Effects of Various Antiepileptics on Behavioral and Electroencephalographic Seizures Induced by Maximal Electroshock in Mice

Aya Murakami, Yusuke Watanabe, Kenshi Takechi, Akinori Fujiwara, and Chiaki Kamei

1Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8530, Japan

Received August 30, 2007; Accepted November 12, 2007

Abstract. The changes of electroencephalogram induced by maximal electroshock were studied in comparison with behavioral seizures in mice. After electroshock, mice showed tonic flexor (TF) seizure, tonic extensor (TE) seizure, and clonic (CL) seizure, in this order of occurrence. At the same time, high frequency spike or spike and wave complex in the cortex was observed, and thereafter, the frequency of the spike or spike and wave complex gradually fell. The antiepileptics used in the present study, except for ethosuximide, caused a dose-dependent shortening of the TE seizure duration. In addition, phenobarbital and carbamazepine at relatively high doses also showed a significant shortening of the duration of CL seizure. Although the duration of total electroencephalographic (EEG) seizures was not influenced by all the antiepileptics used in the present study, the duration of high frequency spike or spike and wave complex observed for 7 – 8 s after electroshock was dose-dependently shortened by all the antiepileptics used. From these findings, it may be concluded that high frequency spike or spike and wave complex induced by maximal electroshock is a useful index to assess antiepileptics that are effective in not only tonic-clonic seizures but also absence seizures in humans.

Keywords: maximal electroshock, tonic extensor (TE) seizure, clonic (CL) seizure, electroencephalogram

Introduction

It is well known that tonic extensor (TE) seizure induced by maximal electroshock is widely used as an index of the effect of antiepileptics used for grand mal epilepsy (1, 2). We have also reported that TE seizure induced by maximal electroshock was selectively inhibited by phenytoin, phenobarbital, topiramate, and carbamazepine, which are clinically effective in generalized tonic-clonic seizures (grand mal) (3). In addition, it has also been demonstrated that electromyographic seizure patterns recorded in the hind legs are more reliable and more highly sensitive than TE seizure to assess the potential activity of antiepileptics (3).

On the other hand, it is well known that electroencephalographic (EEG) changes were observed not only in generalized seizures but also partial seizures in humans (4, 5); however, there is little information about the EEG changes induced by maximal electroshock in animals except for a few papers. Stille and Sayers (6) reported that clinically proven antiepileptic drugs (phenytoin, phenobarbital, and carbamazepine) had no influence on EEG convulsive activity in the rat cortex, even in doses up to 8-fold the ED50 that showed TE seizure. Toman et al. (7) also described that phenytoin caused no inhibition on EEG response to supramaximal electroshock in the rabbit. Kamei et al. (8) reported previously about the effects of antiepileptics on both behavioral and electrographic seizure patterns induced by maximal electroshock, but classification of EEG seizure patterns was rather complex, although almost all antiepileptics showed no influence on electrographic convulsive activity in rats. There are some reports that rats were less sensitive than mice in the potency of...
antiepileptics on TE seizure induced by maximal electroshock (9). In the present study, therefore, the changes of EEG as well as behavioral seizures induced by maximal electroshock were studied using mice. The effects of certain antiepileptics on both EEG changes and behavioral seizures were also studied.

Materials and Methods

Animals

Male ddY mice, 4-week-old (body weight, 20 – 24 g) were purchased from Japan SLC, Shizuoka. Animals were maintained in an air-conditioned room with controlled temperature (24 ± 2°C) and humidity (55 ± 15%). They were housed in plastic cages with sawdust under a light–dark cycle (lights on from 07:00 to 19:00). The mice were allowed free access to food and water, except during the experiments. All procedures involving animals were carried out in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center. Groups of 8 mice each were used for each dose of the drugs in the study.

