EFFECT OF ANTICONVULSANTS ON THALAMIC
AFTERDISCHARGE IN RATS AND CATS

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Abstract—Effects of anticonvulsants were determined on thalamic afterdischarge in
gallamine-immobilized cats and d-tubocurarine-immobilized rats in order to clarify
the participation of anticonvulsants in the thalamus. Thalamic afterdischarge was
induced by electrical stimulation of cat nucleus centralis lateralis and rat nucleus
reticularis at 50 Hz, 1 msec for 4 sec. In cats, diphenylhydantoin, carbamazepine,
phenobarbital, and diazepam raised afterdischarge threshold, and shortened its duration
induced at twice the threshold voltage. Trimethadione and dipropylacetate raised the
threshold, but did not change the duration. In rats, diphenylhydantoin, phenobarbital,
and diazepam raised the threshold, and shortened the duration with comparable dose
ranges used for cats. Dipropylacetate and acetazolamide raised the threshold, although
did not change the duration except for shortening action with a higher dose of dipropyl-
acetate. Trimethadione was without effect. These results suggest that the depressive
effect of anticonvulsants on the thalamus is, at least in part, associated with control
of the epilepsies.

Krupp and Monnier (1) have demonstrated that anticonvulsants affect the non-specific
thalamo-cortical system which plays an essential role in the pathophysiology of epilepsy.
From this point of view, we have studied the involvement of anticonvulsants in cortical
excitability by examining the action on cortical focal seizure (2). We found that
trimethadione differed from diphenylhydantoin in the mode of action. In the present
experiments, attention was directed to the thalamus, the other part of the thalamo-cortical
system, and effects of anticonvulsants were determined on the afterdischarge in cats and
rats. There are apparently few pharmacological reports on thalamic afterdischarge since
studies by Schallek et al. (3) and Gangloff and Monnier (4). In addition, there appear to
be species differences regarding the anticonvulsive effects of the drugs (3-6) and it has yet to
be elucidated whether drug-induced change in thalamic afterdischarge is associated with the
control of generalized seizure. A preliminary report of these findings has been summarized
(7).

MATERIALS AND METHODS

Cats of both sexes weighing 2.5 to 4.0 kg and male rats (Wistar HLA strain) weighing
250 to 380 g were used. All surgical procedures were carried out under ether anesthesia.
Cat: The animal was fixed on a stereotaxic apparatus (Todai Noken type), immobilized with
gallamine triethiodide (i.m.), and artificially ventilated at the rate of 26 strokes/min. For the electroencephalographic (EEG) recording and electrical stimulation, a silver ball electrode was placed on the left cortex (anterior suprasylvian), and stainless steel needle electrodes (external diameter 0.5 mm), insulated except at the tips, were implanted in the right nucleus centralis lateralis of the thalamus, right dorsal hippocampus, and left mesencephalic reticular formation according to the atlas of Snider and Niemer (8). Bipolar stimulating electrodes (electrode distance 1.5 mm), insulated except at the tips, were implanted in the left nucleus centralis lateralis. Coordinates of the electrode positions in mm were as follows: centralis lateralis, anterior (A) 8.0-9.5, lateral (L) 4.0, horizontal (H) 4.0; hippocampus, A 0.5, L 11.0, H 7.0; mesencephalic reticular formation, posterior (P) 0.5, L 4.0, H 3.0. A reference electrode was implanted in the neck muscle.

*Rat:* The animal was fixed on a stereotaxic apparatus (Todai Noken type), immobilized with d-tubocurarine chloride (i.m.), and artificially ventilated at the rate of 90 strokes/min. A silver ball electrode was placed on the left motor cortex according to the map of Terzuolo and Adey (9), and stainless steel needle electrodes (external diameter 0.3 mm), insulated except at the tips, were implanted in the right nucleus reticularis and right dorsal hippocampus according to the atlas of De Groot (10). Bipolar stimulating electrodes (electrode distance 0.5 mm), insulated except at the tips, were implanted in the left nucleus reticularis. Coordinates of the electrode positions were as follows: nucleus reticularis, A 5.4-6.2, L 2.0, H 1.0-0.5; hippocampus, A 2.6, L 4.0, H 2.5. A reference electrode was implanted in the neck muscle.

