The Effect of Antihistamines on Seizures Induced by Increasing-Current Electroshocks: Ketotifen, but Not Olopatadine, Promotes the Seizures in Infant Rats

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Clinical reports have shown that some antihistamines, such as ketotifen, occasionally produced seizures, especially in pre-school age children or young patients with epilepsy. The purpose of this study was to investigate whether olopatadine, one of the most efficacious antihistamines, promotes seizures induced by electroshocks in young rats. We investigated the seizures induced by electroshock using increasing-current delivery in 3- or 4-week-old rats, and found that the threshold-current of tonic extensor seizures was elevated with age in weeks in the vehicle-treatment groups. While caffeine decreased the threshold-current in every age group of rats, pentyleneetetrazole, a γ-aminobutyric acid (GABA) receptor antagonist, significantly decreased them only in 4-week-old rats. On the other hand, ketotifen decreased them only in 3-weeks-old rats. In the 3-week-old rats, neither olopatadine nor fexofenadine had any effect on the threshold-currents of tonic extensor seizures. These results showed that histaminergic neuro-transmission in the brain plays a crucial role in inhibiting seizures in rats soon after weaning, but is no longer effective in rats as they approach sexual maturation. In addition, unlike ketotifen, olopatadine, as well as fexofenadine, do not promote the occurrence of seizures in infant rats.

Key words olopatadine; seizure; infant rat; increasing-current electroshock; ketotifen

Histamine H₁ receptor antagonists, generally referred to as antihistamines, have been the established treatment for the symptomatic relief of allergic disorders such as allergic rhinitis, chronic urticaria, allergic conjunctivitis and so on. In addition to providing strong beneficial effects, antihistamines must be completely safe drugs more so than other agents, because antihistamines are taken primarily in the individual patient’s home, not in the hospital and also because they are taken every day and for extended periods of time. However, some antihistamines, such as diphenhydramine, chlorphenylamine and ketotifen, are known to occasionally promote seizures in clinical use, especially in infants or young patients with epilepsy. In addition, these drugs have been reported to induce or worsen the seizures in various animal models: electrically-induced seizures in rats or mice, pentyleneetetrazole (PTZ)-induced seizures in mice, amygdala-kindled rats, and EL mice which are a model for hereditary temporal lobe epilepsy.

Olopatadine hydrochloride (hereafter referred to as olopatadine) is one of the most selective and efficacious antagonists of histamine H₁ receptors. An ophthalmic solution of olopatadine (Patanol®) has been approved in the U.S.A., the European Union and Japan for the treatment of seasonal and perennial allergic conjunctivitis. Furthermore, oral administration of the agent (Allelock®) was approved in Japan for the treatment of allergic rhinitis, urticaria, and pruritus concomitant with dermatosis in infants and young children, as well as in adults. Although, to the best of our knowledge, there have been no reports in which olopatadine showed pro-convulsant effects in either clinical or non-clinical use, it is important to determine whether olopatadine affects the frequency and/or intensity of seizures.

Therefore, in this study, we first examined whether ketotifen, as well as caffeine and PTZ, each of which is known to have a pro-convulsant activity, could induce seizures in 3- and 4-week-old rats, since this age range corresponds to the age soon after weaning and the age just before sexual maturation, respectively. Next, the effects of olopatadine were compared with ketotifen and fexofenadine, latter of which have already been shown to have no harmful influence on the duration of seizure induced by the maximal electroshock in rats.

MATERIALS AND METHODS

Drugs All test drugs were prepared just before administration. Olopatadine hydrochloride (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) and ketotifen fumarate (Sigma-Aldrich Co., LLC, St. Louis, MO, U.S.A.) were dissolved in 0.5 w/v% of methylcellulose 400cP (MC) (Wako Pure Chemical Industries Co., Ltd., Kyoto, Japan). Fexofenadine hydrochloride (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was ground with a pestle in an agate mortar and was suspended in 0.5 w/v% of methylcellulose 400cP (MC) (Wako Pure Chemical Industries Co., Ltd., Kyoto, Japan). Caffeine (Sigma-Aldrich Co., LLC) and PTZ (1,5-pentamethylenetetrazole, Tokyo Chemical Industry Co., Ltd.) were dissolved in sterile water and physiological saline, respectively. A 0.5 w/v% dose of MC and all test drugs, except PTZ, were administered by oral gavage, and PTZ was given intraperitoneally at a volume of 5mL/kg.

