EFFECTS OF ELECTROCONVULSIVE SHOCK ON
MOUSE-KILLING BEHAVIOR (MURICIDE) IN
OLFACTORY BULBECTOMIZED RATS

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Abstract—We investigated the influence of electroconvulsive shock (ECS), regarded to possess an antidepressant effect clinically, on muricide in olfactory bulbectomized rats (OB rats). Muricide in these rats was markedly inhibited by ECS treatment. Five and 10 min after the termination of ECS-induced convulsions, muricide was inhibited by 100%. Even after intervals of 20 and 60 min, inhibition rates of 80% and 30% were obtained, respectively. ECS-induced muricide inhibition was remarkably antagonized by pretreatment with the α-blocker phenoxybenzamine but not by pretreatment with the β-blocker sotalol. ECS-induced suppression of muricide was potentiated by repeated ECS treatment once daily for 10 days. After several applications of ECS treatment, muricide was inhibited in muricide tests done 24 hours after ECS treatment; this state persisted for up to 10 days thereafter. The results of this experiment demonstrated that ECS treatment specifically inhibited muricide in OB rats and further suggested that the cerebral noradrenergic α-receptor system plays an important role in this ECS-induced inhibition of muricide. Similar to findings in the case of antidepressant administration, inhibition of muricide was potentiated by chronic ECS treatment. Specific inhibition of muricide in OB rats by antidepressants indicated that this phenomenon may serve as an animal model for the evaluation of antidepressant activity. Our results reiterate the usefulness of muricide in OB rats as an excellent experimental model for the assessment of antidepressant activity.

A characteristic pattern of hyperemotionality, including mouse-killing behavior (muricide), is induced in rats by bilateral olfactory bulbectomy. This hyperemotionality of the olfactory bulbectomized rat (OB rat) is useful in evaluating the taming effect of various psychotropic drugs since this phenomenon is suppressed by certain drugs (1). The tricyclic antidepressants specifically suppress muricide, without affecting other manifestations of hyperemotionality, as doses which do not cause a muscle relaxation or ataxia (1–3). The suppression of muricide by antidepressants is strongly antagonized by simultaneous administration of phenoxybenzamine, an α-adrenergic blocker while β-adrenergic blockers such as propranolol and sotalol have no effect (4). These
observations suggest that adrenergic α-receptors are involved in the suppression of muricide by antidepressants in OB rats. Clinically, however, electroconvulsive shock (ECS) often is an excellent form of therapy for depression (5, 6). In the rat, muricide behavior induced by isolated housing is reportedly suppressed by ECS treatment (7).

The present experiment was designed to examine the effect of ECS on muricide in OB rats. The influence of continuous ECS treatment once daily for 10 days, and the effects of α- and β-adrenergic blocking agents on the effectiveness of ECS were also investigated.

MATERIALS AND METHODS

Materials: The male Wistar King A strain rats supplied by Kyushu University Institute of Laboratory Animals weighed 250–300 g at the beginning of the experiments. Rats showing no evidence of muricide were selected by the tests done once prior to olfactory bulb ablation. The olfactory bulbs were bilaterally removed by suction as described previously (1) and the animals were then housed in individual cages (18×17×18 cm) in a room maintained at a temperature of 23±2°C with a 12 hr light/dark cycle (lights on at 7:00). Rats showing muricide behavior within 7 days after olfactory bulbectomy were chosen for further experiments.

Experimental procedure: The apparatus described by Woodbury and Davenport (8) with some modifications was used for the ECS treatments. A constant current of 60 Hz, 150 mA was applied for 200 msec through both eyes of the rat via corneal electrodes. In experiments involving continuous ECS treatment, identical ECS treatment was given once daily for 10 successive days and repeated once after an interval of 1 week. Corneal electrodes were applied to the eyes of control animals. The time required to recover righting reflex was regarded as the duration of convulsions. Muricide tests were carried out at 5, 10, 20, 30 and 60 min after the termination of convulsions. In the rats given continuous ECS treatment for 10 days, muricide tests were conducted immediately before and 60 min after convulsions. Muricide activity was considered positive if the rat bit a mouse to death within 3 min after introduction into the home cage.

The sotalol hydrochloride (Mead-Johnson) and phenoxybenzamine hydrochloride (Tokyo Kasei) dissolved in isotonic saline solution and given i.p. 30 min before ECS treatment. Motor activity of the rat was quantified using Hall's open-field apparatus (9) to determine the number of ambulations during 3-min periods 20 and 60 min after convulsions. Electroencephalogram was recorded in the rat with chronically implanted electrodes to investigate the effects of ECS treatment. Bipolar stainless steel electrodes (tip diameter, 0.2 mm, uninsulated length, 0.4 mm, polar distance, 0.8 mm) were stereotaxically implanted into the frontal cortex and medial amygdaloid nucleus according to König and Klippel (10) as reported previously (11). Electroencephalogram was recorded on a polygraph (Nihon Kohden) before and after ECS treatment.