After surgical procedures, anesthesia was discontinued, and EEG was recorded 90 min after. EEG recordings were made monopolarly using a Nihonkohden recticorder (RJG-3006) with power units (R-I-5). For the electrical stimulation, a Nihonkohden SEN-1101 stimulator with an isolator was used. Thalamic after-discharge was induced by stimulation of the thalamus at 50 Hz square wave pulses with the duration of 1 msec for 4 sec. The voltage by which an afterdischarge was, first, evoked in the contralateral thalamus was taken as the threshold voltage. Ten min later, a stimulus of twice the threshold voltage was applied, and duration of the afterdischarge was determined. The same procedures were followed at 30-min intervals. Under these conditions, little change in threshold voltage occurred, but afterdischarge duration was prolonged.

All wound edges and pressure points were infiltrated with repeated injection of procaine hydrochloride, and the ear bars and other contact points of the stereotaxic frame were slightly loosened to minimize possible sources of pain. Gallamine or tubocurarine was injected repeatedly during the course of experiments. Exposed neural structures were covered with warm liquid paraffin, and body temperature was maintained constant using an infrared lamp. After the experiments, the animals were sacrificed with an overdose of pentobarbital, and the location of electrodes was confirmed histologically.

Drugs used were diphenylhydantoin Na (Aleviatin Na, Dainippon), phenobarbital Na (Fujinaga), carbamazepine (Tegretol, Geigy), diazepam (Cerene, Takeda), trimethadione (Minoaleviatin, Dainippon), acetazolamide Na (Diamox, Lederle), and dipropylacetate
Diphenylhydantoin Na and trimethadione were dissolved in a solvent (40%, propylene glycol, 10.5%, ethanol). Drugs were cumulatively administered by a slow i.v. injection into a cannulated forelimb vein at 30-min intervals. Unless otherwise specified, the dose represents a cumulative amount. Saline or the solvent (cumulative volume 0.1-0.8 ml/kg, i.v.) did not significantly affect afterdischarge threshold and its duration.

RESULTS

Thalamic afterdischarge in cats

Electrical stimulation of the nucleus centralis lateralis of the thalamus produced thalamic afterdischarge in gallamine-immobilized cats (Fig. 1). With stimulation at threshold voltage, seizure was induced in the ipsilateral thalamus, and propagated to the contralateral thalamus, and afterward to the hippocampus (Figs. 1A and 1B). Threshold voltage, when examined in the ipsilateral thalamus, was almost the same as that in the contralateral one, although seizure activity in the ipsilateral thalamus was stronger than that in the contralateral one (Fig. 1B). As shown in Fig. 1C, stimulation at twice the threshold voltage produced a potent afterdischarge. After the stimulation, tonic seizure consisting of high frequency components (≤ 8 Hz) was induced in the contralateral thalamus, hippocampus, and ipsilateral mesencephalic reticular formation, and was maintained with a tendency toward increase. Afterwards, the seizure activity changed to spikes, and abruptly disappeared in all recording areas. Under these conditions, mean threshold voltage necessary for producing afterdischarge was 9.2 ± 0.6 V (mean ± S.E. of 36 examples), and afterdischarge duration induced

![Fig. 1. Thalamic afterdischarge in cats. Thalamic afterdischarge was induced by electrical stimulation of the left nucleus centralis lateralis applied during the period indicated by a horizontal line. A and B: Stimulation at threshold voltage. C: Stimulation at twice the threshold voltage. Abbreviations: R-TH = right thalamus, L-TH = left thalamus, L-ASS = left anterior suprasylvian (cortex), R-HIP = right hippocampus, L-MRF = left mesencephalic reticular formation.](image-url)
by stimulation at twice the threshold voltage was 76.2 ± 4.9 sec.

