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Effect of dipotassium clorazepate on amygdaloid-kindling and comparison between amygdaloid- and hippocampal-kindled seizures in rats

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Abstract

We examined the effect of dipotassium clorazepate (7-chloro-1,3-dihydro-2-oxo-5-phenyl-1*H*-1, 4-benzodiazepine-3-carboxylate potassium hydroxide), an antianxiety drug, on amygdaloid kindling and compared its effects for 7 successive days on amygdaloid- versus hippocampal-kindled seizures, using the rat kindling model of epilepsy. Dipotassium clorazepate at 5 mg/kg significantly delayed amygdaloid kindling. The contralateral cortical after-discharge duration in the dipotassium clorazepate-treated group was significantly shorter than the after-discharge duration in the amygdala in the first seven stimulations, whereas it was significantly shorter only in the first three stimulations in the control group, indicating that dipotassium clorazepate suppressed the spread of seizure activity from focus to contralateral cortex. Dipotassium clorazepate suppressed amygdaloid-kindled seizures at 2 and 5 mg/kg, while 1 mg/kg or more suppressed hippocampal-kindled seizures. Thus, differences in effective dosages in both amygdaloid- and hippocampal-kindled seizures may suggest a difference in the neuronal mechanisms involved in this kindling.

Keywords: Dipotassium clorazepate; Anticonvulsive; Amygdaloid; Hippocampal; Kindling

1. Introduction

Dipotassium clorazepate (potassium 7-chloro-2,3-dihydro-2-oxo-5-phenyl-1*H*-1, 4-benzodiazepine-3-carboxylate potassium hydroxide) was developed in the mid-1960s as an antianxiety drug. It is a carboxylated form of desmethyldiazepam, which is considered to be the primary active metabolite of diazepam (Mattson., 1972). In a kindling model, the benzodiazepines suppressed amygdaloid-kindled seizures (Aston and Wauquier, 1979; Albertson et al., 1980). Dipotassium clorazepate exerted an anticonvulsive effect on both pentylenetetrazol-induced seizures and in electroshock tests (Brunaud et al., 1970; Plotnikoff and Obrien, 1974). We reported the anticonvulsive effect of this drug on hippocampal-kindled seizures in rats (Amano et al., 1998). Its effect on kindling, however, has not been evaluated. Moreover, differences in the anticonvulsive effects of conventional anticonvulsants between amygdaloid- and hippocampal-kindled seizures have been reported (Kamei et al., 1981; Ichimaru et al., 1987; Ishida et al., 1992). We evaluated the anticonvulsive effect of dipotassium clorazepate on kindling, and compared it in amygdaloid- and hippocampal-kindled seizures in rats.

2. Materials and methods

2.1. Surgery and kindling

Male Wistar rats (supplied by Kyusyu Animal, Kumamoto, Japan) weighing 220–330 g were used. They were allowed unlimited access to food and water. They were 10 weeks of age at the time of the operation.

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Following the rat brain atlas of Paxinos and Watson (1986), a tripolar electrode was implanted stereotaxically into the left basolateral amygdala (anterior, 3 mm; lateral, 4.5 mm; vertical, 8.5 mm) or the left dorsal hippocampus (anterior, 2.8 mm; lateral, 2.0 mm; vertical, 3 mm) under pentobarbital anesthesia (Nembutal® 50 mg/kg). Screw electrodes were placed in the bilateral frontal scalp, the right upper orbital scalp, and the posterior scalp 3.0 mm dorsal to the lambda suture for electroencephalogram (EEG) recording at the motor cortex, to ground the subject's body, and as a reference for the EEG recording, respectively.

One week after the operation, the rats were subjected to kindling. Bipolar stimulation consisting of 60 Hz sine waves lasting 1 s was delivered through a constant current stimulator (NIPPON KOHDEN S-6207 A). The stimulation was begun at 10 μA and then increased stepwise by 10 μA with 10-min interstimulus intervals until an after-discharge was induced. No more than four stimulations were given in a day. The stimulus current at the lowest intensity triggering after-discharge was regarded as the after-discharge threshold. Thereafter, the after-discharge threshold current was kept constant and delivered once a day.

