

Only certain antiepileptic drugs prevent seizures induced by pilocarpine

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1. INTRODUCTION

The use of anticonvulsant drugs in the therapy of human seizure disorders is governed by their efficacy against specific seizure type^{7,21,60}. The potential mechanism of anticonvulsant action is much less useful for therapeutic decision with regard to seizure control in man^{7,13,19,21,54,64,81}.

Based on the effectiveness in the maximal electroshock test and pentylenetetrazol seizure threshold test in rodents, anticonvulsants are classified as those preferentially active against generalized tonic-clonic convulsions (diphenylhydantoin and carbamazepine), as those active against generalized absence seizures (ethosuximide and trimethadione), or as those with mixed efficacy (clonazepam, phenobarbital and valproate)²¹. Antiepileptic drugs active against amygdala kindled convulsions are reported to be useful in the control of complex partial seizures in humans (carbamazepine, diphenylhydantoin, clonazepam, phenobarbital and valproate)^{2,46,50,54,64,67}.

The mechanisms through which antiepileptic drugs might aid in the control of seizures are not known. One possibility is that antiepileptic drugs enhance inhibitory and/or reduce excitatory synaptic neurotransmission^{13,53,54,80,82}. Alternatively, antiepileptic drugs might affect ion conductances and reduce repetitive firing non-synaptically⁴⁸ or presynaptically influence release of neurotransmitters^{13,48,53,80,81}.

Seizures elicited by the cholinergic muscarinic agonist pilocarpine in rodents have been proposed,

on the basis of electroencephalographic (EEG) monitoring, behavioral analysis and morphological sequelae, as an animal model resembling some aspects of human temporal lobe epilepsy^{24,57–59,87,90,92–94}. Temporal lobe or ‘psychomotor’ epilepsy is the most common form of epilepsy in man^{27,64}. This type of seizure is particularly resistant to anticonvulsant medication and represents a major therapeutic problem⁷².

Electrical kindling of the amygdala in rodents serves currently as a pharmacological model for complex partial seizures^{28,46,49–51}. Although some similarities between amygdala kindling and human ‘psychomotor’ epilepsy may be found, this pharmacological procedure does not satisfactorily resemble morphological sequelae of complex partial seizures, which are commonly detected in brains from humans suffering from temporal lobe epilepsy⁵⁶. Thus, no satisfactory pharmacological model resembling behavioral, EEG and morphological aspects of temporal lobe epilepsy is available.

This formed the foundation for challenging the effectiveness of commonly utilized antiepileptic drugs in a pilocarpine model of limbic convulsions in rats. This approach would validate this seizure type as a drug-testing model. In the following we describe the efficacy of 8 clinically utilized antiepileptic drugs (clonazepam, phenobarbital, trimethadione, valproate, carbamazepine, diphenylhydantoin, ethosuximide and acetazolamide) in the control of seizures elicited by pilocarpine in rats.

Some of these data have been communicated to

the Congress of the Polish Pharmacological Society in Lublin (Poland)¹² and to the Meeting of the Society for Neuroscience in Washington (DC, U.S.A.)⁸⁹.

2. MATERIALS AND METHODS

2.1. Animals

The experimental subjects were adult male Wistar rats, 250–280 g in weight. The rats were housed individually and maintained on a standard light–dark cycle with ad libitum access to chow pellets and water. The assignment of rats to experimental groups and the determination of subsequent time of behavioral and electroencephalographic (EEG) testing for different treatment groups were performed by means of a randomized method. The behavioral observations and monitoring of the EEG took place between 8.00 and 20.00 h. Experimental groups consisted of 3–12 animals.

2.2. Drugs

Pilocarpine hydrochloride (PILO), methylscopolamine nitrate, carbamazepine (CARB) and acetazolamide (ACT) were obtained from Sigma (St. Louis, MO, U.S.A.). Diphenylhydantoin (DPH), ethosuximide (ETX) and valproic acid (sodium salt) (VPA) were acquired from Desitin (Hamburg, F.R.G.). Phenobarbital (PHB) and clonazepam (CLO) were purchased from Polfa (Poznan, Poland), while trimethadione (TMD) was obtained from Labor (Warsaw, Poland). The drugs were dissolved in saline or suspended in a 3% solution of Tween 80 (Loba, Vienna, Austria) and administered i.p. or s.c. in a volume of 0.5 ml/100 g b. wt.

PILO was administered i.p. in doses of 200 and 380 mg/kg. Methylscopolamine, 1 mg/kg, was administered s.c. 30 min prior to the injection of either dose of PILO.

CLO was administered in doses of 0.1, 0.25, 0.5 and 1 mg/kg, PHB in doses of 12.5, 18, 25 and 50 mg/kg, VPA was given in doses of 100, 200, 300 and 400 mg/kg, while TMD in doses of 100, 200, 300 and 500 mg/kg. DPH was injected in doses of 10, 50, 100 and 200 mg/kg, whereas CARB was administered in doses of 10, 25 and 50 mg/kg. ETX was given in doses of 100, 150, 200 and 500 mg/kg, and ACT in doses of 100, 250, 500, 750 and 1000 mg/kg.

VPA was administered 15 min, CLO and PHB

were given 30 min, while CARB, DPH and TMD 60 min prior to the injection of 380 mg/kg PILO. ETX was administered 30 min, while ACT 60 min prior to 200 mg/kg PILO.

The timing intervals chosen for each anticonvulsant were related to maximal efficacy of the respective drugs in suppressing seizures in other experimental models of epilepsy^{9,14,26,39,41,71,75}. The dose of the drug required to block or trigger the seizure response in 50% of rats (ED_{50} ; effective dose) given 380 and 200 mg/kg of PILO, respectively, was determined by computer analysis of the data obtained from 3–4 experiments with different dosages. The criterion used to indicate convulsive response was status epilepticus defined as continuous motor seizures (stage IV/V according to Racine⁶⁸) persisting for a period of at least 30 min before spontaneous termination. The incidence of seizure response (probit transformed percentages) was plotted vs log dose of either drug tested. The ED_{50} and the confidence limits were estimated by fitting the data by linear regression analysis^{43,88}.

2.3. Surgery and electrophysiological procedures

For depth recordings, bipolar twisted electrodes (tip diameter 100 μm , interelectrode distance 500 μm) were stereotactically positioned in the dorsal hippocampus (AP 4.0, L +2.6, V +1.7) and amygdala (AP 4.6, L +4.0, V -3.6)⁴⁰ under sodium pentobarbital (Nembutal, Ceva, Neuilly-sur-Seine, France; 50 mg/kg i.p.) anesthesia and anchored to the skull with dental acrylic. Surface recordings were led from jeweller screws positioned bilaterally over the occipital cortex. An additional screw placed in the frontal sinus served as a reference (indifferent) electrode. Signals under investigation were amplified by a Beckman model RM polygraph (time constant 0.03 s, high cut off filter 15). EEG recordings and behavioral observations were carried out in a plexiglass compartment (30 × 30 × 45 cm). Before the monitoring of the EEG, animals were individually placed in the recording compartment and allowed 30 min for habituation to the recording setup. The baseline EEG recordings were made for 30 min and then animals received an i.p. injection of antiepileptic drugs or solvent. Subsequently, the rats received PILO, 380 or 200 mg/kg i.p. EEG recordings were made continuously and behavior noted for periods ranging from 4

to 8 h following the injection of PILO. Additional recordings were made between 10–12, 20–24, 36–48 h and 5–7 days. The correct location of implanted electrodes was controlled histologically in Cresyl violet-stained serial sections.

2.4. Morphological techniques

The brains were processed for morphological analysis by light microscopy 24–72 h, 5–14 days and 21–31 days after the administration of PILO and antiepileptic drugs. The details for histological processing are described elsewhere^{33,85}. The rats were anesthetized with an overdose of sodium pentobarbital and perfused by the fixative containing 10% acetic acid, 10% formaldehyde and 80% methanol. The brains were allowed to fix in situ at 4 °C for 24 h, then removed and processed for paraffin embedding. Subsequently, serial sections of the entire brain were cut coronally at 10 µm, with every tenth section being mounted on a glass slide, and stained with Cresyl violet or according to the Fink and Heimer technique²³.

RESULTS

3.1. Behavior

3.1.1. Pilocarpine. PILO, 380 mg/kg ($n = 9$), presented an array of persistently recurring behavioral alterations in rats. Akinesia, ataxic lurching, gustatory automatisms and head tremor dominated the animals' behavior immediately following the injection. After 20–30 min this behavior progressed to motor limbic seizures with rearing, forelimb clonus, salivation, intense masticatory jaw movements and falling. Motor limbic seizures commenced after 26.4 ± 5.3 min ($n = 9$), recurred every 2–8 min, and led to status epilepticus at 50–60 min. Three animals died in the course of seizures elicited by PILO, 380 mg/kg (3/9).

PILO, 200 mg/kg ($n = 8$), rendered animals akinetic and cataleptic immediately following the injection. Mild tremor of the head, occasional myoclonic movements or head bobbing, scratching and teeth chattering comprised the behavior for up to 1–2 h postinjection. This activity subsided within the following 30–45 min and behavior was indistinguishable from that in saline-treated control rats ($n = 5$). None of the rats given PILO, 200 mg/kg, developed generalized seizures and status epilepticus.

3.1.2. Clonazepam. CLO suppressed motor lim-

bic seizures, protected the rats from status epilepticus and decreased the lethal toxicity of PILO, 380 mg/kg, in a dose-dependent manner with an ED₅₀ of 0.35 mg/kg (0.25–0.49; $n = 19$). CLO, 0.1 mg/kg, did not protect against seizures elicited by PILO, 380 mg/kg (4/4), while the dose of 0.25 mg/kg retarded the development of convulsive activity in two out of 5 rats, and totally prevented seizures in one animal (4/5). CLO, 0.5 and 1 mg/kg, protected against the convulsant action of PILO, 380 mg/kg. The behavioral patterns presented by 3 out of 6 rats pretreated with 0.5 mg/kg, were limited to akinesia, tremor of the head and body, and gustatory automatisms. One out of 6 rats treated with CLO, 0.5 mg/kg, displayed motor limbic seizures and status epilepticus (1/6). CLO, 1 mg/kg, prevented seizures and completely eliminated lethal toxicity of PILO, 380 mg/kg (0/4). CLO, 0.1–0.5 mg/kg, slightly depressed exploratory and locomotor activity in the rats, whereas the animals receiving 1 mg/kg were deeply sedated and immobile.

3.1.3. Phenobarbital. PHB protected the rats from behavioral features of seizures produced by PILO, 380 mg/kg, with an ED₅₀ of 23.4 mg/kg (18.5–29.6; $n = 17$). PHB, 12.5 mg/kg, failed to affect seizures and did not decrease the lethal toxicity elicited by PILO, 380 mg/kg (3/3). One out of 4 rats pretreated with PHB, 18 mg/kg, did not develop motor limbic seizures and status epilepticus (3/4). PHB, 25 mg/kg, suppressed all components of motor limbic seizures induced by PILO in 4 out of 6 rats (2/6), and prevented the development of status epilepticus in all 6 animals (0/6). The behavioral changes elicited by PILO, 380 mg/kg, in rats pretreated with PHB, 25 mg/kg, were limited to prominent tremor, infrequent myoclonic movements of hindlimbs and rearing. Two animals displayed motor limbic seizures after a long-lasting period of akinesia (after 65 and 93 min, respectively). The convulsive activity in these animals rapidly subsided within 30–60 min and did not evolve into status epilepticus. None of 4 rats given PHB, 50 mg/kg, and PILO, 380 mg/kg, developed motor limbic seizures or status epilepticus (0/4). The lethal toxicity of PILO, 380 mg/kg, was totally abolished by PHB, 25 and 50 mg/kg. The highest dose of PHB, 50 mg/kg, produced slight sedation and muscle relaxation.

3.1.4. Valproic acid. VPA conferred efficient pro-

tection against PILO-induced seizures in rats with an ED₅₀ of 286 mg/kg (202–405; n = 21). Application of PILO, 380 mg/kg, in rats pretreated with VPA, 200 mg/kg, resulted in motor limbic seizures in 6 out of 7 (6/7) and in status epilepticus in 5 out of 7 animals. The seizures led to the death of 3 rats (3/7). VPA, 300 mg/kg, decreased the range and severity of seizures induced by PILO, 380 mg/kg. Three out of 6 rats receiving VPA, 300 mg/kg, developed motor limbic seizures and one progressed in the status epilepticus. One out of 6 rats died in the course of seizures (1/6). VPA, 400 mg/kg, conferred protection against status epilepticus elicited by pilocarpine in 7 out of 8 rats (1/8). However, 3 rats in this group displayed an attenuated pattern of convulsive activity which comprised myoclonic jerks, gustatory automatisms and single episodes of motor limbic seizures. VPA, 400 mg/kg, did not significantly reduce lethal toxicity of PILO, 380 mg/kg, since two out of 8 animals died in the course of or following seizures (2/8). VPA, 200 and 300 mg/kg, induced a transient period of locomotor stimulation, piloerection and wet dog shakes. When VPA, 400 mg/kg, was administered all animals displayed sedation, ataxia and deep muscle relaxation.

