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Epileptiformic activity induced by antidepressants in amygdala-kindled rats

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Abstract

The present study was undertaken to investigate the spontaneous epileptiformic activity induced by antidepressants using amygdala-kindled rats. The intraperitoneal injection of imipramine and amitriptyline resulted in potent behavioral and electroencephalogram (EEG)-detected seizures in amygdala-kindled rats compared with those seen in sham rats (non-kindled rats). Almost the same findings were observed with clomipramine and maprotiline. On the other hand, paroxetine caused no or little behavioral or EEG seizures in either amygdala-kindled or sham rats, even at a dose of 50 mg/kg. In conclusion, our results indicate that epileptiformic activity induced by kindling increases the susceptibility to cyclic antidepressant-induced seizures.

Keywords: Epileptiformic activity; Antidepressant; Amygdala kindling; (Rat)

1. Introduction

Depression is the most common psychiatric disorder in patients with epilepsy. Functional abnormalities in patients with epilepsy may be responsible for severe depressive symptoms (Gilliam and Kanner, 2002; Lambert and Robertson, 1999). The rate of suicide in patients with epilepsy is significantly higher than that in the general population, and a high risk was observed especially in patients with temporal lobe epilepsy (Barraclough, 1987; Jones et al., 2003). Therefore, antidepressants were used to treat patients with epilepsy and to reduce the risk for suicide (Gilliam and Kanner, 2002; Jones et al., 2003).

It is well recognized that the tricyclic and tetracyclic antidepressants have convulsant activity at high doses. In fact, tonic–clonic convulsions or generalized seizures occurred in both patients and animals treated with imipramine, amitriptyline, clomipramine and maprotiline (Amabeoku, 1993; Koella et al., 1979; Krijzer et al., 1984; Rosenstein et al., 1993; Trimble et al., 1977). In addition, the incidence of antidepressant-related seizures is higher in patients with a history of previous seizures or family history of a seizure disorder (Rosenstein et al., 1993). Therefore, it is generally believed that seizure risk in patients with epilepsy is increased by antidepressant administration compared with the general population.

The amygdala-kindling model in animals is widely accepted as the model for human temporal lobe epilepsy (Racine, 1978). In the amygdala-kindling studies, it has been reported that the pretreatment with low doses of tricyclic antidepressants decreased the behavioral seizure or afterdischarge induced by electric stimulation of the rat amygdala (Knobloch et al., 1982; Nakamura et al., 1993; Yacobi and Burnham, 1991). However, little work has been done to study whether or not high doses of antidepressant drugs caused an increase in epileptiformic activity in amygdala-kindled rats. Therefore, the present study was undertaken to investigate the spontaneous epileptiformic activity induced by antidepressants using amygdala-kindled rats.

2. Materials and methods

2.1. Animals

Male Wistar rats, 7–8 weeks old and weighting 200–250 g, were used (Nippon SLC, Shizuoka, Japan). All animals were maintained in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). They were group housed for five rats each in aluminum cage with sawdust and

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Table 1
Scoring system used for estimation of seizure intensity

Score	Behavior	EEG
0	No convulsion	No change
1	Twitching	Spike
2	Generalized myoclonus	Spikes frequently occurred
3	Generalized convulsions of short (<15 s) duration	Spike and wave complex of short (<15 s) duration
4	Generalized convulsions of long (>15 s) duration	Spike and wave complex of long (>15 s) duration

kept under a light/dark cycle with lights on from 7:00 to 19:00. The animals were given food and water ad libitum. Eight rats for each group were tested. Drug tests were repeated four times in the same animal, therefore, 80 rats were used altogether in this study. When the tests were repeated in the same animal, the drugs were administered at intervals of at least 7 days.

2.2. Surgery

Under pentobarbital anesthesia (35 mg/kg, i.p., Nembutal, Abbot Laboratories, North Chicago, IL, U.S.A.), the rats were fixed to a stereotaxic apparatus (Narishige, SR-5, Tokyo, Japan), and bipolar electrodes were implanted into the right amygdala (A: 5.0, L: 5.0, H: −2.5), and monopolar electrodes were implanted into the frontal cortex (A: 6.9, L: ±5.0), hippocampus (A: 3.0, L: ±2.5, H: 2.5) and left amygdala (A: 5.0, L: −5.0, H: −2.5) according to the atlas of De Groot (1959). A reference electrode was implanted into the occipital part of the cranium. The bipolar electrodes were twisted stainless steel wires 200 μm in diameter. Electrodes were connected to a miniature receptacle, which was embedded in the skull with dental cement. At least 2 weeks were allowed for recovery from the surgery.

