Existence of carcinine, a histamine-related compound, in mammalian tissues. *Life Sci*., **47**, 1587–1593.

Fukagawa, K. & Sakata, T. (2001) Monoaminergic anorectic agents. *Folia Pharmacol. Jpn*., **118**, 303–308. (in Japanese)

Hasenoehrl, R.U. & Huston, J.P. (2001) Neural Histamine: Physiology, Behavior and Brain Diseases. *Behav. Brain Res*., **124**, Special Issue, 103–253.

Hayashi, H., Takagi, H., Takeda, N., Kubota, H., Tohyama, M., Watanabe, T. & Wada, H. (1985) Fine structure of hsitamine neurons in the caudal magnocellular nucleus of the rat as demonstrated by immunocytochemistry using histidine decarboxylase as a marker. *J. Comp. Neurol*., **229**, 233–241.

Higuchi, M., Yanai, K., Okamura, N., Meguro, K., Arai, H., Itoh, M., Iwata, R., Ido, T., Watanabe, T. & Sasaki, H. (2000) Histamine H1 receptors in patients with Alzheimer's disease assessed by positron emission tomography (PET). *Neuroscience*, **99**, 721–729.

Hough, L.B. (2001) Genomics meet histamine receptors: New subtypes, new receptors. *Mol. Pharmacol*., **59**, 415–419.

Huang, S.-L., Qu, W.-M., Li, W.-D., Mochizuki, T., Eguchi, N., Watanabe, T. & Hayaishi, O. (2001) Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Nat. Acad. Sci. USA*, **98**, 9965–9970.

Iinuma, K., Yokoyama, H., Ootuki, T., Yanai, K., Watanabe, T., Ido, T. & Itoh, M. (1993) Histamine H1 receptors in complex partial seizure. *Lancet*, **341**, 238.

Inagaki, N., Yamatodani, A., Shinoda, K., Shiotani, Y., Tohyama, M., Watanabe, T. & Wada, H. (1987) The histaminergic innervation of the mesencephalic nucleus of the trigeminal nerves in rat brain: a light and electron microscopic study. *Brain Res*., **418**, 388–391.

Inagaki, N., Yamatodani, A., Shinoda, K., Panula, P., Watanabe, Shiotani, Y. & Wada, H. (1988a) Histaminergic nerve fibers in the median eminence and hypophysis of rats demonstrated immunocytochemically with antibodies against histidine decarboxylase and histamine. *Brain. Res*., **439**, 402–405.

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

Inoue, I., Yanai, K., Kitamura, D., Taniguchi, I., Kobayashi, I., Watanabe, T. & Watanabe, T. (1996) Impaired locomotor activity and exploratory behaviour in mice lacking histamine H1 receptors. *Proc. Nat. Acad. Sci. USA*, **93**, 13316–13320.

Ishigaki-Suzuki, S., Numayama, K., Kuramasu. A., Simura, S., Shirato, K., Watanabe, T. & Ohtsu, H. (2000) The mouse L-histidine decarboxylase gene: Structural and transcriptional regulation by GpC methylation in the promotor region. *Nucl. Acid Res*., **28**, 2627–2633.

Ito, C, Sato, M., Onodera, K. & Watanabe, T. (1996) The role of brain histaminergic neuron system in methamphetamine-induced bahavioral sen-

sitization in rats. *Ann. New York Acad. Sci*., **801**, 353–360.

Ito, C., Onodera, K., Watanabe, T. & Sato, M. (1997a) Effects of histaminergic agents on methamphetamine-induced stereotyped behavior and behavioral sensitization in rats. *Psychopharmacology*, **130**, 362–367.

Ito, C., Onodera, K., Yamatodani, A., Watanabe, T. & Sato, M. (1997b) The effect of methmphetamine on histamine release in the rat hypothalamus. *Psychol. Clin. Neurosci.*, **51**, 79–81.

Itowi, N., Nagai, K., Nakagawa, H., Watanabe, T. & Wada, H. (1988) Changes in feeding behaviour of rats elicited by hitamine infusion. *Physiol. Behav*., **44**, 221–226.

Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, G.A.R., Malolepszy, J., Zak-Nejmmark, T., Koga, R., Kobayashi, T., Blaser, K. & Akdis, C.A. (2001) Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature*, **413**, 420–425.

Kamei, C. (2001) Involvement of central histamine in amygdaloid kindled seizures in rats. *Behav. Brain Res*., **124**, 243–250.

Karunushina, I., Suzuki, R., Padgett, W. & Daly, W. (1983) Degeneration of CA1 neurons in hippocampus after ischemia in Mongolian gerbils: cyclic AMP systems. *Brain Res*., **268**, 87–94.

Kiyono, S., Seo, M.L., Shibagaki, M., Watanabe, T., Maeyama, K. & Wada, H. (1985) Effects of α-fluoromethylhistidine on sleep-waking parameters in rats. *Physiol. Behav*., **34**, 615–617.

Kobayashi, T., Tonai, S., Ishihara, Y., Koga, R., Okabe, S. & Watanabe, T. (2000) Abnormal functional and morphological regulation of the gastric mucosa in histamine H_2 receptor-deficient mice. *J. Clin. Invest*., **105**, 1741–1749.

Koehler, C.H., Ericson, H., Watanabe, T., Polak, J., Palay, S., Chan-Palay, V. & Palay, V. (1986) Galanin-immunoreactivity is present in hypothalamic histamine neuron. Further evidence for multiple chemical messengers in the tuberomammillary nucleus. *J. Comp. Neurol*, **250**, 58–64.

Kollonitsch, J., Patchett, A.A., Marburg, S., Maycock, S.L., Perkins, L. M., Doldouras, G.A., Duggan, D.E. & Aster, S.D. (1972) Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature*, **274**, 906–908.

Kubota, Y., Ito, C., Kuramasu, A, Sato, M. & Watanabe, T. (1999) Transient increases of histamine H1 and H2 receptor mRNA levels in the rat striatum after chronic administration of methamphetamine. *Neurosci. Lett*., **275**, 37–40.

Leurs, R., Watanabe, T. & Timmerman, H. (2001) Histamine receptors are finally “coming out.” *Trends Pharmacol. Sci*., **22**, 337–339.

Li, Z.Y., Mobarakeh, J.I., Sakurai, E., Watanabe, T., Iinuma, K. & Watanabe, T. (1999) Role of histamine H_1 receptor in the development of pentylenetetrazole-kindling: a study of H_1-receptor knockoutmice. *Abstr. Soc. Neurosci*., 29th Annnual Meeting, Miami Beach, Florida.

Lin, J.S., Sakai, K., Vanni-Mercier, G., Arang, J.M., Garbarg, M. & Schwartz, J.-C. (1990) Involvement of histaminergic neurons in arousal mechanisms demonstrated with H3-receptor ligands in the cat. *Brain Res*., **523**, 325–330.

Lin, J.S., Hou, Y., Sakai, K. & Jouvet, M. (1996) Histaminergic descending inputs to the mesopontine tegmentum and their role in the control of cortical activation and wakefulness in the cat. *J. Neurosci*., **16**, 1523–1537.

Lin, J.S., Vanni-Mercier, G. & Parmentier, R. (2001) Histaminergic neurons and sleep-wake regulation. In: *Histamine Research in the New Millennium*, edited by T. Watanabe, H. Timmerman & K. Yanai, Elsevier, Amsterdam, pp. 125–135.

Maeyama, K. & Watanabe, T. (1998) Histamine. In: *Progress in HPLC-HPCE* Vol. 7, edited by G.A. Qureshi, VSP, Utrecht, pp. 285–349.

Mamune-Sato, R., Yamauchi, K., Tanno, Y., Ohkawara, Y., Ohtsu, H., Katayose, D., Maeyama, K., Watanabe, T., Shibahara, S. & Takishima, T. (1992) Regulation of histidine decarboxylase gene expression in human basophilic leukemia cells and functional analysis of alternatively spliced transcripts. *Eur. J. Biochem*., **209**, 533–539.

