Effects of Repeated Electroconvulsive Seizures on Spontaneous Alternation Behavior and Locomotor Activity in Rats

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Epileptic patients are at a significantly higher risk for impairments of cognitive function and behavioral abnormalities. In this study, we investigated the effect of repeated electroconvulsive shock (ECS)-induced seizures on the spontaneous alternation behavior in a Y-maze test and the locomotor activity in an open-field test, and examined the effects of anti-epileptic drugs in rats. ECS was administered for seven consecutive days and the Y-maze and open-field tests were performed 24 h after the last ECS administration. The repeated electroconvulsive seizures significantly impaired the spontaneous alternation and increased the locomotor activity. Moreover, these behavioral changes induced by the seven administrations of ECS persisted for at least 28 d. The inhibition of the ECS-induced seizures through the daily pretreatment of the phenytoin (120 mg/kg, intraperitoneally (i.p.)), phenobarbital (60 mg/kg, i.p.) and valproate (400 mg/kg, i.p.) abolished the locomotor hyperactivity in the open-field test. The impaired spontaneous alternation behavior in the Y-maze test was also significantly suppressed by the treatment with phenytoin and valproate. However, phenobarbital injection produced no significant ameliorating effect in the Y-maze test. These results suggest that the inhibition of ECS-induced seizures through phenytoin and valproate injections suppress the development of impairment of spontaneous alternation and the locomotor hyperactivity.

Key words electroconvulsive seizure; anti-epileptic drug; spontaneous alternation behavior; Y-maze test; locomotor activity

Epilepsy is a chronic neurological condition characterized by recurrent seizures due to the uncontrolled excessive activity of either a part of or all of the central nervous system. These recurrent seizures frequently produce psychiatric disorders in patients, including cognitive deficits, behavioral abnormalities, emotional impairments and attention deficit hyperactivity disorder. 1—3 In general, the risk for psychiatric disorders in people with epilepsy is three to six times higher than that observed in age-matched normal populations. 2 Several factors influence the risk for these psychiatric disorders, including the duration and frequency of the epilepsy, seizure etiology and the patient’s genetic background. 1—4 In addition, anti-epileptic drugs also may cause cognitive and emotional impairments. Patients treated with multiple anti-epileptic drugs or patients with elevated serum levels of anti-epileptic drugs are at an increased risk for cognitive deficits. 5 In particular, the first-generation anti-epileptic drugs, such as phenobarbital, phenytoin, valproate and benzodiazepines, have been associated with several unwanted side effects including sedation, somnolence and distractibility. 5—7

Experimental models provide evidence that both prolonged and brief seizures can cause irreversible impairment in spatial and emotional learning and memory in kindled rats. 8—10 Hippocampus- and amygdala-kindled rats or recurrent seizures in rats induced by pentyleneetetrazole may result in disturbed social behavior and spatial learning and memory. 7—10 The severity of cognitive impairments has been shown to correlate with the number of seizures experienced in kindled rats. 10 Studies have also shown that memory impairment progressively increases in rats as a function of the number of seizures. 6—10,11 These findings indicated that repeated severe seizures lead to cognitive impairments eventually.

Repeated electroconvulsive shock (ECS)-induced seizures, an animal model of recurrent seizures including a brief generalized tonic-clonic seizure, can also cause a spatial learning deficit of the Morris water maze task in rats. 12 However, the effects of anti-epileptic drugs on cognitive impairments and behavioral abnormalities induced by seizures remain poorly understood. The objective of this study was to evaluate the effects of recurrent seizures induced by repeated ECS administrations on the cognitive function and the general behavior in rats. Spontaneous alternation behavior in the Y-maze test is considered to reflect the short-term memory. 13—15 In this study, we first investigated the locomotor activity using an open-field test and the spontaneous alternation behavior using a Y-maze test after repeated seizures in rats through ECS administration. We then evaluated the effects of the anti-epileptic drugs on the impairments of cognitive function and the behavioral abnormalities.

MATERIALS AND METHODS

Animals Male Wistar strain rats (at 6—8 weeks of age) were obtained from Charles River Japan, Inc. (Yokohama, Japan). All animals were housed 2 rats per cage (42 cm×26 cm×15 cm) at 24±2 °C with a 12-h light period (7:00 a.m. to 7:00 p.m.). Food and water were available ad libitum. The animal experiments were performed in compliance with the Guidelines for Animal Experimentation and with the approval of the Committee of Animal Experimentation at Ehime University School of Medicine.

