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## Abstract

This study was conducted to elucidate the role of central histamine (HA) in seizure susceptibility. We stimulated the left amygdala of rats to produce amygdaloid kindling. We sacrificed rats 1 hour, 1 week and 1 month after the last kindled seizure, and measured the histamine contents and the histidine decarboxylase (HDC) activities of various brain regions. One hour after the last kindled seizure, we found significant decreases in HA levels in the bilateral amygdala, hippocampus and diencephalon in the kindled group. The HDC activities of the bilateral amygdala and diencephalon were lower in the kindled group than in the control group. One week after the last kindled seizure, we also found a significant decrease in the HA level in the bilateral amygdala. No significant change was found in HA content or HDC activity 1 month after the last kindled seizure.

These results suggest that kindling suppresses HA synthesis and that the reduced HA content is maintained until 1 week after the last kindled seizure. The reduced HA may play a role in the acquired kindled seizure susceptibility.

Theme: THEME J, DISORDERS OF THE NERVOUS SYSTEM

Topic: Epilepsy: human studies and animal models

*Keywords*: kindling; histamine; histidine decarboxylase; seizure susceptibility

## 1. Introduction

In the central nervous system, histamine (HA) is considered as a neurotransmitter or a neuromodulator in the mammalian brain [3,14,16,20,25]. The cell bodies of HA neurons are located in the posterior hypothalamus and send nerve fibers to almost all brain regions [22,26]. HA is synthesized from L-histidine by histidine decarboxylase (HDC), a histamine synthesizing enzyme [14,16,20,27], which is located mainly in histamine neurons [2,26]. Central HA participates in hypothalamus functions such as sleep, incretion, thermo-control, and circadian rhythm [25].

Recently, central HA has been thought to have an anticonvulsant effect. Tuomisto et al. [23,24] and Yokoyama et al. [31,32,33,36,37] indicated that central HA works as a seizure inhibitory factor. It has been reported that over-dosage of anti-histamine agents causes convulsion, especially in children of pre-school age [1,4,12,19,28]. These clinical reports indicate a conceivable role for central HA in acute convulsions. However, there is no report of the role of the central HA neuron system in the seizure susceptible state.

Repetitive subconvulsive electrical stimulation culminates in major motor seizures, a phenomenon called kindling [6]. And it is well known that the effect is an adequate model of human temporal lobe epilepsy and complex partial seizures [11,18].

In the present study, we investigated the role of central HA in the seizure susceptible brain of kindled rats. We measured the histamine contents and the HDC activities of brain regions 1 hour, 1 week and 1 month after the last kindled seizure.

## 2. Materials and methods

*2.1. Animals and amygdaloid kindling*

Adult male rats of the Sprague-Dawley strain (Funabashi Farm Co., Funabashi, Japan), weighting 250-300g at the time of surgery, were used. The rats were individually caged in a light- and temperature-controlled environment with water and food available *ad libitum*.

Before surgery, each rat was well handled. Rats were anesthetized with pentobarbital sodium (50mg/kg i.p.) and were inplanted with bipolar electrodes, made of Teflon-coated stainless-steel wire, in the left basal amygdaloid nucleus according to the coordinates of Pellegrino et al. [15] (coordinates: 1 mm posterior, 4.8 mm lateral, 7.8 mm ventral from the bregma). Electrode connectors were fixed by dental acrylic and anchor screws on the exposed skull surface. Ground electrodes were attached to anchor screws which were placed on the exposed skull surface. An equal number of rats was prepared with implanted electrodes in the same site but served as non-stimulated controls. These control rats were only handled everyday at the time of kindling-stimulation.

One week after the postoperative recovery period, we determined the afterdischarge threshold of the left amygdala. We stimulated using electric currents with 25 $\mu$A increments from 50 $\mu$A up to 300 $\mu$A. When afterdischarge was observed, we determined the threshold. Then we stimulated rats once a day at each afterdischarge threshold. Stimulations from a stimulator machine (each afterdischarge, biphasic square waves pulses, 1 ms pulse duration, 60 cycles/s, stimulus duration of 1 s) were administrated until 5th “stage 5” motor seizures, classified by Racine [17], were evoked. Electrical activity of the amygdala was recorded on an EEG apparatus. After completion of the kindling, we divided rats into 3 experimental groups for tissue preparation: 1 hour (n=6), 1 week (n=6)

and 1 month (n=6) after last kindled seizure.