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

Miyamoto, M., Kiyota, Y., Yamazaki, N., Nagaoka,

A., Matsuno, J., Nagawa, Y. & Takeda, T. (1986) Age-related changes in learning-memory in the senescence-accelerated mice (SAM). *Physiol. Behav.*, **38**, 399–406.

Mobarakeh, J.I., Sakurada, S., Katuyama, S., Kuramasu, A., Lee, Z.Y., Watanabe, T., Hashimoto, Y., Watanabe, T. & Yanai, K. (2000) Role of histamine H1-receptor in pain perception: A study of H1 knock-out mice. *Eur. J. Pharmacol.*, **391**, 81–89.

Mobarakeh, J.I., Sakurada, S., Hayashi, T., Orito, T., Sakurada, T., Watanabe, T., Watanabe, T. & Yanai, K. (2002) Enhanced antinociception by intrathecally-administered morphine in histamine H1 receptor gene knockout mice. *Neuropharmacology*. (in press)

Monnier, M., Sauer, R. & Hatt, A.M. (1970) The activating effect of histamine on the central nervous system. *Int. Rev. Neurobiol.*, **12**, 265–305.

Morimoto, T., Yamamoto, Y., Mobarakeh, J.I., Yanai, K., Watanabe, T., Watanabe, T. & Yamatodani, A. (1999) Involvement of histaminergic neuron system in leptin-indeced suppression of food intake. *Physiol. Behav.*, **67**, 679–683.

Nagy, A., Rossaut, J., Nagy, R., Abramow-Newerly, W. & Roder, J. (1993) Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc. Nat. Acad. Sci. USA*, **90**, 8424–8427.

Nakagawa, Y., Yanai, K., Ryu, J.H., Kiyosawa, M., Tamai, M. & Watanabe, T. (1994) Marked increase in [^{3}H](R)α-methylhistamine binding in the superior colliculus of visually deprived rats after unilateral enucleation. *Brain Res.*, **643**, 74–80.

Nambu, T., Sakurai, T., Mizukami, K., Hosoya, Y., Yanagisawa, M. & Goto, K. (1999) Distribution of orexin neurons in the adult rat brain. *Brain Res.*, **827**, 243–260.

Nishino, S., Fujiki, N., Ripley, B., Sakurai, E., Kato, M., Watanabe, T., Mignot, E. & Yanai, K. (2001) Decreased brain histamine content in hypocretin/orexin receptor–2 mutated narcoleptic dogs. *Neurosci. Lett.*, **313**, 125–128.

Ohtsu, H., Tanaka, S., Terui, T., Hori, Y., Makabe-Kobayashi, Y., Pejler, G., Tchougounova, E., Hellman, L., Gersenstein, M., Hirasawa, N., Sakurai, E., Buzas, E., Kovacs, P., Csaba, G., Kittel, A., Okada, M., Hara, M., Mar, L., Numayama–Tsuruta, K., Ishigaki-Suzuki, S., Ohuchi, K., Ichikawa, A., Falus, A., Watanabe, T. & Nagy, A. (2001) Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett.*, **502**, 53–56.

Okamura, N., Yanai, K., Higuchi, M., Sakai, S., Iwata, R., Ido, T., Sasaki, H., Watanabe, T. & Itoh, M. (2000) Functional neuroimaging of cognition impaired by a classical antihistamine, *d*-chlorpheniramine. *Br. J. Pharmacol.*, **129**, 115–123.

Onodera, K., Yamatodani, A., Watanabe, T. & Wada, H. (1994) Neuropharmacology of the histaminergic neuron system in the brain and its relationship with behavioral disorders. *Prog. Neurobiol.*, **42**, 685–702.

Onodera, K. & Watanabe, T. (1998) Histamine H3 antagonists as potential therapeutics in the CNS. In: *The Histamine H3 Receptors*, edited by H. Timmerman & R. Leurs, Elsevier Science B.V., Amsterdam, pp. 255-267.

Panula, P., Yang, H.-Y. & Costa, E. (1984) Histamine-containing neurons in the rat hypothalamus. *Proc. Nat. Acad. Sci. USA*, **81**, 2572–2576.