Experimental procedure

For the measurement of EEG, monopolar screw electrodes were implanted in the frontal pole cortex (FP, area 10), the right occipital cortex (RO, area 17), and the left occipital cortex (LO, area 17) under pentobarbital anesthesia (Nembutal®; 50 mg/kg, i.p.). Electroconvulsion was induced by stimulating the animals with 50 Hz, 200 V for 1.0 s (SEN-3301; Nihon Kohden, Tokyo) using ear-clip electrodes. EEG was recorded bipolarly (FP-LO and FP-RO) with an electroencephalograph (EEG-7314, Nihon Kohden). EEG analysis was done using EEG recordings of FP-LO and FP-RO. In the present study, EEG changes were divided into the following 2 stages: high frequency spike or spike and wave complex (6 – 15 Hz, EEG seizures-1) and low frequency spike and wave complex or slow wave (2 – 6 Hz, EEG seizures-2).

Drugs

The following drugs were used: phenobarbital sodium and phenytoin (Wako, Osaka) and carbamazepine, ethosuximide, and topiramate (Sigma, St. Louis, MO, USA). These drugs were suspended in 0.5% carboxymethyl cellulose (CMC) solution and orally administered 1 h before electrical stimulation at a volume of 5 ml/kg of body weight. Control groups were received with 0.5% CMC solution.

Statistical analyses

All data are expressed as the means ± S.E.M. The Kruskal-Wallis and Steel tests were used for statistical analysis of all data, and a P-value less than 0.05 was considered significant.

Results

Effects of various antiepileptics on the duration of TE seizure

Figure 1 shows the effects of various antiepileptics on the duration of TE seizure. The duration of TE seizure in the control group was 15.5 ± 0.42 s (n = 8). The duration of TE seizure induced by maximal electroshock was dose-dependently shortened by phenytoin, phenobarbital, topiramate, and carbamazepine. Significant differences were observed with phenytoin (10, 20, and 50 mg/kg), phenobarbital (10, 20, and 50 mg/kg), topiramate (20, 50, and 100 mg/kg), and carbamazepine (20 and 50 mg/kg). On the other hand, ethosuximide caused no significant inhibition even at a dose of

![Fig. 1. Effects of various antiepileptics on the duration of tonic extensor (TE) seizure. Each column and vertical bar represents the mean ± S.E.M of 8 mice. *: Significantly different from the control group at \( P<0.05 \) and \( P<0.01 \), respectively.](image)
500 mg/kg.

Effects of various antiepileptics on the duration of clonic (CL) seizure

CL seizure observed after TE seizure induced by maximal electroshock was dose-dependently shortened by phenobarbital and carbamazepine. Significant differences were observed with phenobarbital (50 mg/kg) and carbamazepine (50 mg/kg). On the other hand, phenytoin, topiramate, and ethosuximide caused no significant inhibition even at doses of 50, 100, and 500 mg/kg, respectively (Fig. 2).

Behavioral and EEG changes induced by maximal electroshock

A representative example of the behavioral and EEG changes induced by maximal electroshock is shown in Fig. 3. After electroshock, mice showed TF seizure (1.5 s), TE seizure (14 s), and CL seizure (30 s), in this order of occurrence. EEG seizure patterns were as follows: at first, high frequency spike or spike and wave complex (6 – 15 Hz, for 6 s, EEG seizures-1) was observed immediately after maximal electroshock; thereafter, the frequency of spike or spike and wave complex was gradually lowered (2 – 6 Hz, for 5.5 s, EEG seizures-2). Figure 4 shows the relationship between the duration of TE seizure and EEG changes after electroshock. The regression line of the duration of TE seizure and EEG changes was \( Y = 0.579x + 5.760 \). The duration of EEG changes was shorter than that of TE seizure, and a relatively low correlation coefficient (\( r = 0.730 \)) was observed (A); therefore, we also analyzed the relationship between the duration of TE seizure and EEG seizures-1 or EEG seizures-2. The regression line of the duration of TE seizure and EEG seizures-1 was \( Y = 1.419x + 5.106 \) (\( r = 0.892 \), B). The regression line of the duration of TE seizure and EEG seizures-2 was \( Y = 0.641x + 8.879 \) (\( r = 0.576 \), C).

