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Influence of Phenobarbital on ECoG Phenomena Induced by Metrazol in Rats during Ontogenesis

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Summary

Effect of phenobarbital (PhB, 20 and/or 40 mg/kg) on epileptic ECoG phenomena induced by metrazol was studied in acute experiments in rats aged 7, 12, 18, 25 and 90 days. Fractionated administration of metrazol (20 mg/kg i.p. each 300 s) was used to quantify the effects of PhB. First signs of metrazol action (sharp elements and/or rhythmic metrazol activity) were not reliably influenced by PhB. On the contrary, the latency of the first EEG seizures as well as of the first generalized EEG seizures was prolonged and thus a dose necessary for their elicitation was increased in all age groups. These differences reached statistical significance in 12-, 18- and 25-day-old rats. A lack of effect of PhB against the rhythmic metrazol activity supports the adequacy of this activity as a model of human absences. Differences between the development of antiepileptic and hypnotic effects of PhB (described earlier) suggest two different mechanisms of action.

Key words
EEG – Pentylenetetrazol – Phenobarbital – Ontogenesis – Rat

Introduction

Experimental models of human primary generalized seizures of the absence type are under discussion. The common model, minimal metrazol seizures, is probably not adequate (Loscher and Schmidt 1988, Mareš and Zouhar 1988, Kubová and Mareš 1991). Among other models, rat genetic model of absences (Marescaux *et al.* 1984) was used also for testing of antiepileptic drugs. Feline generalized penicillin epilepsy (Gloor 1979, 1984) served primarily for studies of basic mechanisms of generation of this type of seizures. Recently we have described another model – rhythmic metrazol activity, i.e. periods of EEG spike-and-wave rhythm induced by low doses of metrazol. This activity corresponds to human absences in behaviour (Schickerová *et al.* 1989), pharmacological sensitivity (Mareš and Velíšek 1986, Velíšek and Mareš 1987, Brabcová *et al.* – in preparation) and age dependence (Zouhar *et al.* 1980, Schickerová *et al.* 1984). The aim of this study was to describe the action of phenobarbital not only on rhythmic metrazol activity but also on electrographic seizures elicited by higher doses of metrazol and to compare this action with the effects of drugs effective against absence seizures – ethosuximide, valproate (Mareš and Velíšek 1986) and clonazepam (Velíšek and Mareš 1987).

Material and Methods

Experiments were performed on 79 male albino rats of the Wistar strain 7, 12, 18, 25 and 90 days old. The day of birth was taken as zero. Other 56 animals served as control age matched groups. The numbers of animals in individual age and treated groups are given in Table 1.

Table 1

Numbers of animals in individual groups

Age in days	Control group	PhB 20 mg/kg	PhB 40 mg/kg
90	17	8	8
25	9	7	8
18	10	8	8
12	9	8	8
7	11	8	8

Four trephine openings were made bilaterally in the frontal and occipital regions under ether anaesthesia. The diameter of the trephine openings was 3 mm in adult rats and 1–2 mm in young ones. The dura mater was kept intact. Then a tracheostomy was performed and a tracheal cannula was inserted. Wounds and future pressure points were carefully anaesthetized locally by benzocaine powder. The direct contact of benzocaine with the dura mater was avoided to protect the cerebral cortex from the depressant action of benzocaine. The anaesthesia was then disrupted and the animals immobilized with d-tubocurarine (0.2 mg/kg i.p.) were placed into a modified stereotaxic holder (concave head holders instead of ear bars) on an electrically heated pad. Artificial respiration was performed by means of a positive pressure respirator, the depth was adjusted according to the excursions of the chest.