Kindled seizures were classified, essentially in accordance with the classification of Racine (1972), into six stages: stage 0, after-discharge without motor manifestations; stage 1, facial twitching; stage 2, head-nodding accompanied by simultaneous mastication; stage 3, forelimb clonic convulsion; stage 4, rearing on the hindlegs; and stage 5, full-blown generalized clonic convulsion and falling-down. After five consecutive stage 5 seizures were completed, the stimulus current was reduced by half until the rats failed to respond with a stage 5 seizure. Subsequently, the stimulus current was raised stepwise by 10 μA until a stage 5 seizure was induced. The stimulus current inducing the seizure was regarded as the generalized seizure-triggering threshold. The generalized seizure-triggering threshold was redetermined before each treatment session. Electrical stimulation was delivered 30 min after each injection of dipotassium clorazepate, and seizure and EEG alterations were evaluated. When stage 5 seizures regressed to partial seizures (stages 0, 1, and 2) after the injection of dipotassium clorazepate, the trial was regarded as effective. When secondarily generalized seizures (stages 3, 4, and 5) were provoked, the trial was regarded as ineffective.

2.2. Pharmacological experiments

Dipotassium clorazepate (supplied by Dainippon Pharmaceutical, Osaka, Japan) was dissolved in physiological saline for intraperitoneal injection. Physiological saline was administered as the control vehicle.

2.3. Evaluation of anticonvulsive effects

2.3.1. Experiment I: anticonvulsive effect on amygdaloid-kindled seizures

To clarify the anticonvulsive effect of dipotassium clorazepate against amygdaloid-kindled seizures, rats having two stable stage 5 seizures induced at the generalized seizure-triggering threshold current intensity were tested once a day. The dosages of dipotassium clorazepate were

Table 1
Effects of single administration of dipotassium clorazepate on amygdaloid-kindled seizures

		Stages			Inhibition of generalization (%)	After-discharge duration (% of baseline)
		0	1, 2	5		
Control		0	0	7	0	87.2
Dipotassium clorazepate	0.5	0	1	6	14.3	69.1
	1.0	0	3	4	42.9	48.1[a]
	2.0	0	6	1[b]	85.7	35.1[a]
	5.0	1	5	1[b]	85.7	29.1[b]

After-discharge duration is divided by the average of after-discharge duration before the treatment with dipotassium clorazepate and multiplied by 100.
[a] $P < 0.05$, [b] $P < 0.01$, with Fisher's exact probability test or Wilcoxon's signed rank test.

set at 0.5, 1.0, 2.0, and 5.0 mg/kg in a volume of 1.0 ml/kg. Drug administrations in the same rat were separated by an interval of at least 2 weeks.

2.3.2. Experiment II: effect on amygdaloid kindling

Stimulation of the amygdala was applied once a day after an initial after-discharge threshold determination. The rats were stimulated 30 min after the administration of dipotassium clorazepate. The stimulus intensity was kept at the after-discharge threshold throughout the experiment. Dipotassium clorazepate was administered once a day until the first stage 5 seizure occurred or 15 stimulations had been applied after the initial after-discharge threshold determination. The dosage of dipotassium clorazepate was 5 mg/kg; this dosage was determined based on the result of experiment I. The seizure stage and after-discharge duration were analyzed in comparison with those in the control (vehicle treatment) group.

2.3.3. Experiment III: comparison of anticonvulsive effect between amygdaloid- and hippocampal-kindled seizures in rats

Other groups of rats having more than 10 stage 5 seizures induced at the generalized seizure-triggering threshold current intensity were used and tested once a day for 7 consecutive days. The dosages and the schedule of administration were the same as in experiment I. Drug efficacy was evaluated separately on each test day from day 1 to day 7.

2.4. Adverse effects

During each drug session, behavioral changes in rats were observed. Adverse behavioral effects included (a) sedation, (b) ataxia, and (c) muscle weakness, and each effect was quantified with a rating scale, as follows: grade 0, none; grade 1, mild; grade 2, moderate; grade 3, severe. Total scores for adverse effects were also determined for each rat and each dose. In addition, responses to handling were recorded, with special attention to ataxia and sedation. Muscle weakness was examined by palpation of the neck and limb muscles. Sedation was accepted when the rat was flaccid and did not show increased muscle tone as a result of being picked up in the hand.

