Ontogenetic development of sensitivity of the cerebral cortex to an antagonist of $GABA_A$ receptor bicuculline

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Summary

Local application of four concentrations of bicuculline methiodide (a specific antagonist of GABAA receptors) was used to study a sensitivity of somatosensory cortex in four age groups of immature rats with implanted electrodes. Presence and latencies of two epileptic phenomena (focal discharges and seizures) were evaluated. Focal discharges exhibited moderate tendency to a decrease of sensitivity to bicuculline methiodide with maturation. Concentration-effect relation of incidence of focal discharges was observed only in 7- and 12-day-old but not in older animals. Results with incidence and latencies of seizures did not show relations to age or concentration of bicuculline. Neither of the epileptic phenomena can be used as a reliable index of cortical maturation.

Models of cortical epileptic foci are usually formed by local application of convulsant drugs on (or into) cerebral cortex. Not speaking about strychnine foci studied nearly one hundert years ago (for review Ayala et al. 1973), the very first model documented electroencephalographically was elicited by penicillin (Van Hartesveldt et al. 1975). Penicillin acts at $GABA_A$ receptor complex (Macdonald and Barker 1977), therefore more specific drugs were studied among them competitive antagonist of $GABA_A$ receptors bicuculline (Curtis 1973). Its focal convulsant action was demonstrated with a water soluble form bicuculline methiodide in adult as well as immature rodents (Soukupová et al. 1993, Eder et al. 1997). We used this model also for pharmacological studies (Bernášková and Mareš 2010, Mareš et al. 2012). We decided to use generation of epileptic phenomena for a study of development of sensitivity of the cerebral cortex to bicuculline.

Experiments were approved by Animal care and use committee of the Institute of Physiology to be in agreement with Czech Animal Protection Law and European Community Council directives 86/609/EEC. Male Wistar rats 7, 12, 18 and 25 days old were used. Surgical preparation performed under ether anaesthesia consisted from implantation of epidural flat silver electrodes over sensorimotor and visual areas of both hemispheres. Plastic cannula was implanted to the right sensorimotor electrode, an indifferent electrode was implanted into the nasal bone. The whole electrode assembly was fixed to the skull by fast curing dental acrylic (Dentacryl, Dental, Prague). The surgery lasted approximately 15 minutes, then the animals were allowed to rest for one hour and after control of righting, placing and suckling reflexes the registration started.

Bicuculline methiodide (BiMI, purchased from Sigma-Aldrich) was dissolved in water in four different concentrations (0.5; 0.25; 0.125 and 0.06 mM) to have always the same volume of 2.5 µl for epicortical application.

The animals were placed individually into plastic boxes. Body temperature of the three younger groups was maintained by means of a pad electrically heated to 34°C, i.e. the temperature in the nest. EEG activity was amplified and digitalized at a rate of 500 Hz and saved on a harddisc of the system (Kaminskij, Prague). Bicuculline was applied after a 10-min recording of spontaneous EEG activity and then registration continued for at least 30 min. Individual age and concentration groups were formed

by 8-10 rats. Incidence and latencies of two epileptic EEG phenomena were evaluated: focal discharges and ictal activity.

SigmaStat® software (SYSTAT) was used. Incidence of individual phenomena was statistically evaluated with Fisher exact test, latencies by One way ANOVA with subsequent pairwise comparison using Holm-Sidak test. Level of significance was set at 5%.

Focal discharges (Fig.1) were present after three higher concentrations in all 7-day-old animals, the two high concentrations resulted in the same effect in 12-day-old rats. These two age groups exhibited clear dose-response effect. Similar concentration-response effect was observed in 18-day-old animals, only the highest concentration of BiMI failed to induce epileptic focus in one animal. The oldest age group yielded a little erroneous results, the concentration-effect relation is compromised by a low incidence of focal discharges in the 0.25-mM group. No difference in the incidence reached the level of statistical significance. Latencies to the first focal discharge exhibited an outlined decreasing tendency with increasing concentrations of BiMI in all but the youngest group (Fig.2). The latency to the appearance of focal discharges was significantly longer in the 0.125- and 0.25-mM groups than that in the 0.5-mM group. Similar significant result was found only with the 0.125-mM concentration in 12-day-old animals. The 7-day-old rats paradoxically demonstrated significantly longer latency after application of 0.5-mM BiMI than after the two low concentrations (0.06 and 0.125 mM).

Incidence of ictal activity (Fig.1) was irregular in the 7-day-old rats. It increased with increasing BiMI concentration only in 12-day-old rats reaching 100% incidence after the highest concentration (0.5mM). Very low incidence of seizures was found in 18-day-old rats (3 out of 8 rats with the 0.125-mM concentration as a maximum). No clear-cut concentration-effect relation was seen in the oldest age group. None of the outlined difference reached the level of statistical significance. Latencies exhibited a tendency to a decrease with increasing concentration in the 12- and 25-day-old groups. There was not a sufficient number of data in18-day-old rats. There was again a paradoxical increase of the latency after application of the highest concentration of BiMI in the 7-day-old group. Statistically significant difference was found only between 0.125- and 0.5-mM groups.

