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Effects of classical antiepileptics on thresholds for phenomena induced by cortical stimulation in rats

R. Haugvicová, E. Bílková, H. Kubová and P. Mareš

Abstract

Our aim was to study the effects of phenobarbital, phenytoin and ethosuximide on epileptic afterdischarges induced by cortical stimulation in rats. Fifteen-second series of low-frequency (8 Hz) rhythmic stimulation of the sensorimotor cortex were applied in rats with chronically implanted electrodes. Intervals between the stimulation series were at least 10 min and intensity was increased in a step-wise manner. Threshold current intensities were estimated for movements directly induced by stimulation, epileptic afterdischarges of the spike-and-wave type, clonic seizures accompanying this type of afterdischarge and transition into the limbic type of afterdischarge. Phenobarbital, phenytoin and ethosuximide were administered intraperitoneally before the first stimulation series. Phenobarbital (20, 40 and 80 mg kg^{-1}) significantly increased the thresholds for the first three phenomena in a dose-dependent manner. Transition into the limbic afterdischarge was influenced only by the highest dose. Phenytoin (60 mg kg^{-1}) only increased the thresholds insignificantly and ethosuximide (125 mg kg^{-1}) was ineffective. We concluded that our model is useful for testing anticonvulsant effects. Results with three antiepileptic drugs correspond with their efficacy against myoclonic seizures in man.

Introduction

In animal models it is necessary to differentiate mechanisms of seizure generation, spread and arrest. The two basic models obligatory for testing anticonvulsant effects – maximal electroshock and minimal metrazol seizures (Swinyard 1973) – are focused on only one mechanism: maximal electroshock on spread of seizures (tonic hindlimbs extension is used as an endpoint) and clonic seizures induced by pentetrazol on seizure generation, respectively. In addition, these two types of seizures are generated in different structures (Browning & Nelson 1986). Epileptic afterdischarges elicited by rhythmic electrical stimulation of cerebral cortex, used routinely in our laboratory for testing of antiepileptic drugs (Kubová et al 1996, 1999; Bernášková et al 1999) as well as for studies of a role of neurotransmitter systems in epileptogenesis (Koryntová et al 1997; Bernášková & Mareš 2000), offer an advantage in that two of the aforementioned mechanisms may be studied at once: generation of the afterdischarge of the spike-and-wave type and spread of epileptic activity into the motor system (appearance of clonic seizures accompanying afterdischarges) as well as into the limbic system (transition into another type of afterdischarge). Estimation of threshold current intensity may be used to quantify the action of antiepileptic drugs.

Measurement of threshold stimulation intensity has been used to test the action of antiepileptic drugs by several workers (Voskuyl et al 1989, 1992; Hoogerkamp et al 1994, 1996; Della Paschoa et al 1998a, b). In their paradigm, movements elicited by direct activation of cerebral cortex were studied; sometimes they used generation of seizures as an endpoint but they mostly intentionally avoided self-sustained epileptic activity (Della Paschoa et al 1997). Their model was modified in our laboratory to include the ability to estimate threshold intensities for the three above-mentioned epileptic phenomena and, in addition, movements elicited directly by stimulation of the sensorimotor cortex (Makal et al 1993; Koryntová & Mareš 1998; Mareš et al 2002). To obtain basic pharmacological data for this model we started to study the action of

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

R. Haugvicová, E. Bílková, H. Kubová, P. Mareš

Correspondence: P. Mareš, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ 142 20 Prague 4, Czech Republic. E-mail: maresp@biomed.cas.cz

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phenobarbital and, for comparison, phenytoin and ethosuximide. These drugs were chosen on the basis of Woodbury's classification (Woodbury 1980) – phenytoin as a drug blocking spread of epileptic activity, ethosuximide as a drug suppressing seizure generation and phenobarbital influencing both mechanisms. Since Woodbury's review, the role of these three anticonvulsants in the treatment of epilepsy has dramatically decreased but Woodbury's observation that these drugs represent a broad spectrum of anticonvulsant activity is still valid. These three drugs exert their action using different mechanisms: phenobarbital potentiates GABAergic inhibition and has a moderate influence on glutamatergic excitatory transmission (Prichard & Ransom 1995); phenytoin possesses many mechanisms of anticonvulsant action, the most important being an effect on current-gated sodium channels (DeLorenzo 1995); and ethosuximide affects specifically T-type calcium channels (Ferrendelli & Holland 1995).

Materials and Methods

The experiments were approved by the Animal Care Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic to be in agreement with Animal Protection Law of the Czech Republic (fully compatible with the guidelines of the European Community Council directives 86/609/EEC).