Figs. 2 and 3 show a typical effect of anticonvulsants on thalamic afterdischarge. Diphenylhydantoin Na (10 mg/kg) and phenobarbital Na (20 mg/kg) shortened afterdischarge duration, and depressed its patterns as shown in the decrease of high frequency components and of amplitude. Trimethadione and dipropylacetate Na at a dose of 80 mg/kg, however, had no effect. Fig. 4 shows dose-response curves. Changes in afterdischarge threshold and duration are expressed as a percentage of the pre-injection value. Diphenyl-

Fig. 2. Effect of diphenylhydantoin and phenobarbital on thalamic afterdischarge in cats. Left thalamus was stimulated during the period indicated by a horizontal line. Abbreviations: see Fig. 1.

Fig. 3. Effect of trimethadione and dipropylacetate on thalamic afterdischarge in cats. Abbreviations: see Fig. 1.
hydantoin Na (5–40 mg/kg), phenobarbital Na (5–40 mg/kg), and carbamazepine (2.5–20 mg/kg) raised the threshold and shortened the duration. Diazepam also dose-relatedly raised the threshold at 0.25–2.0 mg/kg, but shortening action of the duration was maximum at 0.25 mg/kg. Trimethadione and dipropylacetate Na at 40–160 mg/kg raised the threshold in a dose-related manner, and acetazolamide Na (20–80 mg/kg as free amide) showed a tendency to raise the threshold. Unlike diphenylhydantoin and phenobarbital, however,
trimethadione, dipropylacetate, and acetazolamide did not affect the duration. In experiments where afterdischarge threshold was determined in the ipsilateral thalamus, diphenylhydantoin and phenobarbital were found to raise the threshold in a similar manner to that in the contralateral one.

**Thalamic afterdischarge in rats**

In rats, the nucleus reticularis was stimulated, since the final thalamic neurons of the non-specific thalamo-cortical system probably lie within this nucleus (11). As shown in

![Fig. 5. Thalamic afterdischarge in rats. Thalamic afterdischarge was induced by electrical stimulation of the left nucleus reticularis applied during the period indicated by a horizontal line. A: Stimulation at threshold voltage. B: Stimulation at twice the threshold voltage. Abbreviations: R-TH = right thalamus, R-HIP = right hippocampus, L-MC = left motor cortex.](image)

![Fig. 6. Effect of diphenylhydantoin and trimethadione on thalamic afterdischarge in rats. Left thalamus was stimulated during the period indicated by a horizontal line. Abbreviations: see Fig. 5.](image)
Fig. 5, stimulation of the nucleus produced afterdischarge in d-tubocurarine-immobilized rats. At threshold voltage, seizure induced was localized in the thalamus, and, at twice the threshold voltage, a potent seizure consisting of high frequency components and subsequent spikes was induced. Under the conditions, mean threshold voltage was $3.9 \pm 0.2$ V (mean $\pm$ S.E. of 36 examples), and afterdischarge duration was $90.1 \pm 4.9$ sec.

Diphenylhydantoin Na (10 mg/kg) shortened the duration and depressed the seizure

Fig. 7. Effect of anticonvulsants on afterdischarge threshold and duration in rats. Upper graph: Ordinate represents afterdischarge threshold expressed as a percentage of pre-injection value. Abscissa represents cumulative dose (mg/kg, i.v.) of drugs and the line shown under the abscissa represents cumulative volume (ml/kg, i.v.) of saline. Lower graph: Ordinate represents afterdischarge duration induced by the stimulation at twice the threshold voltage, expressed as a percentage of pre-injection value. □ diazepam, ■ diphenylhydantoin Na, ■ phenobarbital Na, △ trimethadione, △ dipropylacetate Na, ▼ acetazolamide, ○ control (saline). Each point represents the mean obtained in at least four separate experiments with the S.E. indicated. Differences statistically significant from the control: *$p<0.05$; **$p<0.01$. 