3.1.5. Trimethadione. TMD had a marked suppressant action upon the convulsions induced by PILO. The ED₅₀ for TMD against seizures produced by PILO, 380 mg/kg, was 179 mg/kg (116–277; n = 24). TMD, 100 mg/kg, did not affect seizures and lethal toxicity produced by PILO, 380 mg/kg. Three out of 4 rats in this group exhibited severe convulsions and status epilepticus (3/4) which led to the death of two animals (2/4). TMD, 200 mg/kg, protected one out of 3 rats against motor limbic seizures and status epilepticus (2/3), while 300 mg/kg conferred protection of 6 out of 7 rats against status epilepticus induced by PILO, 380 mg/kg (1/7). Three additional animals pretreated with TMD, 300 mg/kg, demonstrated single episodes of motor limbic seizures. TMD, 500 mg/kg, protected 8 out of 10 rats against seizures elicited by PILO, 380 mg/kg (2/10). Two animals in this group developed motor limbic seizures, which proceeded in one of them to the status epilepticus. TMD did not affect lethal toxicity of PILO, 380 mg/kg, since two out of 7 rats (2/7) treated with 300 mg/kg and two out of 10 rats (2/10) receiving 500 mg/kg died in the course of or following seizures.

With TMD, 300 and 500 mg/kg, animals were sedated and showed a lowered muscle tone.

3.1.6. Diphenylhydantoin. DPH did not suppress seizures produced by PILO, 380 mg/kg. DPH, 10 (n = 6), 50 (n = 6), 100 (n = 3) and 200 mg/kg (n = 3) increased the severity of seizures and enhanced the lethal toxicity of PILO, 380 mg/kg. All rats receiving DPH (n = 18) displayed motor limbic seizures and status epilepticus after a short latency of 10–15 min. Fifteen animals (15/18) died in the course of seizures between 30 and 50 min following the injection of PILO. The remaining 3 rats displayed a full pattern of limbic convulsions culminating in severe status epilepticus. An unexpected severity of seizures induced by PILO, 380 mg/kg, in DPH-pretreated rats and an increased lethal toxicity of PILO suggested a decrease in the threshold for seizures induced by the drug. To approach this problem we pretreated rats with either 10 (n = 4) or 50 mg/kg (n = 4) of DPH prior to application of PILO, 200 mg/kg, a dose which does not induce seizures by itself⁹². None of the rats given DPH and PILO, 200 mg/kg, developed motor limbic seizures or status epilepticus. DPH, 50 and 100 mg/kg, produced sedation and slight muscle relaxation in rats, while the dose of 200 mg/kg led to the loss of the righting reflex and deep muscle relaxation.

3.1.7. Carbamazepine. CARB did not protect rats against PILO-induced convulsions. The rats receiving CARB in doses of 10 (n = 3), 30 (n = 3) and 50 mg/kg (n = 6) developed a full array of behavioral alterations usually elicited by PILO, 380 mg/kg, in drug-naïve rats^{83–85,92}. The time course, spectrum and quality of behavioral alterations did not differ from those monitored following application of PILO, 380 mg/kg, alone. The lethal toxicity of PILO, 380 mg/kg, in CARB-treated rats did not differ from that in drug-naïve rats. Although CARB, 10 and 30 mg/kg, produced only slight sedation, the high dose of 50 mg/kg resulted in deep muscle relaxation and akinesia.

3.1.8. Ethosuximide. The seizures elicited by PILO, 380 mg/kg, were not suppressed by ETX, 500 mg/kg. All animals treated with ETX, 500 mg/kg (n = 3), and PILO, 380 mg/kg, developed a full array of convulsive activity and died in the course of seizures. Surprisingly, ETX increased the range and severity of seizures produced by PILO, 200 mg/kg, in a dose-dependent manner. The ED₅₀ for ETX as a factor

triggering seizures in rats treated with PILO, 200 mg/kg, was 196 mg/kg (141–272; $n = 21$). None of 3 animals receiving ETX, 100 mg/kg, developed motor limbic seizures or status epilepticus (0/3). Two out of 6 rats treated with ETX, 150 mg/kg, presented seizures and status epilepticus (2/6). Application of ETX, 200 mg/kg, led to motor limbic seizures and status epilepticus in 5 out of 6 rats (5/6). One animal died in the course of status epilepticus (1/6). In all animals pretreated with ETX, 500 mg/kg ($n = 6$), PILO, 200 mg/kg, elicited motor limbic seizures and severe status epilepticus, which led to the death of 3 rats (3/6). ETX induced a high degree of sedation and muscle relaxation in all doses employed; however, akinesia and muscle relaxation were particularly pronounced with 500 mg/kg.

3.1.9. Acetazolamide. ACT conferred no protection against seizures produced by PILO, 380 mg/kg, in rats. Although the rats pretreated with ACT, 100 ($n = 3$), 250 ($n = 3$) and 500 mg/kg ($n = 3$), showed a typical pattern of convulsive activity usually ob-

served following application of PILO, 380 mg/kg, alone, the lethal toxicity was dramatically increased. Eight out of 9 rats treated with different dosages of ACT and PILO, 380 mg/kg, died in the course of intractable seizures (8/9). Surprisingly, ACT increased the susceptibility of rats to seizures produced by PILO with an ED₅₀ of 505 mg/kg (332–766; $n = 31$). Although none of 3 rats receiving ACT, 100 mg/kg (0/3), developed motor limbic seizures, one out of 6 animals pretreated with 250 mg/kg displayed a full array of convulsant activity culminating in status epilepticus (1/6). The treatment with ACT, 500 mg/kg, and PILO, 200 mg/kg, led to motor limbic seizures and status epilepticus in 6 out of 12 rats (6/12). In this treatment group status epilepticus led to the death of two animals (2/12). In two out of 3 rats pretreated with ACT, 750 mg/kg, PILO, 200 mg/kg, led to severe status epilepticus (2/3) and the death of one animal (1/3). With ACT, 1000 mg/kg, and PILO, 200 mg/kg, 6 out of 7 rats developed status epilepticus (6/7). One rat from this treatment group died in the

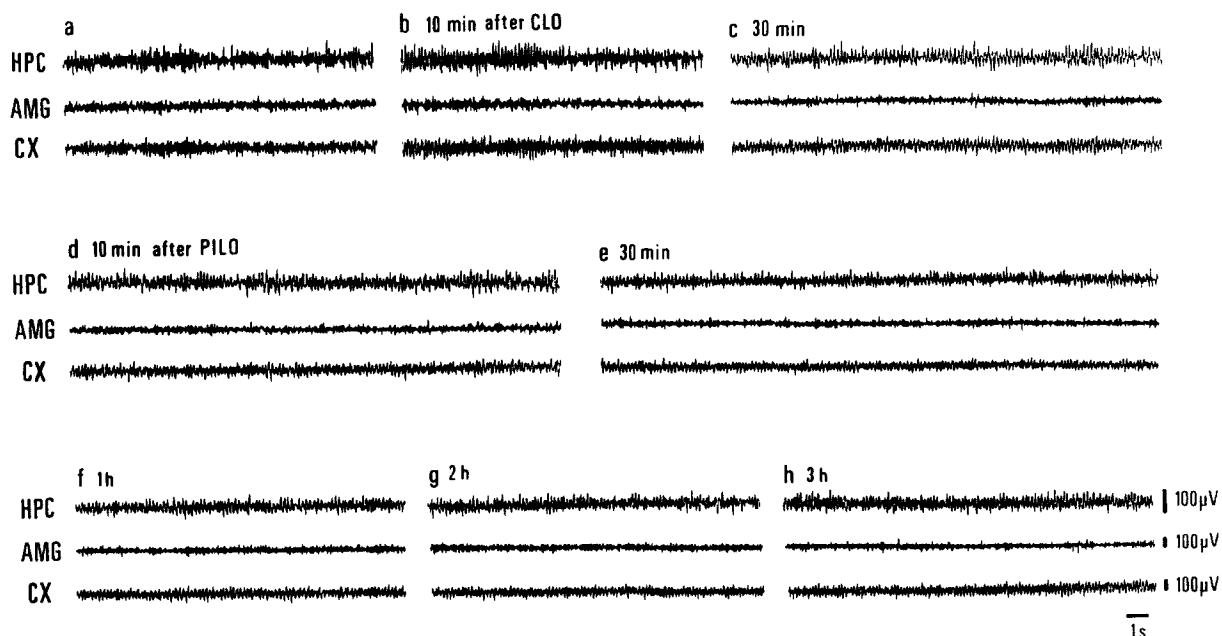


Fig. 1. Electrographic recordings demonstrating the effect of CLO on the convulsant action of PILO. CLO, 1 mg/kg, was administered i.p. 30 min prior to the injection of PILO, 380 mg/kg i.p. a: pre-drug control recordings. b,c: electrographic correlates 10 and 30 min after injection of CLO. High voltage fast activity transiently dominates all recordings immediately after the injection of CLO (b). Subsequently, high voltage slow activity supersedes the hippocampal rhythms and background activity in the cortex (CX), while low voltage slow activity is registered in the amygdala (AMG) (c). d,e: the θ -rhythm reappears in the hippocampal (HPC) recordings 10–30 min after PILO (d). Low voltage activity becomes slower in the AMG (e), while CX recordings remain unchanged (e). f,g: low or high voltage slow activity alternates with θ -rhythm and prevails in the AMG and CX for up to 1–2 h following PILO. h: by 3 h after PILO the EEG progressively returns to the pre-drug background activity (a).

course of protracted seizures (1/7). ACT, 750 and 1000 mg/kg, induced akinesia and slightly lowered the muscle tone in rats.

3.2. Electroencephalography

3.2.1. Pilocarpine. The pattern of electrographic changes elicited by PILO, 380 and 200 mg/kg, has been thoroughly described elsewhere^{83,84}. Briefly, immediately following the injection of pilocarpine, a significant θ -rhythm superseded the background activity in the hippocampus (HPC), and low voltage fast activity occurred in the cortex (CX) and amygdala (AMG). This activity progressed to high voltage fast activity with spiking in the HPC. The spiking activity spread to the AMG and CX, became well synchronized and evolved into electrographic seizures. The ictal periods recurred every 3–5 min and led finally to status epilepticus 60–90 min following the injection of PILO, 380 mg/kg. This pattern of electrographic activity lasted for several hours (4–12 h) and then the seizure activity gradually abated following 10–24 h. The EEG usually returned to the pre-drug patterns within 48–72 h.

The typical pattern of electrographic changes produced by PILO, 200 mg/kg, comprised significant

θ -rhythm in the HPC and low voltage fast activity in the CX and AMG. Isolated spikes and sporadically bursts of polyspiking occurred in the hippocampal recordings 1–2 h following the application of the drug. The EEG returned to the pre-drug background activity within 2–4 h after the administration of PILO, 200 mg/kg.

3.2.2. Clonazepam. The EEG following administration of CLO, 0.5 or 1 mg/kg, was initially characterized by high voltage fast activity typically registered in all records (Fig. 1b,c). This type of transient alterations was gradually replaced by high voltage slow activity in HPC and CX recordings, while low voltage slow activity dominated in the AMG (Fig. 1c). The EEG returned to the background patterns 4–6 h following the application of CLO, 1 mg/kg.