2.3. Experimental procedures in kindled seizures

The procedure to cause kindled seizures was similar to that described previously (Kamei et al., 1998). The animals were placed in a plastic cage (20×35×25 cm). Monopolar electroencephalograms (EEG) were recorded with the electroencephalograph (EEG-7314; Nihon Kohden, Tokyo, Japan). Bipolar stimulation of the amygdala was applied everyday with a constant electric stimulator (SEN-3301, SS-102J; Nihon Kohden, Tokyo, Japan) and continued until a generalized convulsion was obtained. Stimulation parameters were monophasic square pulses with a pulse duration of

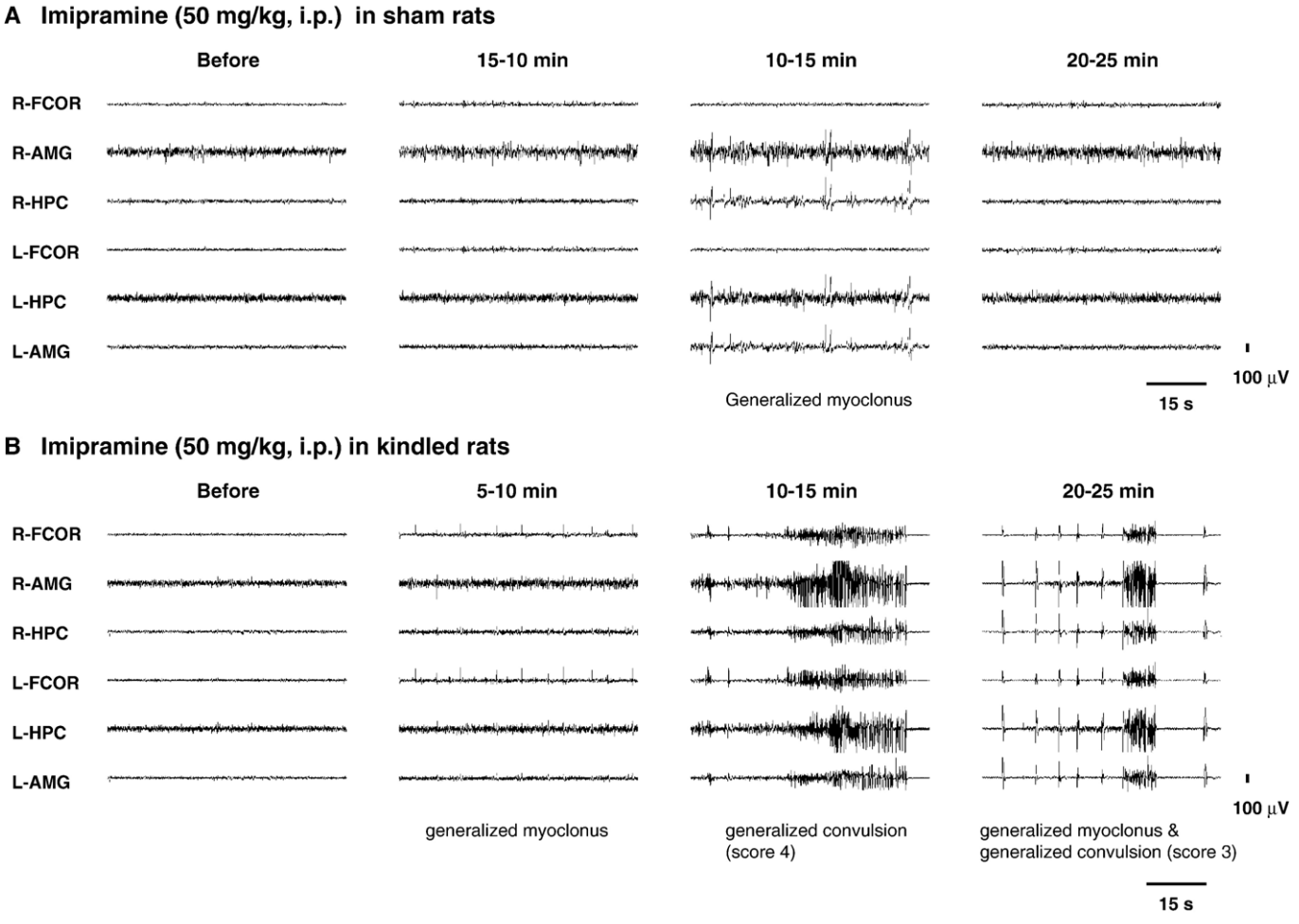

Fig. 1. Representative example of behavioral and EEG seizure patterns induced by imipramine (50 mg/kg, i.p.). Abbreviations: R-FCOR, right frontal cortex; R-HPC, right hippocampus; R-AMG, right amygdala; L-FCOR, left frontal cortex; L-HPC, left hippocampus; L-AMG, left amygdala.