Drugs Sodium valproate, phenobarbital sodium and phenytoin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Valproate and phenobarbital were dissolved in physiological saline (0.9% sodium chloride). Phenytoin was dissolved in a few drops of 0.5 N NaOH (Wako Pure Chemical Industries, Ltd., Osaka, Japan) after which the final volume was mixed with 0.9% physiological saline. Each drug was prepared daily. Valproate and pheno-
barbital were injected in a volume of 1 ml/kg body weight. Phenytoin was injected in a volume of 3 ml/kg body weight.

**Electroconvulsive Shock-Induced Seizures**  Electroconvulsive shock (ECS, 100 V, 50 mA, 0.2 s, 60 Hz) was administered via corneal electrodes once daily for 7 d.15) Rats were administered a drop of 0.9% sodium chloride in each eye before application of the electrodes in order to ensure adequate electrical contact and to reduce the incidence of fatalities to near zero. All of the rats that received ECS exhibited generalized tonic-clonic seizures lasting approximately 5—10 s. The sham rats in the control group were handled and treated in a similar manner to the ECS group except for the application of electroshock.

**Spontaneous Alternation Behavior (Y-maze Test)**  This study examined the continuous spontaneous alternation behavior using the Y-maze apparatus.15) The Y-maze was made of black plastic with three arms (40 cm×15 cm×35 cm) extending from a central platform at a 120° angle. Each rat was placed at the end of one arm and allowed to move freely the three arms of the maze during an 8 min session 24 h after the last ECS administration. Arm entry was defined as the entry of four paws into one arm. The sequence of the arm entries was recorded visually. Alternation was defined as multiple entries into the three different arms on overlapping triplet sets. The percentage of spontaneous alternation was calculated as the ratio of the actual to possible alternations (defined as the total number of arm entries minus 2) multiplied by 100 as shown in the following equation:

\[
\text{alternation } (\%) = \frac{\text{[number of alternation]}}{\text{[total arm entries} - 2]} \times 100
\]

**Locomotor Activity (Open-Field Test)**  In order to investigate general behavioral changes, the rats were individually placed in an acrylic apparatus (69 cm×28 cm) with the gray floor divided into 19 squares. The locomotor activity was recorded with a digital video camera and the total locomotor activity was calculated as the sum of the line crossing during a period of 8 min.

**Experimental Procedures**  To characterize the cognitive impairments which were induced by the repeated ECS administrations, the Y-maze test was performed 24 h after either a single administration or seven administrations of ECS. In addition, to investigate the effect of the cessation of repeated ECS administration, the Y-maze test was performed 1, 7 and 28 d after the seventh ECS administration. The open-field test was performed 24 h after the seventh ECS administration.

In a preliminary study, phenytoin (90—120 mg/kg),17) phenobarbital (40—60 mg/kg)18) and valproate (350—500 mg/kg)19) inhibited the ECS-induced seizures in a dose-dependent manner (data not shown). Based on these results, the minimal doses of phenytoin (120 mg/kg), phenobarbital (60 mg/kg) and valproate (400 mg/kg), which completely inhibited the ECS-induced seizures, were injected intraperitoneally (i.p.) 30 min before the daily ECS and sham administrations for 7 d. The Y-maze and open-field tests were performed 24 h after the last ECS administration. In order to examine the effects of a single treatment of the anti-epileptic drugs, phenobarbital (60 mg/kg, i.p.), phenytoin (120 mg/kg, i.p.) and valproate (400 mg/kg, i.p.) were injected 24 h after the seventh ECS administration, and the Y-maze test and the open-field test were performed 30 min after the treatments.

**Statistical Analysis**  The Student’s t-test was used to analyze the differences between two groups. When more than two groups were compared, the significance of the difference among groups was evaluated through a one-way analysis of variance (ANOVA). When significant differences were obtained, post hoc comparisons within logical sets of means were performed using Bonferroni’s test. p values less than 0.05 were considered to be significant.

**RESULTS**

**The Effects of Repeated ECS Administrations**  Figure 1 shows the effects of the repeated ECS administrations on the spontaneous alternation behavior in the Y-maze test. A one-way ANOVA revealed that the repeated ECS administrations significantly decreased the spontaneous alteration behavior \(F(2,20)=8.564, p<0.01\) and significantly increased the total arm entries \(F(2,20)=5.954, p<0.01\). The post hoc comparisons using Bonferroni’s test showed significant differences in the spontaneous alternation \(p<0.01\) and the total arm entries \(p<0.01\) between the group that received 7 d of ECS administration and the sham control group.