Pretreatment with CLO, 1 mg/kg ($n = 4$), prevented the buildup of convulsive activity in the EEG produced by PILO, 380 mg/kg (Fig. 1). Within 10–30 min following the injection of PILO the θ -rhythm replaced high voltage slow activity in the HPC, while low voltage slow activity was registered in the AMG and CX. The low voltage fast activity alternated with the θ -rhythm in the hippocampal recording 30–60 min following the injection of PILO. The EEG be-

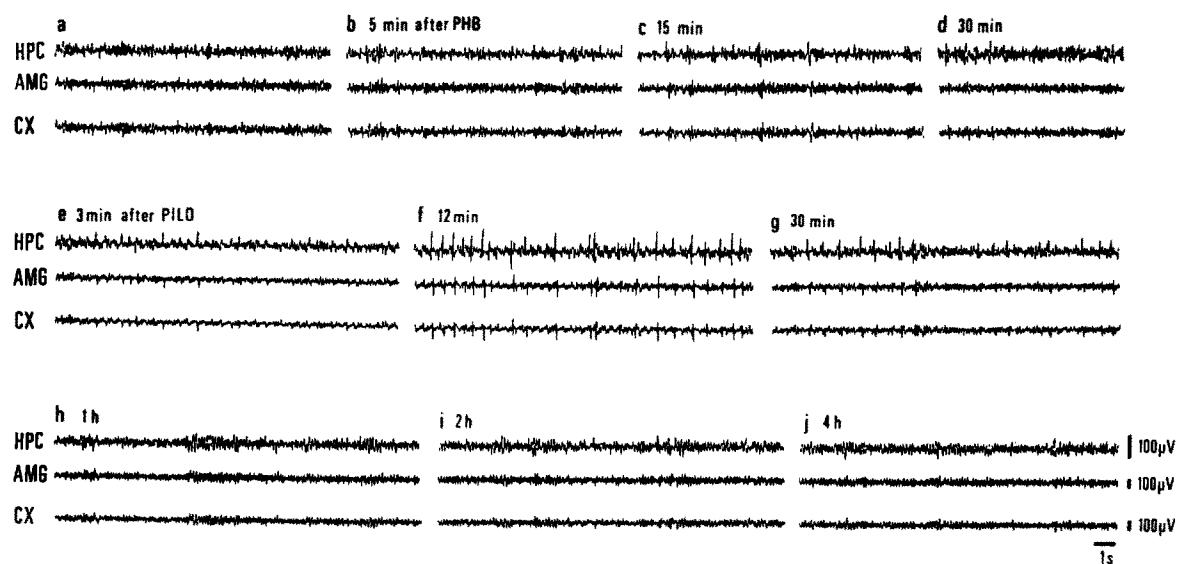


Fig. 2. Recordings to illustrate the electrographic activity elicited by PILO, 380 mg/kg, in rats pretreated with PHB, 50 mg/kg. a: pre-drug control recordings. b: high voltage slow activity superposes over the HPC θ -rhythm and background rhythms in the AMG and CX. c,d: high voltage slow activity and slow waves prevail in all recordings 15 and 30 min following the injection of PHB. e: high voltage fast activity supersedes the electrographic activity in the HPC 2–5 min following the injection of PILO, while low voltage fast activity is concurrently registered in the AMG and CX. f,g: high voltage fast activity and spiking are transiently registered in the HPC, AMG and CX for up to 30–40 min following PILO. h,i: the EEG progressively normalizes in all recordings within 1–2 h. j: the pre-drug pattern is registered 2–4 h after injection of PILO.

came indistinguishable from pre-drug activity by 3–8 h (Fig. 1g,h).

3.2.3. Phenobarbital. The EEG activity following the injection of PHB, 25 or 50 mg/kg, was characterized by high voltage slow activity registered in all records (Fig. 2b–d). The pattern of electrographic changes produced by PILO, 380 mg/kg, in rats pretreated with PHB, 25 mg/kg, is illustrated in Fig. 2. The high voltage fast activity and spiking alternated with θ -rhythm in the HPC for up to 1 h following the administration of PILO, while low voltage fast or spiking activity prevailed in the AMG and CX (Fig. 2e–g). By 1–2 h PILO led to a decrease in the voltage of electrographic activity (Fig. 2i). The EEG returned to the background pattern within 3–4 h (Fig. 2j).

3.2.4. Valproic acid. VPA, 400 mg/kg ($n = 4$), resulted in immediate depression of the EEG-activity

in the HPC, AMG and CX with appearance of low voltage slow activity and slow waves (Fig. 3). This pattern of electrographic activity was interrupted by sudden wet dog shakes. The EEG normalized 3–4 h following the injection of VPA, 400 mg/kg. VPA, 400 mg/kg, prevented the development of paroxysmal activity produced by PILO, 380 mg/kg (Fig. 3). The application of PILO resulted in reappearance of the HPC θ -rhythm and fast activity in the AMG and CX (Fig. 3d,e). The θ -rhythm and periods of high voltage fast activity with isolated spikes were typically registered in the HPC within 1–2 h after PILO application (Fig. 3f–j). AMG and CX recordings displayed minor changes usually limited to the appearance of isolated spikes which were not synchronized with HPC activity (Fig. 3h). The EEG became indistinguishable from the pre-drug activity after 4–6 h (Fig. 3l).

3.2.5. Trimethadione. Immediately after the in-

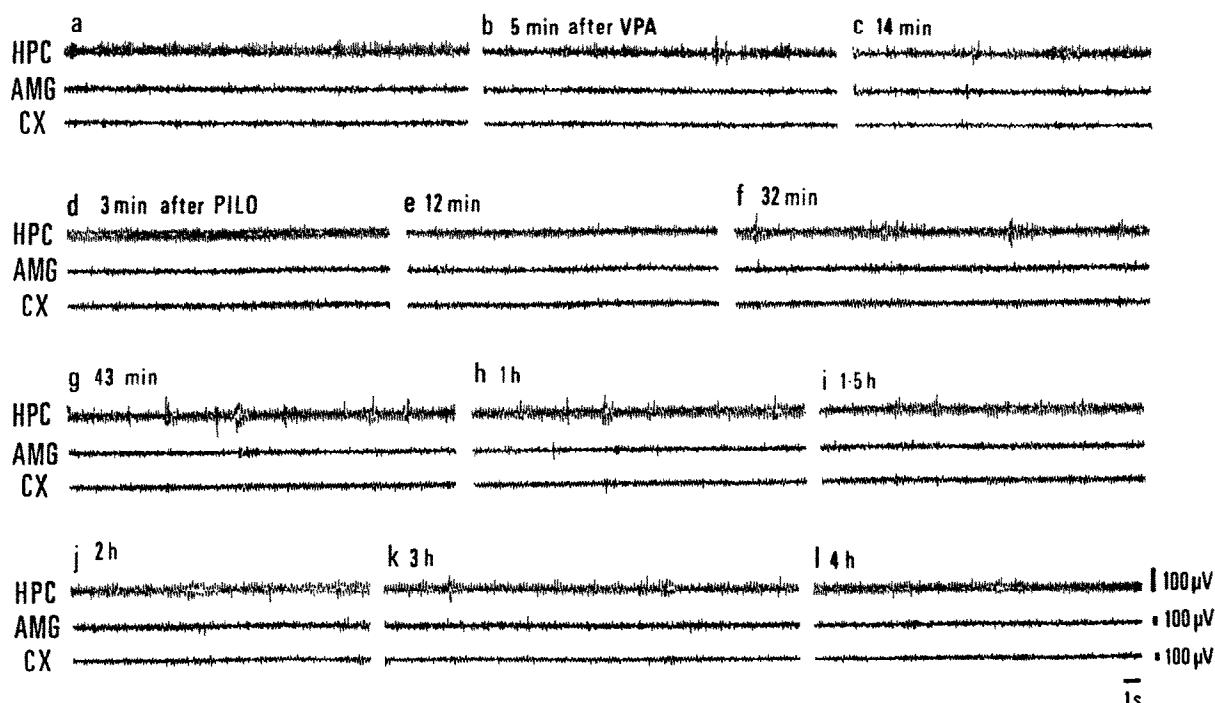


Fig. 3. The effect of VPA on the convulsant action of PILO in rats. VPA, 400 mg/kg, was administered i.p. 15 min prior to the injection of PILO, 380 mg/kg i.p. a: pre-drug control recordings. b,c: electrographic correlates 5 and 14 min after injection of VPA. Low voltage slow activity and slow waves supersede the background activity 2–4 min after administration of VPA (b) and prevail in the EEG for up to 3–4 h (c). This pattern of electrographic activity is frequently interrupted by sudden wet dog shakes (not shown). d: the θ -rhythm in the HPC recordings and background activities in the AMG and CX reappear 2–3 min following the injection of PILO. e,f: high voltage fast activity and isolated spikes registered initially in the HPC alternate with the θ -rhythm (f), while AMG and CX recordings display no or minor changes (f,g) for up to 30–40 min after PILO. g,h: high voltage spiking progressively builds up in the HPC recordings (g) and occasionally spreads to the AMG or CX (h) after a delay of 1–2 h following the injection of PILO. i,j: isolated spikes are infrequently registered within 2–3 h after PILO. k,l: by 3–4 h following the injection of PILO EEG normalizes in all recordings (a).

jection of TMD, 500 mg/kg ($n = 6$), the high voltage slow activity superseded background activity in the HPC and AMG (Fig. 4b) while CX recordings displayed minor or no changes (Fig. 4b). By 10–20 min high voltage slow activity progressed to CX recordings and the EEG activity rapidly evolved into slow wave sleep pattern (Fig. 4c). This pattern of electrographic activity alternated with high voltage slow activity registered typically in all recordings for up to 4–6 h following the injection of TMD, 500 mg/kg.

PILO, 380 mg/kg, induced rapidly a reappearance of the θ -rhythm in the hippocampus and low voltage fast activity in the AMG and CX (Fig. 4e,f) in rats pretreated with TMD, 500 mg/kg ($n = 6$). The θ -rhythm alternated with short-lasting periods of high voltage fast activity and spiking during 1–2 h (Fig. 4g–j). The AMG and CX recordings displayed minor changes limited to the appearance of short-lasting periods of slow activity. This type of transient alterations disappeared within 3–4 h (Fig. 4k,l). The back-

ground pattern in the EEG was registered 4–6 h after the injection of PILO, 380 mg/kg (Fig. 4l,m).

3.2.6. Diphenylhydantoin. DPH, 50 mg/kg ($n = 5$), depressed the electrographic background activity in all records 5–10 min after the injection (Fig. 5b). The θ -rhythm in the HPC and low voltage fast activity in the AMG and CX were replaced by high voltage slow activity (Fig. 5c,d). The EEG normalized 2–4 h after the injection of this dose of DPH.

DPH, 50 mg/kg, did not significantly modify the pattern and quality of EEG changes produced by PILO, 380 mg/kg ($n = 6$) (Fig. 5). Within 2–4 min following the injection of PILO, significant θ -rhythm dominated HPC activity, while low voltage fast activity occurred in the AMG and CX (Fig. 5e). Isolated high voltage spikes usually appeared 5–10 min after the injection of PILO and rapidly spread to CX and AMG recordings (Fig. 5h). The EEG activity progressed into high voltage fast activity with prominent spiking in the HPC (Fig. 5h) and then evolved into

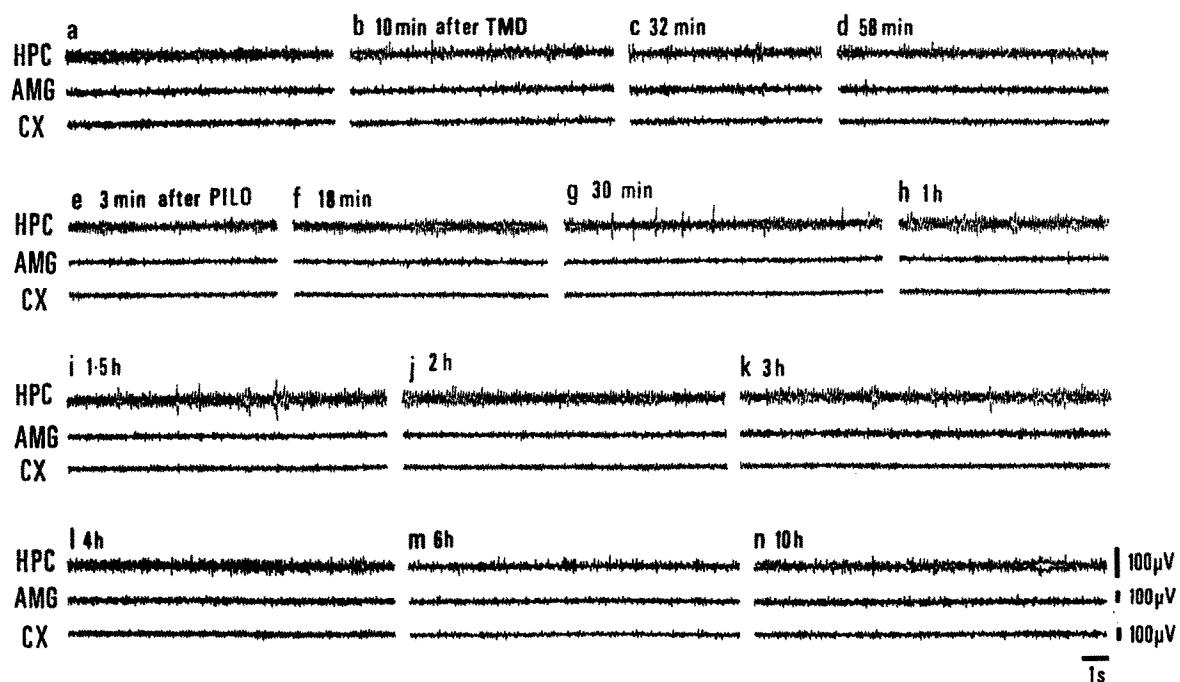


Fig. 4. Electrographic recordings demonstrating the protective effect of TMD against seizures produced by PILO in rats. TMD, 500 mg/kg, was given i.p. 1 h prior to the injection of PILO, 380 mg/kg i.p. a: pre-drug control recordings. b: high voltage slow activity in the HPC and AMG supersedes the background activity 5–10 min after administration of PILO. c,d: high voltage slow activity prevails in all recordings after 20–30 min and progresses to slow wave sleep pattern (c). This type of activity alternates with high voltage slow activity (d) and represents the common EEG feature registered following the application of TMD. e,f: the θ -rhythm reappears in the HPC 2–3 min after administration of PILO, while the AMG and CX recordings display minor changes (f). g,h: high voltage fast activity and spiking originating in the HPC alternate with the θ -rhythm (h). i–k: isolated spikes are infrequently registered in the HPC for up to 2–3 h after PILO (i,k), while AMG and CX recordings remain slightly depressed (i,j). l–n: by 4–10 h after injection of PILO the EEG returns to the pre-drug pattern (a).

