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Hippocampal afterdischarges in rats. I. Effects of antiepileptics

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Summary

Hippocampal afterdischarges (ADs) are considered to be a model of complex partial seizures. To study the pharmacology of these ADs, stimulation electrodes were implanted into the dorsal hippocampus of 33 male Wistar rats. Stimulation (15-s series of monophasic rectangular pulses with a duration of 1 ms and frequency of 8 Hz) was applied four times with interstimulation intervals of 15 min. Drugs (carbamazepine 50 and 100 mg/kg; clonazepam 0.2 and 0.5 mg/kg; ethosuximide 125 and 250 mg/kg; phenobarbital 40 and 80 mg/kg) as well as solvent and isotonic saline were injected intraperitoneally 2 min after the cessation of the first AD. Duration of AD, of the latent period between AD and recurrent AD and duration of recurrent AD and the number of wet dog shakes were measured. ADs were markedly shortened by both doses of clonazepam and phenobarbital and by the higher dose of carbamazepine. The action of ethosuximide was negligible. Wet dog shakes were influenced in the same way as AD duration. Recurrent ADs were more sensitive to antiepileptics than ADs and wet dog shakes.

Key words

Rat • Hippocampus • Epileptic afterdischarge • Antiepileptics • Wet dog shakes

Introduction

Partial complex seizures represent a serious therapeutic problem in clinical epileptology. They form a majority of therapy-resistant cases of human epilepsies (Aicardi and Shorvon 1997). Among adequate models of this type of seizures systemic administration of kainic acid (Ben-Ari 1985) and electrical stimulation of various limbic structures (Naquet and Lanoir 1973) are the most common. Local electrically-induced epileptic after-discharges which may spread to other structures served also as a background of the kindling process (Goddard *et al.* 1969). Usually, amygdala kindled animals were used for testing of antiepileptic drugs. However, fully kindled seizures represent the extensive spread of seizure activity at least to motor system, i.e. secondary generalization (McNamara 1984).

In contrast to stimulation of the amygdala, hippocampal kindling progresses extremely slowly, therefore the hippocampal afterdischarges might be used as a model of limbic seizures for testing of antiepileptics (Mareš *et al.* 1984) or as a marker of brain function in toxicological studies (Burdette and Dyer 1987). Electrically-induced hippocampal afterdischarges consist of several phases: 1) proper afterdischarge (AD); 2) silent, or latent period; 3) recurrent afterdischarge (rAD). These phenomena were repeatedly described (Green and Shimamoto 1953, Dyer *et al.* 1979a,b, Swartzwelder *et al.* 1979, Chocholová and Mareš 1983, Mareš *et al.* 1985, Velíšek *et al.* 1989) both in acute and chronic experiments. Moreover, hippocampal afterdischarge in the rat is accompanied by a constant behavioral correlate

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– wet dog shakes (Lerner-Natoli *et al.* 1984). All these phenomena were used as measures in our study.

We decided to test classical antiepileptics with different spectrum of clinical efficacy as well as mechanisms of action – carbamazepine, phenobarbital, clonazepam and ethosuximide (Holland and Ferrendelli 2002, Macdonald 2002a,b, Olsen 2002). The results of our study might be used as a reference for studies of the action of potential anticonvulsants.

Methods

The experiments were approved by Animal Care and Use Committee of the Institute of Physiology to be in agreement with the Animal Protection Law of the Czech Republic.

Surgery: Thirty-three adult male Wistar rats were surgically prepared under pentobarbital anesthesia (Nembutal® Abbot, 50 mg/kg i.p.). A triple stainless steel electrode (two teflon-coated wires with an outer diameter of 0.1 mm for stimulation, the third one for registration) was inserted stereotaxically into the dorsal left hippocampus, the double recording electrode into the right hippocampus [coordinates: AP=3.5 mm; L=3 mm; H=4 mm according to Fifková and Maršala (1960)]. Moreover, flat silver cortical electrodes were placed epidurally over frontal and occipital areas of both hemispheres. A screw in the nasal bone served as an indifferent electrode. All the electrodes were joined to a connector and fixed to the skull with a dental acrylic. The animals recovered for at least one week.

