DIFFERENTIAL EFFECTS OF ANTIEPILEPTICS ON HIPPOCAMPAL AND PALLIDAL AFTERDISCHARGES IN CATS

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Many investigators reported that the hippocampus (HPC) was involved in cerebral and cerebellar seizure discharges and epileptiform phenomena (1-4), and it was the well known site of histological lesion which was found in approximately 50 per cent of epileptic brains (5, 6). The HPC has the lowest threshold of excitability to electrical stimulus of all the cerebral structures so far studied since reported by Kaada (1). Pathologically and electrophysiologically the sensitive structure of brain is suggested to play an essential part in the development of the epileptic seizures.

Several ablation and electrophysiological experiments on the brains (7-13) have confirmed that extrapyramidal structures play a predominant part in the mechanism of generalized convulsion, and that convulsion has not cortical origin followed with corticospinal propagation. For the relief of tremor and rigidity in extrapyramidal diseases, the surgical lesion is often made in the globus pallidus (GP) (14). Most characteristic response to electrical stimulation of GP is tremor in human (15).

The present investigation was undertaken to evaluate the effects of clinically known antiepileptics quantitatively on the both hippocampal and pallidal seizures with special reference to threshold voltage and afterdischarge propagation induced by electrical stimulation of both HPC and GP.

METHODS

Sixty seven male adult cats weighing 3 to 3.5 kg were used in the experiments. The experiments were performed in unanaesthetized and spinal animals sectioned between C1 and C2. The animals were maintained by artificial respiration, and radiant heating was applied to keep rectal temperature normal.

Concentric bipolar stimulating and recording electrodes were employed. A San’ei Model-2 square wave stimulator was used to deliver stimuli. The stimuli for HPC and GP were 10, 50 and 100 c/s in frequency and 5 msec in pulse width, and the stimulus for midbrain reticular formation (RF) 100 c/s in frequency and 1 msec in width. In all cases the duration of stimulation was for 7 sec. Control threshold voltages were determined by stimulating HPC, GP and RF at unresponsive voltage and by increasing it in step of 0.5 V.

A period of at least 5 min was allowed to elapse between any two successive stimulations. Cortex and subcortex potentials were recorded by San’ei 12 channel electroence-
phalograph. Routine locations of twelve pickup leads were sensory and motor cortices (SC, MC), HPC (Frontal 2, Lateral 9 and Horizontal 6), fornix (Fx; F 4, L 1, H 6.8), amygdala lateralis (AMG; F 10, L 9, H -6), CP (F 14, L 3, H -1.5), caudatus (CD: F 16, L 5, H -2), hypothalmus lateralis (HPT: F 10, L 3, H 1), which were ipsilaterally to stimulation site and determined by reference to the map of Jasper and Ajmon-Marsan (16). The sites of electrodes in the brain were verified histologically.

After each control threshold voltage was obtained, phenobarbital, dilantin, tridione and N-phenyl ethyl-N'-acetyl urea (crampol), which was one of derivatives of phenurone and was more effective for grand mal than the latter drug (17-20), were administered intravenously to the animal in three minutes or more to minimize the effect of the solvent, diluted proylene glycol. The volume of solution injected was less than 0.3 ml and the control experiment of the solvent was included in the study. Doses of drugs employed were 5, 10 and 20 mg/kg for phenobarbital, dilantin and crampol, and 50, 100 and 200 mg/kg for tridione.

The absolute increase in the threshold voltages for HPC, GP and RF, and the effects of the drugs on the seizure propagation caused by stimulations of HPC and GP within 1 or 2 hours after the administration were determined by three different stimulation frequencies as mentioned above, and 15 min were allowed for the onset of the drug effect.

The effects of drugs on both propagations of hippocampal and pallidal afterdischarges in response to stimulus, twice of threshold voltage, were examined. The reticular arousal threshold was determined by measuring of stimulation voltage to inhibit spindle bursts in the neocortex after electrical stimulation of midbrain reticular formation.