Electrocorticogram was registered by means of silver ball electrodes from sensorimotor (frontal) and visual (occipital) cortex (coordinates AP 0; L 2 and AP 6; L 4 in relation to bregma for adult animals; they were recalculated for the youngs according to their bregma-lambda distance) of both hemispheres in reference (an indifferent electrode on the nasal bone) as well as bipolar leads. ECG was recorded to monitor the state of the animal. A short section of spontaneous ECoG was always recorded, then phenobarbital (PhB) was administered intraperitoneally in a dose of 20 or 40 mg/kg. Even the control ECoG exhibited the presence of both waking and slow wave sleep recordings. ECoG was registered for the last five minutes before the metrazol (pentamethylenetetrazol, PTZ) administration. A cannula was introduced intraperitoneally for repeated doses of metrazol (20 mg/kg each 300 s, the first dose was given 25 min after PhB injection). The doses of PTZ were administered till the appearance of generalized seizure activity, then the recording continued for 600 s. If generalized ECoG seizures did not appear, the 15th injection of PTZ was the last one. The duration of the experiment of 80 min at maximum granted that PTZ was accumulated in the brain and no substantial elimination took place (Brink *et al.* 1970).

The latencies of epileptic phenomena and thus a dose necessary for their elicitation were evaluated from EEG recordings: first sign of PTZ action (either single spikes and/or sharp waves or rhythmic metrazol activity, Fig. 1), first RMA (rhythmic metrazol activity, i.e.sections of theta waves with outspoken spikes, Fig. 1), first ECoG seizure (formed by sustained ictal activity, Fig. 2) and first generalized ECoG seizure (Fig. 2). The results were statistically evaluated by analysis of variance, corresponding experimental and control groups were compared by means of the multiple range test of Tukey. The level of statistical significance was set at 5 %.

Results

1. Description of ECoG phenomena (Figs. 1,2 and 3)

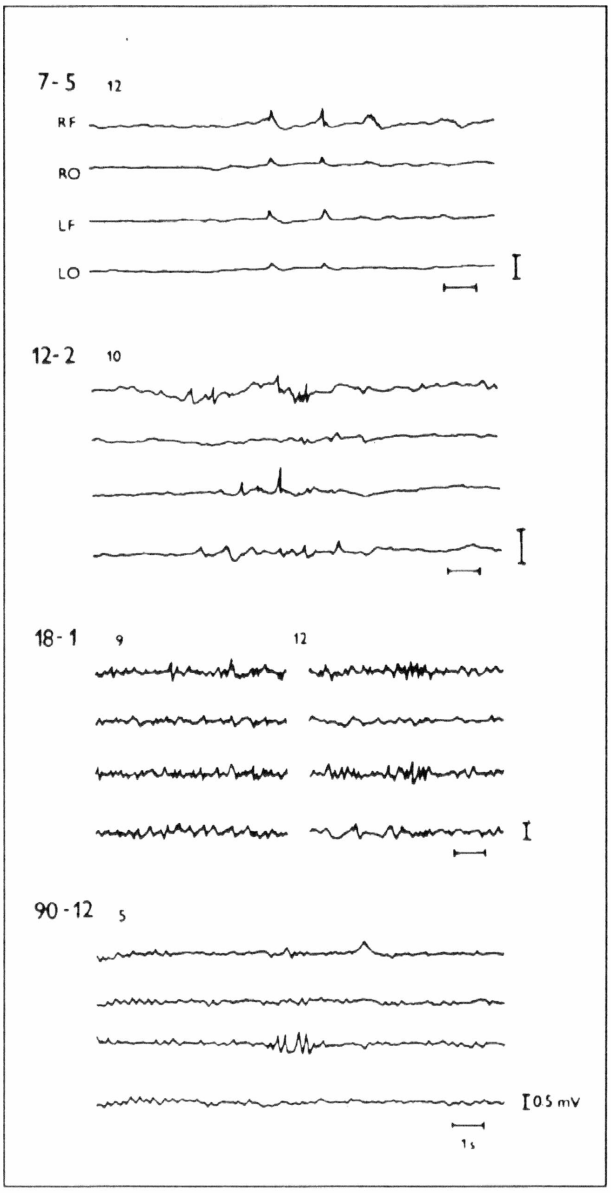

Fig. 1
First signs of PTZ action in EEG from rats aged 7, 12, 18, and 90 days (from top to bottom the second number after the age denotes the serial number of the rats). Individual leads: RF – right frontal, RO – right occipital, LF – left frontal, LO – left occipital cortical region in reference connections. Numbers over individual segments mean time after the first dose of PTZ in min. Time mark – 1 s, amplitude callibration 0.5 mV.