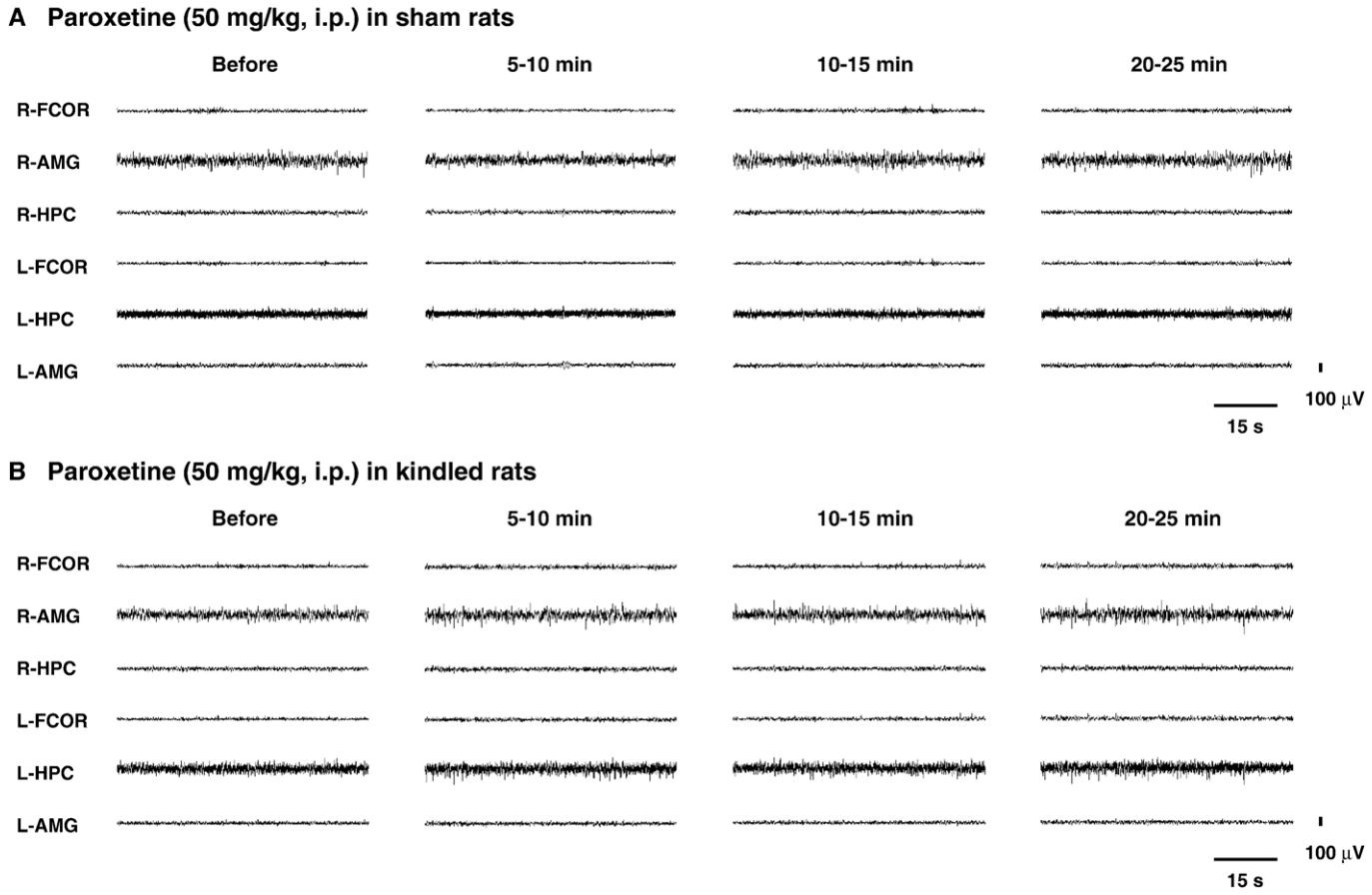

Fig. 2. Representative example of behavioral and EEG seizure patterns induced by paroxetine (50 mg/kg, i.p.).

