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Effect of Phenytoin on Cortical Epileptic Foci in Cerveau Isolé Rats

P. MAREŠ

Institute of Physiology, Academy of Sciences of the Czech Republic and Department of Pathophysiology, Third Medical Faculty, Charles University, Prague, Czech Republic

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Summary

The action of phenytoin was studied in acute experiments in rats with brainstem transection at the midcollicular level. Symmetrical epileptogenic foci were elicited in sensorimotor cortical areas of both hemispheres by local application of penicillin. Seven rats formed a control group, ten animals were pretreated with phenytoin (60 mg/kg i.p., 10 min before penicillin application). Synchronization of interictal discharges in control rats was delayed in comparison to animals with an intact brainstem; phenytoin did not influence this synchronization. Spontaneous transition of interictal into ictal activity was not abolished by phenytoin, i.e. in cerveau isolé preparations phenytoin lost this activity. The loss of anticonvulsant activity was not complete. Ictal episodes were modified; they started as very short ones and their duration progressively increased. Structures localized below the level of transection represent a site of at least one of the mechanisms of phenytoin anticonvulsant action.

Key words

Epileptic focus – Cerebral cortex – Phenytoin – Brainstem – Rat – Cerveau isolé

Introduction

Phenytoin (PHT) is an antiepileptic drug of primary importance used as a drug of choice against simple partial (focal) seizures and one of the possible drugs against complex partial seizures and generalized seizures of the grand mal type (Wilder and Bruni 1981, Porter 1989, Wilder and Rangel 1989). There is a vast amount of data on the mechanisms of antiepileptic action of phenytoin (for review Woodbury 1980, 1982, Macdonald and McLean 1986, de Lorenzo 1989, Rogawski and Porter 1990), serving as evidence for multiple mechanisms of action of this drug at the molecular and cellular levels. The mechanisms described include decreased sodium permeability, decreased calcium entry into cells, effects on Na,K-ATPase, effect on calmodulin-calbindin system, effect on cyclic nucleotides and others (for review see de Lorenzo 1989). To decide which of these mechanisms is of primary importance, the localization of phenytoin action, i.e. determination of the possible target structure or structures, is necessary. The data in this field are scarce. The only series of papers was published by Julien (Julien and Halpern 1972, Julien 1974), who hypothesized that exhibits its main action in the cerebellum by enhancing the firing of Purkynje cells. This lack of data led us to study the possible sites of action of in rats (Buřitová *et al.* – in press, Mareš *et al.* 1993). In the present series of experiments, the model of symmetrical cortical penicillin foci was used. PHT delayed the synchronization of interictal discharges of the two foci and abolished a transition into an ictal phase in this model (Mareš *et al.* 1983). To determine the role of hindbrain structures in the anticonvulsant action of PHT, symmetrical penicillin foci were formed in animals with an intercollicular transection of the brainstem (cerveau isolé preparation – Bremer 1936).

Methods

Experiments were performed on 17 adult male albino rats of the Wistar strain. Surgical preparation was performed under ether anaesthesia: trephining of the skull, tracheostomy and introduction of the tracheal cannula. The trephine openings were made over sensorimotor and visual regions of both hemispheres. The brainstem was transected according