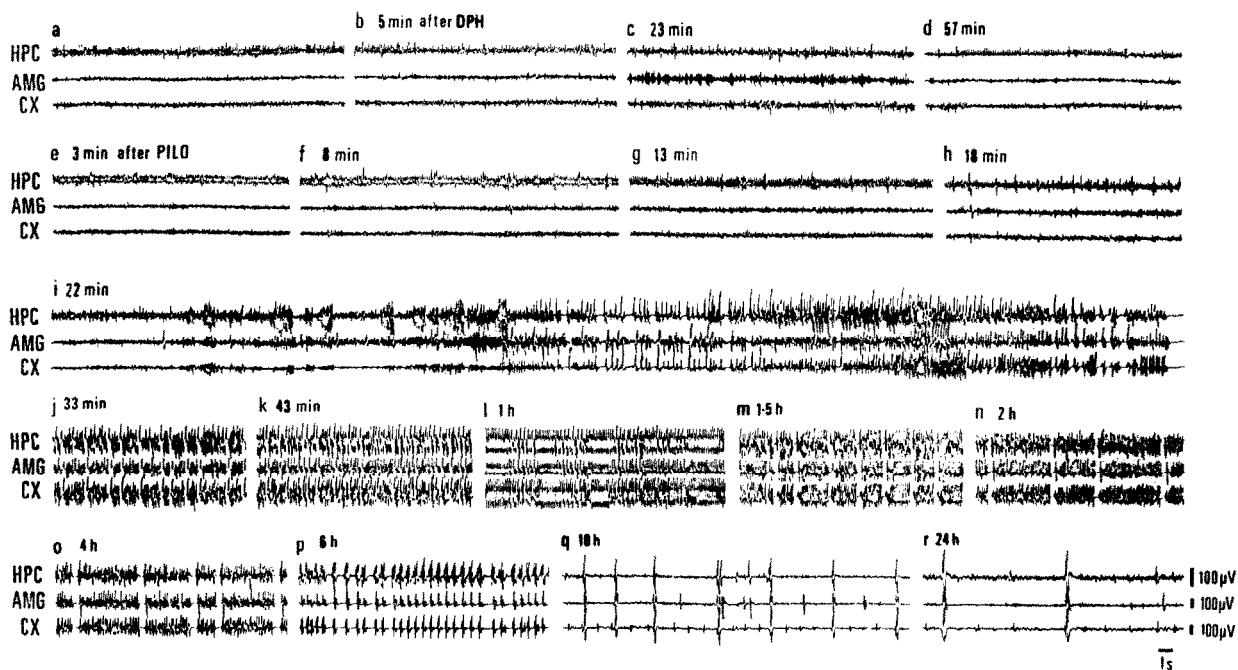


Fig. 5. The effect of DPH on the convulsant action of PILO in rats. DPH, 50 mg/kg, was administered i.p. 1 h prior to the injection of PILO, 380 mg/kg i.p. a: pre-drug control recordings. b,c: electrographic correlates 5 and 23 min after injection of DPH. The high voltage slow activity replaces the background activities in all recordings within 5–10 min (c) and becomes prominent 30–40 min following DPH. d: high voltage slow activity and slow waves prevail in all recordings for up to 1 h. e: θ -rhythm reappears in the HPC recordings immediately after application of PILO, whereas the activity in the AMG and CX becomes faster. f,g: high voltage spiking is initially registered in the HPC records. h: spiking becomes well synchronized in the HPC, AMG and CX 15–20 min after PILO. i: an electrographic seizure registered 22 min after PILO. High voltage fast activity and spiking originating in the HPC precede buildup of the seizure. The waxing of the seizure is slower in the cortex. j: the electrographic activity rapidly progresses to the status epilepticus within 25–35 min after PILO. k-m: electrographic activity registered 40–90 min after injection of PILO during status epilepticus. n-p: the paroxysmal activity slowly abates during the next 4–6 h. q: high voltage spiking with progressive depression of the background activity is registered 10–12 h after PILO. r: isolated spikes and severely depressed background activity are frequently registered 24–72 h after PILO.

electrographic seizures (Fig. 5i). The electrographic seizures rapidly led to severe status epilepticus (first or second seizure evolved into status epilepticus), which occurred 20–30 min after administration of PILO (Fig. 5j), lasted 4–8 h (Fig. 5k–p), and was almost always fatal. In animals which survived, the seizure activity gradually abated during 6–24 h (Fig. 5p,q). By 24 h after the injection of PILO, low voltage slow activity with high voltage spikes was typically registered in all recordings (Fig. 5r). The EEG returned to the pre-drug pattern within 72–120 h; however, the isolated spikes occurred up to 5–7 days after the administration of PILO.

DPH, 50 mg/kg ($n = 3$), did not alter the spectrum of electrographic alterations elicited by PILO, 200 mg/kg.

3.2.7. Carbamazepine. CARB, 50 mg/kg ($n = 4$) produced an electrographic pattern which resembled

well that elicited by DPH. Shortly after the injection CARB depressed the electrographic activity in all recordings (Fig. 6b). Slow waves and high voltage slow activity prevailed in the EEG for up to 4–5 h.

The pattern of electrographic changes induced by PILO, 380 mg/kg, in rats pretreated with CARB ($n = 4$) did not differ from that registered in drug-naive rats (Fig. 6). Within 3–5 min following the injection of PILO, the θ -rhythm reappeared but was rapidly replaced with high voltage fast activity and spiking (Fig. 6e,f). This type of activity spread to the AMG and HPC (Fig. 6g) and became well synchronized in all recordings (Fig. 6g,h). The electrographic seizures were repeatedly registered between 20 and 60 min after the injection of PILO (Fig. 6h–j). The electrographic activity progressed into status epilepticus within 1–1.5 h post-injection (Fig. 6k). This pattern persisted for 6–8 h and then the EEG gradually

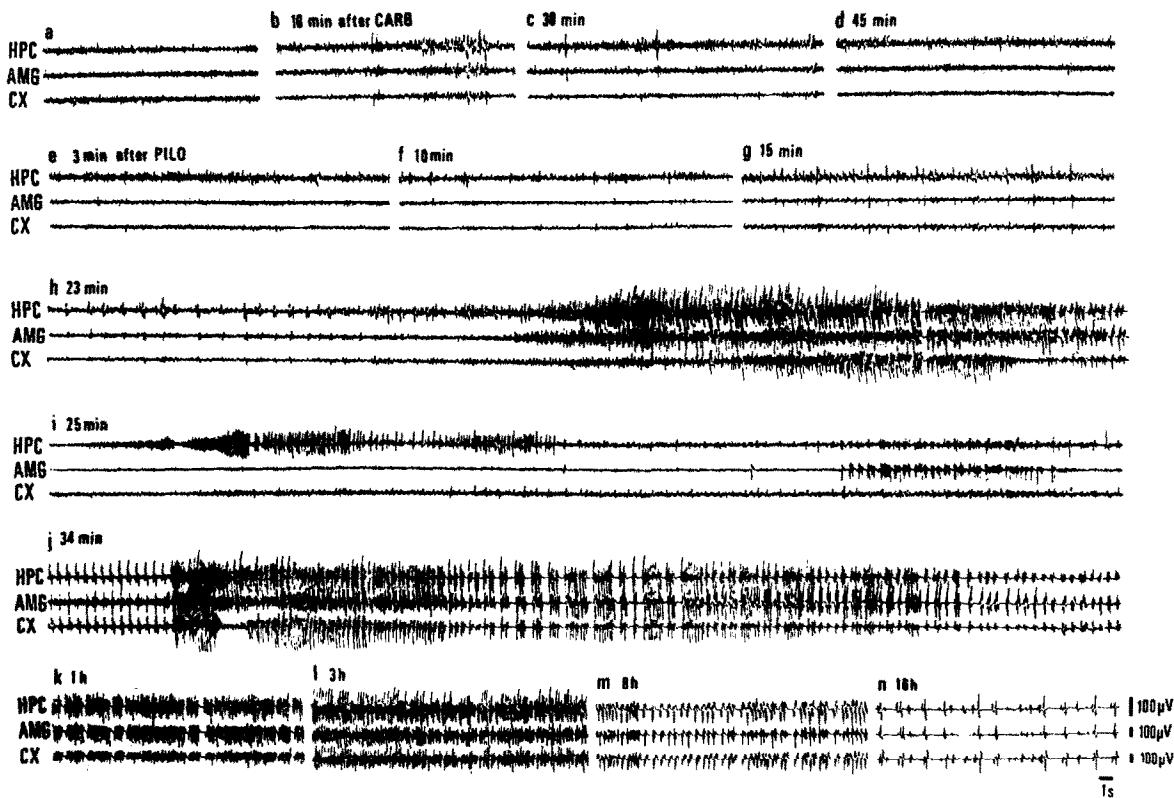


Fig. 6. The effect of CARB on the convulsant action of PILO in rats. CARB, 50 mg/kg, was administered i.p. 1 h prior to the injection of PILO, 380 mg/kg i.p. a: pre-drug control recordings. b-d: electrographic correlates 10, 30 and 45 min following the injection of CARB. High voltage slow activity (b,c) and slow waves (b) prevail in all recordings up to 1 h after CARB. e: high voltage fast activity alternating with significant θ -rhythm represents the characteristic pattern of the EEG registered 2–10 min following the injection of PILO. f: high voltage spiking is initially registered in the HPC, while low voltage activity dominates CX and AMG recordings 10 min after PILO. g: spiking activity becomes highly synchronized in all 3 recordings within 15–20 min. h: the first electrographic seizure 23 min after the injection of PILO. The waxing of the seizure is faster in the HPC and AMG (h). The seizure activity wanes in the HPC and AMG slower relative to CX records (h). i: an electrographic seizure initially registered in the HPC precedes the development of seizures in the AMG and CX. j: high voltage spiking, well synchronized in all recordings, precedes the evolution of a seizure registered 34 min after PILO. k-m: electrographic activity recorded 1–8 h after administration of PILO during status epilepticus. n: high voltage spiking and progressive depression of the background activity characterize the EEG 16–24 h following the injection of PILO.

normalized (Fig. 6l,m). The EEG became indistinguishable from the pre-drug activity after 3–5 days.

CARB, 50 mg/kg ($n = 3$), did not increase the susceptibility to seizures produced by PILO and did not affect the pattern of EEG elicited by PILO, 200 mg/kg.

3.2.8. Ethosuximide. ETX, 500 mg/kg ($n = 5$), transiently reduced the voltage but did not change the morphology of the electrographic activity in all recordings (Fig. 7b–d).

Fig. 7 demonstrates the electrographic alterations produced by PILO, 200 mg/kg, in rats pretreated with ETX, 500 mg/kg. Significant θ -rhythm in the HPC and CX (Fig. 7e) rapidly evolved into spiking, bursts of polyspiking (Fig. 7g,h) and electrographic

seizures (Fig. 7i), which commenced 20–30 min following the injection of PILO, 200 mg/kg. The ictal periods recurred every 3–10 min and led to status epilepticus by 45–60 min (Fig. 7k). The seizure activity gradually abated during 8–24 h (Fig. 7n,o) and the EEG returned to the pre-drug pattern within 72–96 h.