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patterns, while trimethadione (80 mg/kg) had no effect (Fig. 6). Fig. 7 shows dose-response curves in rats. Diphenylhydantoin Na, phenobarbital Na, and diazepam raised the threshold and shortened the duration with comparable dose ranges used for cats. Dipropylacetate Na (40–160 mg/kg) and acetazolamide Na (20–80 mg/kg as free amide) dose-relatedly raised the threshold, although did not change the duration except for the shortening action with a higher dose of dipropylacetate Na. Trimethadione (20–160 mg/kg) had no effect.

**Effect on electrocardiogram (ECG)**

A single dose of diphenylhydantoin Na (10, 20 mg/kg in cats; 20, 40 mg/kg in rats), phenobarbital Na (40 mg/kg in cats and rats), diazepam (2 mg/kg in cats), or dipropylacetate Na (160 mg/kg in cats) increased the amplitude of QRS complexes with a concomitant decrease of the heart rate. Carbamazepine (10 mg/kg in cats) decreased the amplitude of QRS complexes. Lower doses of these drugs, and others used in this experiment, had little or no effect on the ECG.

**DISCUSSION**

Different modes of actions on thalamic afterdischarge were evident between the anti-convulsants. Diphenylhydantoin, phenobarbital, and diazepam raised afterdischarge threshold and shortened its duration in cats and rats. Carbamazepine also showed similar effects in cats. On the other hand, trimethadione, acetazolamide, and dipropylacetate, at doses showing no effect on its duration, raised the threshold in rats and/or in cats. The effects of diphenylhydantoin, phenobarbital, and diazepam were similar to those in cats reported by Schallek et al. (3) and Schallek and Kuehn (12), but different from those in rabbits by Krupp and Monnier (1) and Gangloff and Monnier (4). However, there are no species differences between cats and rats so far as the actions of diphenylhydantoin, phenobarbital, diazepam, and dipropylacetate are concerned.

It has been tentatively concluded that the thalamus is probably the site of action of trimethadione from the implication of the thalamo-cortical system in genesis of petit mal epilepsy (13). This conception was supported by Schallek and Kuehn (12) reporting that the drug is most effective in raising the threshold for afterdischarge induced by stimulation of the nucleus centralis lateralis of the thalamus. Our results herein confirmed such action of the drug, at a smaller dose of 40 mg/kg in cats. In addition, trimethadione had no effect on the threshold of the nucleus reticularis in rats. It has been reported that the drug has no effect on cortical focal seizure (2, 14) and cortical afterdischarge (15). Furthermore, propagation of the seizure from the cortical foci to the thalamus is known to be depressed with the drug, and this depression has been attributed to action on the nucleus centralis lateralis (16, 17). These findings indicate the localization of action of trimethadione in the nucleus centralis lateralis. Dipropylacetate also affects the thalamus in cats and rats without acting on cortical focal seizure (2).

However, diphenylhydantoin, being ineffective against petit mal epilepsy (18), was found to raise afterdischarge threshold of the nucleus centralis lateralis. Namely, the drug at a dose of 5 mg/kg raised the threshold without affecting its duration. The result was different
from the findings reported by Schallek and Kuehn (12) that the drug at 10 mg/kg affected the duration but not its threshold, and by Toman and Goodman (19) that the drug differs from trimethadione in lacking any threshold raising ability in animals. The threshold raising effect was also obtained with phenobarbital and carbamazepine which are ineffective against petit mal epilepsy (18). Therefore, it cannot be always accepted that the threshold raising effect in the nucleus centralis lateralis is directly related with the control of this type of epilepsy.

The present results indicate that all anticonvulsants examined affect the thalamus, one part of the thalamo-cortical system which plays an essential role in the pathophysiology and genesis of different kinds of epilepsies in human pathology (1). Accordingly, it is likely that the depressive effect of anticonvulsants on thalamic activity is, at least in part, associated with control of these epilepsies, although other sites of action of the drugs have to be considered. In order to evaluate distinct antiepileptic action, a determination of the epileptiform activity in animals with brain dysfunction, e.g., change in seizure susceptibility (20, 21), is required for elucidation.

REFERENCES