Effects of various antiepileptics on total EEG seizures

As shown in Figs. 5 and 6, phenytoin and phenobarbital caused a dose-dependent shortening of the duration of total EEG seizures, but no significant difference was observed even at a dose of 50 mg/kg of

![Fig. 2. Effects of various antiepileptics on the duration of clonic (CL) seizure. Each column and vertical bar represents the mean ± S.E.M of 8 mice. *, **: Significantly different from the control group at \( P<0.05 \) and \( P<0.01 \), respectively.](image)

![Fig. 3. Representative example of behavioral and electroencephalographic changes induced by maximal electroshock. E.S., electroshock stimulation; TF, tonic flexor; TE, tonic extensor; CL, clonic convolution; FP, frontal pole cortex; LO, left occipital cortex; RO, right occipital cortex; EEG seizures-1, high frequency spike or spike and wave complex; EEG seizures-2, low frequency spike and wave complex or slow wave.](image)
both drugs. Topiramate, carbamazepine, and ethosuximide also caused no significant effects even at doses of 100, 50, and 500 mg/kg, respectively. As described previously, EEG changes can be divided into 2 stages, that is, high frequency spike or spike and wave complex (6–15 Hz, EEG seizures-1) and low frequency spike and wave complex or slow wave (2–6 Hz, EEG seizures-2); therefore, we studied the effects of various antiepileptics on both EEG seizure patterns.

Effects of various antiepileptics on EEG seizures-1

The results are shown in Fig. 6. The duration of high frequency spike or spike and wave complex was dose-dependently shortened by phenobarbital and ethosuximide, but no significant differences were observed even at doses of 50 and 500 mg/kg, respectively. Phenytoin, topiramate, and carbamazepine also showed no significant inhibition even at doses of 50, 100, and 50 mg/kg, respectively.

Discussion

In the present study, the duration of TE seizure induced by maximal electroshock in mice in the control group was $15.5 \pm 0.42$ s. Vohora et al. (10) reported that the duration of TE seizure in male Swiss-strain mice was $14.0 \pm 0.72$ s. Previously, we (11) have also demonstrated that the duration of TE seizure in male ddY mice was $15.2 \pm 0.5$ s; therefore, we confirmed that the maximal electroshock seizure test used in the present study was a suitable method for estimating TE seizure. In addition, as shown in Fig. 1, all the antiepileptics, except ethosuximide, inhibited TE seizure, and the potency of antiepileptics was almost the same as in our previous work (3). It is well recognized that ethosuximide showed no inhibition of TE seizure induced by maximal electroshock in mice and rats (9, 12). Next, as shown in Fig. 2, both phenobarbital and carbamazepine also caused an inhibition of CL seizure. The dose that showed a significant difference was relatively high in both drugs. Kamei et al. (8) reported that phenobarbital at a dose of 100 mg/kg, p.o. is effective in inhibiting CL seizure induced by maximal electroshock in rats. As for carbamazepine, Novelli et al. (13) described that the drug at a dose of 10 mg/kg, i.p. significantly inhibited CL seizure in mice. On the other hand, phenytoin showed no significant inhibition of CL seizure even at a dose of 50 mg/kg. It is well known that CL seizure induced by maximal electroshock was not inhibited (9) or was rather prolonged by phenytoin (14). Different from the CL seizure induced by pentetrazol that observed with maximal electroshock was weak and unclear. At the present time, therefore, we assume that CL seizure induced by maximal electroshock in mice is not a reliable index for estimating the potency of antiepileptics.

It was found in the present study that EEG changes, that is, spike or spike and wave complex or slow wave (10, 20, and 50 mg/kg), phenobarbital (10, 20, and 50 mg/kg), topiramate (20, 50, and 100 mg/kg), carbamazepine (20 and 50 mg/kg), and ethosuximide (200 and 500 mg/kg).