Animals The experiments on animals were approved by the Committee for Animal Experiments of Kyowa Hakko Kirin Co., Ltd. Male Wistar rats (Charles River Japan, Yokohama, Japan) were used at 3 or 4 weeks of age. They were housed in a controlled environment (19–25°C, 30–70% humidity) and allowed food and water ad libitum. The room lights were kept on between 7:00 a.m. and 7:00 p.m. Behavioral experiments were carried out in a sound-attenuated and

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air-regulated experimental room.

**Experimental Procedure. The Maximal Electroshock, or Constant-Voltage Electroshock, Seizure Test** The maximal electroshock seizure test was carried out as described previously. Briefly, electroshocks were applied via ear-clip electrodes by an electric stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan) and an isolator (SS-104J, Nihon Kohden) using a constant voltage of 200 V delivered with a pulse frequency of 50 Hz (square wave, 9 ms) for 1.0 s. The duration of the tonic extensor seizure was regarded as the period between the onset of hindlimb extension and the beginning of the clonic seizure. The duration of the tonic extensor seizure was measured for the determination of seizure intensity.

All drugs used were administered 1 h before electric stimulation. Seven rats were used in each group.

**Increasing-Current Electroshock Seizure Test** The increasing-current electroshock seizure test was carried out as described previously. Briefly, electroshocks were applied via ear-clip electrodes (AE-80, Muromachi Kikai, Tokyo, Japan) by an electric stimulator (MK-800, Muromachi Kikai). The output current, which consisted of a single train of pulses (square wave, 9 ms, 50 Hz), was gradually stepped up at the rate of 0.1 mA/0.1 s. The initial current was 5 mA, and the current at which a tonic extensor seizure was exhibited in the hindlimb was recorded as the seizure threshold-current for each rat. If no tonic hindlimb extension was observed by a current of 20 mA, the electroshock was terminated. In this case, the data were omitted from the analysis, because electrical shorts were suspected.

All drugs used were administered 1 h before electric stimulation. Ten rats were used in each group, and the effects of the age (in weeks) of the rat on the threshold-current of seizures induced by ketotifen, PTZ and caffeine were determined. Nineteen to 20 rats were used in each group to examine the effects of ketotifen, fexofenadine, olopatadine and caffeine on 3-week-old rats.

**Statistical Analyses** The results are expressed as the mean values and standard errors (S.E.). The statistical analyses were performed using the SAS Institute StatView 2.0 software program (SAS Institute Inc., NC, U.S.A.). The differences between the vehicle- and a drug-treatment group (one dose) were analyzed using an F-test, followed by Student’s t-test or the Aspin–Welch test. For the comparison between vehicle- and different drug-treatment groups (two doses), after a Bartlett’s test, a one-way analysis of variance (ANOVA) test was conducted. Differences were considered to be statistically significant at \( p < 0.05 \).

**RESULTS**

**The Effects of Ketotifen on the Seizures Induced by Maximal Constant-Voltage Electroshocks in 3-Week-Old Rats** Constant-voltage electroshocks were loaded in 3-week-old rats. In the vehicle-treatment group, the tonic flexor seizures were observed immediately after the application of the electroshocks. Thereafter, the rats expressed the tonic extensor seizures, which lasted for \( 11.8 \pm 0.6 \text{s} \). The rats were then shifted to the phase of relaxation, and subsequently went into a coma. Oral administration of ketotifen (3 or 10 mg/kg) did not have any significant effects on the duration of the tonic extensor seizure (Fig. 1).

**The Effect of Ketotifen, PTZ and Caffeine on the Seizures Induced by Increasing-Current Electroshocks in 3- or 4-Week-Old Rats** Increasing-current electroshocks were loaded in 3- or 4-week-old rats. In the vehicle-treatment group, the threshold-current required to induce the tonic extensor seizure was increased with age in weeks (3-week-old; \( 13.3 \pm 0.4 \text{mA, } 4\text{-week-old: } 16.9 \pm 0.5 \text{mA, } p<0.001 \)). Caffeine (100 mg/kg, orally) significantly reduced the threshold current in both the 3- and 4-week-old rats (3-week-old; \( 11.0 \pm 0.3 \text{mA, } p<0.001 \), 4-week-old; \( 14.5 \pm 0.6 \text{mA, } p<0.01 \)). PTZ (30 mg/kg, intraperitoneally) also significantly reduced the threshold-current in 4-week-old rats (14.3 \pm 0.3 mA, \( p<0.001 \)), while the agent only showed a tendency to reduce it in the 3-week-old rats (12.7 \pm 0.4 mA, \( p=0.254 \)). On the other hand, ketotifen (30 mg/kg, orally) significantly reduced the threshold-current in 3-week-old rats (12.0 \pm 0.3 mA, \( p<0.05 \)), while it had no effect in 4-week-old rats (Fig. 2).

