Influences of Histamine H\textsubscript{1} Receptor Antagonists on Maximal Electroshock Seizure in Infant Rats

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Histamine H\textsubscript{1} antagonists are widely used for the treatment of allergic symptoms, such as allergic rhinitis and atopic dermatitis.\(^{1,2}\) Although some histamine H\textsubscript{1} antagonists showed central depressant effects via histamine H\textsubscript{1} receptors in clinical use,\(^{1,3,4}\) high doses of first-generation histamine H\textsubscript{1} antagonists, such as diphenhydramine and triprolidine occasionally produce convulsions and toxic encephalopathy with convulsions in human beings, especially in children under 2 years of age.\(^{5}\) In animal studies, we have reported that higher doses of histamine H\textsubscript{1} antagonists caused behavioral and EEG seizures in rats when injected intravenously.\(^{6}\) Yokoyama et al.\(^{7}\) also demonstrated that the centrally acting histamine H\textsubscript{1} antagonists, pyrilamine, ketotifen and chlorpheniramine, increased the duration of both the tonic extensor (TE) seizure and clonic (CL) seizure induced by maximal electroshock in mice. On the other hand, second-generation histamine H\textsubscript{1} antagonists, such as fexofenadine and loratadine, caused no sedation at usual clinical doses.\(^{1}\) In addition, Epinastine and fexofenadine are reported to hardly enter the brain.\(^{8,9}\) From this point of view, it is reasonable to presume that second-generation histamine H\textsubscript{1} antagonists cause no convulsion.

In the meantime, it has been reported that seizure susceptibility decreases with maturity in experimental animals. For instance, Davenport and Davenport\(^{10}\) found that the electroshock threshold for maximal seizures gradually increased from day 1 to 120 after birth in rats. Besides, the rats that were younger than 30 d were recognized to be more sensitive to the epileptogenic activity.\(^{11}\) On the other hand, it is well recognized that histamine H\textsubscript{1} antagonists are widely prescribed to infant patients.

In general, it is considered that individual organ systems develop at different rates in different species, such as human and rat. For example, as a percentage of mature weight, the human brain at about 2 years of age is similar to the rat brain at about 3 weeks of age, that is a weanling period of rat.\(^{12}\) Therefore, the present study was undertaken to clarify whether or not second-generation histamine H\textsubscript{1} antagonists cause epileptogenic activity as an index of electroencephalogram (EEG) seizure as well as TE seizure induced by maximal electroshock using infant rats (3 weeks old).

**Key words** maximal electroshock; tonic extensor seizure; electroencephalogram seizure; epinastine; fexofenadine; infant rat

**MATERIALS AND METHODS**

**Animals** Male Wistar strain, 3 weeks old and weighing 50—60 g, were used (Nippon SLC, Shizuoka, Japan). All animals were maintained in an air-conditioned room with a controlled temperature (24±2°C) and humidity (55±15%). They were housed in aluminum cages with sawdust and kept under a light–dark cycle (lights on from 7:00 to 19:00). The animals were given food and water ad libitum except during the experiments.

**Surgery** The animals were anesthetized with pentobarbital sodium (Nembutal\textsuperscript{18}, 35 mg/kg, i.p., Abbott Laboratories, North Chicago, IL, U.S.A.) and then fixed to a stereotaxic apparatus (SR-5, Narishige, Tokyo, Japan). For EEG recording, stainless steel screw electrodes (200 μm in diameter) were chronically implanted into the right and left frontal cortex (FCOR, 0.5 mm anterior to the bregma and 3.0 mm lateral to the midline) and the right and left occipital cortex (OCOR, 7.0 mm posterior to the bregma and 3.0 mm lateral to the midline) according to the atlas of Paxinos and Watson.\(^{13}\) The electrodes were connected to a miniature receptacle and the whole assembly was fixed to the skull with dental cement. At least 5 d were allowed for recovery from the surgery.

**Procedure for Maximal Electroshock Seizure** Maximal electroshock seizure was induced by an electric stimulator (SEN-3310, SS-102J; Nihon Kohden, Tokyo, Japan) using a voltage of 200 V delivered with a pulse frequency of 50 Hz for 1 s through ear-clip electrodes according to the method of Woodbury and Davenport.\(^{14}\) After the electroshock, the durations of TE seizure and CL seizure were measured. TE seizure was regarded as the period between the onset of hindlimb extension and the beginning of CL seizure. EEG was recorded bipolarly (LFCOR-RFCOR, LFCOR-LCOR, RFCOR-OCOR, LOCOR-ROCOR) with an elec-
troencephalograph (EEG-7213; Nihon Kohden, Tokyo, Japan).