Effects of various antiepileptics on EEG seizures-2

The results are shown in Fig. 6. The duration of low frequency spike and wave complex and slow wave was dose-dependently shortened by phenobarbital and ethosuximide, but no significant differences were observed even at doses of 50 and 500 mg/kg, respectively. Phenytoin, topiramate, and carbamazepine also showed no significant inhibition even at doses of 50, 100, and 50 mg/kg, respectively.

Fig. 4. Relationship between the durations of tonic extensor seizures and EEG seizures. A: Total EEG seizures and tonic extensor seizure, B: EEG seizures-1 and tonic extensor seizure, C: EEG seizures-2 and tonic extensor seizure. TE, tonic extensor; EEG seizures-1, high frequency spike or spike and wave complex; EEG seizures-2, low frequency spike and wave complex or slow wave.
were observed after maximal electroshock. In tonic seizure in humans, Gastaut et al. (15) reported that the EEG shows a rapid synchronization of increasing amplitude (i.e., high frequency spike or spike and wave complex) ending with slow waves (i.e., low frequency spike and wave complex). These EEG changes observed in humans have a striking resemblance to the EEG seizures in our animal study. In our study, both TE seizure and EEG changes were observed at the same time; however, no clear relationship was observed between the duration of TE seizure and total EEG seizures. In addition, total EEG seizures induced by maximal electroshock were not inhibited by any antiepileptics used. For these reasons, it can be assumed that the occurrence mechanism between high frequency EEG seizures and low frequency EEG seizures was essentially different. When total EEG seizures induced by maximal electroshock were investigated in detail, it was found that they were divided into 2 patterns, that is, high frequency spike or spike and wave complex (EEG seizures-1) and low frequency spike and wave complex (EEG seizures-2); therefore, the effects of...
antiepileptics on these 2 patterns of EEG seizures were studied. As a result, EEG seizures-1 was significantly inhibited by all antiepileptics used. Previously, Toman et al. (7) reported that phenytoin at 100 mg/kg, s.c. showed a reduction of both the voltage and frequency of convulsive discharge induced by electroshock in rabbits. As for phenobarbital, there are many reports that this drug inhibited EEG seizures; high voltage rhythmic spike or sharp wave induced by maximal electroshock was inhibited by phenobarbital in rats (8), rabbits (7), and cats (7). Carbamazepine is also reported to inhibit rhythmic spike or spike and wave complex induced by maximal electroshock in rats (8). There are no reports about the effect of topiramate on EEG seizures induced by maximal electroshock; however, Amano et al. (16) found that topiramate at a dose of 12.5 and 25 mg/kg, i.p. showed a significant reduction of discharge duration and seizure stage, respectively, in amygdaloida kindled seizure in rats. As shown in the present study, it was first found that ethosuximide at a dose of 200 mg/kg is effective in inhibiting high frequency spike or spike and wave complex (EEG seizures-1) observed with maximal electroshock. It is well recognized that ethosuximide caused no remarkable inhibitory effect on TE seizure induced by maximal electroshock. As we previously reported (3), TE seizure induced by maximal electroshock was closely related with potent muscle contraction, and neither the duration nor magnitude of EMG seizures induced by maximal electroshock was inhibited by ethosuximide, even at a dose of 1000 mg/kg. Kreindler et al. (17) and Bergmann et al. (18) demonstrated that the mesencephalic reticular formation is responsible for the occurrence of TE seizure in rats and rabbits. As shown in the present data, ethosuximide caused no inhibition of TE seizure because the action site of ethosuximide was the thalamus and cortex as reported by Pellegrini et al. (19). From these findings, it is reasonable to presume that ethosuximide acts on the cerebral cortex, not on the brain stem reticular formation.

Our results indicate that high frequency spike or spike and wave complex induced by maximal electroshock in mice appears to be a useful index to assess the potential of antiepileptic activity.

References