ANTI-CONVULSANT EFFECT OF PHTHALAZINO-[2,3b]-PHTHALAZINE-5(14H), 12(7H)-DIONE (L-5418).
II. ELECTROENCEPHALOGRAPHIC STUDY

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Abstract—L-5418 has an anti-convulsant effect which is similar to that of diphenylhydantoin. The effects of L-5418 on EEG activity in rabbits with acute and chronic implantation of electrodes were studied in comparison with those of currently available anti-convulsants. Intravenous administration of L-5418 increased a slow-wave sleep pattern in the spontaneous EEG, which was also induced by diphenylhydantoin. With respect to the focal seizure in the cerebral cortex induced by local application of penicillin, L-5418 showed suppressive effects on the frequency and duration of seizure discharge, and on the spread of seizure discharge to other parts of the brain. The efficacy was about twice that of diphenylhydantoin. L-5418 and diphenylhydantoin did not increase the threshold of seizures induced by bemegride while trimethadione raised the threshold. L-5418 also showed suppressive effects twice as active as diphenylhydantoin on after-discharge induced by electrical stimulation of the hippocampus and amygdala. This suppressive effect on after-discharge of the limbic system may be parallel with the suppressive effect on psychomotor seizure. From these results of L-5418 on an experimental model of epilepsy, it is suggested that L-5418 has suppressive effects similar to that of diphenylhydantoin on convulsion and the efficacy proved to be twice that of diphenylhydantoin in the EEG study.

We reported that L-5418 is useful for grand mal epilepsy with the suppressive effect selectively on tonic convulsions (1, 2). L-5418 has proved to be a drug similar to diphenylhydantoin without hypnotic effect, tranquilizing effect and muscle relaxation. Following the behavioral pharmacological studies concerning influence on the central nervous system, the present investigation was related to the electroencephalographic study of experimental epilepsy in rabbits.

MATERIALS AND METHODS

Test drug, L-5418 was provided by Dow Lepetit (Japan) Limited. Its chemical structure and physical properties have been reported in a previous paper (1). Diphenylhydantoin (Aleviatin, Dainippon Seiyaku, DPH), trimethadione (Minealevitan, Dainippon Seiyaku, TMD) and carbamazepine (Tegretol, Fujisawa Yakuhin Kogyo, CMA) were used as control drugs. Although L-5418 is insoluble in water, it was administered intravenously in a form of suspension in 0.3% tragant gum solution. DPH was diluted with distilled water after dissolution with addition of 1% NaOH solution. TMD was dissolved in water and CMA in 30% propylenglycol.

White male rabbits weighing 2.5 to 3.5 kg were used in all experiments. The electro-
encephalogram was recorded by electroencephalograph recorder (Nihon Kohden, EEG-4109 type 9 ch). The influence of the test drug on the spontaneous EEG activity and various types of seizure discharges in brain were observed. The seizure discharges were induced by intravenous administration of beinegire, local application of penicillin to the surface of the cortex and electrical stimulation of the limbic system. The electrical stimulation was applied by pulse generator (Nihon Kohden MSE-3 type).

*Experiment in animals with chronic implantation of electrodes*

The rabbit was anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and fixed on a stereotaxic apparatus (Narishige made) and electrodes were implanted through a drilled hole in the skull into the hippocampus (A:-3, L:5, H:5) and amygdala (A:2, L:6, H:-6), according to a stereotaxic atlas of Sawyer et al (3). The electrodes were the bipolar electrodes of 0.2 mm in diameter, made from insulated stainless steel wires having naked tips, with a distance of 0.5 to 1.0 mm between two electrotips. For recording the EEG in deep parts of the brain, monopolar electrode of the above mentioned bipolar one was used. The electrodes were connected to the connector socket, which was fixed with dental cement on the skull. To prevent infection, iodine tincture was applied on the operated region and 100,000 units of penicillin were injected intramuscularly once a day for three days after operation. The experiment was carried out later than 1 week after operation.

*Experiment in animals with acute placement of electrodes*

Under ether anesthesia, the concentric bipolar electrodes (outside diameter of 0.5 mm) were inserted stereotaxically into the hippocampus and amygdala. The silver ball electrodes (diameter of 1.0 mm) were placed on the dura mater to record the cortical EEG. The reference electrode was placed on the frontal skull. The experiment was started after the influence of anesthetic agent on the EEG had disappeared. The animals were immobilized with an intramuscular injection of 1.0 ml of 2% gallamine, and were given artificial respiration through a tracheal cannula. Gallamine was injected as needed during the experiment.