Development of either phenomenon evaluated (focal discharges and seizures) is irregular. There is an outlined tendency to a decrease of the incidence of focal

discharges with age indicating the highest sensitivity of cerebral cortex to convulsant action of BiMI in the 7-day-old group. There was no developmental tendency in the incidence of seizures. On the other hand, the youngest age group (7-day-old rat pups) exhibited longer latencies of both phenomena after application of the highest concentration of BiMI than after lower concentrations. A hypothetical explanation might be in a presence of a part of cortical neurons still possessing GABA-induced depolarization therefore the highest concentration of bicuculline antagonizes not only GABA-elicited hyperpolarization but also depolarization. Depolarizing action of GABA was demonstrated up to postnatal day 5 in hippocampus (Ben-Ari 2014), the cortical development is approximately parallel to development of hippocampus (Kirmse et al. 2011). Another explanation might be in very strong GABA-induced depolarization so that many neurons are no longer able to generate action potentials. Immaturity of the neocortical connections should be also taken into account (Luhmann et al. 2014). Future experiments must show if some of these hypothetical explanations is right.
It is possible to conclude that neither focal discharges nor seizures can be taken as a reliable index of cortical sensitivity to $GABA_A$ receptor antagonist bicuculline.

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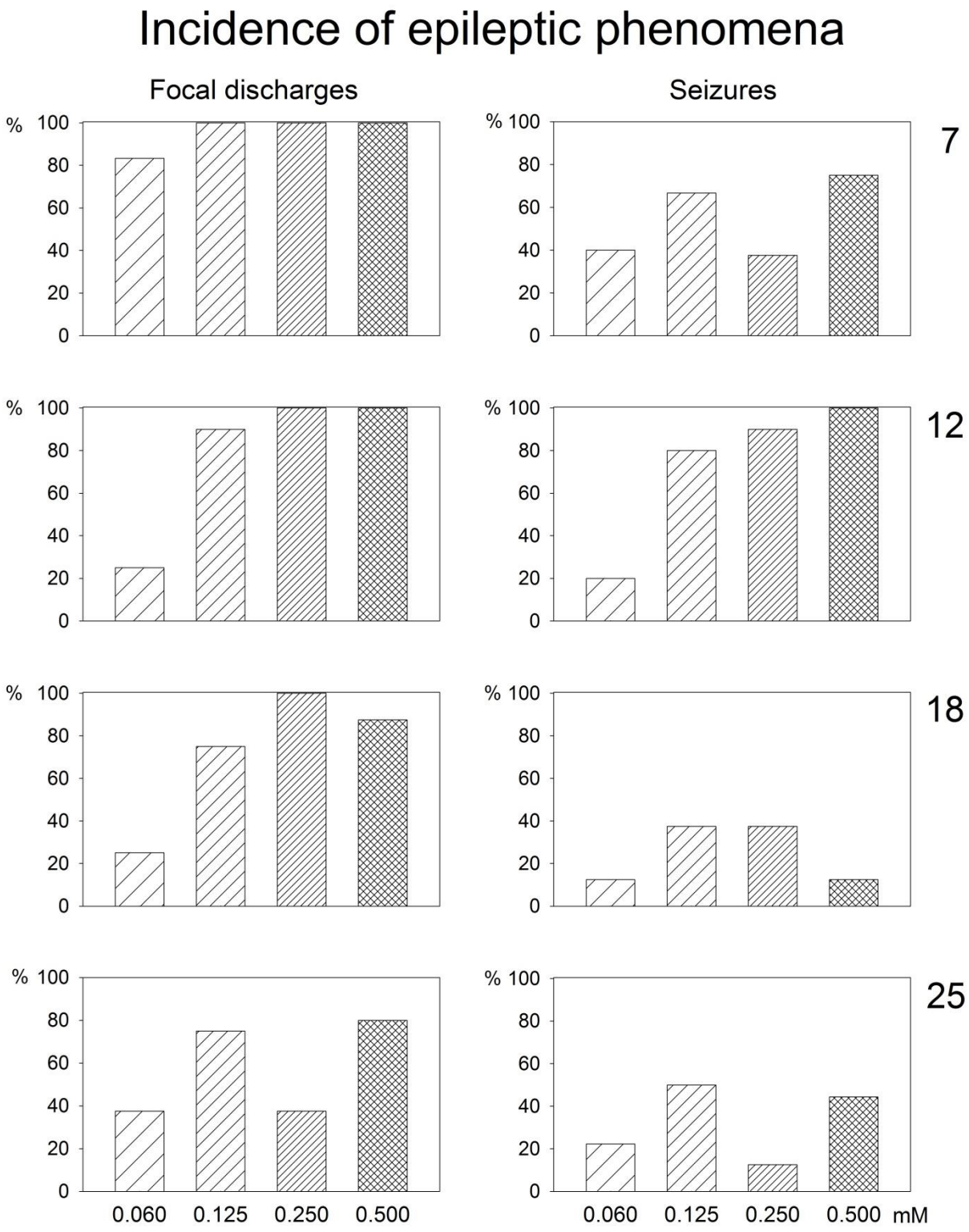