Figure 2 shows the spontaneous alternation behavior after the cessation of the seventh ECS administration. The impairment of the spontaneous alteration after the 1-d cessation period persisted for 28 d \(F_{(3,32)}=6.236, p<0.01,\) one-way ANOVA. The increase of the total arm entries after the 1-d cessation period also persisted for 28 d \(F_{(3,32)}=9.559, p<0.001,\) one-way ANOVA.

**The Effects of the Anti-epileptic Drugs**  Figure 3 shows
the effects of the repeated administration of the anti-epileptic drugs in rats. A one-way ANOVA revealed that a 7-d administration of phenytoin (120 mg/kg), phenobarbital (60 mg/kg) and valproate (400 mg/kg) had no effect on the spontaneous alternation behavior \[F(3,28) = 0.482, p > 0.05\] or the total arm entries \[F(3,28) = 2.409, p > 0.05\] in the Y-maze test or locomotor activity \[F(3,28) = 0.396, p > 0.05\] in the open-field test by itself.

The Effects of Inhibition of the ECS-Induced Seizures by Anti-epileptic Drugs  

Figure 4 shows the effects of the daily pretreatment of the anti-epileptic drugs on the impaired spontaneous alternation behavior in the Y-maze test. The daily pretreatment of phenytoin \((p < 0.01)\) and valproate \((p < 0.05)\) significantly reversed the lowered alternation which was induced by the repeated ECS administrations for 7 d (Bonferroni's test). However, phenobarbital had no significant effect on the impaired alternation behavior. On the other hand, all of the anti-epileptic drugs significantly \((p < 0.05\) or \(p < 0.01,\) Bonferroni's test) suppressed the increase of the total arm entries induced by the repeated administration of ECS for 7 d.

Figure 5 shows the effects of the daily pretreatment of the anti-epileptic drugs on the locomotor hyperactivity in the rats that received repeated ECS administration. The 7-d ECS administrations markedly increased the locomotor activity in the open-field test. The daily pretreatment of phenobarbital \((p < 0.05)\), phenytoin \((p < 0.01)\) and valproate \((p < 0.05)\) significantly suppressed the locomotor hyperactivity (Bonferroni's test).

Figure 6 shows the effect of a single administration of the anti-epileptic drugs in the rats that received the repeated ECS administrations for 7 d. The repeated ECS administrations produced a significant impairment of the spontaneous alternation \((p < 0.05)\) and a significant increase in the total arm entries \((p < 0.01)\) in the Y-maze test. In addition, the administrations significantly \((p < 0.05)\) increased the locomotor activity in the open-field test. To evaluate the effect of a single administration of the anti-epileptic drugs, ANOVA was applied to all ECS-treated groups (four groups). Phenobarbital, phenytoin and valproate had no effect on either the lowered alternation behavior \([F(3,28) = 1.125, p > 0.05] or the increased total arm entries \([F(3,28) = 1.058, p > 0.05\] induced by the repeated ECS administrations. In addition, these anti-epileptic drugs also showed no effects on the increased locomotor hyperactivity \([F(3,24) = 1.266, p > 0.05]\).
DISCUSSION

In this study, seven administrations of ECS produced the impairment of the spontaneous alternation and locomotor hyperactivity, but a single administration of ECS did not produce any results. Moreover, the behavioral changes induced by the seven administrations of ECS persisted for at least 28 d. Lukoyanov et al. reported that six ECS seizures could cause a spatial learning deficit of the Morris water maze task following a 2-month recovery period in rats.12) Smith and Sharp reported that five ECS-induced enhancements of dopamine-mediated behavior were observed for up to 3 weeks after the cessation of ECS treatments in rats.20) These findings suggest that repeated ECS-induced seizures can produce a number of morphological and functional changes in the brain. In fact, previous studies have shown that the electrical or pentylenetetrazole-induced kindling seizures resulted in long-lasting changes in mossy fiber sprouting and cell loss.9,21)

In the present study, the seven consecutive ECS administrations significantly increased the total number of arm entries in the Y-maze test and the locomotor activity in the open-field test. Previous studies have shown that the ECS administration increased the dopamine-mediated behavior, such as locomotion, in rats and mice.7,20,22,23) An in vivo brain microdialysis study showed that both single and repeated ECS treatments increased the extracellular dopamine concentrations in rat striatum and that the baseline concentration of 3,4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite, was increased after the eighth ECS administration.24) These findings suggest that repeated ECS administrations increase the dopaminergic system of the rat brain and that the dopaminergic system appears to be responsible for the locomotor hyperactivity induced by the repeated ECS treatments.