In rats receiving ETX, 500 mg/kg ($n = 3$), PILO, 380 mg/kg, led to rapid development of fatal status epilepticus.

3.2.9. Acetazolamide. ACT, 500–1000 mg/kg ($n = 6$), slightly depressed the normal background activity in all recordings (Fig. 8b–d). Isolated high voltage spikes were registered in the HPC up to 2 h post-injection in one rat. This pattern of activity usually

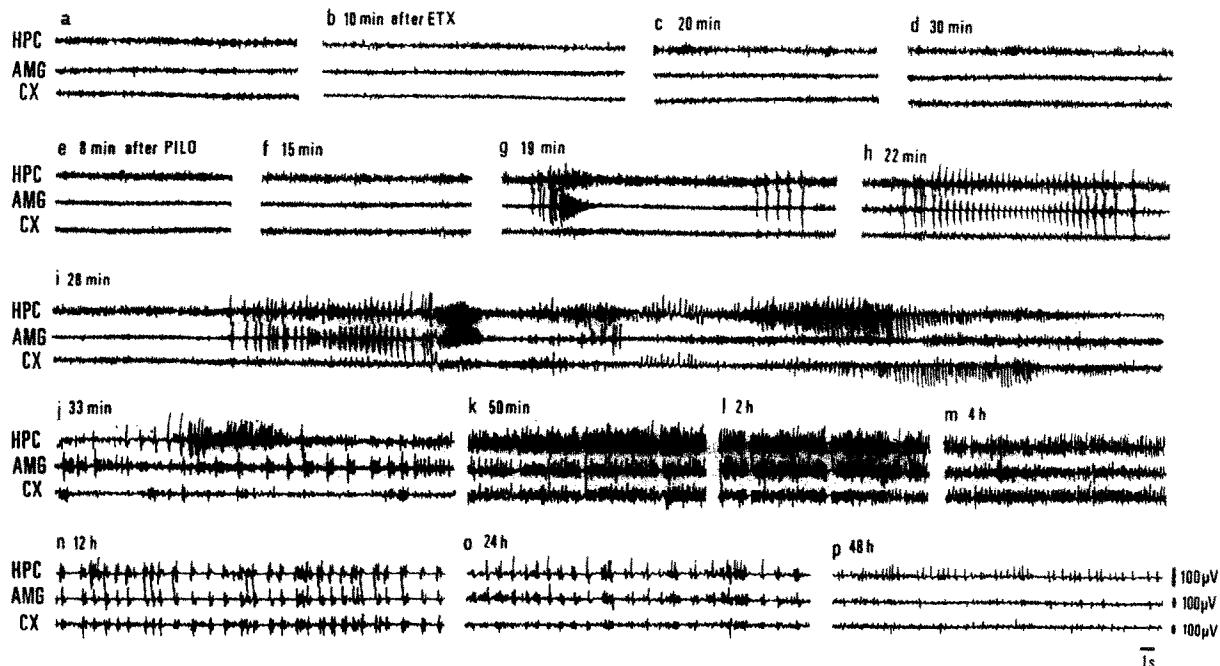


Fig. 7. Electrographic recordings demonstrating the effect of ETX on the convulsant action of PILO in rats. ETX, 500 mg/kg, was administered i.p. 30 min prior to the injection of PILO, 200 mg/kg i.p. a: pre-drug control recordings. b: transient depression of the EEG registered 10 min after ETX. c,d: unchanged records 20 and 30 min after ETX. e,f: significant θ -rhythm in the HPC and low voltage fast activity in the AMG and CX are registered 8 and 15 min after PILO. g: high voltage spiking and bursts of polyspiking are registered in the HPC and AMG, while CX recordings display no or minor changes. h: first electrographic seizure highly synchronized in the HPC and AMG 22 min after injection of PILO. i: an electrographic seizure registered 28 min following the injection of PILO. The seizure activity builds up concurrently in the HPC and AMG, whereas the CX record remains initially unchanged. j: high voltage spikes and bursts of polyspikes represent characteristic EEG pattern registered during the interictal periods. k-m: electrographic activity registered during status epilepticus 1–6 h after PILO. n,o: by 12–24 h after injection of PILO the EEG progressively normalizes, the paroxysmal activity gradually abates and the background activity becomes depressed. p: the EEG returns to the pre-drug patterns after a delay of 3–7 days.

persisted up to 3–4 h following the injection of the drug.

The EEG activity following the injection of PILO, 200 mg/kg, in rats pretreated with ACT, 500 or 1000 mg/kg ($n = 8$), was characterized by rapid development of high voltage fast activity with spiking and bursts of polyspiking, which progressed to electrographic seizures within 10–20 min (Fig. 8e,f), and led to status epilepticus within 20–40 min (Fig. 8h). Status epilepticus lasted for up to 6–8 h (Fig. 8j). The EEG progressively normalized within the next 24–48 h (Fig. 8l,m) and was indistinguishable from the pre-drug pattern within 3–5 days (Fig. 8n). In rats treated with PILO, 380 mg/kg, ACT 250 ($n = 3$) and 500 mg/kg ($n = 3$), resulted in rapid development of status epilepticus which led to the death of all animals within 1–2 h.

3.3. Neuropathology

3.3.1. Pilocarpine. The brains of rats subjected to convulsant action of PILO, 380 mg/kg, presented a characteristic damage pattern involving the amygdaloid complex, thalamus, pyriform and entorhinal cortex, hippocampus, neocortex and substantia nigra. The extent of the damage to the rat forebrain following seizures elicited by PILO, 380 mg/kg, its topography and neuroanatomical characteristics have been thoroughly described elsewhere^{83,85,92}.

PILO, 200 mg/kg, did not induce detectable damage to the forebrain in rats.

3.3.2. Clonazepam. With the highest dose of CLO, 1 mg/kg ($n = 4$), which reproducibly suppressed the electrographic activity produced by PILO, 380 mg/kg, no morphological alterations were detected throughout the entire brain (Fig. 9A). After

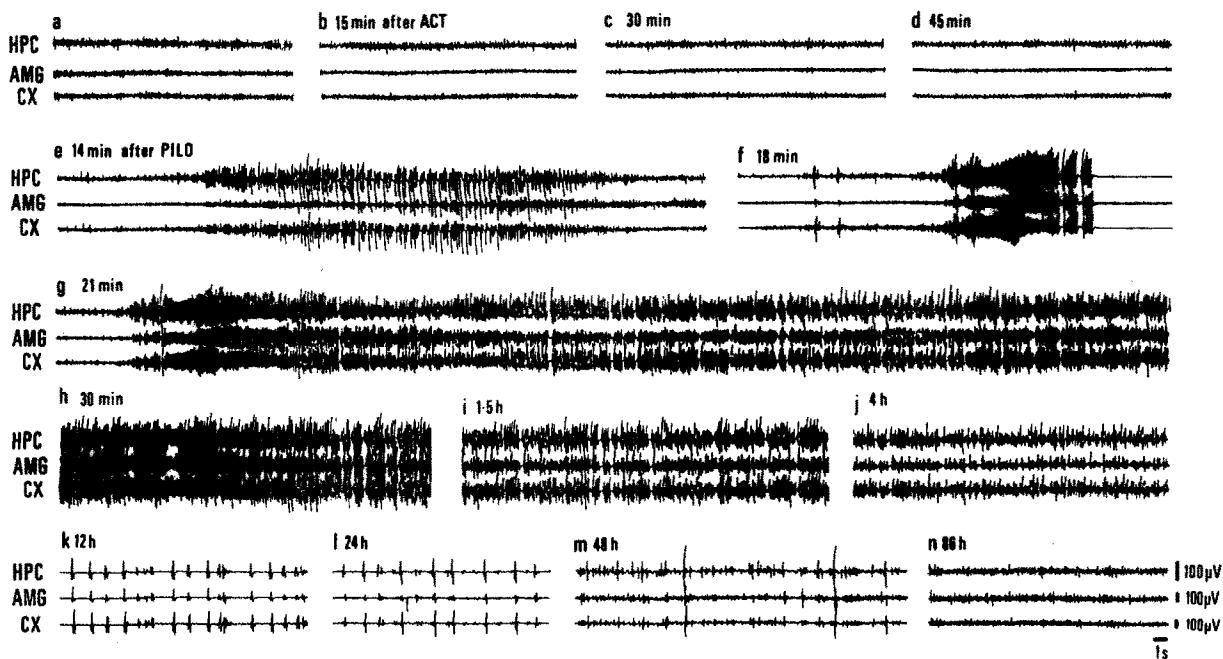


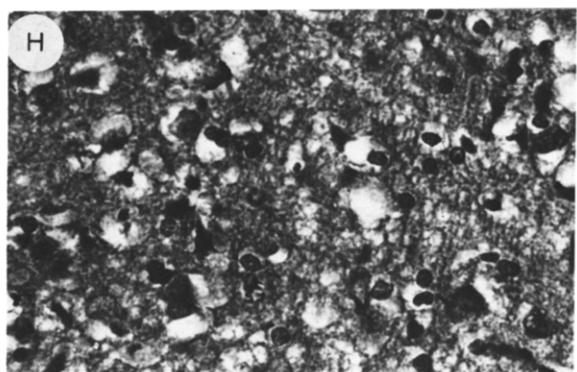
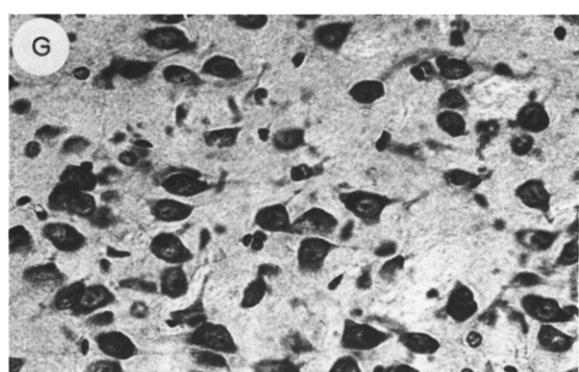
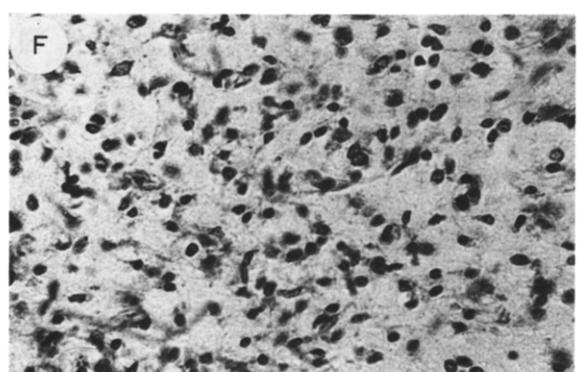
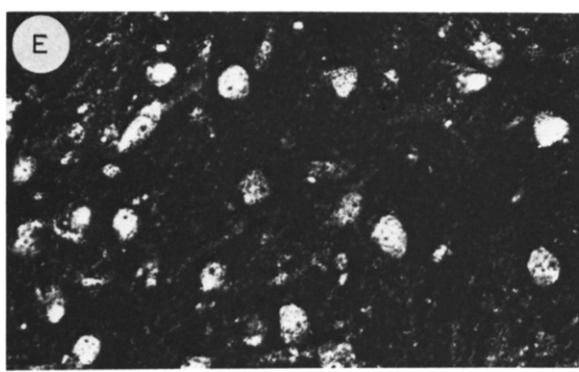
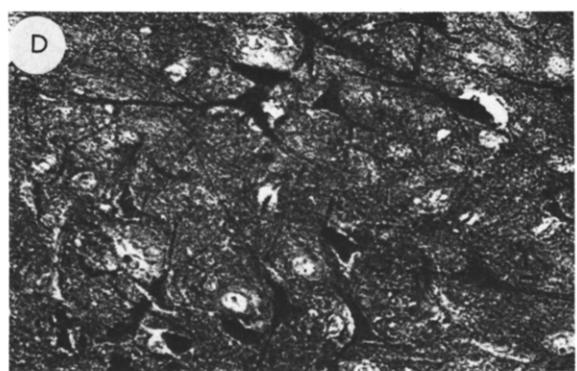
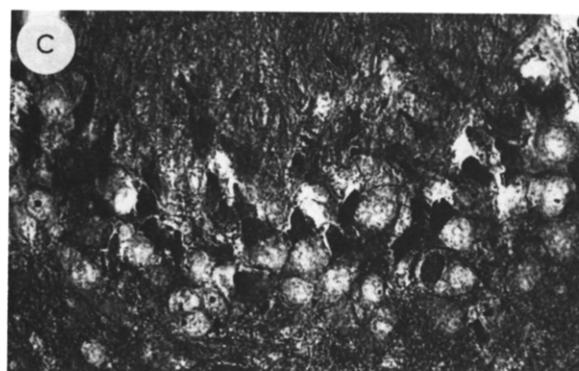
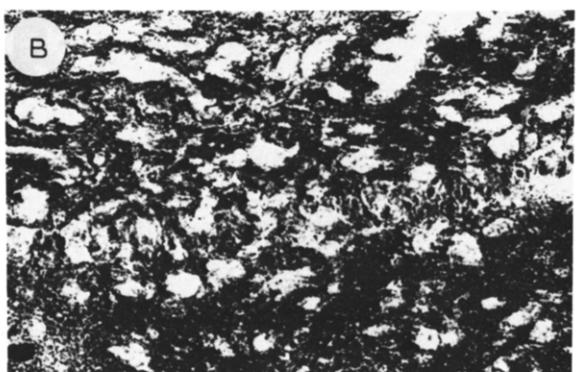
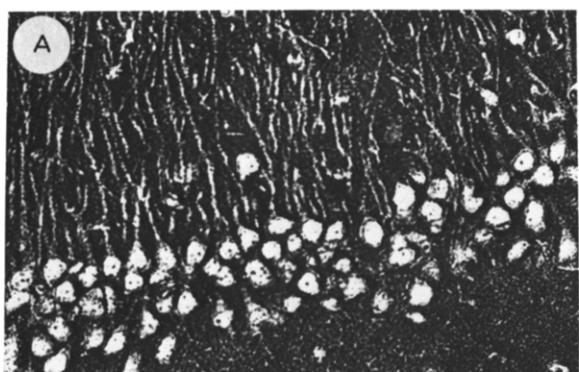
Fig. 8. Electrographic recordings demonstrating the effect of ACT on the convulsant action of PILO in rats. ACT, 500 mg/kg, was administered i.p. 1 h prior to the injection of PILO, 200 mg/kg i.p. a: pre-drug control recordings. b-d: electrographic correlates 15–45 min after the injection of ACT. e: the first electrographic seizure registered 14 min following the injection of PILO. The seizure activity builds up faster in the HPC relative to the AMG and CX recordings. f,g: paroxysmal activity becomes highly synchronized in all recordings (f). Electrographic seizures of long-lasting duration are frequently recorded in the course of the development of convulsant action of PILO in rats pretreated with ACT. h-j: electrographic recordings to illustrate the alterations registered during the status epilepticus 30 min to 4 h after the injection of PILO. k-m: the EEG activity progressively normalizes 12–48 h after PILO. Severely depressed background activity and high voltage spikes represent the common characteristic of the EEG for up to 48–72 h. n: the EEG returns to the pre-drug patterns within 4–7 days following the administration of PILO.