Surgical preparation of adult male Wistar albino rats was performed under pentobarbitone anaesthesia (Nembutal, Abbot, 50 mg kg^{-1} i.p.). Silver ball electrodes were placed epidurally, two stimulation electrodes over the right sensorimotor cortical area (AP = −1 and +1; L = 2.5 mm in relation to bregma), recording electrodes over the left sensorimotor cortex (AP = 0; L = 2.5 mm) and over the occipital, visual cortical areas of both hemispheres (AP = 6; L = 4 mm). An indifferent electrode was placed into the nasal bone. All electrodes were attached to the female plug and fixed to the skull by a fast-curing dental acrylic.

Electroencephalogram (EEG) was recorded one week later. Paper registration was used and all behavioural changes were marked directly onto the recording. Rectangular biphasic pulses of 1-ms duration were generated by a constant-current stimulator. Stimulation series of pulses with an 8-Hz frequency, lasting 15 s, were repeated with an inter-series interval of 10 min. Intensity of pulses increased with repetition of stimulation series. The first stimulation was realized with 0.4-mA intensity, then intensities were applied as follows: 0.8; 1.2; 1.6; 2; 3; 4; 5; 6; 8; 10; 12; and 15 mA. At the moment the transition of the afterdischarge into the limbic type was present the experiment was stopped. Even if all stimulation intensities had to be used the experiment lasted approximately 2 h.

Thresholds for four different phenomena were established: movements accompanying stimulation; spike-and-wave-type of afterdischarge; clonic seizures accompanying this type of afterdischarge; and transition into the second limbic type of afterdischarge.

Phenobarbital (Spofa) and ethosuximide (Gerot Pharmazeutica) were dissolved in distilled water and phenytoin (Parke Davis) was dissolved in a mixture of propylene glycol, ethanol and water (5:2:3), always in such concentration that a volume of 1 mL kg^{-1} was administered intraperitoneally. Phenobarbital was administered in three different doses (20, 40 and 80 mg kg^{-1}), phenytoin as a 60-mg-kg^{-1} dose and ethosuximide as a 125-mg-kg^{-1} dose. Rats were pre-treated with antiepileptics and stimulation series started either 30 min (phenobarbital) or 15 min (phenytoin, ethosuximide) after drug administration. Doses and time intervals were chosen on a basis of our previous data for pentetrazol-induced motor seizures (Mareš et al 1981; Staňková et al 1992). Metabolism of all three drugs was sufficiently slow to keep plasma levels within the usual therapeutic range (for man) for this whole time period (phenobarbital – Kapetanovic et al (1982); phenytoin – Woodbury & Swinyard (1972), ethosuximide – Bachman et al (1988)). We have data (unpublished) showing plasma concentration for phenytoin in the therapeutic range – 12.6±5.71 g mL^{-1} (mean±s.d.) – three hours after administration. The number of rats in individual groups is given in Table 1; the rats were used only once. Controls were always examined on the same days as treated rats therefore the number of controls in the phenobarbital group was higher than in other groups.

Statistical evaluation was performed by Mann–Whitney test and in the case of phenobarbital analysis of variance on Ranks with subsequent comparison by Dunnet's test (SigmaStat SPSS) was used. $P < 0.05$ was taken as a level of statistical significance.

Results

All rats in the three control groups (two injected with saline, one with a three-component solvent) exhibited all four phenomena registered. The solvent did not change the thresholds, patterns or incidence of transition to the mixed type of afterdischarges in comparison with saline-injected groups. An example of the two types of afterdischarges is given in Figure 1.

Only the highest dose of phenobarbital resulted in clear changes in the spontaneous EEG – there were barbiturate spindles and an increased amount of slow waves. Phenobarbital exhibited a dose-dependent effect on generation of stimulus-bound movements, spike-and-wave afterdischarges and clonic seizures (Table 1). Transition into the limbic type of afterdischarges was significantly influenced only by the 80-mg-kg^{-1} dose. The EEG pattern of the afterdischarges was not changed by phenobarbital and the intensity of motor phenomena was decreased (e.g. only movements of digits). The percentage of rats exhibiting the transition into the limbic afterdischarges was not significantly changed.

With phenytoin, the dose used did not induce obvious changes in spontaneous EEG. No significant effect was observed despite a slight increase of thresholds, especially that for the mixed type of afterdischarges (Table 1). EEG, motor patterns and incidence of the mixed afterdischarges were not influenced by phenytoin.