**RESULTS**

**Stimulation of dorsal hippocampus**

The characteristic HPC afterdischarge was obtained after the stimulation. The afterdischarge patterns, which appeared in archiocortex, were classified into two types, tonic and clonic types from the patterns at the termination of the discharges. The tonic type, of which duration differed markedly (15 sec to 2 min), consisted of 20 to 25 c/s in frequency and about 120 μV in amplitude, and thereafter the wave disappeared. Whereas, the clonic type had a train of the discharge with 15 to 25 c/s in frequency and 150 to 200 μV in amplitude, and after the sudden disappearance of the discharge, the typical grouping seizure discharges appeared at intervals of 1 to 2 sec. The latter discharge consisted of 10 to 15 c/s in frequency and 150 to 200 μV in amplitude. However, the threshold voltage did not differ between tonic and clonic types. The mean control seizure threshold voltages ±S.E. were 5.79±0.415 V (39 cats) for 100 c/s stimulation, 4.55±0.546 V (27 cats) for 50 c/s and 10.6±0.864 V (25 cats) for 10 c/s, which are shown in Figs. 1-A and B.

The threshold voltages were significantly different in three frequency stimulations. The discharge propagations to other ten areas varied considerably following the stimula-
tion of three different frequencies (see Fig. 3). However, the pattern of HPC after-discharges following threshold stimulation resembled one another, as shown in Fig. 2.

The seizure duration varied every time at threshold stimulation and so it was not utilized as index for drug actions.

Stimulation of globus pallidus

Stimulation of GP evoked the afterdischarge of spike complexes or spike and wave
with 2 to 4 c/s in frequency and 100 to 200 μV in amplitude.

The control afterdischarge threshold voltages ±S.E. were 6.80 ± 0.480 V (30 cats) for 100 c/s, 4.90 ± 0.457 V (30 cats) for 50 c/s and 8.55 ± 1.25 V (21 cats) for 10 c/s, as shown in Figs. 4-A and B.

These threshold voltages at each frequency were statistically significant. However, the duration of pallidal afterdischarge varied everytime at threshold stimulation. The
afterdischarge at threshold voltage propagated most frequently to RF, putamen, cortices and AMG (see Fig. 5). The pattern of discharge in response to each stimulation of GP did not markedly differ as shown in Fig. 6.

**Effects of drugs on hippocampal seizure threshold**

After the administrations of drugs each hippocampal threshold showed an elevation in proportion to dose. However, analysis of variance for each drug revealed no signi-
significant difference in the threshold between doses irrespective of the frequency applied ($P > 0.05$). Assuming this may be due to an insufficient number of trials.

The approximate doses required to elevate the threshold voltage to all three stimulation frequencies by 25 and 50% revealed that dilantin was the most potent among the four drugs used, while the effect of phenobarbital in 100 c/s stimulation was approximately 70% of that of dilantin, and in 50 and 100 c/s 60 to 50%. The effect of crampol was 30% of that of dilantin in 100 c/s stimulation, 40% in 50 c/s and 30% in 10 c/s. Crampol was the only drug which reduced the hippocampal afterdischarge in voltage and frequency. Tridione was not effective in 50 c/s stimulation and slightly suppressive in 10 and 100 c/s stimulations, as shown in Fig. 7.

These four drugs showed a common aftereffect by which the threshold subjected to decrease below that before the injection of drugs.

*Effects of drugs on hippocampal seizure propagation*

The suppression patterns of propagation caused by each drug varied with the frequency of stimulation of twice of the threshold voltage. Dilantin showed a considerable suppression on the seizure propagation throughout the stimulation frequencies applied. Phenobarbital was less active at low frequency of 10 c/s, while crampol was less active at high frequency of 50 and 100 c/s. Tridione was the lowest effect among four drugs used at high frequency of 50 and 100 c/s.

These results are summarized in Table 1.

*Effect of drugs on pallidal discharge threshold and propagation*

The suppression of the pallidal seizure induced by 100 c/s stimulation was in the order of description, tridione, crampol, dilantin and phenobarbital. However, statistically analysis revealed that only tridione in 100 mg/kg increased the threshold voltage by 66% ($0.01 > P > 0.005$).

The threshold at 50 c/s stimulation was increased by drugs in the following descending order, phenobarbital, crampol and tridione. Phenobarbital was the only drug to
show a significant increase ($0.05 > P > 0.025$). Dilantin and crampol were not effective on the pallidal threshold at 10 c/s. The parallel relation was observed between an increase in pallidal threshold and suppression of the propagation induced by twice of threshold stimuli.

Atropine and benadryl (diphenhydramine) are clinically used for Parkinsonian tremor. Atropine of 0.5 mg/kg i.v. had no effect on both pallidal threshold and seizure propagation at 10, 50 and 100 c/s stimulations of each threshold in 5 cats. Also, suppressions of propagations to all areas studied were not obtained at twice of each threshold voltage.