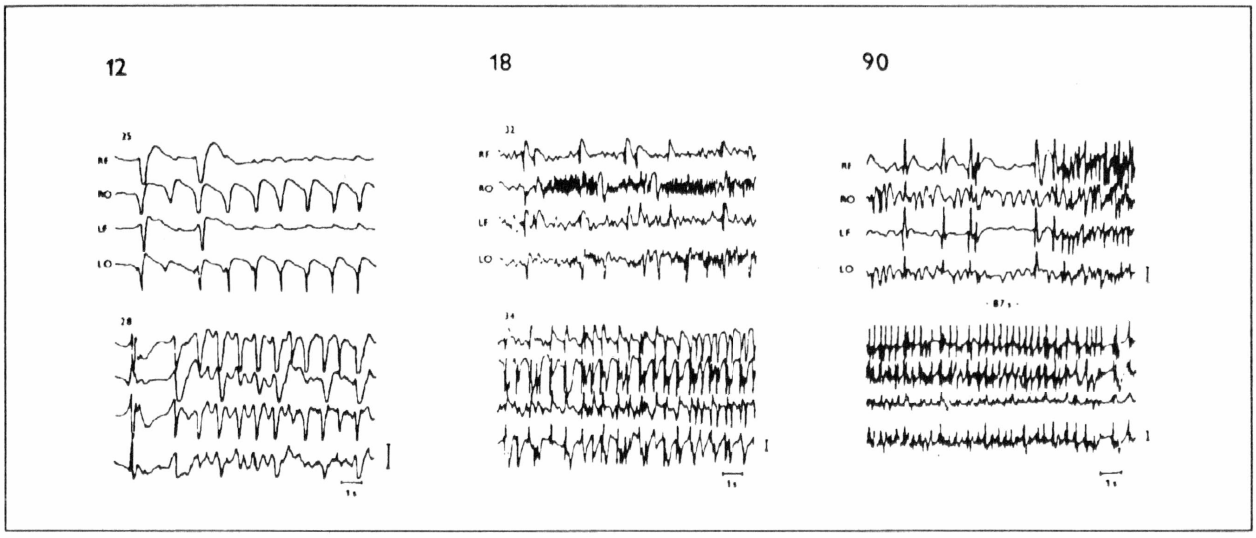

Fig. 2
ECoG seizures in rats aged 12, 18 and 90 days (from left to right). Details as in Fig. 1. −87 s- between the two parts of recording from an adult rat denotes duration of the omitted record.

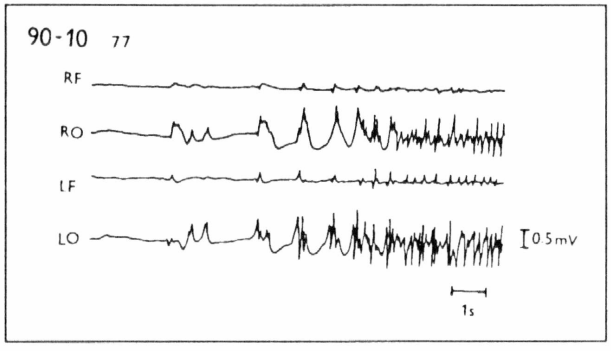

Fig. 3
EEG recording of a "limbic-like" pattern of seizures in a 90-day-old rat. Details as in Fig. 1.