Ryu, J.H., Yanai, K. & Watanabe, T. (1994) Histamine H3 receptors in the striatonigral neurons are markedly increased after 6-hydroxydopamine-induced denervation of nigrostriatal dopaminergic neurons: an autoradiographic study. *Neurosci. Lett.*, **178**, 19–22.

Ryu, J.H., Yanai, K., Sakurai, E., Kim, C.Y. & Watanabe, T. (1995) Ontogenetic development of histamine receptor subtypes in rat brain demonstrated by quantitative autoradiography. *Dev. Brain Res.*, **87**, 101–110.

Ryu, J.H., Yanai, K., Zhao, X.L. & Watanabe, T. (1996) The effect of dopamine D1 receptor stimulation on the up-regulation of histamine H3 receptors following destruction of the ascending dopaminergic neurons. *Br. J. Pharmacol.*, **118**, 585–592.

Sakai, N., Onodera, K., Maeyama, K., Yanai, K. & Watanabe, T. (1991) Effects of thioperamide, a histamine H3 receptor antagonist, on locomotor activity and brain histamine levels in mast cell-deficient W/W^v mice. *Life Sci.*, **48**, 2397–2404.

Sakai, N., Onodera, K., Yanai, K., Maeyama, K. & Watanabe, T. (1992) Effects of (S)α-fluoromethylhistidine and metoprine on locomotor activity and brain histamine con-

tent of ICR mice. *Life Sci*., **51**, 397–405.

Sakai, N., Yamazaki, S., Onodera, K., Yanai, K., Maeyama, K. & Watanabe, T. (1993) Effects of (S)-α-fluoromethylhistidine and (R)-α-methylhistamine on locomotion of W/W^v mice. *Pharmacol. Biochem. Behav*., **46**, 95–99.

Sakai, N., Sakurai, E., Onodera, K., Sakurai, E., Asada H., Miura, Y. & Watanabe, T. (1996) Long-term depletion of brain histamine induced by α-fluoromethylhistidine increases feeding-associated locomotor activity in mice with a modulation of brain amino acid. *Behav. Brain Res*., **72**, 83–88.

Sakata, T., Yoshimatsu, H. & Kurokawa, M. (1997) Hypothalamic neuronal histamine: Implications of its homeostatic control of energy metabolism. *Nutrition*, **13**, 403–411.

Sato, M., Numachi, Y. & Hamamura, T. (1992) Relaps of paranoid psychotic state in methamphetamine model of schizophrenia. *Schiz. Bull*., **18**, 115–122.

Scammell, T., Gerashchenko, D., Urade, Y., Onoe, H., Saper, C. & Hayaishi, O. (1998) Activation of ventrolateral preoptic neurons by the somnogen prostaglandin D2. *Proc. Nat. Acad. Sci. USA*, **95**, 7754–7759.

Senba, E., Daddona, P.E., Watanabe, T., Wu, J.Y. & Nagy, J.I. (1985) Coexistence of adenosine deaminase, histidine decarboxylase and glutamate decarboxylase in hypothalamic neurons in the rat. *J. Neurosci*., **5**, 3393–3402.

Steinbusch, H.M.W. & Mulder, A.H. (1984) Immunohistochemical localization of histamine in neurons and mast cells in the rat brain. In: *Classical Transmitters and Tranmitter Receptors in the CNS*. Part II, *Handbool of Chemical Neuroanatomy*, Vol. 3, edited by A. Björklund, T.M. Höckfelt & M.J. Kumar, Elsevier, Amsterdam, pp. 126–140.

Steinbusch, H.W.M., Sauren, Y., Groenewegen, Watanabe, T. & Wada, H. (1986) Histaminergic projections from the premammillary and posterior hypothalamic region to the caudate-putamen complex in the rat. *Brain Res*., **368**, 389–393.

Sugimoto, K., Abe, K., Lee, T.-H., Sakurai, E., Yanai, K., Kogure, K., Itoyama, Y. & Watanabe, T. (1994) Histamine depletion in brain caused by treatment with (S)-α-fluoromethylhistidine enhances ischemic damage of gerbil hippocampal CA2 neurons. *Brain Res*., **666**, 279–283.