Whereas, 1 to 5 mg/kg i.v. of benadryl showed a slight increase in threshold voltage by 10 to 25% at all of three frequency stimulations and suppressed the propagation to all pickup areas at 50 and 100 c/s stimulations of threshold voltage in 5 cats.

**TABLE 1. Effects of drugs on hippocampal seizure propagations at twice of threshold voltages.**

<table>
<thead>
<tr>
<th>Propagation to</th>
<th>Stimulation frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 c/s</td>
</tr>
<tr>
<td>MC</td>
<td>(D) (P)</td>
</tr>
<tr>
<td>SC</td>
<td>(D) (T)</td>
</tr>
<tr>
<td>CM</td>
<td>D C</td>
</tr>
<tr>
<td>HPT</td>
<td>D C</td>
</tr>
<tr>
<td>RF</td>
<td>D C T</td>
</tr>
<tr>
<td>HPC</td>
<td>D C</td>
</tr>
<tr>
<td>FX</td>
<td>D C</td>
</tr>
<tr>
<td>AMG</td>
<td>D (P) C T</td>
</tr>
<tr>
<td>CD</td>
<td>D (P) (C)</td>
</tr>
<tr>
<td>GP</td>
<td>D P C</td>
</tr>
<tr>
<td>PUT</td>
<td>D (P) C</td>
</tr>
</tbody>
</table>

P, D, C and T mean phenobarbital, dilantin, crampol and tridione which showed the suppression more than 50%.

( ) means doubtful because of small number of samples.

Doses and number of cats: same as shown in Fig. 7.

**Fig. 8. Effects of drugs on pallidal thresholds.**

Doses: dilantin, 10 mg/kg 9 cats; phenobarbital, 10 mg/kg 7 cats; crampol, 10 mg/kg 8 cats and tridione, 100 mg/kg 9 cats.

$V/V_0$: threshold voltage after drug/control threshold voltage.
Effects of drugs on the midbrain reticular arousal response

The effect of intravenously administered dilantin at 5, 10 and 20 mg/kg was negligible in 8 cats (0.20 > P > 0.10).

The application of 10 mg/kg of phenobarbital (9 cats) increased in the threshold voltages ± S.E. from 3.16 ± 0.397 V to 3.82 ± 0.448 V but the elevation was not significant (0.20 > P > 0.10). In doses of 5, 10 and 20 mg/kg of phenobarbital, the probit regression curve of the threshold voltages upon log dose of drug showed no significant slope.

If higher doses were applied, significant slope might have been obtained.

The effect of crampol was negligible at doses of 5 and 10 mg/kg. However, following the dose of 20 mg/kg the slight threshold elevation was observed from 2.78 ± 0.27 V to 3.21 ± 0.291 V in 8 cats, though the difference was not significant (0.20 > P > 0.10). Tridione had no demonstrable effect upon reticular threshold up to 200 mg/kg in 11 cats.

The above results are all summarized in Table 2.

**Table 2. Effects of drugs on hippocampal, pallidal and reticular arousal thresholds.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dilantin</th>
<th>Phenobarbital</th>
<th>Crampol</th>
<th>Tridione</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses in mg/kg</td>
<td>5-20</td>
<td>5-20</td>
<td>5-20</td>
<td>50-200</td>
</tr>
<tr>
<td>Stimulation frequency in c/s</td>
<td>10 50 100</td>
<td>10 50 100</td>
<td>10 50 100</td>
<td>10 50 100</td>
</tr>
<tr>
<td>Hippocampal threshold</td>
<td>↑ ↑ ↑</td>
<td>0 ↑ ↑</td>
<td>0 (↑) 0 (↑) 0 0</td>
<td></td>
</tr>
<tr>
<td>Pallidal threshold</td>
<td>0 0 0</td>
<td>↑ ↑ 0</td>
<td>(↑) (↑) 0 0 0 ↑</td>
<td></td>
</tr>
<tr>
<td>Reticular arousal</td>
<td>0 (↑)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clinical use</td>
<td>grand mal</td>
<td>psychomotor</td>
<td>grand mal</td>
<td>psychomotor</td>
</tr>
</tbody>
</table>

↑ : means elevation of threshold voltage.
( ) : means no significant elevation of threshold.
0 : no effect.