**The Effects of Various Antihistamines or Caffeine on the Seizures Induced by Increasing-Current Electroshocks in 3-Week-Old Rats** Increasing-current electroshocks were loaded in 3-week-old rats. In the vehicle-treatment group, the threshold-current of the tonic extensor seizure was \( 14.0 \pm 0.6 \text{s} \).
0.3 mA. Both caffeine (100 mg/kg, orally) and ketotifen (10 or 30 mg/kg, orally) reduced the threshold-current significantly (caffeine, 100 mg/kg, 11.6±0.2 mA, p<0.001; ketotifen, 10 mg/kg, 13.1±0.3 mA, p<0.05; 30 mg/kg, 12.5±0.2 mA, p<0.001). On the other hand, neither olopatadine (10 or 30 mg/kg, orally) nor fexofenadine (30 or 100 mg/kg, orally) significantly affected the threshold-current (Fig. 3).

**DISCUSSION**

In this study, we demonstrated that olopatadine did not affect the threshold of seizures induced by increasing-current electroshocks in infant rats, whereas ketotifen made the rats more sensitive to the occurrence of seizures. This is the first study which showed that olopatadine can be prescribed for allergic diseases in young children without risking the possibility of seizures.

Ishikawa et al. had previously reported that some antihistamines, including ketotifen at a dose of 2 to 10 mg/kg, caused a dose-dependent and significant prolongation of tonic extensor seizures induced by maximal electroshocks which were loaded by constant-voltage. In the present study, we could not detect any significant effects of ketotifen treatment on the seizures at first, although the same method, the same ages of rats and the same experimental conditions were used as they described in their paper. While it remains uncertain why the pro-convulsant effects of ketotifen could not be detected in our study, we abandoned this method using maximal electroshocks. There was another method that had been previously reported using an increasing-current delivery system, which allowed for the determination of the threshold-current for seizures in individual animals, and was useful for the detection of both anti- and pro-convulsant activities and might be more sensitive for detecting the subtle activities than methods using maximal electroshocks. Therefore, we next examined the effects of the compounds on convulsions induced by this increasing-current seizure test.

It has been empirically known that excessive consumption of caffeinated beverages, such as a tea or cola, may cause a lowering of the threshold for seizures in humans. Moreover, high dose caffeine has been suggested to promote convulsions during electro-convulsive therapy in patients with depression. While the mechanism(s) of action by which caffeine contributes to seizures in humans, have been unknown, the pro-convulsant effects of caffeine might partially depend on the inhibition of signal transmission through adenosine A1 receptors, because caffeine is an adenosine A1/A2 receptor antagonist and adenosine A1 receptor-knockout mice are prone to convulsions. Furthermore, in studies using model animals, it had also been reported that the duration of the convulsions in the maximal electroshock seizure test in rats was lengthened by the administration of caffeine. In the present study, oral administration of caffeine at a dose of 100 mg/kg decreased the threshold-current of tonic extensor seizures in the hind limbs of both 3- and 4-week-old rats. Therefore, we regarded the increasing-current electroshock seizure test as a suitable method for the evaluating the pro-convulsant activity of agents, since the predictive validity of this test was guaranteed.

In vehicle-treatment groups in the present study, the threshold-current of the tonic extensor seizures was higher in 4-week-old rats than in 3-week-old rats. Although we also performed a similar experiment using younger (2-week-old rats), the pattern of seizures was considerably different from that in older rats in many respects. For example, many individuals exhibited incomplete, not typical and sufficient, extension of their hindlimb upon competent stimulations. However, we did not consider that the 2-week-old rats were resistant to the electroshock-induced convulsions, because clonic convulsion-like symptoms could still be seen in these rats. Similar age-specific changes in the pattern of seizures have also been reported in the case of constant-voltage electroshock testing in mice. Therefore, the threshold-currents were considered to be inappropriate for the comparison of rats less than 2 weeks of age with older rats.

On the other hand, as the results of our preliminary test using a smaller number of animals indicated, no sign of the convulsion could be detected in almost all of the 6-week-old rats, even after increasing the current to 20 mA. We therefore regarded the 6-week-old rats as resistant to electroshock-induced seizures, although no further tests using higher limits of currents were performed for ethical reasons. These findings may suggest that the seizures were less likely to be occurred as a result of increasing age among 3- to 6-week-old rats, the periods which correspond to the age soon after weaning and sexual maturation, respectively.