2.5. Measurement of serum desmethyldiazepam concentration and histology

Venous blood was collected immediately after the kindled convulsions. The serum samples, separated quickly from the collected whole blood, were kept at −20°C. The serum desmethyldiazepam concentration was determined by high-performance liquid chromatography (HPLC) (Dainippon Pharmaceutical). Added to 0.5 ml of the serum was 2 ml of 1 N KOH and 8 ml of $CHCL_3$. The mixture was shaken for 10 min and then centrifuged at 2500 rpm for 10 min. The organic layer was evaporated to dryness at 40°C. The residue was dissolved with 0.2 ml of internal standard (IS) solution (2 mg of diazepam in MeOH), and 10 ml of the solution was injected for HPLC. HPLC was performed with a Shimadzu LC-6A using a Shimadzu STR ODS-M column, 150 × 4.6 mm i.d. Conditions were: mobile phase, 1% AcOH: i-PrOH: CH_3CN (20:5:10); flow rate, 1.0 ml/min; and detector, UV 230 nm. In the 7 consecutive trials, venous blood was collected on test day 7 and on other test days when a seizure stage was altered.

After the completion of each experiment, each rat was given an overdose of sodium pentobarbital, and the brain was perfused with saline followed by 10% formalin. The location of each implanted electrode was histologically verified. All electrodes were confirmed to be located in the amygdala and hippocampus.

2.6. Statistical analysis

Statistical comparisons were made with Student's *t*-test to evaluate the after-discharge duration and Fisher's exact

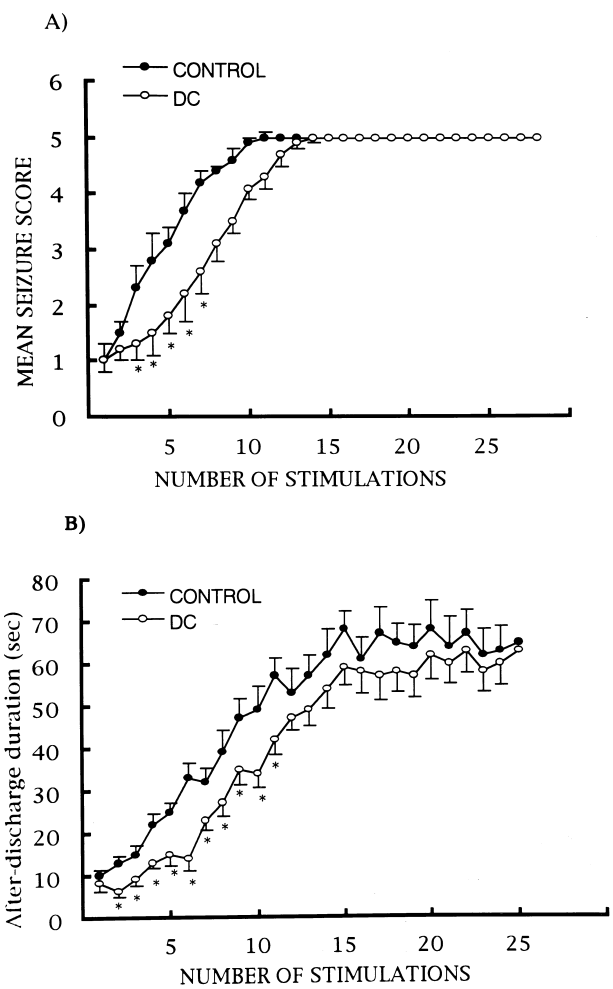

Fig. 1. Pretreatment with dipotassium clorazepate (5 mg; open circles) significantly retarded kindling. Each point represents the mean ± S.D. * $P < 0.05$ with Student's *t*-test compared with control group (closed circles).

Table 2
A comparison of after-discharge duration between the contralateral cortex and the stimulated amygdala

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
(A) Dipotassium clorazepate-treated group							
Contralateral cortex	2.1 ± 0.6^a	1.9 ± 0.8^a	2.3 ± 1.0^a	5.5 ± 0.9^a	9.1 ± 1.5^a	8.5 ± 1.3^a	11.4 ± 2.1^a
Stimulated amygdala	8.9 ± 0.8	7.5 ± 0.5	9.3 ± 0.7	12.2 ± 1.2	14.5 ± 1.0	13.5 ± 2.2	22.2 ± 1.7
(B) Control group							
Contralateral cortex	3.5 ± 0.5^a	6 ± 0.4^a	8.5 ± 0.5^a	17.2 ± 1.7	20.3 ± 0.8	28.3 ± 1.4	29.3 ± 1.7
Stimulated amygdala	10 ± 0.41	2.2 ± 0.61	5.1 ± 0.7	21.3 ± 1.2	23.2 ± 1.1	32.2 ± 1.0	30.5 ± 2.2