CLO, 0.5 mg/kg ($n = 6$), one rat demonstrated a full pattern of epilepsy-related brain damage, while 5 out of 6 animals showed protection against neuronal degeneration. This protection was complete in 4 rats, whereas one animal demonstrated an attenuated pattern largely confined to the pyriform cortex. The apparent threshold dose for the occurrence of the protection against brain damage produced by PILO in rats pretreated with CLO was 0.25 mg/kg. One out of 5 animals treated with CLO, 0.25 mg/kg, failed to develop neuronal degeneration in the forebrain, while two additional rats revealed an attenuated pattern of the damage which was limited to the anterior and medial pyriform cortex, some few pyramidal cells in the CA₁ subfield of the hippocampus and thalamus. One rat in this group died in the course of status epilepticus and was discarded from the morphological analysis. CLO, 0.1 mg/kg ($n = 3$), did not protect against

neuropathological sequelae of PILO, 380 mg/kg.

3.3.3. Phenobarbital. The rats pretreated with PHB, 50 mg/kg ($n = 4$), and subsequently subjected to the convulsant action of PILO, 380 mg/kg, exhibited no brain damage (Fig. 9E,K). With the dose of 25 mg/kg ($n = 6$) no neuropathological alterations were detected in 4 rats, whereas an attenuated pattern was seen in two animals. The threshold dose for the occurrence of the protective action of PHB was 18 mg/kg, since one rat did not present morphological alterations throughout the brain. With doses of 12.5 mg/kg or less no protection against seizure-related brain damage was found.

3.3.4. Valproic acid. The brain damage produced by PILO, 380 mg/kg, was moderately suppressed by VPA. VPA, 200 mg/kg ($n = 4$), conferred no efficient protection against pathological sequelae of PILO, 380 mg/kg. The topography and extent of the

Fig 9A-H

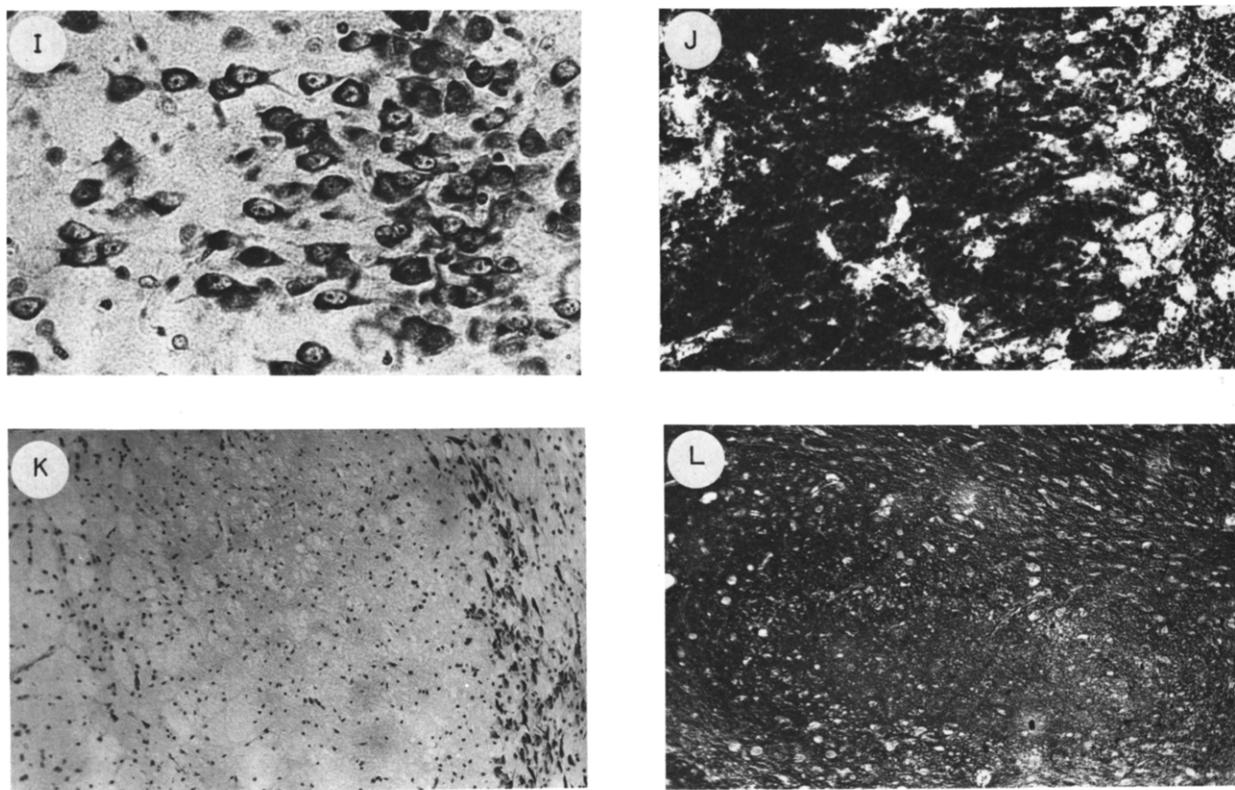


Fig. 9. The distribution and extent of the damage to the forebrain produced by PILO in rats subjected to the action of antiepileptic drugs. Survival times: 72 h to 30 days. A: protection from neuropathological sequelae of seizures elicited by PILO, 380 mg/kg, in a rat pretreated with CLO, 1 mg/kg. No signs of neuronal degeneration are discernible in the CA₁ subfield of the dorsal hippocampus. Survival time: 7 days. Fink-Heimer stain. $\times 186$. B: advanced breakdown of the morphological structure of the CA₁ subfield of the dorsal hippocampus of a rat pretreated with ETX, 500 mg/kg, and subsequently given PILO, 200 mg/kg. Almost all pyramidal cells have disappeared. Survival time: 30 days. Fink-Heimer stain. $\times 186$. C: shrunken argyrophilic neurons surrounded with dilated clear spaces are visible in the CA₃ subfield in the dorsal hippocampus of a rat pretreated with ACT, 500 mg/kg, and subjected to seizures elicited by PILO, 200 mg/kg. Survival time: 72 h. Fink-Heimer stain. $\times 186$. D: extensive disruption of the CA₄ subfield with a cluster of degenerating cells in a rat treated with DPH, 100 mg/kg, and PILO, 380 mg/kg. Survival time: 5 days. Fink-Heimer stain. $\times 186$. E: photomicrograph of the lateral thalamic nucleus of the rat treated with PHB, 50 mg/kg, and PILO, 380 mg/kg. Note the normal cytoarchitecture of the lateral thalamus. No signs of neuronal degeneration are discernible throughout the entire lateral thalamic nucleus. Survival time: 12 days. Fink-Heimer stain. $\times 186$. F: massive necrosis of the lateral thalamic nucleus in a rat treated with CARB, 50 mg/kg, and PILO, 380 mg/kg. Neuronal depopulation, total disruption of the neuropil and infiltration of glial cells is present throughout the entire lateral thalamic nucleus. Survival time: 30 days. Cresyl violet stain. $\times 186$. G: photomicrograph of the basolateral amygdala of a rat receiving VPA, 400 mg/kg, followed by systemic injection of PILO, 380 mg/kg. This section shows normal cytoarchitecture of the basolateral nucleus. No sign of neuronal injury is seen throughout the basolateral amygdaloid nucleus. Survival time: 72 h. Cresyl violet stain. $\times 186$. H: extensive destruction of the basolateral amygdaloid nucleus in the rat treated with ACT, 750 mg/kg, and PILO, 200 mg/kg. Nearly total neuronal depletion with edema and disruption of the neuropil comprise the pathological tissue reaction within the basolateral amygdala. Survival time: 7 days. Cresyl violet stain. $\times 186$. I: TMD, 500 mg/kg, fully protects this rat from morphological sequelae of seizures elicited by PILO, 380 mg/kg. This section demonstrates normal morphology of the pyriform cortex. Survival time: 17 days. Cresyl violet stain. $\times 186$. J: a portion of anterior pyriform cortex of a rat receiving ETX, 500 mg/kg, followed by PILO, 200 mg/kg. Shrunken and intensely argyrophilic neurons are widely interspersed among normal appearing cells. Extensive disintegration of the neuropil with extensive edema is prominent throughout all layers of the pyriform cortex. Survival time: 21 days. Fink-Heimer stain. $\times 186$. K: substantia nigra of the rat treated with PHB, 50 mg/kg, and PILO, 380 mg/kg, showing protection against morphological sequelae of seizures. Survival time: 4 days. Cresyl violet stain. $\times 38$. L: the substantia nigra is undergoing grave breakdown of the morphological structure after treatment with DPH, 50 mg/kg, followed by PILO, 380 mg/kg. Extensive neuronal loss and edema are evident in the pars reticulata. Survival time: 10 days. Fink-Heimer stain. $\times 38$.

damage to the forebrain in two out of 4 rats which developed status epilepticus resembled those found in the brains of drug-naive rats treated with PILO, 380 mg/kg. In two remaining rats in this treatment group the damage had an attenuated pattern. With VPA, 300 mg/kg ($n = 6$), 3 rats presented no morphological alterations, whereas in two animals an attenuated pattern of the damage was detected. One rat treated with VPA, 300 mg/kg, and PILO, 380 mg/kg, died in the course of seizures. With the highest dose of VPA, 400 mg/kg ($n = 8$), the brain damage was absent in 4 (Fig. 9G) or limited to an attenuated pattern resembling that detected after treatment with CLO or PHB in two rats. Two animals in this treatment group died following the seizures and their brains were not analyzed.

3.3.5. Trimethadione. TMD also had a marked suppressant effect upon the morphological sequelae of seizures produced by PILO, 380 mg/kg. None out of 8 rats pretreated with TMD, 500 mg/kg, developed morphological alterations in the brain (Fig. 9I). Two out of 10 animals treated with TMD, 500 mg/kg, died following seizures induced by PILO, 380 mg/kg, and their brains were not analyzed for epilepsy-related brain damage. After TMD, 300 mg/kg, 3 out of 7 animals showed a protection against neuronal degeneration and two developed morphological alterations confined to the pyriform cortex, amygdala and hippocampus. Two rats in this treatment group died in the course of seizures. TMD, 100 and 200 mg/kg, provided no protection against seizures induced by PILO. One of 3 rats, which survived the seizure insult in these treatment groups had a typical, in terms of extent and topography, damage to the forebrain, while two additional animals displayed an attenuated pattern of the damage.