1.0 ms, a frequency of 60 Hz, and a train duration of 1.0 s. The current intensity was just sufficient to induce the after-discharge (AD, 100–300 μA). The convulsive behavior was divided into 5 stages, 1) jaw movement, 2) head nodding, 3) forelimb clonus, 4) kangaroo posture, and 5) kangaroo posture and falling back (Kamei et al., 1998).

After the animals developed the final stage of generalized seizures (kangaroo posture and falling back), stimulation was repeated for 5 more days to establish completely kindled rats.

On the other hand, the sham rats (non-kindled rats) were also placed in a plastic cage, and monopolar EEG were recorded with the electroencephalograph everyday. Experimental procedures were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

2.4. Evaluation of epileptiformic intensity induced by antidepressants

The intensity of epileptiformic symptoms induced by antidepressants was evaluated using the scoring system shown in Table 1. At least 7 days were allowed between establishing the amygdala-kindled rats and the beginning of antidepressant administration.

The animals were continuously observed for alterations in behavior and EEG for 30 min after intraperitoneal injection. For the comparative evaluation of drug effects in kindled and sham rats, the behavioral and EEG alterations determined at 0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 min after drug administration were scored and each score was summed over the six observation periods.

2.5. Drugs

The drugs were imipramine hydrochloride (Wako, Osaka, Japan), amitriptyline hydrochloride (Sigma, St. Louis, MO, U.S.A.), clomipramine hydrochloride (Sigma), maprotiline hydrochloride (Sigma) and paroxetine hydrochloride hemihydrate (GlaxoSmithKline, Uxbridge, UK). All drugs except paroxetine were dissolved in saline, whereas paroxetine was suspended in 1% Tween 80. They were injected intraperitoneally at a volume of 5 ml/kg of body weight. Doses of the drug were expressed in terms of free base.

2.6. Histological experiments

After the experiments, the animals were killed, and the localization of the electrodes in the brain was verified histologically. In this experiment, localization of the electrodes in