to Burešová *et al.* (1962) by means of an L-shaped spatula introduced stereotaxically from occipital trephine openings of both hemispheres at an AP coordinate 7 mm. All somatosensory inputs remained below the level of transection so that perception of pain was abolished. Anaesthesia was discontinued immedialy after the transection, the rats were curarized (d-tubocurarine, 0.2 mg/kg i.p.), connected to a positive pressure respirator and placed on a pad heated electrically to 35 °C. The trephine openings were then covered with warm physiological saline, the animals were allowed to recover for at least 30 min. After this period of rest, silver ball recording electrodes were placed over both sensorimotor and occipital areas with an indifferent electrode on the nasal bone. Subcutaneous electrodes were used for monitoring ECG. Spontaneous ECoG was recorded for two minutes and then a small amount of sodium salt of penicillin (PNC) was applied onto both sensorimotor areas. Seven animals served as controls, other ten were pretreated by phenytoin (Epanutin^{R,} Parke and Davis) in a dose of 60 mg/kg i.p. 10 min before the PNC application. The ECoG was recorded in reference as well as bipolar connections for 30 min after PNC application. The incidence and latency of focal discharges, the incidence and latency of their spontaneous transition into ictal activity as well as the duration of ictal episodes were evaluated. The synchronization of discharges of the two symmetrical foci was quantified in 5 min sections of recordings (always 50 discharges were evaluated and they were taken as synchronous when the difference did not exceed 100 ms) and the mean values for the control and treated group were compared. The unpaired t-test was used for statistical comparison of corresponding sections as well as of the duration of ictal episodes in the two groups. The incidence of ictal phases was evaluated by means of Fisher's exact test (four pole table). The results were compared to previously published data from rats with an intact brainstem (Mareš *et al.* 1983).

After the end of recording, the rats were killed by an overdose of pentobarbital and their brains were fixed in formaldehyde. Sagittal sections of the brainstem were prepared by means of a freezing microtome and the extent of brainstem transection was documented macrophotographically. All 17 rats forming the control and phenytoin groups had a complete midcollicular transection, other 7 animals with an incomplete transection were discarded.

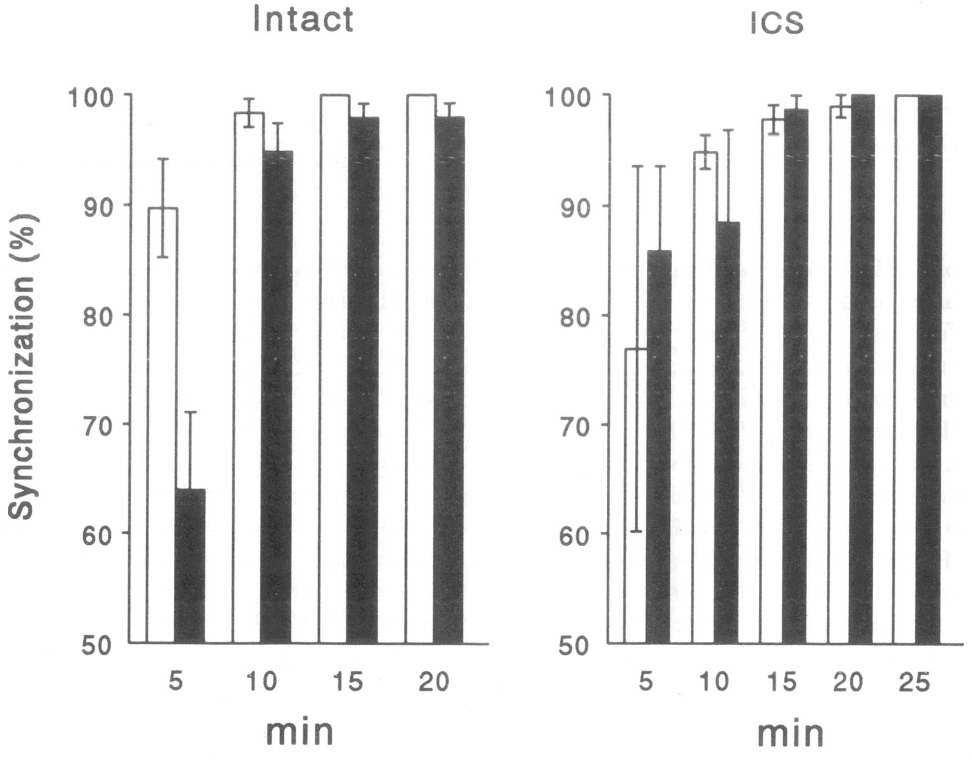

Fig. 1
Synchronization of interictal discharges of symmetrical penicillin foci in intact rats (left panel, data from Mareš *et al.* 1983, with permission) and in rats with brainstem transection (ICS, right panel) expressed as a mean percentage of synchronous bilateral discharges (±S.E.M.). White columns – control animals; black columns – rats pretreated with phenytoin. Abscissa – 5 min intervals after the application of penicillin; ordinate – incidence of synchronous discharges (percentage of animals).