**Drugs** The drugs used were diphenhydramine hydrochloride (Sigma, St. Louis, MO, U.S.A.), chlorpheniramine maleate (Sigma), cyproheptadine hydrochloride (Periactin®, Banyu Pharmaceutical Co., Ltd., Tokyo, Japan), ketotifen fumarate (Zaditen®, Novartis-Pharma K.K., Tokyo, Japan), epinastine hydrochloride (Alesion®, Nippon Boehringer Ingelheim Co., Ltd., Hyogo, Japan) and fexofenadine hydrochloride (Allegra®, Sanofi-Aventis K.K., Tokyo, Japan). All drugs were suspended in 0.5% carboxymethyl cellulose (CMC) solution. The vehicle control for them was 0.5% CMC solution. They were orally administered 1.0 h before electrical stimulation. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

**Statistical Analyses** All data are expressed as the means±S.E.M. One-way analysis of variance (ANOVA) with Dunnett’s test was used for assessing the significant effects on both behavioral and EEG seizures. A probability value of less than 0.05 was considered significant.

**RESULTS**

**Effects of Some Histamine H₁ Antagonists on EEG Seizure Induced by Maximal Electroshock in Infant Rats**

A representative example of EEG seizure pattern induced by maximal electroshock is shown in Fig. 1. After the electroshock, the EEG seizure characterized by high voltage spike or spike & wave complex lasted about 17 s, and thereafter silence EEG was observed. The duration of EEG seizure was more than that of TE seizure. Figures 2 and 3 show the representative examples for the effects of histamine H₁ antagonists on EEG seizure induced by maximal electroshock in infant rats. The duration of EEG seizure was prolonged by 10 mg/kg of ketotifen up to about 24 s (Fig. 2). On the other hand, 50 mg/kg of epinastine had no effect on EEG seizure (Fig. 3). Figure 4 shows the effects of some histamine H₁ antagonists on EEG seizure induced by maximal electroshock in infant rats. The duration of EEG seizure was significantly prolonged by diphenhydramine (10, 20 mg/kg, p.o.), chlorpheniramine (20 mg/kg, p.o.), cyproheptadine (5, 10 mg/kg, p.o.) and ketotifen (5, 10 mg/kg, p.o.). On the other hand, epinastine and fexofenadine caused no effect on EEG seizure, even at a dose of 50 mg/kg.

**Effects of Some Histamine H₁ Antagonists on TE Seizure Induced by Maximal Electroshock in Infant Rats**

Figure 5 shows the effects of some histamine H₁ antagonists on TE seizure induced by maximal electroshock in infant rats. The duration of TE seizure was significantly prolonged by diphenhydramine (5, 10, 20 mg/kg, p.o.), chlorpheniramine (10, 20 mg/kg, p.o.), cyproheptadine (2, 5, 10 mg/kg, p.o.) and ketotifen (2, 5, 10 mg/kg, p.o.). On the other hand, epinastine and fexofenadine had no effect on TE seizure, even at a dose of 50 mg/kg.

**Effects of Some Histamine H₁ Antagonists on CL Seizure Induced by Maximal Electroshock in Infant Rats**

Figure 6 shows the effects of histamine H₁ antagonists on CL seizure induced by maximal electroshock in infant rats. All drugs used in the experiment showed no significant effect on CL seizure induced by maximal electroshock.

**DISCUSSION**

It is well known that TE seizure induced by maximal electroshock is widely used as a criterion for the assessment of
the potential activity of new compounds against generalized tonic-clonic seizures.\(^{14,15}\) On the other hand, it was found in the present study, that high voltage spike or the spike and wave complex at the cortex was observed not only during tonic phase (tonic flexion and TE), but also in a part of clonic phase (CL) after electroshock. It is common knowledge that there is an intimate relationship between EEG spike and epilepsy in human beings. In the present study, therefore, EEG seizure was also used as an index for judgment of the effectiveness of certain drugs. As a result, it was found that diphenhydramine, chlorpheniramine, cyproheptadine and ketotifen caused a significant increase of the duration of EEG seizure induced by maximal electroshock. Almost the same results were obtained with TE seizure. Although these histamine \(H_1\) antagonists induced diminished alertness, slowed reaction time and somnolence in normal volunteers,\(^{16}\) convulsion and toxic encephalopathy with convulsion in children were observed with higher doses.\(^{5}\) Yokoyama et al.\(^{7}\) also reported that ketotifen produced a convulsion in a 5 year old boy with secondary generalized epilepsy and allergic rhinitis. There are many reports that histamine has an anticonvulsant effect acting through histamine \(H_1\) receptors. We have found in a previous study that histidine, a precursor of histamine, and metoprine, an inhibitor of histamine \(N\)-methyltransferase, inhibited amygdala kindled seizures in rats at doses causing an increase in histamine contents of the brain and these effects were antagonized by histamine \(H_1\) antagonists, diphenhydramine and chlorpheniramine.\(^{17}\) Tuomisto and Tacke also showed that metoprine raised brain histamine concentrations and inhibited maximal electroshock seizures.