[a] $P < 0.05$ with Student's *t*-test compared with after-discharge duration in the stimulated amygdala.

probability test to analyze the regression of seizure stage. The ED_{50} value, the calculated dose required to block the amygdaloid kindled seizure in 50% of the rats tested, was determined by the method of Litchfield and Wilcoxon (1948).

3. Results

3.1. Experiment I: anticonvulsive effect on amygdaloid-kindled seizures

Dipotassium clorazepate suppressed amygdaloid-kindled seizures in a dose-dependent manner (Table 1). Regression of seizures to stage 3 or 4 was not observed. Regression to stage 1 or 2 seizures was observed in 14 sessions (50%), and complete clinical seizure suppression occurred in one session (3.6%) at 5 mg/kg. The ED_{50} and the confidence limits for 95% probability of secondary generalization were 1.45 and 0.7–2.8 mg/kg, respectively. Significant reduction of after-discharge duration compared with the control group was observed at dosages of 2 mg/kg or more, but complete suppression of after-discharge was observed in only one of seven sessions at the highest dose.

3.2. Experiment II: effect on amygdaloid kindling

Fig. 1 illustrates the effects of dipotassium clorazepate on the seizure stage reached and after-discharge duration in amygdaloid kindling. The control rats developed the first stage 2 seizure after an average of 2.4 ± 0.6 stimulations, whereas the average in rats receiving dipotassium clorazepate at 5 mg/kg was 5.3 ± 0.9 stimulations. The number of stimulations required for the first stage 5 seizure in the 5-mg/kg dosage group was 14.1 ± 1.4 stimulations, which was significantly greater than the 10.2 ± 1.7 stimulations in the control group ($P < 0.01$). The contralateral cortical after-discharge in the group treated with dipotassium clorazepate was significantly shorter than the after-discharge in the amygdala in the first seven stimulations, whereas it was significantly shorter only in the first three stimulations in the control group (Table 2).

3.3. Experiment III: comparison of anticonvulsive effect in amygdaloid- and hippocampal-kindled seizures in rats

When the number of effective trials on a day of treatment was significantly greater than that of the controls (χ^2 test), the drug was considered effective (Table 3). The drug was effective on day 3 at 2 mg/kg of dipotassium clorazepate and on day 4 at 5 mg/kg in the amygdaloid-kindled rats (Table 3). In contrast, the drug was effective on day 4 at 2 mg/kg, and on day 5 at 5 mg/kg in the hippocampal-kindled rats (Table 3). In comparison with that for the controls, the number of days on which the drug was effective was significantly greater at 5 mg/kg of dipotassium clorazepate in the hippocampal-kindled rats (χ^2 test, $P < 0.05$).

Table 3
Number of effective trials of dipotassium clorazepate in amygdaloid-kindled rats and hippocampal-kindled rats
The figure in the column indicates the number of effective trials on each day.

	Amygdaloid-kindled rat Dipotassium clorazepate (mg/kg)					Hippocampal-kindled rat Dipotassium clorazepate (mg/kg)				
	Control	0.5	1	2	5	Control	0.5	1	2	5
Day 1	0	1	2	6^a	6^a	0	1	3	6^a	7^a
Day 2	0	1	2	6^a	6^a	0	0	3	6^a	6^a
Day 3	0	0	2	5^b	5^b	0	1	2	5^b	6^a
Day 4	0	1	1	4	5^b	0	0	3	5^b	5^b
Day 5	0	0	1	2	4	0	1	2	4	5^b
Day 6	0	0	0	2	2	0	1	2	3	4
Day 7	0	0	0	2	2	0	0	1	3	3

[a] $P < 0.01$.
[b] $P < 0.05$.