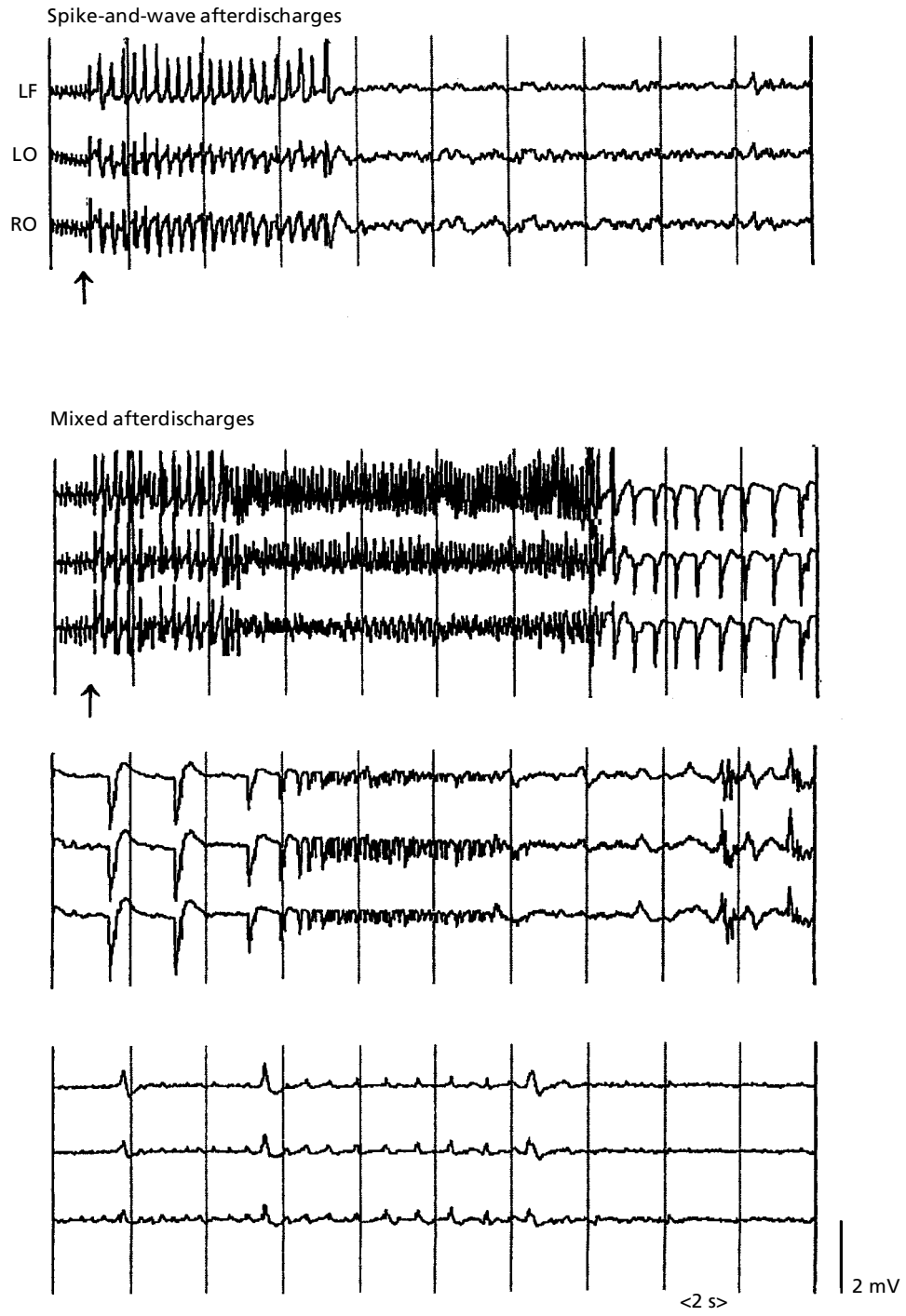

Figure 1 EEG recordings of the two types of afterdischarges from one control rat. Upper part, spike-and-wave type; lower part, transition from spike-and-wave rhythm into the limbic type. The first two sections represent a continuous recording; between the second and the third section 64 s are omitted. Arrows indicate the end of stimulation. Individual leads: LF = left frontal, LO = left occipital, RO = right occipital cortical area in reference connection. Time mark 2 s, amplitude calibration 2 mV.

Ethosuximide influenced neither the spontaneous electrocorticogram nor any of the four thresholds (Table 1). The EEG pattern of spike-and-wave afterdischarges was not changed and motor phenomena and incidence of mixed afterdischarges also remained untouched.