*Histological study*

After direct electrical current (3 mA) was sent through the electrodes for 10 sec to mark the electrode positions, the animal was sacrificed and 10% buffered formalin solution was perfused intra-arterially. Thereafter, the brain was removed, frozen and sliced. Brain slices were stained with Nissl's dye and the region of each electrode position was confirmed histologically.

**RESULTS**

*Effect on spontaneous EEG activity*

The effect of L-5418 on spontaneous EEG activity was studied in the acute experiment. Five to 10 mg/kg of L-5418 given i.v. had no influence on the spontaneous EEG activity. In the case of 20 mg/kg of L-5418, as shown in Fig. 1, the basic rhythm of cortical EEG become slightly irregular a few minutes after administration, and slow waves increased. In the hippocampus, arousal waves disappeared and the irregular pattern of a mixture of
FIG. 1. Effect of L-5418 on spontaneous EEG activity. A: Control. B: 5 min after 20 mg/kg i.v. injection of L-5418. FC: frontal cortex. OC: occipital cortex. HPC: hippocampus. AMG: amygdala. The vertical scale indicates 200 μV and the horizontal scale sec at right hand bottom in the figure. Abbreviations and scales are the same for all figures.

FIG. 2. Effect of diphenylhydantoin on spontaneous EEG activity. A: Control. B: 5 min after 20 mg/kg i.v. injection of diphenylhydantoin.
fast and slow waves appeared. However, those changes gradually decreased and regular waves returned after 2 hr. DPH showed almost the same pattern as L-5418, as shown in Fig. 2 and normal waves appeared after 1.5 hr.

**Effect on seizure discharge induced by penicillin**

When a filter paper of 3 mm in diameter that contained 5 μl of 5,000 units of penicillin was placed on the motor cortex, typical solitary spikes appeared within a few minutes and the amplitude reached 1 mV. Low amplitude spikes were also observed in other regions.

![Fig. 3. Penicillin induced seizure discharges in EEG of rabbits. 20 S, 50 S and 6 m indicate time (sec, min) after the appearance of seizure discharges by topical application of penicillin. PC: parietal cortex.](image)

![Fig. 4. Effect of L-5418 on penicillin induced seizure discharge. A: 10 min after 10 mg/kg i.v. injection of L-5418. B: 30 min after. C: 40 min after.](image)
and such increased gradually in amplitude and appeared repeatedly for a few hours with a
frequency of once every few seconds spreading over the whole brain. On the other hand,
the waves of seizure discharge started with the pattern of continuous rhythmic spikes and
then developed into a mixture pattern with irregular spikes and slow waves. At a late
stage, the pattern was that of postictal depression and brain activity was suppressed. This
seizure discharge lasted for 50 to 60 sec and occurred with a frequency of once every 5 to
8 min. A few seconds after the seizure discharge in a focal region, another discharge appeared
mainly with spikes. Thereafter, both discharges simultaneously disappeared as shown in
Fig. 3. The test drug was given after observation of the fixed seizure discharge at least 3
to 4 times by penicillin application. Five mg/kg of L-5418 did not suppress solitary spikes,
but delayed the onset of the seizure discharge and showed a tendency toward prolongation
of intervals, at the onset. The seizure discharge with a duration of about 12 sec appeared
15 and 30 min after administration and about 40 min later, the same seizure discharge as
control appeared. Ten mg/kg of L-5418 completely suppressed the seizure discharge at
an early stage, as shown in Fig. 4A. The seizure discharge at 30 min after the drug admin-
istration appeared but was short in duration, as shown in Fig. 4B. This discharge at 40 min
later also was of short duration. The same length of seizure discharge as before the admin-
istration appeared about 60 min later. In the case of 20 mg/kg, the suppressive effect

![Diagram](image-url)

**Fig. 5.** Effect of diphenylhydantoin on penicillin induced seizure discharge. A:
10 min after 20 mg/kg i.v. injection of diphenylhydantoin. B: 20 min after.
C: 40 min after.
appeared more markedly and the first seizure discharge did not appear until 60 min. A discharge the same as seen in the control appeared about 80 min later.

On the other hand, 5 mg/kg of DPH did not show a suppressive effect. In the case of 10 mg/kg, moderate suppressive effect on the onset of seizure and slight decrease of frequency of seizure appearance were demonstrated. In the case of 20 mg/kg, the onset of the seizure discharge was markedly delayed and the first one appeared 40 min later. A discharge the same as seen in the control appeared about 60 min later, as shown in Fig. 5. The results of L-5418 and DPH on seizure discharge induced by penicillin application are summarized in Fig. 6. L-5418 proved to have an suppressive effect on seizure discharge and to be twice as potent as DPH.