### *2.2. Measurement of HA content*

The rats were killed by decapitation. Brains were removed rapidly and divided into cortex, amygdala, hippocampus, diencephalon, brain stem and cerebellum by the method of Glowinski and Iversen [5]. The brain tissues were stored at –80℃ until used.

Brain tissue was homogenized in 3% perchloric acid containing 5mM $Na_2$–EDTA by a Polytron homogenizer (Kinematica, Lucern, Switzerland) at a maximum setting for 10 s in ice bath, and the homogenate was centrifuged at 10,000 ×g for 10 min at 4℃ to obtain a clear supernatant. The HA content was measured fluorometrically with *o*-phthalaldehyde after separation on an HPLC system as described by Yamatodani et al. [30]. Briefly, HA was separated on a cation exchanger, TSK gel SP2SW (Tosoh, Tokyo, Japan; particle size 5 μm) eluted with 0.25 M $KH_2PO_4$ at a flow rate of 0.6 ml/min using a constant flow pomp (Model CCPM, Tosoh, Tokyo, Japan). The HA eluate was derivatized using an on-line automated Shore's *o* –phthalaldehyde method [21], and the fluorescence intensity was measured at 450 nm with excitation at 360 nm in a spectrofluorometer equipped with a flow cell (model C-R3A, Shimadzu, Kyoto, Japan) and a chromatographic data processor (Model C-R3A, Shimadzu, Kyoto, Japan).

### *2.3. Measurement of HDC activity*

Brain tissue obtained as described above was homogenized in the HDC solution (100mM potassium phosphate buffer, pH 6.8, 0.2mM dithiothreitol, 0.01 mM pyridoxal

5'-phosphate, 1% polyethyleneglycol and 100 μg/ml phenylmethanesulfonylfluoride) in a Polytron homogenizer (Kinematica, Lucern, Switzerland) at a maximum setting for 10 s in an ice bath. The homogenates were centrifuged at 10,000 × g for 30 min and the supernatants were dialyzed against HDC solution overnight. The HDC reaction was started at 37℃ by adding 0.5 mM L-histidine (final concentration) in a total volume of 1.0ml. Three hours later, the reaction was stopped by adding 0.03 ml of 60% perchloric acid. The mixtures were briefly centrifuged, and the supernatants were used for HA analysis. HDC activities were expressed as pmol histamine produced per min per mg of protein. Proteins were determined by the method of Lowry et al. [10] with bovine serum albumin as standard.

### *2.4. Statistical analysis*

Statistical analysis of the data was carried out using one-way ANOVA followed by Duncan's test. In all cases, $P$ values less than 0.05 were considered statistically significant.

## 3. Results

### *3.1. Changes of HA contents in kindled rat brains*

As shown in Fig. 1, we found significant decreases of HA contents of the bilateral amygdala, bilateral hippocampus and diencephalon of kindled rats compared with those of control rats 1 hour after the last kindled seizure. One week after the last kindled

seizure, the histamine content of the bilateral amygdala was significantly lower than that of control rats (Fig.2). There were no significant differences in HA contents between kindled and control rat's brains 1 month after the last kindled seizure (data not shown).

### *3.2. Changes in HDC activities in kindled rat brains*

Table 1 shows significant decreases of HDC activities in the bilateral amygdala and diencephalon 1 hour after the last kindled seizure. One week after the last kindled seizure, we found no significant changes in HDC activities between kindled and control rat's brains (Table 2). There were no significant changes between kindled and control rat's brains 1 month after the last kindled seizure (data not shown).