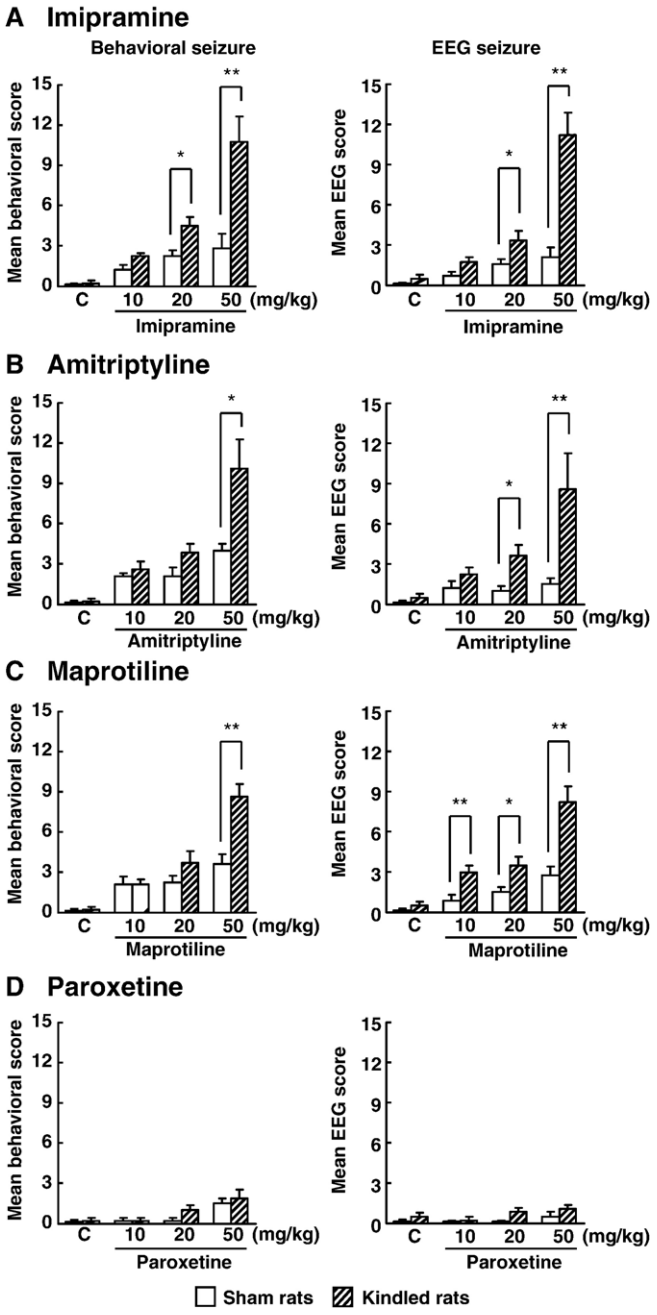

Fig. 3. Comparison of the epileptiformic activity induced by intraperitoneal injection of antidepressants in sham rats and kindled rats. The behavioral and EEG alterations determined at 0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 min after drug administration were scored and each score was summed. Each value represents the mean±S.E.M. of eight rats. Open column: sham rats. Closed column: kindled rats. *, **: Significantly different from the sham group ($P<0.05$ and $P<0.01$, respectively).

the brain was checked under the microscope and reconstructed according to the atlas of De Groot (1959).

2.7. Statistical analysis

All data are expressed as the mean±S.E.M. The Mann–Whitney *U*-test was used for assessing significant dose effects on both behavioral and EEG seizures. The chi-squared test was used for the incidence of generalized seizure.

3. Results

3.1. Characteristics of the behavioral and EEG seizures induced by antidepressants

The spontaneous epileptiformic activities induced by imipramine in sham and kindled rats are shown in Fig. 1. At a dose of 50 mg/kg, imipramine showed a generalized myoclonus, together with high voltage EEG spikes in the bilateral hippocampus and amygdala in the sham rat 10–15 min after injection (Fig. 1A). In the kindled rat, generalized convulsion (score 4) was observed followed by generalized myoclonus 10–15 min after imipramine administration (Fig. 1B). At this time, a high frequency spike and wave complex of a long duration (about 30–40 s) was observed. The generalized convulsions with the high frequency spike and wave complex of a long duration were observed one or two times for each rat. Thereafter, the generalized myoclonus and convulsions (score 3) were observed together with spiking activity or a high frequency spike and wave complex, 20–25 min after imipramine administration. Almost identical behavioral and EEG changes were observed with amitriptyline and maprotiline at a dose of 50 mg/kg (data not shown). The effect of paroxetine in sham and kindled rats is shown in Fig. 2. Paroxetine caused no observable changes in either behavior or EEG activity in both sham (Fig. 2A) and kindled rats (Fig. 2B), even at a dose of 50 mg/kg.

3.2. Comparison of the epileptiformic seizures induced by antidepressants in sham and kindled rats

Imipramine caused behavioral seizure dose-dependently in sham and kindled rats (Fig. 3A). However, behavioral seizure in

Table 2
Development of generalized convulsions induced by antidepressants in sham rats and kindled rats

Drugs (mg/kg, i.p.)		No. generalized convulsion/no. tested	
		Sham rats	Kindled rats
Imipramine	10	0/8	0/8
	20	0/8	0/8
	50	0/8	4/8[a]
Amitriptyline	10	0/8	0/8
	20	0/8	0/8
	50	0/8	4/8[a]
Clomipramine	10	0/8	0/8
	20	0/8	0/8
	50	0/8	0/8
Maprotiline	10	0/8	0/8
	20	0/8	0/8
	50	0/8	2/8
Paroxetine	10	0/8	0/8
	20	0/8	0/8
	50	0/8	0/8

The 'number of animals showing generalized convulsion (score 4)/number of animals tested' are represented. [a]: $P<0.05$ as compared with sham rats.

kindled rats was more potent than that in sham rats, and a significant difference was observed at doses of 20 and 50 mg/kg. Almost the same findings were observed with EEG seizure; a significant difference between the sham rats and kindled rats was observed with the doses of 20 and 50 mg/kg. Amitriptyline also caused behavioral seizures in both group of rats, and behavioral seizures in kindled rats were more potent than in sham rats. The significant difference was observed at the dose of 50 mg/kg (Fig. 3B). In EEG seizure, a significant difference between sham and kindled rats was observed at doses of 20 and 50 mg/kg (Fig. 3B). The same findings were observed with clomipramine (data not shown). Maprotiline at a dose of 50 mg/kg also caused a more potent behavioral seizure in kindled rats than in sham rats. In EEG activity, maprotiline caused a potent EEG seizure in kindled rats, and a significant difference was observed between kindled rats and sham rats, even at a dose of 10 mg/kg (Fig. 3C). Paroxetine caused slight behavioral and EEG abnormalities in both the sham and kindled rats. However, no significant difference was observed between the sham and kindled rats in either the behavior or EEG activity, even at a dose of 50 mg/kg (Fig. 3D).