Tagawa, M., Kano, M., Okamura, N., Higuchi, N., Matsuda, M., Mizuki, Y., Arai, H., Iwata, R., Fujii, T., Komemushi, S., Ido, T., Itoh, M., Sasaki, H., Watanabe, T. & Yanai, K. (2001) Neuroimaging of histamine H1 receptor occupancy in human brain by positron emission tomography (PET): A comparative study of ebastine, a second-generation antihistamine, and d-chlorpheniramine, a classical antihistamine. *Br. J. Clin. Pharmacol*., **52**, 501–509.

Takeda, N., Inagaki, S., Taguchi, Y., Tohyama, M., Watanabe, T. & Wada, H. (1984a) Origins of histamine-containing fibers in the cerebral cortex of rats studied by immunohistochemistry with histidine decarboxylase as a marker and transection. *Brain Res*., **323**, 55–63.

Takeda, N., Inagaki, S., Shiosaka, S., Taguchi, Y., Oertel, W.H., Tohyama, M., Watanabe, T. & Wada, H. (1984b) Immunohistochemical evidence for the coexistence of histidine decaboxylase-like and glutamate decarboxylase-like immunoreactivities in nerve cells of the magnocellular nucleus of the posterior hypothalamus of rat. *Proc. Nat. Acad. Sci. USA*, **81**, 7647–7650.

Toyota, H., Ito, C., Ohsawa, M., Sakurai, E., Sato, M. & Watanabe, T. (1998) Decreased central histamine in the amygdaloid kindling rats. *Brain Res*., **802**, 241–246.

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

Watanabe, T., Taguchi, Y., Hayashi, H., Tanaka, J., Shiosaka, S., Tohyama, M., Kubota, H., Terano, Y. & Wada, H. (1983) Evidence for the presence of a histaminergic neuron system in the rat brain: an immunocytochemical analysis. *Neurosci. Lett*., **39**, 249–254.

Watanabe, T., Taguchi, Y., Shiosaka, S., Tanaka, J., Kubota, H., Terano, Y., Tohyama, M. & Wada, H. (1984) Distribution of the histaminergic neuron system in the central nervous system of rats: A flurorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res*., **295**, 13–25.

Watanabe, T., Yamatodani, A., Maeyama, K. & Wada, H. (1990) Pharmacology of α-fluoromethylhistidine, a specific inhibitor of histidine decarboxylase (a histamine-forming enzyme). *Trends Pharmacol. Sci*., **11**, 363–367.