Stimulation: Four 15-s stimulation trains of 1-ms monophasic rectangular pulses with an 8 Hz frequency were applied to the left hippocampus in each session. An interval between the end of AD and start of the subsequent stimulation was always 15 min. The voltage of the stimulation was twofold the threshold for elicitation of the evoked response in homolateral sensorimotor cortex by a single stimulus (Velíšek *et al.* 1989).

Drugs: Drugs as well as vehicles were administered intraperitoneally 2 min after the end of the first AD. The doses of antiepileptics used were based on published data (for review see Levy *et al.* 1995) as well as on our data from experiments with pentetrazol-induced motor seizures. Phenobarbital sodium (PhB, Spofa, 40 or 80 mg/kg) and ethosuximide (ESI, a gift from Gerot Pharmazeutika, 125 or 250 mg/kg) were dissolved in physiological saline, carbamazepine (CBZ, Sigma, 50 or 100 mg/kg) and clonazepam (CZP, Rivotril® Roche, 0.2 or 0.5 mg/kg) were dissolved in a mixture of propyleneglykol, ethanol and water in a ratio 5:2:3. All the drugs were injected in a volume of 1 ml/kg, only the higher dose of CBZ had to be administered in a volume of 2 ml/kg. Therefore, four control groups had to be formed: one without any treatment (always the first experimental session), the second treated with physiological saline (1 ml/kg) to make clear that the injection itself did not influence the ADs, the third and the fourth were injected with a mixture of propyleneglykol, ethanol and water in doses of 1 (for CZP and lower dose of CBZ) and 2 (for the higher dose of CBZ) ml/kg, respectively. Individual rats were used for two (exceptionally only one) control sessions and two sessions with the same drug. Four animals first received the lower and in the next session the higher dose of the antiepileptic drug and the order was reversed in the remaining four (or five in the case of phenobarbital) rats. Individual dose groups consisted of 8-9 rats, control groups were substantially higher because all animals underwent control stimulation (see Table 1).

Recording procedure: EEG was recorded in reference as well as bipolar connections 15 s before stimulation, during the stimulation, AD and at least three min after the end of AD. The animals were allowed to recover after each session for at least three days, mostly for one week. Three or four sessions were performed on each animal because of the stability of hippocampal ADs. No significant differences were found when comparing the data from the first control session with those from the fourth session with physiological saline.

Evaluation of results: Duration of the afterdischarge (AD), duration of the latent period (L) and of recurrent afterdischarge (rAD) were measured in the recording from the stimulated hippocampus. Moreover, wet dog shakes were registered by an observer. All values were expressed as a percentage of the given value to the corresponding value measured after the first, i.e. pretreatment stimulation in the ongoing session. This method enabled standardization of the responses for further comparison.

Statistical evaluation was performed by means of ANOVA (BMDP, University of California, Los Angeles). The results concerning the AD and rAD durations and the number of wet dog shakes (WDS) were compared by Student-Newman-Keuls procedure (Miller 1981). The incidence of all registered phenomena (i.e. AD, rAD and WDS) was compared using Fisher´s exact

test (Lehman and d'Abrera 1975). The data for carbamazepine and clonazepam were compared to those obtained with the appropriate dose of the solvent, whereas phenobarbital and ethosuximide results were compared with the data from saline sessions.

Histological control: After the last session the resistance of the stimulation electrodes was measured and the values of currents previously used were calculated. The computed values ranged from 140 to 200 μA. The rats were then killed by an ether overdose and their brains were removed for histological control of electrode localization. The 33 rats included in this study had electrode tips in dorsal hippocampus in CA1 area.

