THALAMIC GENERALIZED SEIZURE INDUCED BY TUNGSTIC ACID GEL IN CATS AND ITS SUPPRESSION BY ANTICONVULSANTS

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Abstract—The experiments were performed electroencephalographically on gallamine-immobilized cats with the thalamic foci induced by application of tungstic acid gel (gel). The gel (50 µl) applied to n. centralis lateralis (CL) caused generalized seizure (GS) with high frequency components triggered by slow wave, and GS recurred with a regular interictal period. The application to n. centralis medialis or n. medialis dorsalis did not induce recurring GS, indicating the heterogeneity in the epileptogenesis of the thalamus. The GS induced by the gel application to the CL was of thalamic origin. Anticonvulsants used were found to prolong the interictal period of the GS, without modifying its duration. There was a difference between the drugs effective against grand mal and petit mal epilepsies in that the prolongation by the former drugs, diphenylhydantoin and phenobarbital, was more pronounced at low doses than that by the latter drugs, trimethadione and dipropylacetate. These results suggest that the gel-induced epileptic model with thalamic foci is useful for analyzing the pathophysiological process of epilepsy and for evaluating the drugs effective against grand mal epilepsy.

Grand mal epilepsy is characterized by occurrence of bilaterally synchronous generalized seizure (GS) with high frequency components on electroencephalogram (EEG) and peripheral seizure manifestation with loss of the consciousness. Such epilepsy is generally referred to as “centrencephalic epilepsy”, based on the concept of Penfield and Jasper (1) that its origin is in the centrencephalic system lying in the rostral brainstem including the thalamus. From this point of view, thalamic epileptiform activity, induced by electrical stimulation or application of the foreign substances, has been intensively studied (2–6). However, most of these activities show the spike and wave complexes on EEG unless they are induced by high frequency stimulation, and do not seem to be a suitable model for grand mal epilepsy. Schallek and Kuehn (7) have demonstrated that thalamic afterdischarge with high frequency components, induced by electrical stimulation, is suppressed not only with the anticonvulsants of grand mal type, but also with those of petit mal type. Thus, the role of the thalamus in centrencephalic epilepsy does not seem to have sufficiently been determined. Against the stream of the centrencephalic concept, several lines of experimental and clinical evidence have been suggested that the cortical mechanism plays an important role in the genesis of epilepsy (8–11). We have pharmacologically studied the role of the cortex in
experimental seizures, using several means (12–16), however, it was felt necessary to examine the involvement of the thalamus, a part of centrencephalic system in the pathophysiological process of epilepsy, since the cortical and thalamic structures are highly connected with and functionally controlled by each other.

One purpose in this experiment was to induce the thalamic GS with high frequency components, similar to that of grand mal epilepsy, by applying a foreign substance to the thalamus. We used tungstic acid gel (gel) which produces intensive neuronal hyperactivity at the foci (17), and examined whether or not GS could be induced by the gel application to three different structures of the thalamus, in an attempt to determine the heterogeneity of the structure. Application of the gel to the n. centralis lateralis (CL) induced GS which originated from the thalamus. Therefore, by examining the effect of anticonvulsants on the gel-induced GS, we attempted to clarify the pharmacological significance of this experimental epileptic model in the light of the centrencephalic concept. A preliminary report of these findings has been made (18).

MATERIALS AND METHODS

Cats of both sexes weighing 2.4 to 4.0 kg were used. All surgical procedures were carried out under ether anesthesia. 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 EEG recordings, silver ball electrodes were placed on the left and right corticies (anterior suprasylvian), and stainless steel needle electrodes (external diameter 0.5 mm), insulated except at the tips, were implanted in the CL, n. centralis medialis (CM) or n. medialis dorsalis (MD) of the thalamus, and in the dorsal hippocampus, according to the atlas of Snider and Niemer (19). Coordinates of the electrode positions in mm were as follows: CL, anterior (A) 9.0, lateral (L) 4.0, horizontal (H) +4.0; CM, A 9.0, L 0, H +2.0; MD, A 8.5, L 2.0, H +4.5; hippocampus, A 0.5, L 11.0, H +7.0. A reference electrode was implanted in the neck muscle. EEG was recorded 90 min after surgical procedures. EEG recordings were made monopolarly using an ink-writing oscillograph (Nihon-Kohden RJG-3006).

Tungstic acid gel was prepared according to the method described by Blum and Liban (20). After normal EEG was recorded, the gel (50 μl) was injected unilaterally into the CM, left CL or MD, with the aid of a stereotaxically driven cannula.

All wound edges and pressure points were infiltrated with repeated injection of procaine hydrochloride, and ear bars and other contact points of the stereotaxic frame were slightly loosened to minimize possible sources of pain. Gallamine 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. At the end of the experimental period, the animals were sacrificed with an overdose of pentobarbital Na and the location of electrodes and gel-injected site were confirmed histologically.