Watanabe, T. & Wada, H. (1991) *Histamine*

Neurons: Morphology and Function, CRC Press, Boca Raton, pp. 1–415.
Watanabe, T. (1992) Prof. Hiroshi Wada —— A retrospective personal view. *Annuario Psichiatrico*, **3**, 1–11.
Watanabe, T. (2001) Studies on histamine with L-histidine decarboxylase (a histamine-producing enzyme) as a probe: from purification to gene knockout. *Folia Pharmacol. Jpn.*, **118**, 159–169. (in Japanese)
Watanabe, T., Timmerman, H. & Yanai, K. (2001) Histamine Research in the New Millennium, ICS 1224, Elsevier, Amsterdam, pp. 1–521.
Wouterlood, F.G., Gaykema, R.P.A., Steinbusch, H.W.M., Watanabe, T. & Wada, H. (1988) The connections between the septum-diagonal band complex and histaminergic neurons in the posterior hypothalamus of the rat. Anterograde tracing with *Phaseolus vulgaris-leucoagglutinin* combined with immunocytochemistry of histidine decarboxylase. *Neuroscience*, **26**, 827–845.
Wyngaartan, J.B. & Seevers, M.H. (1951) The toxic effects of antihistaminic drugs. *JAMA*, **145**, 277–283.
Yamamoto, J., Yatsunami, K., Ohmori, E., Sugimoto, Y., Fukui, T., Katayama, T. & Ichikawa, A. (1990) cDNA-derived amino acid sequence of L-histidine decarboxylase from mouse mastocytoma P-815 cells. *FEBS Lett.*, **276**, 214–218.
Yamatodani, A., Inagaki, N., Panula, P., Itowi, N., Watanabe, T. & Wada, H. (1991) Structure and functions of histaminergic neuron system. In: *Handbook of Experimental Pharmacology*, Vol. 97, edited by B. Uvnas, Springer, Berlin, pp. 145–163.
Yanai, K., Watanabe, T., Yokoyama, H., Hatazawa, J., Iwata, R., Ishiwata, K., Meguro, K., Itoh, M., Takahashi, T., Ido T. & Matsuzawa, T. (1992a) Mapping of histamine H_1 receptors in the human brain using [^{11}C]pyrilamine and positron emission tomography. *J. Neurochem.*, **59**, 128–136.
Yanai, K., Watanabe, T., Meguro, K., Yokoyama, H., Sato, I., Sasano, K., Itoh, M., Iwata, R., Takahashi, T. & Ido, T. (1992b) Age-dependent decrease in histamine H1 receptor in human brains revealed by PET. *Neuro Report*, **3**, 433–436.
Yanai, K., Ryu, J.H., Watanabe, T., Iwata, R., Ido, T., Sawai, Y., Ito, K. & Ito, M. (1995) Histamine H1 receptor occupancy in human brains after single oral doses of histamine H1 antagonists measured by positron emission tomography. *Br. J. Pharmacol.*, **116**, 1649–1655.
Yanai, K., Son, L.Z., Tadano, T., Kisara, K., Watanabe, T., Sakurai, E. & Watanabe, T. (1998) Behavioral characterization and amounts of brain monoamines and their metabolites in mice lacking histamine H1 receptors. *Neuroscience*, **87**, 479–487.
Yanai, K., Okamura, N., Tagawa, M., Itoh, M. & Watanabe, T. (1999) New findings in pharmacological effects induced by antihistamines: From PET to knockout. *Clin. Exp. Allergy*, **29**, 29–36.
Yang, Z.-B., Tanaka, T., Kiso, Y., Sakurai, E., Watanabe, T. & Yanai, K. (2001) Anti-stress effects of the chicken essence in food-deprived activity stress: Possible involvement of histaminergic neurons. In: *Histamine Research in a New Millennium*, edited by T. Watanabe, H. Timmerman & K. Yanai, Elsevier, Amsterdam, pp. 491–492.
Yatsunami, K., Ohtsu, H., Tsuchikawa, M., Higuchi, T., Ishibashi, K., Sida, A, Shima, Y., Nakagawa, S, Yamauchi, K., Yamamoto, M., Hayashi, N., Watanabe T. & Ishikawa, A. (1994) Structure of the L-histidine decarboxylase gene. *J. Biol. Chem.*, **269**, 1554–1559.
Yokoyama, H., Onodera, K., Maeyama, K., Yanai, K., Iinuma, K., Tuomisto, L. & Watanabe, T. (1992) Histamine levels and maximal electroshock seizure in mice: The effects of α-fluoromethylhistidine and metoprine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **346**, 40–45.
Yokoyama, H., Onodera, K., Iinuma, K. & Watanabe, T. (1993) Effect of thioperamide, a histamine H3 antagonist, on electrically-induced convulsions in mice. *Eur. J. Pharmcol.*, **234**, 129–133.
Yokoyama, H., Onodera, K., Iinuma, K. & Watanabe, T. (1994) 2-Thiozolylethylamine, a selective H1 antagonist, decreases seizure susceptibility. *Pharmacol. Biochem. Behav.*, **47**, 503–507.
Yokoyama, H. & Iinuma, K. (1996) Histamine and seizures. Implications for the treatment of epilepsy. *CNS Drugs*, **5**, 321–330.
Zhao, X.-L., Yanai, K., Ryu, J.H., Hashimoto, Y., Steinbusch, H.W.M. & Watanabe, T. (1996) Effects of unilateral vagotomy on nitric oxide

synthase and histamine H3 receptors in the rat dorsal vagal complex. *J. Chem. Neuroanat.*, **11**, 221–229.