Statistical analysis: Student's t-test was used for the statistical processing of the results for the effects of drugs on duration of convulsions and ECS treatment on spontaneous motor activity (ambulation). The results obtained for the effects of drugs on the suppression of muricide by ECS were statistically analyzed by Fisher's exact probability test.

Histology: After completion of all experiments, the animals were anesthetized with ether and the brains were perfused through the carotid arteries with saline and 10% formalin. The brains were then removed,
stained with cresyl violet, and the extent of olfactory bulbectomy and electrode placement were verified histologically. If the extent of olfactory bulbectomy was not appropriate, the results were discarded.

RESULTS

Single ECS and muricide: The characteristics and duration of ECS-induced convulsions in this experiment were as follows: tonic flexion 15-20 sec, tonic extension 15-20 sec, clonic convulsions 24-40 sec, and coma 15-17 sec. The total duration of convulsions was defined at the time from immediately after ECS to the termination of coma.

Table 1 shows the total duration of convulsions and the effects of the drugs. Sotalol, a β-blocker, in a dose of 20 mg/kg had no effect on the duration of convulsions. However, the duration of convulsions was markedly prolonged by administration of the α-blocker phenoxybenzamine, 20 mg/kg. All rats lived throughout these experiments.

Figure 1 shows the effect of ECS on muricide in OB rats and the influence of drug treatment. Muricide was suppressed 100% at 5 and 10 min, 80% at 20 min, and 30% at 60 min after convulsions. No suppression of muricide was observed in control animals in which electrodes were in contact with the cornea only. Sotalol, 20 mg/kg, had no effect on the inhibition of muricide by ECS. Phenoxybenzamine, 20 mg/kg, strongly antagonized inhibition by ECS (p<0.001). In the phenoxybenzamine treated group, muricide was inhibited only at 5 min after the termination of convulsions but almost every rat showed muricide behavior after 10 min.

Single ECS and locomotor activity: Ambulation in ECS treated and control animals was quantified at 17.8±14.7 and 44.5±9.3 at 20 min after convulsions, respectively; ambulation was significantly lower in the ECS treated group (p<0.01). No significant difference was found, however, 60 min after ECS treatment between the ECS treated and the control group which displayed values of 30.2±12.3 and 39.8±13.1, respectively.

Single ECS and EEG: Electroencephalogram of the frontal cortex and medial amygdaloid nucleus recorded 5 min after the termination of convulsions showed a lower amplitude than that before ECS treatment. However, abnormal patterns such as the appearance of a spike wave were not apparent. The electroencephalogram of these areas returned to a normal pattern after

| Table 1. Effects of sotalol and phenoxybenzamine on the duration of convulsion in the rat |
|---------------------------------------------|-----------------|--------------------|
| Treatment      | Dose (i.p.) | Number of rats | Duration (sec±S.E.) |
| Saline         | 0.2 ml/100 g | 11               | 69.5±12.4           |
| Sotalol        | 20 mg/kg   | 10               | 61.6±22.0           |
| Phenoxybenzamine | 20 mg/kg | 10               | 140.6±49.8          |

Fig. 1. Effect of electroconvulsive shock (ECS) on muricide in olfactory bulbectomized rats (OB rats) and the influence of drug treatment. Ordinate: incidence of muricide. Abscissa: time after ECS. ***p<0.001: significant difference VS drug untreated group.
10 min and there were no noticeable changes thereafter.

Repeated ECS and muricide: The duration of convulsion was about 70 sec after the first application of ECS. Following repeated ECS treatment once daily, the duration of coma, in particular, was gradually shortened. After 4–5 days of ECS treatment, there were no appreciable signs of coma, and the rats resumed an upright posture immediately after the termination of clonic convulsions. The duration of convulsions was 69.5±12.4 sec on the first day, 50.0±18.8 sec on the 5th day, and 42.4±7.4 sec on the 10th day.

Figure 2 shows the incidence of muricide before and 60 min after convulsions. Muricide was suppressed by 30% 60 min after the first ECS treatment. The inhibition of muricide was increased to about 50% after ECS treatment on day 2 and this level was maintained thereafter. Suppressions of muricide were completely alleviated 24 hr after the first ECS and all animals showed muricide before the second ECS. Recovery of muricide was not complete after the second ECS and this tendency remained in about 25% of all the rats. Muricide suppression 24 hr after ECS further increased to 40% by the third ECS and 50% by the seventh ECS.

Fig. 2. Effect of repeated ECS treatment on muricide in OB rats. ECS treatment was given once daily for 10 successive days and repeated once after an interval of 1 week. Muricide tests were conducted immediately before and 60 min after daily ECS treatment.