Table 1. Incidence of recurrent ADs and wet dog shakes

	rAD stimulation			WDS stimulation		
Drug	**2nd**	**3rd**	**4th**	**2nd**	**3rd**	**4th**
None	22/27	23/27	22/27	19/27	25/27	25/27
Saline	9/11	10/11	11/11	10/11	10/11	11/11
Solvent 1	6/6	5/6	6/6	6/6	5/6	6/6
Solvent 2	4/11[a]	7/11	5/11	7/11	8/11	6/11
PhB 40	2/9 [a]	2/9 [a]	5/9	6/9	5/9 [a]	8/9
PhB 80	0/9 [a]	0/9 [a]	1/9 [a]	1/9 [a]	0/9 [a]	0/9 [a]
ESI 125	5/8	8/8	6/8	7/8	7/8	7/8
ESI 250	2/8 [a]	6/8	5/8	1/8 [a]	4/8 [a]	2/8 [a]
CZP 0.2	2/8	3/8	3/8	1/8 [a]	4/8	3/8
CZP 0.5	0/8 [a]	1/8 [a]	4/8	0/8 [a]	0/8 [a]	2/8
CBZ 50	1/8 [a]	2/8	1/8 [a]	2/8	6/8	7/8
CBZ 100	0/8	1/8	0/8	1/8	1/8	1/8

The incidence of rAD and WDS following repeated stimulations of the hippocampus expressed as a number of rats exhibiting the phenomenon/number of animals in the group. Left part - incidence of rAD; right part - incidence of WDS. Rows represent individual groups in the experiment. Columns represent 2nd, 3rd, and 4th stimulation. [a] - indicates the significant difference in comparison with the appropriate controls (controls + NS for PhB and ESI; Solvent 1 for both doses of CZP and lower dose of CBZ; Solvent 2 for higher dose of CBZ). For abbreviations see Method section.

Results

Incidence and duration of ADs

Afterdischarges were recorded after the first stimulation in all animals, its average duration was 21.0±1.4 s (Mean ± S.E.M.). The EEG pattern of the first phase of hippocampal AD was different in individual animals. ADs formed by fast series of spikes superimposed onto slow waves (serrated waves) represented the most common type; fast spikes sometimes with an intermingled slow wave were also frequently registered. ADs formed by spike-and-wave complexes were extremely rare. All these types might be combined. The activity was clearly expressed in the hippocampus, but it could be recorded in cortical recordings as well (Fig. 1).

The treatment with saline, solvent or drugs did not influence the incidence of ADs significantly. The tendency to a decrease of the incidence was observed following treatment with the 100 mg/kg dose of carbamazepine after the second and third stimulation (data not shown). The relative durations of ADs (Fig. 2) did not differ in non-treated controls and controls injected with physiological saline. Lower dose of solvent did not lead to significant changes in ADs duration, the 2 ml/kg dose shortened the duration of AD after the second and fourth stimulation. Phenobarbital was found to be very efficient; both doses used decreased the duration of ADs after all postdrug stimulations. Ethosuximide in a lower dose (125 mg/kg) was ineffective, but an extremely high dose of 250 mg/kg decreased the duration of the second and third AD. Both doses of clonazepam exhibited only a moderate effect which was apparent only after the third stimulation. A lower dose of carbamazepine (50 mg/kg) did not affect AD duration at all, the higher dose shortened the third and fourth ADs.