DISCUSSION

In the present studies all four drugs employed showed a tendency to increase in the threshold voltage at 10, 50 and 100 c/s stimulations for hippocampal afterdischarge. Hippocampal seizures caused by all frequency stimulations were significantly suppressed by dilantin and then by phenobarbital, except of 10 c/s in phenobarbital. These results confirm the work of Aston and Domino (21) who used a stimulation protocol of 100 c/s and 5 msec pulse width in monkey.

However, no effect of dilantin on rhinencephalic seizure threshold was reported in rabbit using the stimulation of 30 to 40 c/s with 1 to 7 msec width (22). These negative results might have been accounted by species difference and different route of administration.

No significant influence on the hippocampal threshold at 50 and 100 c/s frequencies...
was observed in crampol and tridione. However, crampol, which is clinically in use for psychomotor and grand mal types of epilepsy, modified the pattern of afterdischarge. No modification of the pattern was obtained by tridione at the doses administered. Whereas, Gangloff and Monnier (22) showed the depressing action of tridione on the rhinencephalic seizure in rabbit and this effect was obtained by high oral doses of 1.5 to 2.0 g/kg.

Stimulation of the basal ganglia has been reported to induce many types of motor phenomena including epileptic convulsion. The pallidal stimulation in the Parkinsonian patients induced tremor (15, 23), tremor augmentation (24–26) or suppression of tremor (27).

Pallidal afterdischarge evoked by 50 and 10 c/s stimulations was significantly inhibited by phenobarbital but any dose of drug was without effect on the seizure induced by 100 c/s stimulation. However, clinical use of this drug showed occasionally a worse effect on Parkinsonian disease (28).

Another two drugs, dilantin and crampol showed no effect on the seizures at three frequency stimulations. The pallidal discharge was not significantly affected by atropine and benadryl, which were clinically useful for Parkinsonian disease. This result suggests that the pallidal afterdischarge used here might have no relationship with Parkinsonian disease.

One of the essential factors for an ideal antiepileptics requires without anesthetic action at the dose which is effective for epilepsy. The requirement was further investigated to evaluate the drugs effect on the reticular activating system, which was one of the most sensitive sites of hypnotics as reported by French et al. (30).

Phenobarbital, being different from another barbiturates such as pentobarbital, markedly increased the convulsive threshold in non-anesthetic doses (21, 31). The presents results confirmed the clinical usefulness of phenobarbital in this point.

SUMMARY

The effects of dilantin, phenobarbital, crampol and tridione on hippocampal and pallidal afterdischarges evoked by a series of frequency stimulations (10, 50 and 100 c/s) of HPC and GP were studied in unanesthetized cats.

1. Significant difference was observed in hippocampal threshold voltages between 10, 50 and 100 c/s stimulations. The intensity of the afterdischarge increased progressively by the order of 10, 100 and 50 c/s frequencies.

2. Dilantin (5 to 20 mg/kg) was most suppressive on hippocampal seizure threshold and propagation at 10, 50 and 100 c/s frequencies. Phenobarbital (5 to 20 mg/kg) was the nextly potent suppressant on the seizures at frequencies of 50 and 100 c/s. Crampol (5 to 20 mg/kg) and tridione (50 to 200 mg/kg) showed no significant effect at all stimulations of 10, 50 and 100 c/s frequencies. Only crampol modified the pattern of hippocampal afterdischarge.

3. Pallidal afterdischarge was obtained by electrical stimulation of GP at 10, 50
and 100 c/s frequencies. The discharge increased along with stimulation frequencies of 10, 100 and 50 c/s in this order.

4. Phenobarbital was most potent in suppressing the pallidal discharges evoked by stimulations of 10 and 50 c/s frequencies. Tridione showed a significant elevation of the pallidal threshold at 100 c/s. Crampol had a trend to elevate all thresholds at 10, 50 and 100 c/s. Atropine (0.5 to 2.5 mg/kg) and benadryl (1 to 5 mg/kg) did not affect upon threshold and propagation.

5. Dilantin, crampol and tridione showed no effect on reticular arousal threshold. Phenobarbital showed no elevation of the threshold at the dose which was suppressive on hippocampal seizure.

6. Functional relation between hippocampus and globus pallidus was discussed concerning with the comparative sensitivity of both afterdischarges to clinically known anti-epileptics.

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