Adult rats

Rhythmic metrazol activity was always recorded in occipital regions as the first sign of PTZ action in control animals, later the RMA became generalized. Isolated spikes appeared in ECoG in all cases but later than RMA. Seizures were always generalized since the very beginning and were formed by spike-and-wave rhythm.

PhB-pretreated animals exhibited either RMA and/or isolated spikes as the first PTZ-induced phenomenon. RMA was recorded in all animals. Seizures were again generalized from the onset, the pattern of ictal activity was more variable than in controls. Higher dose of PhB led invariably to ictal activity formed by sharp delta waves with superimposed fast activity ("limbic-like" seizures, Fig. 3) as the final pattern of ECoG seizures.

Twenty five-day-old rats

First signs of PTZ action were formed by RMA and/or isolated frontal spikes; RMA was registered in all cases. Ictal activity could start in one cortical region but generalization was fast and constant.

Rats given PhB exhibited nearly the same ECoG phenomena as the controls, the only difference was the predominantly generalized onset of ECoG seizures.

Eighteen-day-old rats

Isolated frontal spikes formed the first ECoG change in all control animals. Somewhat later, generally after the same dose of PTZ, RMA appeared in frontal regions. Spikes spread to the occipital region soon, meanwhile RMA was recorded in this area with a substantial delay. ECoG seizures, characterized again by spikes and the spike-and-wave rhythm, started either as incomplete paroxysms with subsequent generalization or as generalized ones. Seizures usually terminated with the "limbic-like" pattern.

The only difference in PhB-pretreated rats was absence of "limbic-like" ictal activity towards the end of seizures.

Twelve-day-old rats

Isolated spikes and/or sharp waves formed the first change after PTZ administration, typical RMA

was never registered. Spikes increased in amplitude and frequency and then progressed into ictal activity. Seizures could start as localized or generalized, the presence of a "limbic-like" seizure pattern in later stages of ictal activity was a rule. In some cases two independent seizure patterns (huge spike-and-wave complexes and the "limbic-like" pattern) might be recorded simultaneously.

Isolated sharp elements as the first sign were present in PhB-pretreated rats and they progressed into ictal activity. Generalized beginning of seizures clearly predominated, the "limbic-like" pattern was recorded only exceptionally.

Fast sinusoid waves (with a frequency of 10 to 15 Hz) with waxing and waning, but generally of very low amplitude, represented a special phenomenon recorded only at this age in PhB-pretreated rats.