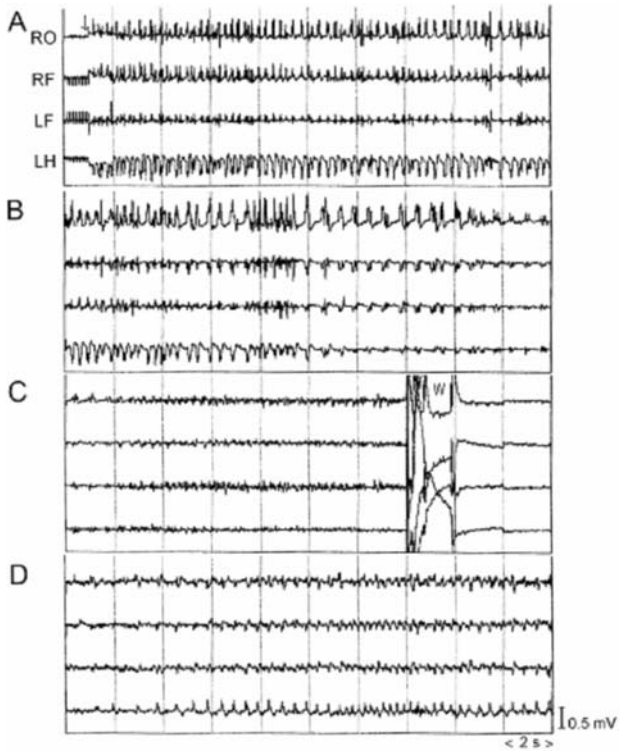

Fig. 1. Hippocampal afterdischarges in an adult rat – from top to bottom: the first three continuous sections (**A,B,C**) demonstrate the afterdischarge; the fourth section (**D**) recorded with a delay of 40 s after section C represents recurrent afterdischarge. Traces from top to bottom: RO - right occipital, RF - right frontal, LF - left frontal, LH - left hippocampus. An arrow indicates the end of the electrical stimulation; W denotes wet dog shake. Time mark 2 s, calibration 0.5 mV.

Incidence, latency and duration of rAD

Recurrent afterdischarge was always recorded after the first stimulation in the control group. This phase of the afterdischarge appeared with a latency of 32.6±2.9 s following the end of the AD. The duration of rAD was 24.4±2.3 s on the average. The latent period was characterized by a profound attenuation of the EEG activity. The pattern of rAD was uniform and rather different from that of AD. It was formed by slow sharp waves with the frequency of about 2 Hz decreasing toward the end of rAD and with an amplitude between 0.5 and 1 mV (Fig. 1).

The incidence of rAD was not influenced by saline treatment. On the contrary, a higher dose of tricomponent solvent had depressant effects, which was statistically significant after the second stimulation. Pharmacological experiments demonstrated a high effectiveness of phenobarbital and a moderate action of high doses of ethosuximide and clonazepam. The data for the other drugs did not significantly differ from the corresponding solvent groups, though both doses of carbamazepine as well as the lower dose of clonazepam exhibited a tendency to suppression of rADs.

The evaluation of the latencies of rAD and of the rAD duration was performed only in the case if a sufficient number of data was available. Physiological saline was without effect on both parameters, whereas the solvent increased the latencies (data not shown) and shortened the rAD duration (Fig. 3). The pretreatment with antiepileptics had no effects but ethosuximide delayed rAD without affecting its duration.

Behavior accompanying ADs

The animals pretreated with the solvent, phenobarbital, carbamazepine and clonazepam were quiet, they usually lay in the test cage without rapid movements or orienting reaction which always formed a constant correlate of the first (predrug) afterdischarge. Occasional movements of the rat with pretreatment mentioned above were slow and sometimes atactic. These changes were more pronounced after the antiepileptics than after the solvent.

Wet dog shakes (WDS) were observed in 81.5 % of animals in the control group after the first stimulation. The mean number of WDS was 7.5±1.2. Normal saline was without effect, whereas phenobarbital and a higher dose of ethosuximide suppressed the incidence of WDS (Table 1). Carbamazepine (100 mg/kg) and clonazepam (0.5 mg/kg) exerted some effects in comparison with the appropriate control groups, but these differences did not reach the level of statistical significance because the mean number of WDS was also very effectively suppressed by the 2-ml/kg dose of the solvent. Moreover, phenobarbital and ethosuximide were found to be effective (Fig. 4). Occasionally, the other drugs tended to depress the mean number of WDS, but these differences were not significant.

Discussion

Our study demonstrated that EEG as well as behavioral phenomena of hippocampal afterdischarges fully correspond to those observed in patients with complex partial seizures (Wieser 1987). As far as the EEG pattern is concerned, different types were described not only in our study but also in the literature. Correl and Ingram (1956) divided hippocampal ADs into five groups according to their frequency bands and amplitude. This classification has up to now only a descriptive value, because the structures involved in the generation and spread of different types of ADs are not known.