Since almost all antiepileptic drugs, such as the enhancers of the g-aminobutyric acid (GABA)-ergic inhibitory neuronal system, inhibitors of sodium and/or T-type calcium channels and N-methyl-d-aspartate (NMDA) channel blockers, can suppress the electroshock-induced seizures in rats, these channels in the brain are thus suggested to be closely associated with the seizure reaction in this system. Therefore, the changes in the activity and/or expression of these receptors during the developmental period may be responsible for the lower seizure-thresholds in juvenile rats. Indeed, it has been suggested that such differences in the vulnerability to seizures during the different developmental periods were due to the immaturity of excitatory or inhibitory neuronal systems in the brain, especially NMDA receptors and GABA.
contribution of the histaminergic neuronal system to the suppression of seizures made these older rats resistant to ketotifen. Moreover, it was considered that the relatively smaller eara than the GABAergic neuronal system, occupies a large space. In contrast to ketotifen, oral administration of olopatadine at a dose of 10–30 mg/kg, or 30–100 mg/kg of fexofenadine, did not decrease the threshold-current of seizures in 3-week-old rats.

Olopatadine at doses of 0.03 mg/kg or higher has been reported to ameliorate the symptoms of experimental allergic cutaneous responses and rhinoconjunctivitis in sensitized animals. After the oral administration of 14C-olopatadine to adult rats, the radioactivity was the highest at 0.5 h in most tissues, including the brain and it thereafter decreased gradually over several hours, although the relative activity in the brain was less than that in the other organs. It was therefore concluded that even at doses high enough to exert anti-allergic effects, this drug did not induce seizures in infant rats.

It has been suggested that some antihistamines can elicit or exacerbate seizures, as mentioned above, and that these effects were closely correlated with their activity in the central nerve systems, or their ability to cross the blood-brain barrier. Most of the non-centrally active antihistamines are known to be substrates for P-glycoprotein, a membrane protein which plays a crucial role in brain penetration. Olopatadine was also reported to be a substrate for the P-glycoprotein. In addition, it was reported that orally administered olopatadine in humans at the usual dose for antiallergic effects occupied the histamine H1 receptors in the brain at a fairly lower rate than ketotifen and at a somewhat higher rate than fexofenadine.

In our present study, although ketotifen lowered the threshold-current of seizures, neither olopatadine nor fexofenadine could do so in 3-week-old rats. Moreover, the fact that only antihistamines with centrally-active properties could induce or exacerbate seizures had also been reported in some other animal models.

Such centrally-active antihistamines, the so-called first-generation antihistamines, often have a high affinity for the muscarinic M1 receptor and/or other receptors, as well as the histamine H1 receptor. For this reason, these compounds have been reported to produce anticholinergic side effects, such as dry mouth and constipation. On the other hand, olopatadine and fexofenadine, which are so-called second-generation antihistamines, do not show affinities for most of the neurotransmitter receptors in the brain including the M1 receptor, but they instead show a selective affinity for the H1 receptor. Therefore, we could not rule out the possibility that the effects on the seizures depended on differences in the receptor binding profiles among these compounds, although it is unclear at this time as to whether or not there is any relationship between the antagonistic activities with regard to M1 receptor and the induction of seizures.

In this study, olopatadine was determined to have no effects on the sensitivity of infant rats to seizures by using the increasing-current electroshock test, which was thought to be more sensitive than the conventional maximal electroshock test. As mentioned above, the criteria used for the assessment in the increasing-current electroshock test are the threshold-currents of the seizures. Since the greatest interest in the clinic may be whether or not patients become more seizure-prone as a result of their medication, this test would...
be superior to the maximal electroshock test with regard to the validity related to clinical conditions. We and other groups have previously confirmed the better predictive validity of the increasing-current electroshock test using both anti- and pro-convulsive agents, which have been shown to be effective in human.\(^{33}\) We therefore believe that the results of the present study generally reflect the clinical impact of antihistamines on the induction of seizure, although juvenile and normal animals, not pathological model animals were used. However, we are currently trying to perform the same kind of experiments using such model animals, since there are now several animal models of epilepsy and/or seizures that are available, including a variety of genetically engineered animals.\(^{33}\)

In conclusion, our results suggest that, unlike ketotifen, olopatadine, as well as fexofenadine, would be a safer antihistamine for use in children because it would not increase the risk of seizures.

REFERENCES