3.3. Development of generalized convulsions induced by antidepressants in sham rats and kindled rats

None of the antidepressants used in this study showed generalized convulsions in sham rats, even at a dose of 50 mg/kg. On the other hand, in kindled rats, imipramine, amitriptyline and maprotiline caused generalized convulsions at a dose of 50 mg/kg. However, clomipramine and paroxetine caused no generalized convulsions in kindled rats at a dose of 50 mg/kg (Table 2).

4. Discussion

In the present study, it was confirmed that tricyclic and tetracyclic antidepressants (cyclic antidepressants) caused spontaneous epileptiformic activity characterized by a spike and twitching in sham rats. Almost the same findings were observed by Koella et al. (1979) and Amabeoku (1993) in cats and mice, respectively. In addition, we also found that the cyclic antidepressants used in the present study caused a potent epileptiformic activity in the amygdala-kindled rats compared with sham rats. The animals having an epileptiformic property, like baboons with photosensitive epilepsy (*Papio papio*), showed a lowered seizure threshold with imipramine, clomipramine and maprotiline (Trimble et al., 1977). Clinically, it was also demonstrated that antidepressant-related seizures have a high incidence in patients with a history of previous seizures or a family history of seizure disorders (Rosenstein et al., 1993). These findings clearly indicate that cyclic antidepressants are liable to produce epileptiformic activity in both humans and animals having an epilepsy prone property.

As shown in the present study, although the cyclic antidepressants caused epileptiformic activity, there are some differences in the potency among these drugs. The potency of these drugs in their epileptiformic activity, from greatest to least, was as follows: imipramine, amitriptyline, maprotiline and clomipramine. It is well known that histamine H_1 receptor antagonists produced convulsions both clinically (Wyngaarden and Seevers, 1951; Yokoyama et al., 1993) and in animal studies (Kamei et al., 2000), and particularly first-generation histamine H_1 receptor antagonists having a higher affinity for histamine H_1 receptors, had more potent convulsive activity (Kamei et al., 2000). Cyclic antidepressants antagonize the several types of neurotransmitter receptors, especially histamine H_1 receptors (Richelson and Nelson, 1984). In fact, imipramine, amitriptyline and maprotiline had a higher affinity for histamine H_1 receptors compared with the classic antihistamine, d-chlorpheniramine (Richelson and Nelson, 1984). In Richelson and Nelson's (1984) study, it was also reported that clomipramine showed a lower histamine H_1 receptor binding activity compared with the above three antidepressants. From these results, it is reasonable to presume that the epileptiformic activity induced by cyclic antidepressants is mainly due to their histamine H_1 receptor antagonistic activity. On the other hand, Amabeoku (1993) reported that central noradrenergic and cholinergic neurotransmission might be involved in imipramine-induced seizures in mice. Besides, Malatynska et al. (1988) described that an inhibition of the GABA-receptor chloride uptake by some antidepressants (imipramine, amitriptyline and mianserine) correlated with the frequency of convulsive seizures in rats. From these findings, the noradrenergic, cholinergic and GABAergic innervations were considered as the other possible mechanisms of antidepressant action beside histamine H_1 receptor antagonistic activity.

In contrast, the results of the present study confirmed that paroxetine, a specific blocker of the reuptake of serotonin, showed a lower epileptiformic activity compared with the other cyclic antidepressants. Paroxetine has been shown not to affect the seizure frequency or the EEG in human reports (Andersen et al., 1991; Boyer and Blumhardt, 1992; Sedgewick et al., 1987). Similar findings were also reported by Krijzer et al. (1984) that fluvoxamine hardly induced any epileptiformic signs in rats. It has been reported that the specific blockers of the reuptake of serotonin, such as fluvoxamine and fluoxetine, have the extremely weak affinity for histamine H_1 receptor and muscarinic acetylcholine receptor (Kanba and Richelson, 1986).

As shown in the present study, imipramine caused both behavioral and EEG seizures to the same extent. On the other hand, EEG seizure was induced by maprotiline, amitriptyline and clomipramine more potently than behavioral seizure. These findings suggest that the intensity of behavioral and EEG seizures induced by these drugs is not always the same, though the detailed mechanism is obscure. In the present study, worthy of special mention is that generalized convulsions were observed with imipramine, amitriptyline and maprotiline in high doses.

From these findings, therefore, it is concluded that particular attention should be given to cyclic antidepressant therapy in epileptiformic patients.

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