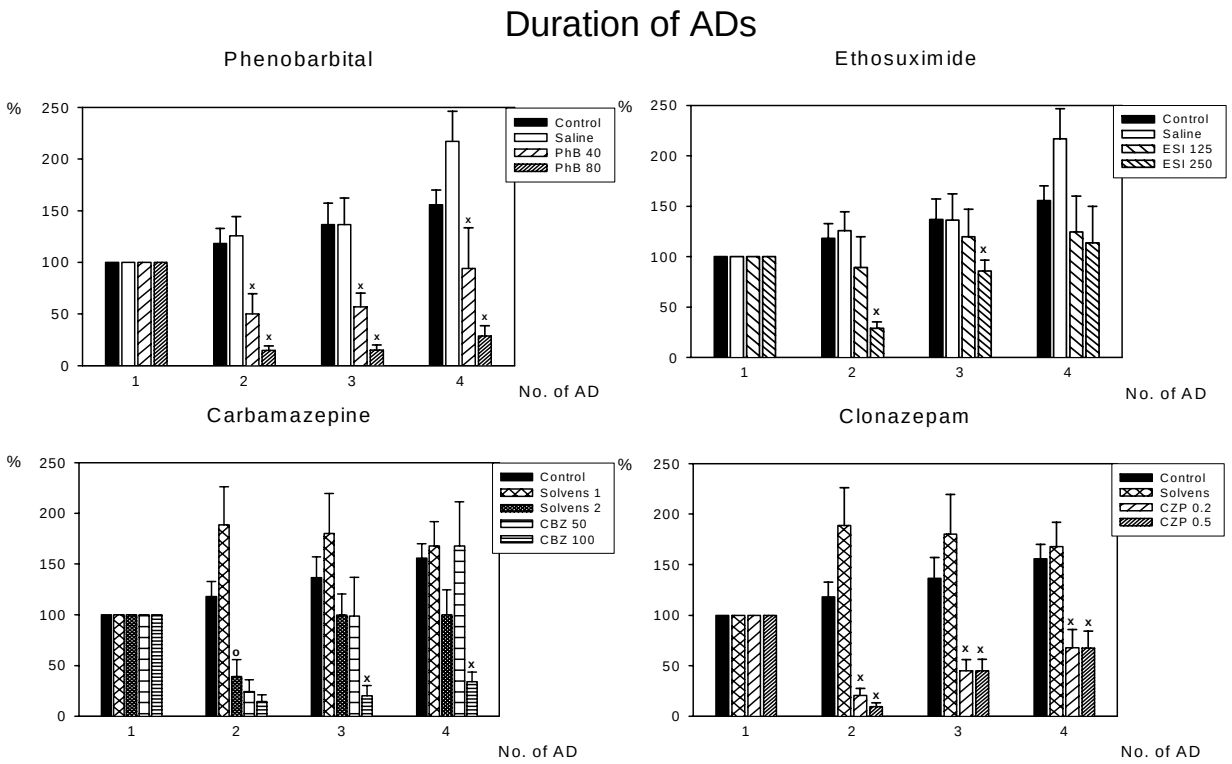

Fig. 2. Effects of antiepileptics on relative duration (Mean ± S.E.M.) of the first phase of hippocampal afterdischarge (proper AD) following repeated stimulations. Values are related to the duration of the first afterdischarge, which is taken as 100 %. Upper left graph – effects of phenobarbital (40 and 80 mg/kg); upper right graph – effects of ethosuximide (125 and 250 mg/kg); lower left graph – effects of carbamazepine (50 and 100 mg/kg); lower right graph – effects of clonazepam (0.2 and 0.5 mg/kg). Abscissa – first, second, third and fourth afterdischarges; ordinate - relative duration in %. Asterisks denote significant differences in comparison with the corresponding controls, circle denotes significant difference between non-injected rats (control) and higher volume of the tricomponent solvents used only for the 100-mg/kg dose of carbamazepine (solvent 2, i.e. 2 ml/kg).