## 4. Discussion

It is well known that the activation of some neurotransmitter systems prevents development of kindling and induction of kindled seizure after its completion [9]. And it has been reported that there were some changes in regional neurotransmitter contents during kindling development and after completion of kindling [9]. In this paper, we investigated the role of central HA after the completion of kindling.

At first, 1 hour after the last kindled seizure, the reduced HA contents of the amygdala, hippocampus and diencephalon might have resulted from the suppressed HDC activity, mainly in the diencephalon where the HA nerve cell bodies are located. Kindling may suppress HA synthesis. These results may reflect not only the developed seizure susceptibility, but also the seizure itself and the effect of the electrical stimulation.

Secondly, in the steady state at 1 week after the last kindled seizure, restoration of the HDC activity to the control level indicates that the HA synthesis in the neuron may recover. It is natural to find normal HA levels in the diencephalon and hippocampus. On the other hand, the HA content is still depressed in the bilateral amygdala. This reduced HA is consistent with the report of Onodera et al. [13] that HA levels in the central nervous systems of genetically Epilepsy-Prone rats were lower than those of resistant rats. Kairiss et al. [8] and Yamada et al. [29] reported the functional importance of the amygdala in the electrical rat kindling model. The lasting HA depletion in the left amygdala may indicate a strong effect of the kindling on the HA system in the kindling foci. This lasting HA depletion may play a role in the maintenance of high sensitivity to electrical re-stimulation in the kindling foci after completion of the kindling. Goddard et al. [6] reported a "positive transfer effect" which means that prior kindling through one electrode would facilitate subsequent kindling through a second electrode located elsewhere in the limbic system. They reported that the contra-lateral amygdala required fewer stimulations to reach a full kindled seizure following prior amygdaloid kindling. Therefore, this reduced histamine level of the contra-lateral amygdala 1 week after the last kindled seizure may partly contribute to the "positive transfer effect". The lasting HA depletion in the bilateral amygdala may suggest transient functional change of the central HA nervous system after acquisition of seizure susceptibility. These results may reflect the seizure susceptible state obtained after kindling. Iinuma and his colleagues [7,34] reported an increased histamine $H_1$ receptor binding in epileptic foci and surrounding regions in patients with complex partial seizure by positron emission tomography. Yokoyama et al. [35] reported that the blockade of histamine $H_1$ receptor by anti-histamine agent increased the epileptic discharge in an infantile epileptic patient.

These lines of clinical evidence, which indicate the involvement of the central HA nervous system in the human epileptic seizure vulnerable state, could partly support our results. Furthermore, Yokoyama et al. [38] reported that histamine $H_1$ antagonists accelerate the development of kindling rate. It is likely that the central HA system acts as an inhibitory factor to the development of brain susceptibility to the kindling stimulation.

We could not, however, observe any changes in HA turnover 1 month after the last kindled seizure.

In conclusion, our present study suggests that kindling reduces central HA synthesis, and that persisting low HA level in the amygdala until 1 week after the last kindled seizure may play a role in the maintenance of kindled seizure susceptibility.

## References


[1] E. Bernstein, B.M. Diskant, Phenylpropanolamine: A potentially hazardous Drug, Ann. Emerg. Med. 11 (1982) 311-315.

[2] S. Bischoff, J. Korf, Different localization of histidine decarboxylase and histamine-N-methyl-transferase in the rat brain, Brain Res. 141 (1978) 375-379.

[3] R. Cacabelos, Histaminergic regulation of the neuroendocrine system(NES). In: T. Watanabe, H. Wada, (Eds.), Histaminergic Neurons: Morphology and Function, CRC Press, New York, 1991, pp. 241-270.

[4] J.A. Churchill, G.D. Gamonn, The effect of antihistaminic drugs on convulsive seizures, J. Amer. Med. Assoc. 141 (1949) 18-21.

[5] J. Glowinski, L.L. Iversen, Regional studies of catecholamines in the rat brain I. The disposition of [$^3$H]dopamine and [$^3$H]DOPA in various regions of the brain, J. Neurochem. 13 (1966) 655-669.