3.3.6. Diphenylhydantoin. DPH, 10–200 mg/kg ($n = 18$), had no protective efficacy against brain damage produced by PILO, 380 mg/kg. In 3 rats treated with different doses of DPH and PILO, 380 mg/kg, the topography of the damage to the forebrain resembled that usually detected in the brains of rats treated with PILO, 380 mg/kg (Fig. 9D,L). By contrast, the extent of the damage in rats pretreated with DPH was greater relative to that seen in drug-naive rats receiving PILO, 380 mg/kg. Unusually severe destructions were detected in the thalamus, temporal, frontal, parietal and occipital cortices, CA₁ sub-

field of the dorsal hippocampus, dentate gyrus and substantia nigra. One animal displayed additional damage to the globus pallidus and dorsolateral parts of the caudate-putamen.

3.3.7. Carbamazepine. CARB did not affect the evolution of the damage to the forebrain induced by PILO, 380 mg/kg. All rats treated with CARB in doses of 10, 30 or 50 mg/kg ($n = 9$), and subjected to seizures produced by PILO, 380 mg/kg, developed brain damage comparable to that seen in drug-naive rats receiving PILO (Fig. 9F). The incidence of an unusual severity of the damage to the frontal and parietal cortex, the hippocampus and the thalamus was seen in the brains of two rats.

3.3.8. Ethosuximide. Extensive morphological changes were found throughout the amygdala, thalamus, pyriform and entorhinal cortex, hippocampus, neocortex and substantia nigra in rats pretreated with ETX and PILO, 200 mg/kg (Fig. 9B,J). The topography of the damage to the forebrain produced by PILO, 200 mg/kg, in rats pretreated with ETX resembled that encountered in rats subjected to the convulsant action of PILO in doses exceeding 350 mg/kg⁹¹. Two out of 6 rats receiving ETX, 150 mg/kg, and PILO, 200 mg/kg, presented a widespread damage to the brain. With ETX, 200 mg/kg, 4 out of 5 rats developed typical damage to the forebrain, while all rats ($n = 3$) which survived the treatment with ETX, 500 mg/kg, and PILO, 200 mg/kg, underwent prominent morphological injury and cell loss.

3.3.9. Acetazolamide. ACT increased the susceptibility of rats to the convulsant and brain damaging action of pilocarpine (Fig. 9C,H). One rat which developed motor limbic seizures and status epilepticus following the treatment with ACT, 250 mg/kg, and PILO, 200 mg/kg ($n = 1$), had morphological alterations in the limbic forebrain. In 5 other rats from this treatment group no neuropathological alterations were seen throughout the entire forebrain. Four rats which survived seizures and status epilepticus following the injection of ACT, 500 mg/kg, and PILO, 200 mg/kg, displayed a widespread damage to the limbic forebrain and the substantia nigra. The topography of this damage was similar to that encountered following seizures elicited by PILO, 380 mg/kg, in drug-naive rats. A similar distribution of the damage to the forebrain was presented by rats, which survived status epilepticus following the injection of ACT, 750 (n

= 1) and 1000 mg/kg ($n = 5$); however, the extent of the damage in rats pretreated with lower doses of ACT was less relative to that encountered in rats subjected to moderate doses of the drug.

4. DISCUSSION

A key problem to be solved concerning the action of anticonvulsant drugs in humans and experimental animals is the identification of the precise mechanisms that are operative in the control of seizure spread and the elucidation of morphological networks active in the motor expression of generalized seizures. The present study was undertaken to explore the action of antiepileptic drugs on the emergence of seizures elicited in rats by the muscarinic cholinergic agonist PILO. The principal finding of this study is that anticonvulsant drugs do not affect seizures produced by PILO in a manner which correlates well with the action profile in other experimental models of epilepsy^{65,70}. The benzodiazepine, CLO, which is efficient in terminating either status epilepticus or complex partial seizures in humans and blocks pentylenetetrazole (PTZ), maximal electroshock (MES) or amygdala kindled seizures in rodents^{7,9,21,60,65}, was able to prevent seizures induced by PILO in rats.

PHB and VPA, two other antiepileptic drugs active against generalized tonic-clonic and complex partial seizures in man^{7,21}, also protected rats against PILO-induced seizures. TMD, which is preferentially used against absence seizures and much less potent against tonic-clonic convulsions^{7,21,41} was found to be effective against seizures produced by PILO.

These findings prompted us to challenge the action of drugs of choice for absence seizures in the PILO model. Surprisingly, ETX and ACT, both successfully used in the management of absences in humans⁷, dramatically decreased the threshold for seizures induced by PILO in rats. In contrast, DPH and CARB, both drugs regarded clinically as effective against generalized tonic-clonic seizures and partial complex seizures but not against absences^{21,65,102}, did not affect the course of seizures produced by PILO. DPH and CARB are known to block MES- and the tonic phase of PTZ-induced seizures, while both drugs are ineffective against the clonic phase of chemical convulsions^{17,18,36,75}.

A concurrent protection against or an augmentation of the electrographic and morphological sequelae of seizures produced by PILO in rats subjected to adequate doses of antiepileptic drugs provides evidence that the effect of anticonvulsants on PILO-induced neuropathology is a function of a seizure prevention or the seizure exasperation. This is immediately established by the fact that the anticonvulsant drugs, which prevent behavioral and electrographic seizures produced by PILO, (CLO, PHB, VPA and TMD) protected rats also against epilepsy-related brain damage. ETX and ACT exacerbated PILO-induced seizures and dramatically enhanced the extent of the brain damage. DPH and CARB showed no apparent protection neither against behavioral and electrographic seizures nor against epilepsy-related brain damage.

4.1. *γ -Aminobutyrate-mediated inhibition and anticonvulsant drug action: clonazepam, phenobarbital, valproic acid and trimethadione prevent against pilocarpine-induced seizures*

Our results demonstrate a remarkable anticonvulsant efficacy against PILO-induced seizures of drugs acting to potentiate GABA-mediated inhibition in the CNS. The GABA receptor and associated chloride ionophore compose parts of a macromolecular protein complex at which GABA, benzodiazepines and barbiturates act to achieve their pharmacological effects²⁹. Benzodiazepines, which are clinically approved for therapy of status epilepticus, generalized tonic-clonic seizures, absence seizures and myoclonic seizures in humans^{7,21} and which protect against both PTZ- and MES-induced seizures in rodents⁹, are active against seizures and epilepsy-related brain damage produced by PILO. The mechanism of anticonvulsant action of benzodiazepines may be related to alterations of the binding properties of the GABA receptor following the interaction of benzodiazepines with its binding site²⁹. The potency of the anticonvulsant action of CLO in the PILO model fits well that in amygdala kindling and chemically induced seizures^{2,3,9}. There is also no apparent tendency for anticonvulsant action of PHB and VPA to be weaker than in either kindling model of epilepsy or in chemically induced seizures^{2,3,14,44,46}. PHB, a phenyl-substituted 'anticonvulsant' barbiturate, effective in therapy of generalized tonic-clonic and complex

partial seizures²¹ as well as against MES- and PTZ-induced seizures in rodents²⁶, only slightly modulates postsynaptic responses to GABA as justified by a great deal of neurochemical and electrophysiological evidence⁴⁸. Although multiple actions of barbiturates (reduction of amino acid-mediated excitation, reduction of voltage-dependent Na⁺ and K⁺ conductances, decreases of neurotransmitter release and limitation of repetitive firing) may well contribute to the anticonvulsant effect, enhancement of GABA-mediated synaptic inhibition cannot be entirely dismissed in the action of PHB. Electromyographic monitoring of the action of PHB on the muscle tone in genetically spastic rats provides persuasive evidence on the GABAergic component in the muscle relaxant effect of the barbiturate³⁴.

At free-serum concentrations relevant in anticonvulsant therapy, PHB enhances postsynaptic GABA and blocks glutamate-mediated responses⁴⁸. This may be indicative of participation of both mechanisms to the anticonvulsant effect of the barbiturate in humans.

There are basic discrepancies^{14,35,44}, but the weight of the evidence favors the hypothesis that VPA enhances GABA-mediated synaptic inhibition and reduces sustained repetitive firing of mouse cortical and spinal cord cells in dissociated cultures⁴⁸. This predicts the effectiveness of VPA in the therapy of generalized absence, myoclonic and generalized tonic-clonic seizures in humans and against PTZ- and MES-induced seizures in animals^{7,14,21,44}. VPA does not seem to affect glutamate-mediated excitation^{14,48}. In the PILO seizure model VPA provides efficient protection against seizures and brain damage in doses only slightly exceeding those shown to be effective in blocking amygdala kindled or MES-seizures in rats^{2,46}.

Although mystery surrounds the mechanism of action of oxazolidinedions, GABA is believed to play a role in the anticonvulsant action of TMD^{45,65}. TMD is particularly useful in the treatment of absence seizures and prevents rodents against MES-, PTZ-induced and amygdala kindled seizures^{1,41}.

In the present experiments, TMD attenuated seizures and protected against brain damage induced by PILO. A resolution of the issue of whether a presumed GABAergic mechanism contributes to the mechanism of anticonvulsant action of TMD in the PILO

seizure model is certainly provocative and awaits further studies.

4.2. Seizures produced by pilocarpine are resistant to the treatment with diphenylhydantoin and carbamazepine

DPH and CARB, both clinically regarded as effective against complex partial and generalized tonic-clonic seizures but not against absences^{7,21}, did not consistently affect the convulsant action of PILO in rats. DPH and CARB also lack efficacy against the clonic phase of PTZ-induced seizures in mice^{17,18} and limbic seizures induced by systemic application of kainate in rats^{15,25,39}. DPH is also of a doubtful efficacy in preventing electrical and chemical kindling of the amygdala^{1-3,10,96} and appears to be ineffective in blocking the development of amygdala kindling⁶⁹.

The action on the movement of Na⁺ and Ca²⁺ across biological membranes is presumably responsible for the membrane stabilizing and anticonvulsant effect of DPH^{20,102}. Another possible factor that should be considered is a DPH-induced, time- and voltage-dependent limitation of high-frequency repetitive firing of action potentials^{48,102}. It is assumed that the anticonvulsant action of CARB also resides in depression of high-frequency repetitive firing⁴⁸. This implies interpretation of the anticonvulsant action of both DPH and CARB in terms of limitation of the seizure spread and not in terms of seizure arrest.

There is evidence for a purinergic component in the anticonvulsant action of CARB^{16,76,77,98}. CARB was found to inhibit the binding of the adenosine analogue L-N⁶-phenylisopropyladenosine to rat brain synaptosomal membranes^{78,79,98}. Aminophylline, an antagonist at purinergic receptors, reverses the anticonvulsant action of CARB in the MES-test in mice¹⁶. The anticonvulsant efficacy of DPH in the MES seizure model in mice is also reduced by aminophylline¹⁶.

Interestingly, 2-chloroadenosine, an agonist at A₁ purinergic receptors, raises the threshold for seizures produced by pilocarpine⁹¹ and curtails the motor expression of amygdaloid and hippocampal kindled seizures⁶. An apparent lack of anticonvulsant efficacy of DPH and CARB against PILO-induced seizures demonstrates that a purinergic mechanism does not seem to contribute to the action of DPH and CARB in this seizure model.

4.3. Proconvulsant action of ethosuximide and acetazolamide in pilocarpine model of seizures

Demonstrating the proconvulsant effect of ETX and ACT does not necessarily insure that these drugs increase synaptic excitability. It is also impossible, based on these experiments, to determine if ETX or ACT are acting by blocking inhibitory or by enhancing excitatory transmission. Thus, it is necessary to determine which neurotransmitter system is involved in this action and how these processes affect synaptic functions. Research on the action of ETX and ACT has documented that ETX is effective against PTZ-induced seizures but nearly inactive against MES convulsions⁴¹, while ACT has a wide spectrum of anti-convulsant activity abolishing MES convulsions and protecting against chemically induced seizures⁷¹.

ETX possesses variable actions on synaptic transmission, release of neurotransmitters, Ca^{2+} metabolism and Na^+, K^+ -ATPase activity^{20,48}. Although the mechanism of anticonvulsant action of ETX is uncertain and there exists no hypothesis that is actually advanced, two properties of this drug might be thought to have contributed to the decrease of the threshold for limbic seizures we observed.