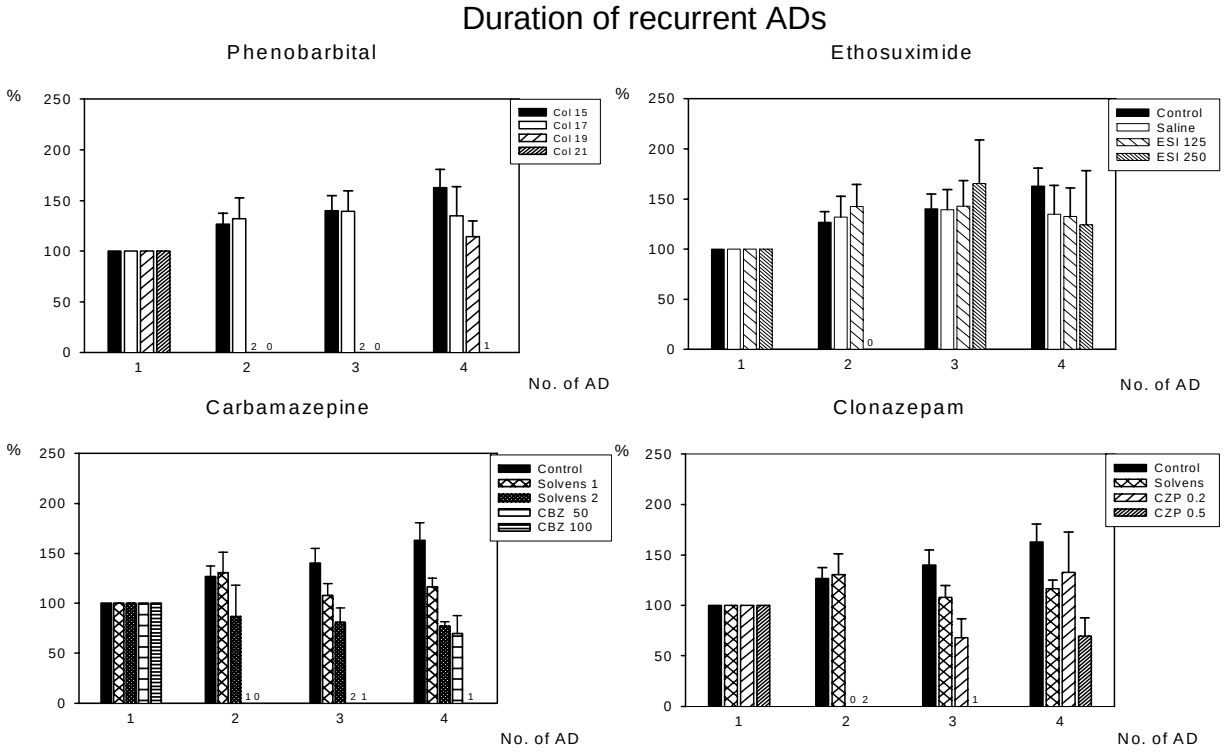

Fig. 3. Effects of antiepileptics on relative duration of recurrent hippocampal afterdischarges following repeated stimulations (Mean ± S.E.M.). Columns are presented only if there are four or more values in one group; numbers instead of columns denote number of animals exhibiting recurrent ADs. Other details as in Fig. 2.