[6] G.V. Goddard, D.C. Mcintyre, C.K. Leech, A permanent change in brain function resulting from daily electrical stimulation, Exp. Neurol. 25 (1969) 295-330.

[7] K. Iinuma, H. Yokoyama, T. Otsuki, K. Yanai, T. Watanabe, T. Ido, M. Itoh, Histamine $H_1$, receptors in complex partial seizure. Lancet 341 (1993) 238 pp.

[8] E.W. Kairiss, R.J. Racine, G.K. Smith, The development of interictal spike during kindling in the rat, Brain Res. 322 (1984) 101-110.

[9] M.W. Kalichman, Neurochemical correlates of the kindling model of epilepsy, Neurosci. Behav. Rev. 6 (1982) 165-181.

[10]O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265-275.

[11] J.O. Mcnamara, L.C. Rigsbee, L.S. Butler, C. Shin, Intravenous phenytoin is an effective anticonvulsant in the kindling model, Ann. Neurol. 26 (1989) 675-678.

[12] M.S. Mueller, Phenylpropanolamine: a nonprescription drug with potentially fatal side effects, N. Engl. J. Med. 308 (1983) 653 pp.

[13] K. Onodera, L. Tuomisto, U. Tacke, M. Airaksinen, Strain differences in regional brain histamine levels between genetically epilepsy-prone and resistant rats, Methods Find. Exp. Clin. Pharmacol. 14 (1992) 13-16.

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

[15] L.J. Pellegrino, A.J. Pellegrino, A.J. Cushman, (Eds.), A stereotaxic atlas of the rat brain. Plenum Press, New York, 1979, pp 1-122

[16] G.D. Prell, J.P. Green, Histamine as a neuroregulator. Ann. Rev. Neurosci. 9 (1986) 209-254.

[17] R.J. Racine, Modification of seizure activity by electrical stimulation. Ⅱ. Motor seizure, Electroenceph. Clin. Neurophysiol. 32 (1972) 281-294.

[18] M. Sato, R.J. Racine, D.C. Mcintyre, Kindling: basic mechanisms and clinical validity, Electroencephalogr. Clin. Neurophysiol. 76 (1990) 459-472.

[19] J.C. Schwartz, J.H. Patterson, Toxic encephalopathy related to antihistamine-barbiturate antiemetic medication, Amer. J. Dis. Child. 132 (1978) 37-39.

[20] J.C. Schwartz, J.M. Arrang, M. Garbarg, H. Pollard, M. Rust, Histaminergic transmission in the mammalian brain, Physiol. Rev. 71 (1991) 1-51.

[21] P.A. Shore, A. Burkhalter, A. V.H. Cohn, A method for the fluorometric assay of

histamine in tissues, J. Pharmacol. Exp. Ther. 127 (1959) 182-186.

[22] H. Takagi, Y. Morishima, T. Matsuyama, H. Hayashi, T. Watanabe, H. Wada, Histaminergic Axons in the Neostriatum and Cerebral Cortex of the Rat: a Correlated Light and Electron Microscopic Immunocytochemical Study Using Histidine Decarboxylase as a Marker, Brain Res. 364 (1986) 114-123.

[23] L. Tuomisto, U. Tacke, Is histamine an anticonvulsive inhibitory transmitter? Neuropharmacology 25 (1986) 955-958.

[24] L. Tuomisto, U. Tacke, A. Willman, Inhibition of sound-induced convulsions by metropine in the audiogenic seizure susceptible rat, Agents Actions, 20 (1987) 252-254.

[25] H. Wada, N. Inagaki, A. Yamatodani, T. Watanabe, Is the histaminergic neuron system a regulatory center for whole-brain activity? Trends Neurosci. 14 (1991) 415-419.

[26] T. Watanabe, Y. Taguchi, S. Shiosaka, J. Tanaka, H. Kubota, Y. Terano, M. Tohyama, H. Wada, Distribution of the Histaminergic Neuron System in the Central Nervous System of Rats; A Fluorescent Immunohistochemical Analysis with Histidine Decarboxylase as a Marker, Brain Res. 295 (1984) 13-25.