Drugs used were diphenylhydantoin Na (Aleviatin Na, Dainippon), phenobarbital Na (Fujinaga), carbamazepine (Tegretol, Geigy), diazepam (Cercine, Takeda), trimethadione (Minoalevatin, Dainippon), acetazolamide Na (Diamox, Lederle), dipropylacetate Na (Depakene, Kyowa Hakko), and taurine (Nakarai). All the drugs were dissolved in saline or in another solvent (40% propylene glycol and 10.5% ethanol), depending on their solubility. Drugs were cumulatively administered by a slow i.v. injection into a cannulated forelimb vein at 15 min intervals.
Control studies were done with saline or another solvent in volumes identical with those used for the drugs.

RESULTS

(I) Epileptiform EEG activity induced by gel application to the thalamus: Application of the gel (50 µl) to the CL caused GS. The time course was as follows: several min after the application, epileptiform burst waves and slow waves were induced in the contralateral thalamus, and propagated to other brain sites (Fig. 1A). Thereafter, the seizure patterns, triggered by slow wave, were induced in the contralateral thalamus and hippocampus (Fig. 1B and C). These epileptiform activities were propagated to the cortex and developed to GS within 2 hr after the gel application (Fig. 1D). Once the GS occurred, its activity frequently recurred with stable interictal period over 5 hr (status epilepticus). When measured at 3 hr after the application, interictal period and seizure duration were 265.6±39.6 sec and 119.3±8.4 sec, respectively (mean±S.E. of 30 examples). On the other hand, the application to the CM or MD did not induce any GS with an exception of one animal. The epileptiform activities such as slow waves or focal seizure were observed several min after the application, and these were similar to those induced by the application to the CL. However, a striking difference was observed between epileptiform activities in the CL and those in the two nuclei. Namely, focal seizure and GS induced by the application to the CM or MD did not recur unlike those in the CL. Figure 2 shows comparison of epileptiform activities induced by the gel application to three different thalamic structures. The gel application to the CL caused the recurring GS in 31 out of 45 animals (68.9%).

To determine whether GS induced by the gel application to the CL originates from the application site or not, EEG in the region close to application site was recorded by implanting the electrode into the position 1 mm distant from the application site.

Fig. 1. Tungstic acid gel (50 µl)-induced epileptiform activity in each cat. Tungstic acid gel was applied to the left n. centralis lateralis. Right upper tracings show the magnified epileptiform EEG from each point (●). Abbreviations: L-ASS, left anterior suprasylvian gyrus; R-ASS, right anterior suprasylvian gyrus; R-TH, right thalamus (n. centralis lateralis); R-HIP, right dorsal hippocampus.
Several min later, the epileptiform activity with slow waves and subsequent burst-like spikes of short-lasting was induced (Fig. 3A). Thereafter, burst-like spikes were increased in duration and frequency. Finally, focal seizure or GS developed (Fig. 3C). The fact indicates that GS induced by the gel application to the CL originates from the application site, namely, in the thalamus.

(II) Effect of anticonvulsants on GS: The GS induced by the gel application to the CL recurred with a regular interictal period. So, the effect of anticonvulsants on GS could be precisely examined. Anticonvulsants effective against grand mal epilepsy, diphenylhydantoin Na (1.25–5 mg/kg, i.v.), carbamazepine (1.25, 2.5 mg/kg), phenobarbital Na (2.5–20 mg/kg), and diazepam (0.063, 0.125
mg/kg) dose-relatedly decreased the frequency of occurrence of GS and prolonged the interictal period (Fig. 4). However, the average duration of GS and its patterns were little changed by the drugs. For example, as shown in Fig. 5, diphenylhydantoin Na (5 mg/kg, cumulative dose) completely blocked the occurrence of GS in the early period after the injection, but, the GS which reappeared 43 min later had almost the same pattern as that observed before the drug injection. Similar actions were observed with phenobarbital and diazepam.

The drugs effective against petit mal epilepsy, trimethadione (40-160 mg/kg), dipropylacetate Na (40-160 mg/kg) and acetazolamide Na (5-40 mg/kg, as a free amide), prolonged the interictal period (Fig. 4), and these drugs did not alter the average duration of GS and its patterns, even when the interictal period was prolonged. However, trimethadione and dipropylacetate necessitated higher doses for prolonging the interictal period, and even at high doses the prolongation was less than that induced by diphenylhydantoin and phenobarbital. Taurine (50-200 mg/kg) was without effect in this aspect (Fig. 4).