Fig. 1. Incidence of epileptic phenomena. Left column – focal discharges, right column – seizures; from top to bottom – 7-, 12-, 18- and 25-day-old rat pups. Abscissae – four concentrations of bicuculline methiiodide; ordinate – percents on animals exhibiting epileptic phenomena.

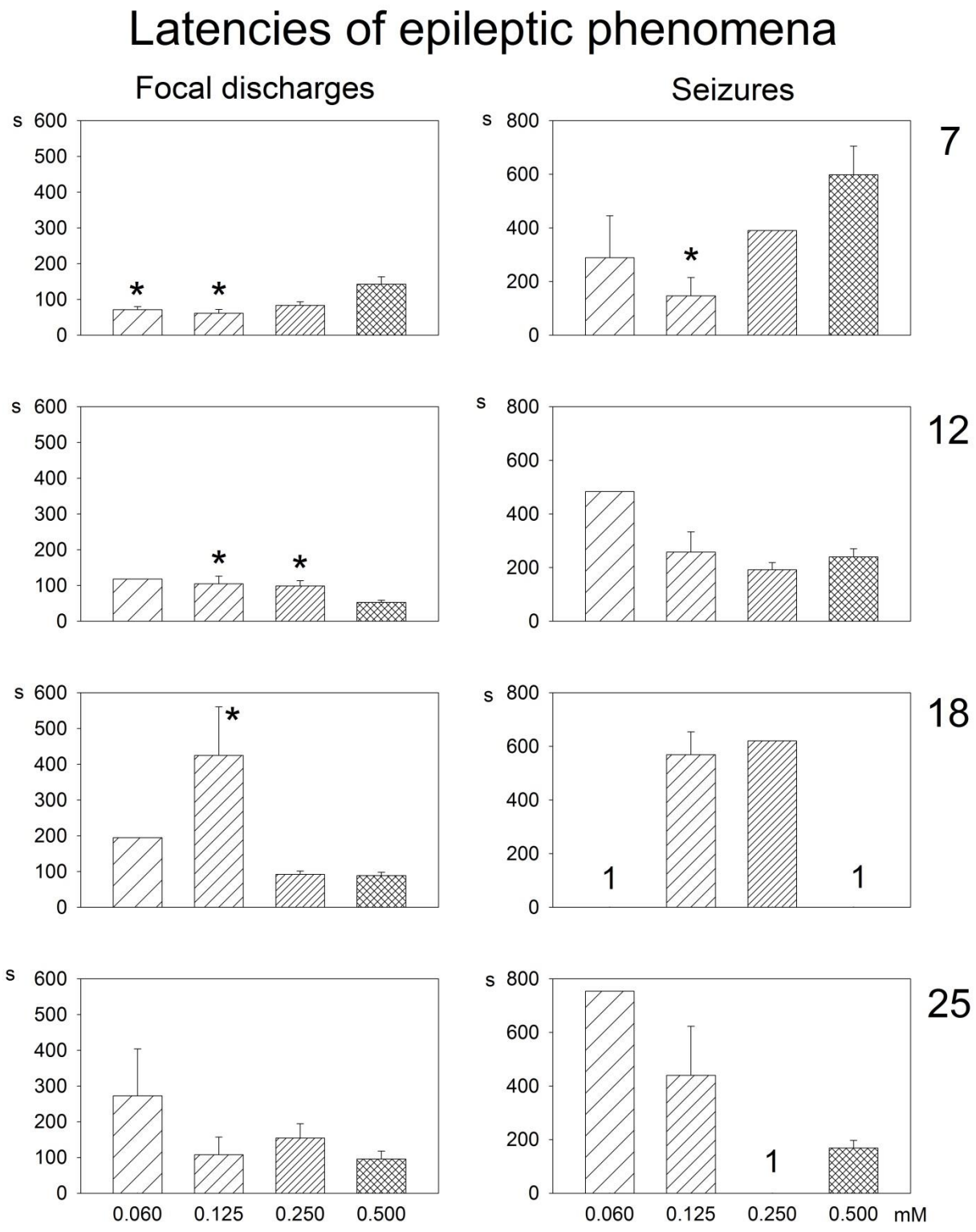

Fig. 2. Latencies of epileptic phenomena. Asterisks denote a significant difference in comparison with 0.5-mM group. Number 1 in 18- and 25-day-old rats denotes the presence of seizures in only one rat. Other details as in Fig.1.