This behavior recovered almost completely with a seven day intermission period after 10 successive days of ECS application. Application of ECS at this time resulted in 60% inhibition of muricide.

DISCUSSION

As shown in the present experiment, muricide in OB rats was significantly suppressed by ECS treatment. In tests up to 10 min after the termination of convulsions, muricide was suppressed in almost all animals. Muricide recovered with the passage of time and suppression was found in 25% of the animals 60 min after convulsions. Vogal and Haubrich (7) reported that the muricide induced by individual and isolated housing was suppressed by ECS treatment. It was found that ECS treatment inhibited muricide following isolated housing and also that by olfactory bulbectomy, although different methods were used for the induction. It can be assumed that a strong ECS caused disturbances in motor ability thereby suppressing muricide unselectively. Although there was no significant muscle relaxation or ataxia, the ambulation of rats was reduced to about 50% of the control group at 20 min after the convulsions. In tests with phenoxycbenzamine administration, muricide was observed in almost every animal 10 min after the termination of convulsions, despite the fact that ECS-induced convulsions were significantly enhanced by phenoxycbenzamine. The electroencephalogram recovered to normal 10 min after the termination of convulsions. Based on the above-mentioned fact, suppression of muricide occurring 10 min after convulsions can be considered to be specific.

The effects of α- and β-adrenergic blockers on the inhibition of muricide by ECS were also examined. ECS-induced convulsions were not affected by sotalol, a β-blocker given in a dose of 20 mg/kg. Inhibition of
muricide also was not affected. The α-blocker phenoxybenzamine significantly augmented ECS-induced convulsions at 20 mg/kg and the duration of the convulsions was doubled. This seems to be a natural consequence since it has been reported that the noradrenergic system of the central nervous system is suppressive to convulsions and that the ECS-induced convulsions are enhanced by reserpine which causes a remarkable reduction in the noradrenaline content of the brain (12). On the other hand, the inhibition of muricide by ECS was remarkably antagonized by phenoxybenzamine. In this case, muricide was suppressed to a similar level as that seen without the administration of phenoxybenzamine in tests done 5 min after the termination of convulsions. However, muricide was re-manifested in 9 out of 10 rats in tests done 10 min after and by all animals 30 min after the termination of convulsions. Inhibition of muricide 5 min after convulsions is considered to be non-specific because the electroencephalogram did not show normal findings, the rats were lethargic and there was some ataxia during this period.

The turnover rate of noradrenaline in the brain is increased by ECS treatment (13). Thus, it can be assumed that noradrenergic function was enhanced by ECS treatment and muricide was suppressed by a mechanism involving adrenergic α-receptors. We have reported that muricide in OB rats was inhibited by desipramine, and that the action of desipramine antagonized by phenoxybenzamine was not affected by propranolol (4). Muricide in OB rats was suppressed by a microinjection of noradrenaline into the medial amygdaloid nucleus (14) and this action of noradrenaline was antagonized by phenoxybenzamine (4). Furthermore, the induction of muricide by olfactory bulbectomy was increased by destruction of the dorsal bundle through which noradrenergic fibers mainly pass from the locus coeruleus (15). All of these findings indicate that ECS suppressed muricide through α-receptors in the central noradrenergic system.

The effect of chronic ECS treatment on muricide was also examined. In this experiment, muricide tests were carried out 60 min after the termination of convulsions when muricide was inhibited to 30% of control by a single ECS in order to observe the enhancement of the inhibition of muricide. Coma was specifically shortened and the entire duration of convulsions was reduced by repeated ECS treatment once daily. The inhibitory effect of ECS on muricide was enhanced by repeated treatment. In this case, muricide recovered completely 24 hr after the first ECS treatment. However, muricide remained suppressed 25% after the second ECS and 40% after the third ECS even 24 hr post-treatment. Thus, the duration of the effect was markedly prolonged along with an enhancedment of intensity. It has been confirmed that repeated ECS treatment enhances the noradrenaline turnover rate in the brain (16). Therefore, it is likely that the enhancement of muricide inhibition is manifested via a mechanism mediated by α-adrenergic receptors. On the other hand, it may be possible that subsensitivity occurs in postsynaptic receptors when the noradrenaline turnover rate is increased for a long period, thereby suppressing the function of noradrenaline system inversely. When noradrenaline turnover rate was increased by chronic treatment with ECS or antidepressants, subsensitivity reportedly occurs only in the β-receptors (17–19) and not in α-receptors (17, 20). As stated above, suppression of muricide was not attenuated by repeated ECS treatment probably because α-receptors participate in muricide inhibition. The augmentation of muricide inhibition is therefore ascribed to the repeated functioning of an α-receptor...
mediated mechanism.

Muricide in OB rats is a useful model for evaluating antidepressants as this behavior is inhibited not only by antidepressants but also by ECS which is clinically effective for the treatment of depression.

REFERENCES