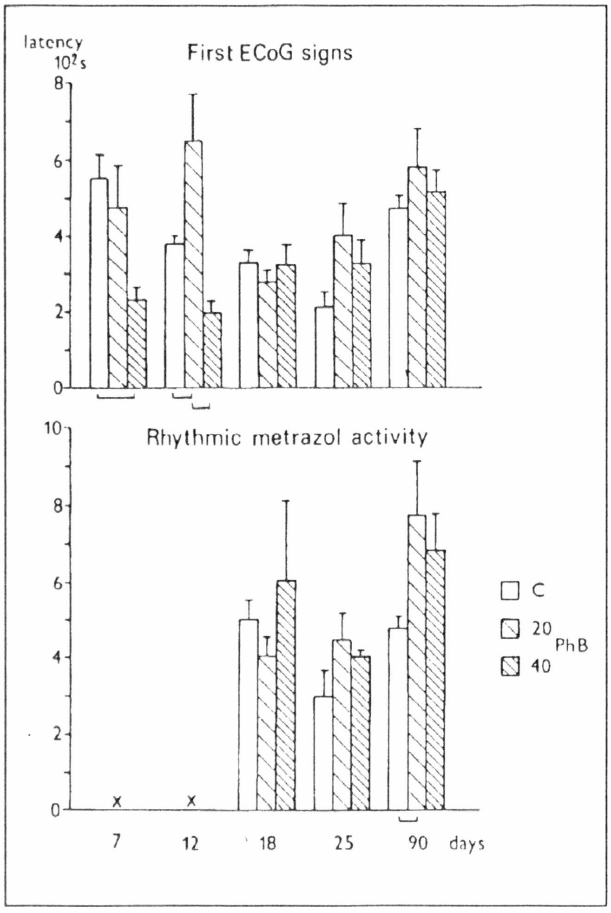

Fig. 4
Latencies (M±S.E.M.) of the first ECoG signs of PTZ action (upper part) and of the first rhythmic metrazol activity (lower part). White columns – control rats, obliquely hatched columns – animals pretreated with two doses of phenobarbital. Abscissa – age groups; ordinate – latencies in hundreds of seconds. Significantly different values are marked by bars under the columns.

Seven-day-old rats

Sharp waves formed the first sign of PTZ action. Their incidence increased and they progressed into ictal activity formed again by sharp waves. Seizures started locally and generalization appeared relatively late. Synchronization of individual graphoelements among different cortical regions was never perfect.

First signs in both PhB groups were not changed. The only change in ictal activity was a possibility of its generalization from the beginning.

2. Statistical evaluation of ECoG epileptic phenomena

First signs of PTZ action (Fig. 4)

Under control conditions, the average latency of the first signs of PTZ action was longer in 7-day-old than in all other groups of young rats. In addition, the latency of the first signs in 25-day-old animals was significantly shorter than that in adult rats. The only significant prolongation of the mean latency was seen after the lower dose of PhB in 12-day-old animals. Paradoxically, PhB in a dose of 40 mg/kg shortened the latency of the first changes in 7-day-old rats. Comparing the effects of PhB among various age groups, no significant changes were found after the 20-mg/kg dose, whereas the higher dose of PhB reversed the sensitivity of different age groups: The small mean latencies in 7- and 12-day-old rats became significantly shorter than those in adult animals.

First RMA (Fig. 4)

Among control rats, the shortest latency was seen in the 25-day-old group, the difference compared to adult animals was statistically significant. There was only one finding with PhB - prolongation of latency of RMA after the 20-mg/kg dose in adult rats. Due to this increase the mean latency of RMA in 18-day-old animals pretreated with the 20-mg/kg dose of PhB was significantly shorter in comparison with the corresponding adult group. No changes were seen after the 40-mg/kg dose.

First seizures (Fig. 5)

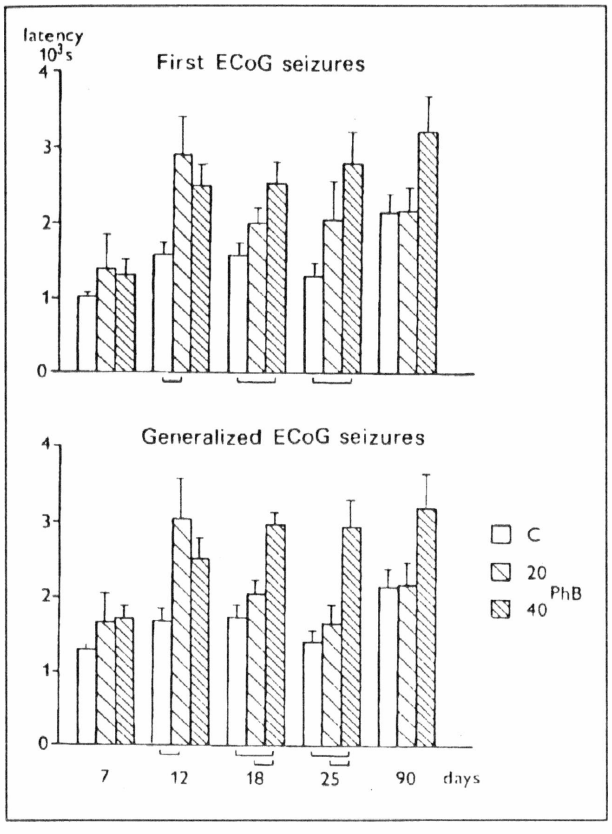

Fig. 5
Latencies (M ± S.E.M.) of the first ECoG seizures (upper part) and of the first generalized ECoG seizures (lower part). Details as in Fig. 4. Ordinates – latencies in thousands of seconds.