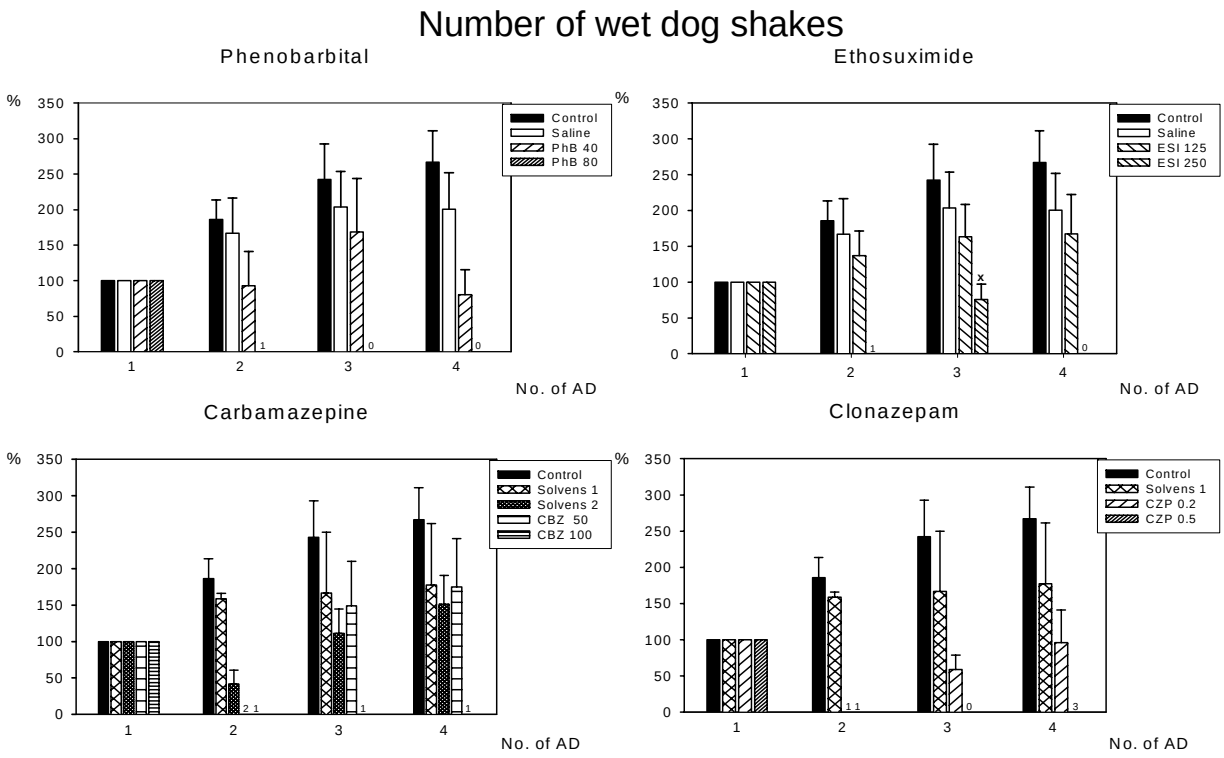

Fig. 4. Effects of antiepileptics on relative number of wet dog shakes following repeated hippocampal stimulations (Mean ± S.E.M.). Details as in Figs 2 and 3, only the numbers denote the number of rats exhibiting wet dog shakes.

The first phase of AD is followed by an EEG depression (Mareš *et al.* 1985, Bragin *et al.* 1997). The depression is interrupted by recurrent ADs first noted in the fifties of last century (Green and Shimamoto 1953). Recurrent afterdischarge proved out to be highly sensitive to the drug therapy. Unfortunately, the significance of rADs has not yet been revealed in spite of the role played by them in an increase of duration of ADs during the kindling process (McNamara *et al.* 1980).

The decreased duration of AD following the treatment with the solvents used for carbamazepine and clonazepam suggests a depressant effect of alcohol which represents 20 % of the solvent. This is in agreement with studies in rats and cats (Swartzwelder *et al.* 1979, Lesse and Harper 1984). These studies described a shortening of ADs following alcohol in doses of 3 g/kg or 0.4-1.6 g/kg, respectively. In our experiment, the dose of alcohol equaled 0.4 g/kg in the case of 2 ml of solvent/kg, i.e. the lowest dose used in the above mentioned studies. This dose shortened the duration of ADs as well as of rAD, decreased the incidence of rAD and the mean number of wet dog shakes. The results with the 1 ml/kg dose of the solvent did not reach the level of statistical significance, but they have to be taken into account at least as a potentiating factor.