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**Fig. 4. Effects of Histamine \(H_1\) Antagonists on EEG Seizure Induced by Maximal Electroshock in Infant Rats**

All drugs used were administered orally 1.0 h before electric stimulation. Each value represents the mean±S.E.M. of eight rats. *, **Significantly different from the control group \((p<0.05\) and \(p<0.01\), respectively).**

**Fig. 5. Effects of Histamine \(H_1\) Antagonists on TE Seizure Induced by Maximal Electroshock in Infant Rats**

All drugs used were administered orally 1.0 h before electric stimulation. Each value represents the mean±S.E.M. of eight rats. *, **Significantly different from the control group \((p<0.05\) and \(p<0.01\), respectively). Abbreviations; TE, tonic extension.

**Fig. 6. Effects of Histamine \(H_1\) Antagonists on CL Seizure Induced by Maximal Electroshock in Infant Rats**

All drugs used were administered orally 1.0 h before electric stimulation. Each value represents the mean±S.E.M. of eight rats. Abbreviations; CL, clonic convulsion.
in rats. These reports suggest that histaminergic mechanisms play an important role in the inhibition of seizures through histamine \( H_1 \) receptors. In addition, Kubo et al. reported that diphenhydramine, chlorpheniramine, cyproheptadine and ketotifen had high affinity for histamine \( H_1 \) receptors in a histamine \( H_1 \) receptor binding study. This is the reason why these histamine \( H_1 \) antagonists caused significant enhancement of EEG seizure and TE seizure induced by maximal electroshock. In conclusion, therefore, particular attention should be given to the prescription of these histamine \( H_1 \) antagonists for children of pre-school age, especially in the epileptogenic patients.

Epinephrine and fexofenadine caused no significant effect on both EEG and TE seizures induced by maximal electroshock, even at a dose of 50 mg/kg. In a previous study, we have reported that epinephrine showed no depressant effect on the central nervous system and produced no changes in the duration of TE seizure induced by maximal electroshock and pentetrazol-induced seizures in mice. These results in the present study clearly indicate that epinephrine as well as fexofenadine caused no effect on not only behavioral seizure but also EEG seizure patterns in infant rats. In addition, Yokoyama et al. found that epinephrine showed no acceleration in the development of the amygdala kindling different from ketotifen in rats. Casale et al. also reported that fexofenadine caused no sedation at the usual clinical doses. In general, it is understood that the mechanism of central nervous system side effects is that histamine \( H_1 \) antagonists, penetrating the blood brain barrier, occupy histamine \( H_1 \) receptors in the brain. It has been reported that epinephrine and fexofenadine hardly cross the blood brain barrier examined by positron emission tomography with \(^{11}C\)-dopexin. This is the reason why these histamine \( H_1 \) antagonists showed no effect on EEG seizure and TE seizure induced by maximal electroshock.

On the other hand, diphenhydramine, chlorpheniramine, cyproheptadine and ketotifen showed no significant effect on the duration of CL seizure. On the contrary, Yokoyama et al. reported that pyrilamine, ketotifen and chlorpheniramine increased the duration of CL seizure as well as TE seizure in mice. The duration of CL seizure in mice in Yokoyama et al.’s study was about 9—10 s. While in our present rat study, the duration of CL seizure was very long at 42.8 ± 2.24 s. This is the reason why first-generation histamine \( H_1 \) antagonists in the present study showed no potentiating effect on the duration of CL seizure. In addition, it seems likely that CL seizure after electroshock is not true CL seizure. Toman et al. reported that CL seizure induced by electroshock is characterized by one or more extensor thrusts followed by complete relaxation. In contrast to this, wild running and repetitive spasm of the hind legs were continuously observed with pentetrazol-induced CL seizure; i.e., true CL seizure. From these results, it is reasonable to conclude that CL seizure induced by maximal electroshock is not a reliable indicator of epilepsy.

In summary, epinephrine as well as fexofenadine may cause no harmful influences on epilepsy, even when used in infants different from first-generation histamine \( H_1 \) antagonists.

REFERENCES