Previous studies have shown the ED$_{50}$ values for phenytoin, phenobarbital and valproate against the electroconvulsive seizures to be approximately 28, 9, and 485 mg/kg in rats, respectively.19) However, the minimum effective doses, which completely abolished the seizures, have been reported to be approximately 100—250 mg/kg (per os (p.o.)) for phenytoin,17,25,26) 30—100 mg/kg (i.p.) for phenobarbital18,26,27) and 1800 mg/kg (p.o.) for valproate26) in rats. In this study, we confirmed that the doses of phenytoin (120 mg/kg), phenobarbital (60 mg/kg) and valproate (400 mg/kg) completely inhibited the ECS-induced seizures. Therefore, in the present study, we examined the effect of high-doses of the anti-epileptic drugs on the impairment of spontaneous alternation and the locomotor hyperactivity to clarify the effect of a complete inhibition of ECS-induced seizures.

First-generation anti-epileptic drugs such as phenobarbital, phenytoin and valproate have been shown to have cognitive deficits.5) Several clinical studies have reported no significant differences in the cognitive deficits elicited by carbamazepine, phenytoin or valproate treatments,28—30) whereas phenobarbital has been reported to produce greater cognitive deficits than both carbamazepine and phenytoin.31) Shannon and Love have reported that a single administration of phenobarbital, but not valproate, 30 min before an operant conditioning test impairs the working memory as assessed by spa-
tial alternation performance in nonepileptic rats. In this study, phenobarbital, phenytoin and valproate had no effect on the spontaneous alternation behavior or locomotor activity, when both Y-maze and open-field tests were performed 24 h after the last treatment for seven consecutive days. However, anti-epileptic drugs are administered for long-time generally, and phenobarbital and phenytoin have long half-lives. Therefore, further investigation is required to evaluate the long-term effect of these anti-epileptic drugs.

The inhibition of ECS-induced seizures by phenytoin and valproate suppressed both the impairment of the spontaneous alternation and the locomotor hyperactivity. Phenobarbital (60 mg/kg), which also abolished the electroconvulsive seizures, suppressed the locomotor hyperactivity. The dose of phenobarbital partially reduced the impaired spontaneous alternation behavior, but there was no significant antagonism. Becker et al. reported that phenobarbital (12.5—25 mg/kg), which was administered prior to each kindling stimulation through repeated injections of pentylenetetrazole, suppressed the development of motor seizures in rats and counteracted the learning deficits in a shuttle-box paradigm. On the other hand, in this study, when the single injections of pheno-barbital, phenytoin and valproate were injected 24 h after the seventh ECS administration, these anti-epileptic drugs had no effect on the impairment of the spontaneous alternation behavior and the locomotor hyperactivity. Taken together, the findings of both previous studies and the results of the current study suggest that at least phenytoin and valproate suppress the impairment of spontaneous alternation and the locomotor hyperactivity induced by the repeated ECS-induced seizures, thus indicating that the inhibition of seizures is closely related to the protection of cognitive function.

Previous studies have shown that seizures induced by ECS or convulsant drugs, such as kainic acid, picrotoxin and pentylenetetrazole stimulate the expression of brain-derived neurotrophic factor (BDNF). In addition, ECS seizure increases the cAMP response element-binding protein (CREB), a transcription factor that is activated by cAMP and Ca^{2+} intracellular pathways. However, pentylenetetrazole seizures have been reported to decrease the expression of CREB in the rat brain. On the other hand, BDNF has been demonstrated to enhance behavioral sensitization by triggering the overexpression of the dopamine D3 receptor. The selective D3 receptor antagonist, nafadotride, has also been reported to improve the scopolamine-induced memory deficit in a passive avoidance test of rats. These findings suggested that the increased BDNF might be responsible for the ECS-induced memory impairment through D3 receptors. However, further studies are necessary to clarify the mechanism of seizure-induced cognitive impairments and to investigate further drug effects.

In conclusion, the results of the present study indicate that repeated electroconvulsive seizures induce a long-lasting impairment of the spontaneous alternation behavior and locomotor hyperactivity in rats and that both phenytoin and valproate suppress the development of the impairments. In addition, the data presented showed that repeated electroconvulsive seizures might be a simple and effective method in detecting both the anticonvulsive action and the side effects of anti-epileptic drugs.

REFERENCES