Results

Interictal focal discharges

The two symmetrical foci were elicited in all control and phenytoin-pretreated rats. The latency to the first focal discharge varied from 90 to 160 s after penicillin application. There were no differences in latencies between control and phenytoin-pretreated animals with brainstem transection.

This transection delayed the progress of synchronization in comparison to rats with an intact brainstem (Mareš *et al.* 1983), the differences for the second and third interval being significant. A complete synchrony was achieved only after the 20th min. Phenytoin did not change the synchronization in animals with the intercollicular transection (Fig. 1).

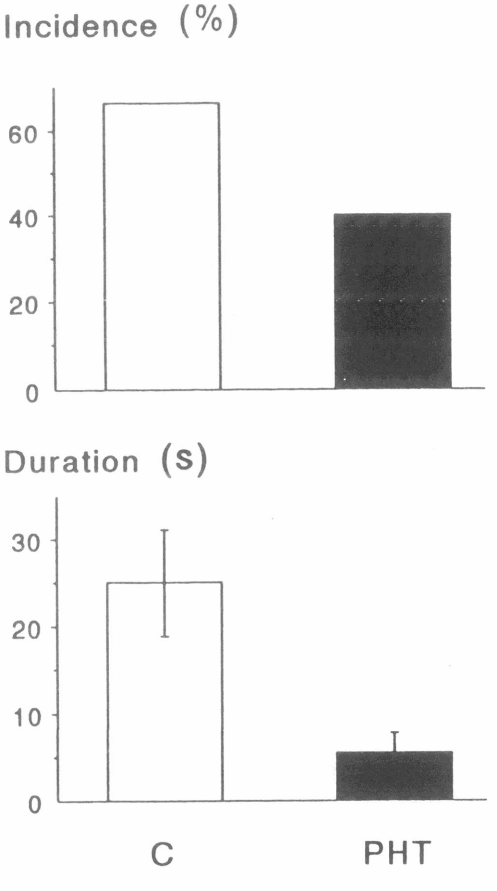

Fig. 2
Incidence (left) and duration (right, mean±S.E.M.) of ictal episodes in rats with brainstem transection. White columns – control animals (C), black columns – rats pretreated with phenytoin (PHT). Upper panel – incidence of EEG seizures (percentage of animals); lower panel – duration of ictal phases in seconds. The PHT-pretreated group shown here are data from four rats exhibiting seizures longer than five seconds.

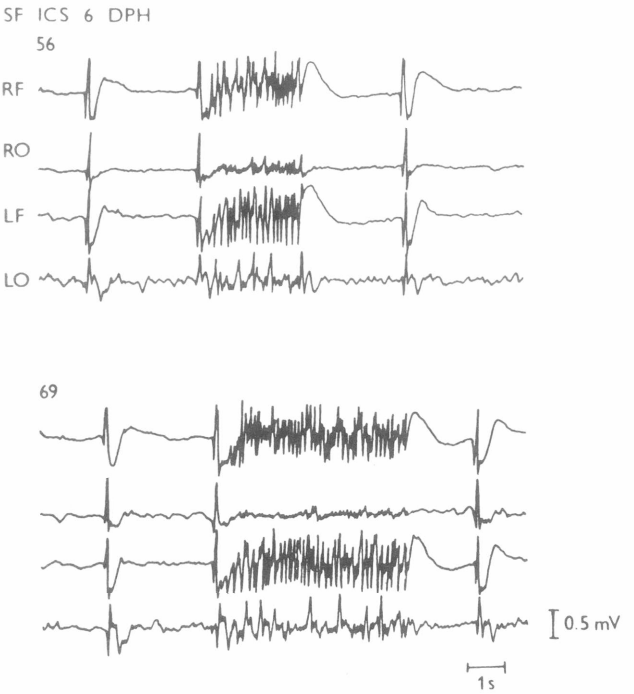

Fig. 3
Electrocorticographic recording of ictal activity in a phenytoin-pretreated rat with brainstem transection. Interictal discharges are recorded before as well as after ictal episodes. Upper part – a brief episode recorded 56 min after phenytoin administration (i.e. 41 min after penicillin application); lower part – ictal phase lasting 6 s recorded 69 min after phenytoin pretreatment. Individual traces from top to bottom: right frontal (RF) and occipital (RO) and left frontal (LF) and occipital (LO) cortical regions in reference connections. Time mark 1 s, amplitude calibration 0.5 mV.