One is a reduction of GABA-mediated inhibition in the forebrain. In cultured cortical neurons ETX decreases responses to exogenously applied GABA and glycine, and enhances the GABA-antagonistic action of picrotoxin⁴. In guinea pig hippocampal slices ETX increases the penicillin-induced synchronous bursting and tends to increase burst duration⁷⁵.

We have found deficits in the activity of the GABA synthesizing enzyme, L-glutamate decarboxylase (GAD), in the brain regions undergoing morphological insult in the course of seizures produced by PILO⁸⁴. We have also observed a protection against PILO-induced seizures in rats after local application of γ -vinyl-GABA, an irreversible inhibitor of the GABA degrading enzyme, GABA transaminase, into the substantia nigra⁸³. Others⁶¹ reported a similar anticonvulsant effect in the pilocarpine seizure model after microinfusions of muscimol into the entopeduncular nucleus. These findings indicate that a decrease in GABA-mediated inhibition could have accounted for the seizure-promoting action of ETX in the PILO model. Another factor that must be considered is an ETX-induced increase of glutamate and

aspartate in the rat brain⁶². Glutamate and aspartate are convulsants in primates and rodents and are regarded as factors triggering epilepsy related brain damage^{19,55,57,58,73,77}.

We have found that 2-amino-7-phosphonoheptanoic acid, an antagonist selectively blocking N-methyl-D-aspartate-mediated excitation⁵⁵, protects against seizures induced by PILO following focal application into the substantia nigra pars reticulata⁸⁵. An involvement of factors other than cholinergic in convulsions presented by rats subjected to PILO implicates the failure of scopolamine to terminate such seizures^{90,93}. This muscarinic cholinergic antagonist protects against, but does not interrupt, pilocarpine-induced seizures^{90,93}. Conceivably, both a decrease in GABA-mediated inhibition and an increase in amino acid-mediated excitation could have contributed to the increased susceptibility of rats to seizures elicited by PILO after treatment with ETX.

A similar controversy surrounds the explanation of the proconvulsant effect of ACT in the PILO seizure model. ACT lowered, however, the threshold for seizures elicited by PILO in relatively high doses, which exceeded those effective in preventing rats from MES-seizures^{63,71}. The only known effect of ACT in the mammalian CNS is inhibition of carbonic anhydrase activity in the glia⁹⁹. This enzyme catalyses the hydration of CO_2 and thus governs the extent to which CO_2 is allowed to accumulate in the brain. In addition, membrane-bound carbonic anhydrase is involved in $\text{HCO}_3^- - \text{Cl}^-$ transfer across glial membranes⁹⁹. Evidently, a functional demand for glial carbonic anhydrase in brain regions of high neuronal activity associated with sustained seizures is related to the supply of metabolites generated in and released from activated neurons. This process actively contributes to the regulation of metabolic neuronal/glial homeostasis and therefore an increase in carbonic anhydrase activity may exert an anticonvulsant effect. A linear relationship between carbonic anhydrase activity and intensity of seizures is reported in mice sensitive to sound-induced seizures²² and in rats subjected to focal seizures induced by cobalt¹⁰¹. ACT, in doses that completely inhibit carbonic anhydrase, decreases the threshold for MES-seizures in mice²² and makes cobalt-induced seizures in rats worse¹⁰¹. The capacity of ACT to decrease the threshold for seizures induced by PILO may perhaps

be explained by a similar mechanism. The most persuasive evidence that ACT may augment seizures and extend seizure-related brain damage after PILO acting on carbonic anhydrase is the excellent correlation between inhibition of the activity of this enzyme and blockade of Cl^- and K^+ entry into glia⁹⁹. Inhibition of both cytosolic and membrane-bound carbonic anhydrase by ACT disturbs HCO_3^- - Cl^- exchange across the membrane and leads to depolarization of glial cells. This results in accumulation of K^+ and Cl^- in extracellular space⁹⁹.

A rise in extracellular K^+ concentration creates the functional conditions for the generation of epileptic activity⁴⁷. Rapid increases in extracellular K^+ and decreases in extracellular Ca^{2+} are recorded during cellular events underlying epileptic activity⁴⁷.

An unregulated passive influx of Cl^- balanced ionically by influx of cations, which leads to osmotic overload and cell lysis, is currently regarded as an essential ionic mechanism responsible for cell death in the course of sustained seizures^{57,58,73}. It seems likely that disruption of Cl^- homeostasis caused by ACT might thus account, in part, for dramatic augmentation of morphological sequelae of PILO seizures.

4.4. Calcium homeostasis and pilocarpine-induced seizures

Experimental evidence suggests that some of the actions of benzodiazepines, DPH and CARB in the CNS are related to the inhibition of Ca^{2+} -dependent processes²⁰. The regulation of the Ca^{2+} -calmodulin kinase system by benzodiazepines, DPH and CARB may underlie the modulatory action of these drugs upon neuronal excitability and contribute to the mechanism of anticonvulsant action.

The involvement of the Ca^{2+} -calmodulin kinase system is implicated in the generation of seizures²⁰. The kindling of amygdala in rats changes the activity of hippocampal Ca^{2+} -calmodulin kinase⁹⁶. In view of the differences in the preventive efficacy of CLO and both DPH and CARB against PILO-induced seizures, it is difficult to arrive at devising a hypothesis which relates the anticonvulsant action in this epilepsy model to regulation of the activity of the Ca^{2+} -calmodulin enzyme system. PHB, VPA and TMD, which all protect rats against seizures induced by PILO, do not affect Ca^{2+} homeostasis and Ca^{2+} -regulated processes²⁰.

4.5. Morphological substrates underlying anticonvulsant efficacy of drugs — a hypothesis

The substantia nigra has been proposed as a site at which anticonvulsant activity related to GABA-mediated inhibition is operative^{30,32,52,74,83}. Electrophysiological observations in rats established that certain, clinically and experimentally effective anticonvulsant drugs share an ability to inhibit firing of non-DAergic neurons of the substantia nigra pars reticulata⁹⁷. Benzodiazepines (diazepam and CLO), barbiturates (PHB and pentobarbital), VPA and TMD consistently inhibited neuronal firing, DPH and CARB remained inactive, whereas ETX markedly elevated firing rate of nigral cells. The effect of anti-epileptic drugs on the firing rate of substantia nigra neurons resembled neither the clinical effectiveness of anticonvulsants nor their action profile in established experimental models of epilepsy.

Surprisingly, careful analysis of the action of diverse antiepileptic drugs on seizures elicited by PILO in rats shows a remarkable similarity between protective efficacy against seizures and ability to affect firing rate of substantia nigra pars reticulata neurons. CLO, PHB, VPA and TMD, anticonvulsants which do enhance GABA-mediated inhibition and inhibit firing of substantia nigra pars reticulata neurons⁹⁷, curtail motor expression of PILO-induced seizures and prevent epilepsy-related brain damage. DPH and CARB, antiepileptic drugs known to possess little or no effect on GABAergic neurotransmission and devoid of depressant action on the firing rate of substantia nigra cells⁹⁷, are ineffective against seizures produced by PILO. Curiously, ETX, an anti-epileptic drug which shares the ability to attenuate GABA-mediated inhibition and elevates the firing rate of nigral cells⁹⁷, increases the susceptibility to seizures induced by PILO.

These observations may have interesting implications for a better understanding of the critical function which the substantia nigra plays in the propagation of seizure activity in the forebrain and of the morphological requirements necessary for anticonvulsant action to occur. However, these findings do not necessarily identify the substantia nigra pars reticulata as an exclusive site of anticonvulsant drugs' action. We actually do not know whether ACT, which also decreases the threshold for PILO-induced seizures, elevates the firing rate of nigral neurons and/

or reduces GABA-mediated inhibition.

4.6. *Seizures induced by pilocarpine — a new animal model of temporal lobe epilepsy*

The present findings demonstrate that seizures produced by PILO in rats represent a unique animal model relevant for delineation of anticonvulsant drug action. Seizures induced by PILO are sensitive to the anticonvulsant action of drugs increasing the GABA-mediated inhibition: CLO, PHB, VPA and TMD. DPH and CARB are inactive in preventing PILO-induced seizures, while ETX and ACT apparently lower the threshold for PILO-induced convulsions. The resistance of seizures elicited by PILO in rats to anti-epileptic drug treatment is consistent with the clinically observed resistance of limbic seizures to anti-epileptic medication^{7,21,65}.

The mechanistic limitation of this experimental approach relates to the generation and temporal appearance of seizures. When convulsions are elicited by systemic application of PILO, as in the present study, the behavioral and electrographic manifestation of the motor limbic seizures proceeds through several stages and finally progresses to status epilepticus. Thus, the seizures monitored under these conditions will, at most, reveal only one component of temporal lobe epilepsy, namely, drug-induced evolution of intractable limbic convulsions. This may be related to the rapid development of electrical kindling, in which seizures eventually result from repeated application of low-intensity electrical stimuli to discrete parts of the forebrain^{28,49–51,67} and their behavioral and electrographic evolution also proceeds through several stages^{28,50,67}. This hypothesis finds support in the results of Buterbaugh et al.⁸ who recently demonstrated that PILO facilitates the development of status epilepticus in amygdala kindled rats. A suprathreshold electrical stimulation of fully kindled rats subjected to definitely subconvulsant doses of PILO triggers an immediate evolution of status epilepticus⁸. The status epilepticus commences after a delay of approximately 30–60 min in drug-naïve rats subjected to a high-dose treatment with PILO, or in lithium-, aminophyline- or morphine-pretreated rats subjected to a low-dose treatment with the drug^{31,37,38,86,91}. Therefore, an inference emerges that amygdala kindling may function to establish neuronal networks necessary for spatial

spread of seizure discharges required for status epilepticus to develop and that kindling may be integral to the development of status epilepticus in the PILO seizure model. Interestingly, the efficacy of anticonvulsant drugs in preventing the development of kindling differs considerably from that against fully developed kindled convulsions^{1–3,11,42,95,100}. Benzodiazepines, diazepam and CLO^{11,100}, PHB^{11,100}, VPA⁴² and TMD^{22,41} retard the development of kindling in rats, while DPH^{69,100} and CARB^{66,95} afford no such activity.

Thus, there is an apparent correlation between anticonvulsant efficacy in the PILO seizure model and ability to retard the development of kindling⁷ and to depress the activity of substantia nigra neurons⁹⁷. The functional importance of this observation is experimentally substantiated by the finding that in rats neurons in the substantia nigra change their firing pattern during amygdala kindling⁵.

The second component of temporal lobe epilepsy relates to spontaneously recurrent motor limbic seizures which are registered during a long-term period following application of PILO⁹³ and which have many features in common with partial complex seizures in humans^{27,93}.

This limitation emphasizes the need for long-term studies during a period of spontaneous motor limbic seizures in order to further assess the usefulness of the PILO model in selecting drugs active against human partial complex seizures.

The data presented here, nonetheless, furnish supportive evidence that PILO-induced seizures provide a new animal model of epilepsy with which therapeutic approaches to drug-resistant forms of complex partial seizures can be investigated.

5. SUMMARY

Seizures produced in rats by systemically administered pilocarpine (PILO) provide a model for studying the generation and spread of convulsive activity in the forebrain. PILO, 380 mg/kg, induces a sequence of behavioral and electroencephalographic alterations indicative of motor limbic seizures and status epilepticus which is followed by widespread damage to the limbic forebrain resembling that occurring subsequent to prolonged intractable seizures in humans. The present study was undertaken to de-

termine whether clinically utilized antiepileptic drugs share an ability to suppress seizures and brain damage elicited by PILO in rats. Clonazepam, ED₅₀ 0.35 mg/kg (0.25–0.49), phenobarbital, 23.4 mg/kg (18.5–29.6), and valproic acid, 286 mg/kg (202–405), prevented the buildup of limbic seizures and protected against seizure-related brain damage. Pre-treatment with trimethadione, 179 mg/kg (116–277), resulted in a moderate protection against PILO-induced seizures, whereas carbamazepine, 10–50 mg/kg, and diphenylhydantoin, 10–200 mg/kg, blocked neither convulsions nor brain damage produced by the drug. Surprisingly, ethosuximide, 196 mg/kg (141–272), and acetazolamide, 505 mg/kg (332–766), both lowered the threshold for seizures

induced by PILO and converted a non-convulsant dose of PILO, 200 mg/kg, into a convulsant one. These results indicate that only certain anticonvulsant drugs elevate the threshold for PILO-induced seizures and prevent the occurrence of epilepsy-related brain damage. The resistance of seizures produced by PILO in rats to antiepileptic drugs reaffirms the clinically obvious lack of effective treatments for limbic convulsions.

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