Phenobarbital was found to be highly potent against hippocampal afterdischarges in our experiments. It influenced all phenomena evaluated, the electrophysiological indices were more affected than the behavioral ones (WDS). The main site of action of barbiturates is the binding site at the chloride ionophore in the supramolecular $GABA_A$ receptor/benzodiazepine receptor/chloride ionophore complex, but other effects were also found (e.g. suppression of glutamatergic excitation – Olsen 2002). Clonazepam which binds to the specific benzodiazepine site at the $GABA_A$ receptor and thus positively modulates this receptor (Macdonald 2002a), exerted similar but less expressed action against hippocampal afterdischarges. The similarity of phenobarbital and clonazepam effects might be due to the same supramolecular complex which is the target for these drugs and it is also in agreement with previous studies with amygdaloid kindling (Albertson *et al.* 1981). Lower activity of clonazepam in our experiments might be related to the fact that benzodiazepines are more effective against fully developed kindled seizures than against focal activity in the archicortex (Burnham 1985). Benzodiazepines are more effective against generalization than against generation of seizure activity (Macdonald 2002a). Our results with a marked suppression of wet dog shakes (which have to be taken as an expression of the spread of epileptic activity from

hippocampus into a generator localized probably in the midbrain and further into the motor system) by clonazepam is in agreement with this action. The role of $GABA_A$ supramolecular complex in the suppression of hippocampal afterdischarges and their behavioral correlates is clearly expressed, but their interpretation is not simple. The increased number of GABA but not benzodiazepine receptors was described after limbic stimulation (Shin *et al.* 1985) and the drugs acting antagonistically at the $GABA_A$ complex were found not to increase AD duration as well as the number of wet dog shakes (Burdette and Dyer 1987). Further analysis of the role of the GABAergic system is necessary.

Carbamazepine exerted a clear-cut action against hippocampal afterdischarges in the present study in a high dose of 100 mg/kg. Its main mechanism of action is a use-dependent blockade of voltage-gated sodium channels (Macdonald 2002b). Carbamazepine is used for treatment of human complex partial seizures (Gupta and Jeavons 1985) and we therefore expected a marked action in our model. This might be explained by an action of carbamazepine primarily against the spread of seizure activity (which is in agreement with its main mechanism of action) with practically no action on the epileptic focus itself as suggested by Hawkins *et al.* (1985). This is in good agreement with our present data and also with an *in vitro* study (Olpe *et al.* 1985) which described a reduction of fiber excitability following carbamazepine treatment. On the contrary, lack of the effect of the 50 mg/kg dose of carbamazepine on wet dog shakes does not support this explanation.

Mechanism of action of ethosuximide is completely different from the above mentioned antiepileptic drugs. It exerts a specific action against T-type calcium channels; these channels are involved in thalamocortical epileptic phenomena (Coulter *et al.* 1989). Weak effects of the 125 mg/kg dose against hippocampal afterdischarges were expected (Mareš *et al.* 1984). However, this dose is able to abolish pentylenetetrazol-induced spike-and-wave rhythm in rats (Brabcová *et al.* 1993). This difference in the action against two different types of seizures corresponds to the clinical profile of ethosuximide; it is inactive against complex partial seizures but highly effective against absence seizures (Sherwin 2002). Our data are also in agreement with the inefficacy of ethosuximide against hippocampal afterdischarges *in vitro* (Ohno and Higashima 2002).

Epileptic hippocampal afterdischarges represent a valuable model of human complex partial seizures. However, it appears that the action of anticonvulsants in this model does not fully correspond to the action of these drugs in clinical practice. This might be due to the mass activation of hippocampus by rhythmic electrical stimulation so that the model is very robust and could not be influenced easily.

Our results confirmed the relevance of hippocampal afterdischarges as a model of human complex partial seizures and may serve as a reference for pharmacological studies with new drugs.

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