Transition of interictal discharges into ictal activity

Spontaneous appearance of ictal episodes was found in five out of seven control rats and in all ten phenytoin-pretreated animals. All five seizing control rats exhibited long-lasting sections of ictal activity from the very beginning of this activity with an average duration of ictal episodes of 25.5±6.2 s (mean±S.E.M.). On the contrary, phenytoin-pretreated rats exhibited very short periods of ictal activity, lasting only 1–2 s. The duration of these episodes progressively increased but up to the end of the recording period (60 min after the application of penicillin) only four rats (40 %) exhibited episodes lasting longer than five seconds, i.e. the limit used in our other studies (Figs. 2 and 3). The average duration of ictal episodes was significantly shorter than in the control group, even in the case when only four rats with

seizures longer than 5 s were used for the evaluation (5.2±0.2 s). The six remaining animals did not surpass this limit. Phenytoin thus did not suppress but only modified ictal activity.

Discussion

Cortical penicillin foci have served as a model of human partial seizures with simple symptomatology. In spite of detailed knowledge of the pathophysiology of these foci (for review see Prince 1978) they were not used for testing antiepileptic drugs. These foci exhibit very strong activity and they thus represent an exaggerated model of human foci, which is therefore insensitive to antiepileptic drugs. The elicitation of two symmetrical foci (Mareš 1973) introduced some additional phenomena, which might be quantified and which are sensitive to antiepileptics – the synchronization of discharges of these foci as an expression of the spread of epileptic acitivity and the transition into ictal activity, i.e. secondary generalization from the epileptic focus. The transition into ictal activity was succesfully used for testing of antiepileptic drugs (Mareš and Pohl 1981, Mareš *et al.* 1983, Marešová and Mareš 1985).

Phenytoin in the dose of 60 mg/kg i.p. was found to delay the synchronization of interictal discharges of the two symmetrical foci and to abolish the transition into ictal activity in animals with an intact brainstem (Mareš *et al.* 1983). It is rather difficult to evaluate the action of phenytoin on the synchronization of interictal discharges in cerveau isolé rats, because of a delayed synchronization in control rats with brainstem transection. Complete synchronization was attained in our present experiments only after the 20th minute after penicillin application, whereas intact animals complete this synchronization before the 10th min after eliciting foci (Mareš *et al.* 1983). On the other hand, the failure of phenytoin to affect the appearance of ictal episodes was clear and this finding speaks in favour of the localization of this action of phenytoin in hindbrain structures. The modification of ictal episodes – their progressive build-up never seen in control transected as well as in intact animals – indicates that some structures anterior to the transection also play a role in the anticonvulsant action of phenytoin. The plurality of mechanisms of phenytoin action was demonstrated by many authors (for review see Woodbury 1982, Macdonald and McLean 1986, de Lorenzo 1989). Our developmental data are also in agreement with at least two mechanisms of the anticonvulsant action of phenytoin with different ontogenetic development (Marešová and Mareš 1983, Staňková *et al.* 1992). These various mechanisms of action might also be localized in different brain structures. The present results have demonstrated that hindbrain structures are responsible for a part of the phenytoin anticonvulsant action. Julien (1974) suggested that cerebellum represents a target structure for phenytoin. Our own results on the influence of phenytoin on cerebellar neurones (Buřitová *et al.* – in press) did not confirm this hypothesis and thus further analysis of the localization of phenytoin anticonvulsant action is necessary.

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P. Mareš, M.D., D.Sc., Institute of Physiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Vídeňská 1083, Czech Republic.