[27] T. Watanabe, H. Wada, (Eds.), Histaminergic neurons: Morphology and Function. CRC Press, Boca Raton 1991 pp. 1-412.

[28] J.B. Wyngaarden, M.H. Seevers, The toxic effect of antihistaminic drugs, J. Amer. Med. Assoc. 145 (1951) 277-283.

[29] N. Yamada, K. Akiyama, S. Otsuki, Hippocampal kindling enhances excitatory amino acid receptor-mediated polyphosphoinositide hydrolysis in the hippocampus and amygdala / pyriform cortex. Brain Res. 490 (1989) 126-132.

[30] A. Yamatodani, H. Fukuda, H. Wada, T. Iwaeda, T. Watanabe, High-performance

liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: Cation-exchange chromatography coupled with postcolumn derivatization fluorometry, J. Chromatog. 344 (1985) 115-123.

[31] H. Yokoyama, K. Onodera, K. Maeyama, K. Yanai, K. Iinuma, L. Tuomisto, T. Watanabe, Histamine levels and clonic convulsions of electrically-induced seizure in mice: the efects of α-fluoromethylhistidine and metoprine, Naunyn-Schmiedeberg's Arch Pharmacol. 346 (1992) 40-45.

[32] H. Yokoyama, K. Onodera, K. Iinuma, T. Watanabe, Effect of thioperamide, a histamine $H_3$ receptor antagonist, on electrically induced convulsions in mice, Eur. J. Pharmacol. 234 (1993) 129-133.

[33] H. Yokoyama, K. Onodera, K. Iinuma, T. Watanabe, Proconvulsive effects of histamine $H_1$-antagonists on electrically-induced seizure in developing mice, Psychopharmacology 112 (1993) 199-203.

[34] H. Yokoyama, K. Yanai, K. Iinuma, T. Watanabe, M. Itoh, T. Fujiwara, R. Iwata, T. Takahashi, T. Ido, Mapping of histamine $H_1$-receptors in patients with complex partial seizure with PET and $^{11}$C-doxepin, in: K. Uemura et al. (Eds.), Quantification of brain function. Tracer Kinetics and Image Analysis in Brain PET, Elsevier, Amsterdam, 1993, pp.297-301.

[35] H. Yokoyama, K. Yanai, T. Watanabe, E. Sakurai, K. Onodera, Proconvulsant effect of ketotifen, a histamine $H_1$ antagonist, confirmed by the use of *d*-chlorpheniramine with monitering electroencephalography, Meth. Find. Exp. Clin. Pharmacol. 15 (1993) 183-188.

[36] H. Yokoyama, K. Onodera, K. Maeyama, E. Sakurai, K. Iinuma, R. Leurs, H. Timmerman, T. Watanabe, Clobenpropit (VUF-9153), a new histamine $H_3$ receptor

antagonist, inhibits electrically-induced convulsions in mice, Eur. J. of Pharmacol. 260 (1994) 23-28.

[37] H. Yokoyama, K. Onodera, K. Iinuma, T. Watanabe, 2-Thiazolylethylamine, a selective histamine $H_1$ agonist, decreases seizure susceptibility in mice, Pharmacol. Biochem. Behav. 47 (1994) 503-507.

[38] H. Yokoyama, M. Sato, K. Iinuma, K. Onodera, T. Watanabe, Centrally acting histamine $H_1$ antagonists promote the development of amygdala kindling in rats, Neurosci. Lett. 217 (1996) 194-196.

## Figure Legend

Figure 1. Histamine contents of brain regions of the kindled rats 1 hour after the last kindled seizure and of the control rats without kindling. Results are mean and standard errors (n=6). Statistical analysis was performed by one-way ANOVA followed by Duncan's test. (* $P<0.05$ vs. the control group)

Figure 2. Histamine contents of brain regions of the kindled rats 1 week after the last kindled seizure and of the control rats without kindling. Results are mean and standard errors (n=6). Statistical analysis was performed by one-way ANOVA followed by Duncan's test. (* $P<0.05$ vs. the control group)