3.4. Adverse effects

Compared with their body weight before the experiment, the control rats gained 5.8%, but the dipotassium clorazepate-treated group gained 4.4% by day 7 of dipotassium clorazepate administration. No significant body weight alterations were observed during the present experiment between the dipotassium clorazepate-treated group and the control group. The 5-mg/kg dose of dipotassium clorazepate produced sedation 30 min after each daily administration. The mean score for sedation on day 1 and day 7 in the dipotassium clorazepate-treated rats was 2.2 ± 0.4 (severe, 3/15 rats; moderate, 12/15 rats) and 1.8 ± 0.6 (severe, 1/15 rats; moderate, 10/15 rats; mild, 4/15 rats), respectively. As for ataxia and muscle weakness, the mean scores on day 1 were 0.4 ± 0.5 (mild, 6/15 rats) and 0.5 ± 0.2 (mild, 8/15 rats), respectively. On day 7, these values were 0.2 ± 0.4 (mild; 2/15 rats) and 0.3 ± 0.5 (mild; 4/15 rats), respectively.

3.5. Serum concentration

The serum desmethyldiazepam concentration in the amygdaloid-kindled rats that were given dipotassium clorazepate at 0.5, 1.0, 2.0, and 5.0 mg/kg doses were 0.17 ± 0.03, 0.35 ± 0.06, 0.48 ± 0.13, and 1.46 ± 0.43 μg/ml, respectively. The serum concentration of the dose for the ED_{50} for secondary generalization was 0.41 μg/ml. The increase in concentrations seemed to be dose-dependent. With seven consecutive administrations of dipotassium clorazepate, the serum desmethyldiazepam concentration in the amygdaloid-kindled rats at the 0.5, 1.0, 2.0, and 5.0 mg/kg dosages were 0.16 ± 0.05, 0.39 ± 0.04, 0.77 ± 0.07, and 1.22 ± 0.55 μg/ml, respectively, on the last day. The serum desmethyldiazepam concentration in the hippocampal-kindled rats at the 0.5, 1.0, 2.0, and 5.0 mg/kg dosages were 0.13 ± 0.03, 0.38 ± 0.10, 0.82 ± 0.22, and 1.41 ± 0.38 μg/ml, respectively, on the last day. No significant differences in serum desmethyldiazepam concentrations between the amygdaloid- and hippocampal-kindled rats were observed. There were no significant differences between the effective and the ineffective trials in either amygdaloid- and hippocampal-kindled rats (Table 4).

Table 4
Serum concentration of dipotassium clorazepate in amygdaloid-kindled rats and hippocampal-kindled rats
Values are expressed as means ± standard deviation

Serum concentration of dipotassium clorazepate (μg/ml)		
Dose	Effective trials	Ineffective trials
Amygdaloid-kindled rats		
2.0 mg/kg	0.62 ± 0.14	0.69 ± 0.19
5.0 mg/kg	1.12 ± 0.63	1.3 ± 0.49
Hippocampal-kindled rats		
1.0 mg/kg	0.33 ± 0.08	0.48 ± 0.11
2.0 mg/kg	0.72 ± 0.04	0.83 ± 0.17
5.0 mg/kg	1.35 ± 0.29	1.42 ± 0.37

4. Discussion

The present study demonstrated that dipotassium clorazepate has modest anticonvulsive effects, i.e., it retards development of amygdaloid kindling. The contralateral cortical after-discharge was significantly shorter than the amygdaloid after-discharge in the dipotassium clorazepate-treated group in the first seven stimulations, whereas the former was significantly shorter than the latter only in the first three stimulations in the control group. Wise and Chinerman (1974) emphasized that the inhibition of motor seizure is due to inhibition of after-discharge occurrence or to suppression of after-discharge propagation. In the present study, perfect suppression of after-discharge at the stimulated site was observed in only one of seven rats treated with a dosage of 5 mg/kg. We previously reported that increased stimulus intensity could not completely reverse the anticonvulsive effects of dipotassium clorazepate (Amano et al., 1998). Accordingly, the principal anticonvulsive effect of dipotassium clorazepate is likely to be related mainly to attenuation of propagation of seizure activity rather than to an elevated seizure threshold.