Discussion

There are many types of epileptic seizure with different pathogenetic mechanisms and sensitivity to antiepileptic drugs (Engel & Pedley 1998) and therefore many ex-

Table 1 Threshold current intensity of cortical stimulation for elicitation of four different phenomena.

	n	Movements	Spike-and-wave	Clonic seizures	Limbic type
Control	16	1.43±0.60	3.53±1.48	3.53±1.48	9.38±3.64
Phenobarbital 20 mg kg^{-1}	9	2.24±0.78*	5.00±2.07*	5.00±2.07*	10.57±5.16
Phenobarbital 40 mg kg^{-1}	13	2.26±1.44*	6.09±3.30*	6.09±3.30*	11.40±6.48
Phenobarbital 80 mg kg^{-1}	10	3.10±0.32*	6.00±2.40*	6.00±2.40*	13.14±2.87*
Control	9	1.70±0.28	4.57±1.90	4.57±1.90	6.85±4.87
Phenytoin 60 mg kg^{-1}	9	2.00±0.67	5.38±3.29	5.38±3.29	10.20±3.49
Control	8	2.20±0.97	5.80±2.97	5.80±2.97	12.33±2.52
Ethosuximide 125 mg kg^{-1}	9	2.23±0.66	6.55±2.42	6.55±2.42	13.50±2.12

Data are presented as means±s.d. *$P < 0.05$ in comparison with the appropriate control.

perimental models are necessary (Sarkisian 2001). The common experimental seizures model one type of human seizure (e.g. maximal electroshock seizures in rodents represent a model of human convulsive generalized seizures (Swinyard 1973)). Cortical afterdischarges allow study of at least two different models in one experiment – spike-and-wave afterdischarges as a model of myoclonic seizures and limbic afterdischarges as a model of complex partial seizures. In addition, direct (stimulation-bound movements) and indirect (clonic seizures accompanying spike-and-wave afterdischarges) activation of the motor system may be measured.

Pilot experiments demonstrated that repetition of the whole stimulation series resulted in a marked decrease of threshold for afterdischarges lasting at least two weeks (Koryntová & Mareš 1998). Similar decrease of seizure threshold was found by Albright (1983) in amygdala and cortical afterdischarges. Repeated stimulation, especially clustered stimuli as in our study, led to potentiation similar to kindling (Racine 1972; Pinel et al 1976; Lothman & Williamson 1994). Therefore the rats might be used only once and parallel examination of control and treated rats increased the number of controls necessary.

Phenobarbital was shown to elevate the threshold for electroshock seizures (Goodman et al 1953) and for cortical afterdischarges induced by 60-Hz stimulation (Vastola & Rosen 1960; Straw & Mitchell 1966). It decreased duration of cortical afterdischarges repeatedly elicited by the same current intensity (Kubová et al 1996). Phenobarbital was efficient against movements elicited by cortical stimulation in monkeys (Aston & Domino 1961) and rats (Hoogerkamp et al 1994). All these three effects were induced even by the lowest dose of phenobarbital used (i.e. by a dose far removed from that needed for a loss of righting reflex – more than 100-mg kg^{-1}) (Marešová & Mareš 1980). The high dose of phenobarbital required to influence the transition into limbic afterdischarges is in contrast with the effects of low doses on amygdala (Wise & Chinerman 1974), hippocampal (Albertson et al 1978) or cortical (Albright & Burnham 1980) kindled seizures. It might be due to progressive epileptogenesis set in action by kindling.

Results with phenytoin were surprising especially in relation to our data demonstrating the action of phenytoin on the threshold for stimulation-bound movements and spike-and-wave afterdischarges induced by 50-Hz stimulation (Kršek et al 1998) and in relation to an increase of the threshold for generalized seizures appearing during a 50-Hz stimulation train (Hoogerkamp et al 1994; Della Paschoa et al 1998a, b). On the other hand, phenytoin did not significantly influence afterdischarges induced repeatedly by 8-Hz cortical stimulation (Kubová et al 1996) or by stimulation of amygdala (Babbington & Wedeking 1973; Wise & Chinerman 1974). Positive findings in kindled rats were obtained with doses of phenytoin up to 100 mg kg^{-1} (Racine et al 1975) and 150 mg kg^{-1} (Albright & Burnham 1980). Again, these differences might be due to activation of different epileptogenic mechanisms.

Negative results with ethosuximide were expected. They are in agreement with results in electroshock seizures (Chen et al 1963), cortically kindled seizures (Albright & Burnham 1980), Voskuyl's model (Hoogerkamp et al 1994) and repeated cortical stimulation (Mareš et al 1997). The anticonvulsant action of ethosuximide is highly specific due to its mechanism of action and even models characterized by spike-and-wave rhythm are influenced differently (Mareš et al 1997).

Our results demonstrated that measurement of the threshold current intensities necessary for elicitation of different phenomena might be used to test anticonvulsant effects.

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