**DISCUSSION**

GS with high frequency components was found to be induced by application of tungstic acid gel to the CL, and to be of thalamic origin since the epileptiform activity was triggered by slow waves induced in the area close to the site of its application. So far, most of the epileptiform activities induced by thalamic application of the foreign substances have been demonstrated to consist of spike and wave complexes, not of high frequency components, although Lange and Julien (21) reported that high frequency poly-spike discharges were induced by injection of conjugated estrogens into the n. ventralis anterior. The gel-induced GS with high frequency components seems to be similar to the paroxysms in grand mal epilepsy, and to be of value for analyzing the pathophysiological process of epilepsy.

The application to the CM or MD failed to elicit the recurring GS, while the application to the CL elicited it in a rate of 68.9%. Thus, there are differences in the manner of response to the gel. Krupp and Monnier (22) demonstrated that the thalamus reveals a heterogeneity of function. Schallek and Kuehn (7) reported that trimethadione had an effect on
the afterdischarge induced by electrical stimulation of the CL without affecting the discharge by stimulation of the MD. Our result supports their view for functional

Fig. 4. Effect of anticonvulsants on GS induced by the gel application in cats. Ordinate represents interictal period of GS, expressed as a percentage of pre-injection value. When GS was suppressed completely with the drug, the interictal period was calculated as 900 sec, approximately 3 times of the mean interictal period. Abscissa represents cumulative dose (mg/kg, i.v.) of the drugs and the line shown under the abscissa represents cumulative volume (ml/kg, i.v.) of saline. Each point represents the mean of 4–6 different experiments with the SE indicated on one direction. Differences statistically significant from the control: *p<0.05, **p<0.01, calculated according to Student's t-test.
heterogeneity of the thalamus. The mechanism underlying this heterogeneity remains obscure. However, in the present study, when the gel was applied to each thalamic structure, the epileptiform activity such as slow wave or focal seizure was found to occur equally in each structure, indicating that the three thalamic regions have much the same responsiveness to the action of the gel. Therefore, the observed difference in the occurrence of recurring GS among the three structures may be due to the difference in the stage of GS development. The transition from interictal stage to seizure has been proposed to be achieved through an elimination of inhibitory mechanisms or to an overpowering of excitatory drive (23, 24). It seems likely that the CL is under less intensive control of such inhibitory mechanisms than other thalamic nuclei, resulting in much easier propagation of epileptiform activity to other brain structures. The increase in seizure susceptibility which is often observed in epileptic models may be explained by a possible change of the inhibitory mechanisms. Thus, the CL, unlike the CM and MD, can be considered to play an important role in the pathophysiological process of epilepsy.

In an attempt to elucidate the significance of this experimental model in relation to the centrencephalic concept, the effects of anticonvulsants were examined on the recurring GS. The drugs effective against grand mal epilepsy, diphenylhydantoin, phenobarbital and carbamazepine, dose-relatedly decreased the occurrence of GS and these effective doses were the same or lower than those effective against the thalamic afterdischarge (25). There was a clear difference between the drugs effective against grand mal and petit mal epilepsies in that the prolongation by the former drugs, diphenylhydantoin and phenobarbital, was more pronounced at low doses than that by the latter drugs, trimethadione and dipropylacetate, and the latter drugs were less effective against GS, even at high doses which pronouncedly raised the threshold for the thalamic afterdischarge (25). In addition, the finding that the duration of GS was not changed with the drugs was different from findings on the gel-induced cortical seizure or thalamic afterdischarge where seizure duration was dose-relatedly shortened (15, 25), and was rather similar to clinical findings that the paroxysms in patients with grand mal epilepsy are completely blocked by the anticonvulsants (26). Therefore, it is suggested that the gel-induced thalamic GS is an epileptic model more closely related to human centrencephalic epilepsy, especially, grand mal.

Whether it is the cortex or thalamus which plays a critical role in the pathogenesis of grand mal epilepsy has to be determined.
Diphenylhydantoin and phenobarbital evenly suppress not only the thalamic but also the cortical seizures (12–16, 25). In addition, the cortical and thalamic structures are anatomically related to and physiologically inter-controlled. Thus, a pharmacological explanation is difficult. In this study, since the gel-induced GS was found to be an epileptic model more closely related to grand mal epilepsy, the drugs effective against grand mal epilepsy would expectedly act more effectively in the case of this particular model. In the present study, the doses of diphenylhydantoin and phenobarbital required to decrease the occurrence of GS were the same or lower than those effective in the case of other models (12–16, 25). The role of the thalamus in epilepsy cannot be clearly determined on the gel-induced thalamic GS.

The GS induced by application of the gel to the CL consisted of high frequency components and subsequently spikes, and was of thalamic origin. The heterogeneity between the CL and other thalamic nuclei, CM and MD, was observed in the transit from the preictal state to seizure. In addition, this GS was completely blocked with low doses of the drugs which are clinically effective against grand mal epilepsy. Accordingly, the present findings suggest that this epileptic model is of value for epilepsy research and evaluation of anticonvulsants effective against grand mal epilepsy.

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