In the present study, dipotassium clorazepate showed suppressive effects in amygdaloid- and hippocampal-kindled seizures. More potent suppressive effects of dipotassium clorazepate, however, were observed in hippocampal-kindled rats than in amygdaloid-kindled rats. This inter-model difference was found for the efficacy of other anticonvulsants such as zonisamide and clobazam. (Kamei et al., 1981; Ichimaru et al., 1987). These differences may indicate differences in neuronal mechanisms involved in amygdaloid- and hippocampal-kindling. Sato (1975) reported that facilitation of amygdaloid kindling following hippocampal kindling was observed, but not the opposite. Racine et al. (1988) demonstrated that cuts that were surgically placed to disrupt the ventral amygdala-fugal pathway had no effect on amygdaloid kindling. Therefore, these results suggest a difference in the neuronal mechanisms involved in amygdaloid and hippocampal kindling, which may support our speculation.

Diazepam, another benzodiazepine derivative, showed an anticonvulsive effect against an amygdaloid kindling model (Wise and Chinerman, 1974; Racine et al., 1975; Löscher and Schwark, 1985). However, it did not necessarily suppress after-discharge duration at the stimulated site with the amygdaloid-kindled seizures (Morita et al., 1982). In the present study, dipotassium clorazepate significantly suppressed the after-discharge duration in a dose-dependent manner. In pentylenetetrazol-induced seizures in rhe-

sus monkeys, dipotassium clorazepate showed more stable anticonvulsive effects than did diazepam (Plotnikoff and Obrien, 1974). The development of tolerance to the anticonvulsants limits the clinical usefulness of benzodiazepines. A slower onset and lack of clear tolerance were found after treatment with dipotassium clorazepate compared to diazepam (Frey et al., 1984; Scherkl et al., 1989). Thus, we speculate that dipotassium clorazepate may have a more consistent anticonvulsive effect and be more useful because of the slower onset of tolerance compared to that with diazepam. Further study may be needed to compare the anticonvulsive effects of, and tolerance to, these two drugs in the kindling model.

The effective serum concentrations of conventional antiepileptic drugs reported for amygdaloid-kindled animals are almost identical to the therapeutic serum concentrations in humans (Wada et al., 1976; Hamada et al., 1990). In humans, there is no direct relationship between serum desmethyldiazepam concentration and clinical response (Booker, 1974; Mimaki et al., 1984). Frey and Scherkl (1988) proposed that the anticonvulsant effect of dipotassium clorazepate is promoted by desmethyldiazepam, since dipotassium clorazepate is devoid of a central nervous system pharmacological effect. In the present study, dipotassium clorazepate suppressed seizure activity in a dose-dependent manner with increases in the concentration of desmethyldiazepam, which supports the results of Frey and Scherkl (1988).

One study showed no adverse effects (Troupin et al., 1977), while other studies found sleepiness or sedation during treatment with dipotassium clorazepate in humans (Booker., 1974; Mimaki et al., 1984). In the present study, sedation was observed in the 5-mg/kg dosage group, but adverse effects of dipotassium clorazepate on the motor systems were slight.

The present study demonstrated that dipotassium clorazepate has a modest anticonvulsive effect without serious adverse effects, which indicates the clinical usefulness of dipotassium clorazepate for treatment of intractable epilepsy.

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References

Albertson, T.E., Peterson, S.L., Stark, L.G., 1980. Anticonvulsant drugs and their antagonism of kindled amygdaloid seizures in rats. Neuropharmacology 19, 643–652.

Amano, K., Takamatsu, J., Kaneyama, H., Miyazaki, C., Deshimaru, M., Sumiyoshi, S., Ogata, A., Miyakawa, T., 1998. Anticonvulsant effects of dipotassium clorazepate on hippocampal kindled seizures in rats. Psychiatry Clin. Neurosci. 52, 459–462.

Aston, D., Wauquier, A., 1979. Behavioral analysis of the effects of 15 anticonvulsants in the amygdaloid kindled rat. Psychopharmacology 65, 7–13.

Booker, H.E., 1974. Clorazepate dipotassium in the treatment of intractable epilepsy. JAMA 229, 552–555.

Brunaud, M., Navarro, J., Salle, J., Siou, G., 1970. Pharmacological, toxicological, and tetratological studies on dipotassium-7-chloro-3-carboxy-1,3-dihydro-2,2-dihydroxy-5-phenyl-2*H*-1,4-benzodiazepine-clorazepate (dipotassium clorazepate, 4306 CB), a new tranquilizer. Arzneim. Forsch. 20, 123–124.

Frey, H.H., Scherkl, R., 1988. Clorazepate, correlation between metabolism and anticonvulsant activity. Eur. J. Pharmacol. 158, 213–216.