![Graph showing the effect of L-5418 and diphenylhydantoin on penicillin induced seizure discharge.](image1)

**Fig. 6.** Effect of L-5418 and diphenylhydantoin on penicillin induced seizure. Each symbol indicates an occasional seizure.

The end of bemegride injection.

**Fig. 7.** Effect of L-5418 on bemegride induced seizure discharge. 40 S indicates time (sec) after bemegride administration. A: Control. B: 5 min after 20 mg/kg i.v. injection of L-5418.
Effect on seizure discharge induced by bemegride

When bemegride of 5 mg/ml was injected i.v. at a speed of 1 ml per 10 sec, the onset of seizure discharge was found with injection of 3.5 to 4.0 ml. The pattern of seizure discharge started with multiple spikes and gradually was mixed with slow waves, then finally was flattened and disappeared. At first, the threshold amount of bemegride which induced the seizure discharge was determined, and 1 hr later the test drug was administered. Five min later, the threshold amount of bemegride was injected to provoke the seizure discharge. As shown in Fig. 7, L-5418 did not suppress the seizure discharge by bemegride. Neither DPH nor CMA had a suppressive effect on the seizure discharge induced by bemegride, as shown in Fig. 8. TMD at 250 mg/kg suppressed completely the seizure discharge, as shown in Fig. 9 and the threshold amount of bemegride required to induce the seizure discharge was 1.5 times that of the control threshold.

![Fig. 8. Effect of diphenylhydantoin on bemegride induced seizure discharge. 40 S and 45 S indicate time (sec) after bemegride administration. A: Control. B: 5 min after 20 mg/kg i.v. injection of diphenylhydantoin.](image)

![Fig. 9. Effect of trimethadione on bemegride induced seizure discharge. 30 S in figure indicates time (sec) after bemegride administration. A: Control. B: 5 min after 250 mg/kg i.v. injection of trimethadione.](image)
Effect on after-discharge induced by limbic system stimulation

Electrical stimulation on the limbic system such as hippocampus and amygdala produced after-discharge on EEG. The influence of L-5418 on the onset and duration of after-discharge and on the spread over to other parts of the brain were studied. After normal after-discharge occurring with electrical stimulation was observed three times, the drug was given and the effect was evaluated. After-discharge by electrical stimulation was readily induced in the hippocampus and amygdala.

Effect on after-discharge of hippocampus: When a rectangular pulse stimulation with a duration of 0.5 to 1.0 msec, an intensity of 2 to 8V and a frequency of 50 Hz was given for 5 sec to hippocampus of rabbit, the after-discharge appeared. The influence of the test drugs on this after-discharge was investigated in acute experiments. As shown in Fig. 10, 10 mg/kg of L-5418 given i.v. shortened by 1/2 the duration of after-discharge, but did not influence the wave shape and the spread over other areas. The suppressive effect lasted for 40 to 60 min. In the case of 20 mg/kg, as shown in Fig. 11, the suppressive effect was more marked than that of 10 mg/kg and lasted for 90 to 120 min. In the case of 10 mg/kg i.v. administration of DPH, the suppressive effect on the after-discharge was not observed. In the case of 20 mg/kg, as shown in Fig. 12, the duration of after-discharge was shortened by 1/2 and the suppressive effect lasted for 40 to 50 min. The same results as in acute experiments were also obtained in chronic experiments.

Effect on after-discharge of amygdala: When the electrical stimulation was applied to the amygdala under the same condition as in the hippocampus, the after-discharge appeared and the influence of the test drugs was studied with acute experiments in rabbits. As shown in Fig. 13, 10 mg/kg of L-5418 given i.v. shortened by 1/2 the duration of after-discharge immediately after administration and the suppressive effect lasted for 50 to 70 min. As shown in Fig. 14, 20 mg/kg of DPH showed the same suppressive effect as that of 10 mg/kg of L-5418 and the effect lasted for 40 to 60 min.

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**Fig. 10**. Effect of L-5418 on after-discharges induced by hippocampal stimulation. Figures show EEG immediately and at some sec(s) after electrical stimulation (50 Hz, 0.5 msec, 6V for 5 sec). A: Control. B: 5 min after 10 mg/kg i.v. injection of L-5418. C: 60 min after.
Fig. 11. Effect of L-5418 on after-discharges elicited by hippocampal stimulation. 
A: Control. B: 5 min after 20 mg/kg i.v. injection of L-5418. C: 60 min after. 
D: 120 min after. The hippocampus was stimulated at 50 Hz, 0.5 msec, 6V.