Control rats exhibited the shortest latencies in the 7- and 25-day groups, the difference compared with adult animals being significant. A tendency to lengthening of latencies after PhB was observed in all age groups, statistical significance was reached with the 20-mg/kg dose in 12-day-old rats and with the higher dose in the 18- and 25-day-old groups. No significant changes were found among the 20-mg/kg groups, the 40-mg/kg dose of PhB resulted in a significantly shorter latency in 7- than in 25-day-old and adult rats.

First generalized seizures (Fig. 5)

Seven- and 25-day-old control rats exhibited significantly shorter latencies than control adult animals. The same significant differences as with first seizures were found. In addition, a dose-dependent increase of latencies was observed in 18- and 25-day-old rats. No differences among various age groups were found after the lower dose of PhB, the 40-mg/kg dose resulted in conservation of the significant difference between 7-day-old and adult rats.

Discussion

Our results demonstrated the inefficacy of PhB against rhythmic metrazol activity. On the contrary, ECoG seizures were delayed in PhB-pretreated young as well as adult animals. Our previous data from the same model demonstrated a specific action of ethosuximide against RMA in adult rats, whereas valproate and clonazepam delayed the appearance of both RMA and ECoG seizures at all developmental stages studied (Mareš and Velíšek 1986, Velíšek and Mareš 1987). These data are in full agreement with clinical efficacy of the four antiepileptics studied: PhB is efficient against generalized tonic-clonic seizures, ethosuximide against absences and valproate and clonazepam against both types of generalized seizures (Engel 1989). This correlation may be taken as indirect evidence for the adequacy of RMA as a model of human absences.

The present results confirmed the finding that generalization of seizures is slow in the youngest groups and that it ameliorates with age (Mareš and Velíšek 1986). The effects of PhB on generalization were rather inconsistent but there was a tendency to an increase in generalization especially in the 7-day-old rat pups. Opposite results were found with clonazepam: even adult rats pretreated with clonazepam exhibited a localized onset of seizures and generalization was slowed down in all age groups (Velíšek and Mareš 1987). Benzodiazepines exert their action through the same supramolecular complex ($GABA_A$ receptor/benzodiazepine receptor/chloride ionophore) as barbiturates but at a different binding site (for review Olsen and Venter 1986). Therefore, different mechanisms of action probably cannot explain their different effects on seizure generalization. The transition of the original spike-and-wave activity into the "limbic-like" pattern towards the end of seizures was also influenced unequivocally by PhB. The relation between these two types of EEG seizures may be better studied in models, where epileptic phenomena are elicited by means of electrical stimulation and may therefore be timed exactly.

No marked differences in the anticonvulsant action of PhB were found among different age groups. Similar results were also obtained with motor seizures induced by metrazol (Kubová and Mareš 1991). This lack of developmental changes is in marked contrast to the hypnotic action of PhB during ontogenesis – sleeping time was very long in 7-day-old rat pups and decreased significantly with age (Marešová and Mareš 1980). The discordance of these two measures of PhB action during development might be explained by two different mechanisms of action of PhB. All data published on the molecular pharmacology of barbiturates have found only one mechanism of action

of barbiturates – binding to a site in the chloride channel (for review Olsen and Venter 1986). Therefore the second explanation is more probable: the difference in the development of anticonvulsant and hypnotic action of PhB may be due to two target structures which differ in their developmental rate. The hypnotic action of barbiturates is ascribed to the reticular formation, the site of anticonvulsant action is unknown (Prichard and Ransom 1989). The pharmacokinetic changes seem very improbable as a cause of the above mentioned developmental differences.

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