Frey, H.H., Philippin, H.P., Scheuler, W., 1984. Development of tolerance to the anticonvulsant effect of diazepam in dogs. Eur. J. Pharmacol. 104, 27–38.

Hamada, K., Ishida, S., Yagi, K., Seino, M., 1990. Anticonvulsant effects of zonisamide on amygdaloid kindling in rats. Neurosciences 16, 407–412.

Ichimaru, Y., Gomita, Y., Moriyama, M., 1987. Effects of clobazam on amygdaloid and hippocampal kindled seizures in rats. J. Pharmacol. Biodyn. 10, 189–194.

Ishida, S., Hamada, K., Yagi, K., Seino, M., 1992. Comparing the anticonvulsive effects of dapsone on amygdala-kindled seizures and hippocampal-kindled seizures in rats. Acta Neurol. Scand. 85, 132–135.

Kamei, C., Oka, M., Masuda, Y., Yoshida, K., Shimizu, M., 1981. Effects of 3-sulfamoy-metyl-1,2-benzisoxazole (AD-810) and some antiepileptics on the kindled seizures in the neocortex, hippocampus and amygdala in rats. Arch. Int. Pharmacodyn. Ther. 249, 164–176.

Litchfield, J.T., Wilcoxon, F., 1948. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Löscher, W., Schwark, W.S., 1985. Development of tolerance to the anticonvulsant effect of diazepam in amygdala-kindled rats. Exp. Neurol. 90, 373–384.

Mattson, R.H., 1972. The benzodiazepines. In: Woodbury, D.M. (Ed.), Antiepileptic Drugs. Raven Press, New York, pp. 497–516.

Mimaki, T., Tagawa, T., Ono, J., Tanaka, J., Terada, H., Itoh, N., Yabuuchi, H., 1984. Antiepileptic effect and serum levels of clorazepate on children with refractory seizures. Brain Dev. 6, 539–544.

Morita, Y., Shinkuma, D., Shibagaki, N., Miyoshi, K., 1982. Effect of benzodiazepine derivatives on amygdaloid-kindled convulsion. Folia Psychiatrica et Neurologica 36, 391–400.

Paxinos, G., Watson, C., 1986. The Rat Brain In Stereotaxic Coordinates. 2nd edn. Academic Press, New York.

Plotnikoff, N.P., Obrien, G.S., 1974. Comparison of anticonvulsant effects of clorazepate dipotassium and diazepam: four week anticonvulsant study in Rhesus monkeys. Dis. Nerv. Sys. 35, 87–90.

Racine, R., 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroenceph. Clin. Neurophysiol. 32, 281–294.

Racine, R.J., Livingston, K.E., Joaquin, A., 1975. Effects of procaine hydrochloride, diazepam, and diphenylhydantoin on seizure development in cortical and subcortical structures in rats. Electroenceph. Clin. Neurophysiol. 38, 355–365.

Racine, R.J., Paxinos, G., Mosher, J.M., Kairiss, E.W., 1988. The effects of various leisions and knife-cuts on septal and amygdala kindling in the rat. Brain Res. 454, 264–274.

Sato, M., 1975. An experimental study of epilepsy with kindling effect procedure: II. Study on the secondary epileptogenesis of hippocampal seizure with transference phenomenon. Psychiatr. Neurol. Jpn. 77, 509–522.

Scherkl, R., Kurudi, D., Frey, H.H., 1989. Clorazepate in dogs: tolerance

to the anticonvulsant effect and signs of physical dependence. Epilepsy Res. 3, 144–150.

Troupin, A.S., Friel, P., Wilensky, A.J., Moretti-Ojemann, A., Levy, R.H., Feigl, P., 1977. Evaluation of clorazepate (Tranxene®) as an anticonvulsant — a pilot study. Neurology 29, 458–466.

Wada, J.A., Osawa, M., Wake, A., Corcoran, M.E., Troupin, A.S., 1976. Acute anticonvulsant effects of diphenylhydantoin, phenobarbital, and carbamazepine: a combined electroclinical and serum level study in amygdaloid kindled cats and baboons. Epilepsia 17, 77–88.

Wise, R.A., Chinerman, J., 1974. Effects of diazepam and phenobarbital on electrically-induced amygdaloid seizures and seizure development. Exp. Neurol. 45, 355–363.