Fig. 12. Effect of diphenylhydantoin on after-discharges induced by hippocampal stimulation. 
Figures show EEG immediately and 15 sec after electrical stimulation (50 Hz, 0.5 msec, 6V for 5 sec). 
A: Control. B: 5 min after 20 mg/kg i.v. injection of diphenylhydantoin. C: 40 min after.
Fig. 13. Effect of L-5418 on after-discharges induced by amygdaloid stimulation. Figures show EEG immediately and 30 sec after electrical stimulation (50 Hz, 0.5 msec, 6V for 5 sec). A: Control. B: 5 min after 10 mg/kg i.v. injection of L-5418. C: 70 min after.

Fig. 14. Effect of diphenylhydantoin on after-discharges induced by amygdaloid stimulation. Figures show EEG immediately and 30 sec after electrical stimulation (50 Hz, 0.5 msec, 6V for 5 sec). A: Control. B: 5 min after 20 mg/kg i.v. injection of diphenylhydantoin. C: 60 min after.

DISCUSSION

We found in previous pharmacological studies on the behavioral effect of L-5418 that this compound had anti-convulsant actions similar to that of DPH. Detailed studies on an anti-convulsant action of L-5418 were carried out herein by electroencepharographical analysis on experimental models of epilepsy. The seizure discharges induced by local application of penicillin to the cortex (4-5) or an electrical stimulation of hippocampus (6) and amygdala (7) in animals were used as models of focal seizure of the cortex and the epilepsy of temporal lobe or limbic system in humans, respectively. Intravenous administration of bemegride induced epilepsy of the grand mal type in many species of animals as
well as in humans.

Though L-5418 could not suppress solitary spikes in focal seizure discharge induced by penicillin, the strong suppressive effect was found in amplitude and duration of the seizure discharge which spread over the entire brain. Julien and Halpern (8) studied the duration and frequency of seizure discharge induced by penicillin application on motor cortex in the cats and reported that DPH and phenobarbital had a suppressive effect, while TMD did not. Our study also support their results. L-5418 has an anti-convulsant effect similar to DPH and was different from TMD, since it suppressed the focal seizure discharge and the spread of seizure discharge over other regions, though solitary spikes remained.

L-5418 did not suppress the seizure discharge induced by i.v. administration of bemegride, but occasionally strengthened the seizure discharge as did DPH or CMA. TMD suppressed completely the seizure discharge by bemegride. Duijin and Visser (9) reported that barbital and diazepam raised the threshold of bemegride, but DPH and CMA did not. The authors previously reported that L-5418 showed no effect on convulsions induced by bemegride. In the present work also, the suppressive effect of L-5418 on bemegride was not observed.

Concerning the limbic system, L-5418 had a suppressive effect on after-discharge of the hippocampus and amygdala by electrical stimulation. The hippocampus as well as the amygdala have been given increasing attention concerning their important roles in epilepsy. As Gibbs et al. (10) reported, it has become the general opinion that psychomotor seizure is discharged from the focus of the hippocampus or amygdala. When the hippocampus or amygdala of experimental animals is stimulated electrically, similar phenomena to psychomotor seizures in humans appeared (11-13). Kato (14) and Inada (15) suggested that after-discharge in experimental epilepsy may be useful for testing new drugs effective for psychomotor seizure, because DPH, TMD and CMA among the anti-convulsants are effective for psychomotor seizures and produce a suppressive effect on after-discharge. In our experiments, L-5418 demonstrated a more potent effect than DPH on after-discharge.

According to Woodbury (16), DPH promotes the outflow of Na⁺ from the neuron and controls excitatory mechanisms by stabilizing the neuron membrane. In the case of epilepsy, this action is considered to protect the normal neuron from abnormal discharge of the seizure focus. Toman (17) stated that there may be three possible mechanisms to be considered regarding anti-epileptic agents, first, the effect of the drug on non-nervous systems, for example, improving action on the blood flow disorders in cortical seizure focus, second, suppressive effect on the excess of seizure discharge due to the direct action on the neuron altered pathologically in seizure focus, and third, suppressive effect on spread of discharge due to the stabilizing action on the normal neuron membrane of other regions. Currently available anti-epileptic agents have the mode of action of the third factor mentioned. The mechanisms of action of anti-convulsants remain to be clarified, however, in the case of L-5418, the mechanism may be due to a stabilizing action on the neuron membrane. Esplin reported that DPH has a decreasing action on post-tetanic potentiation as a factor of anti-epileptic effect (18). Whether or not such an action is possessed also by L-5418 is now
being investigated.

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