

An Antagonist of GABA-B Receptors Potentiates Activity of Cortical Epileptic Foci

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Summary

Cortical epileptic foci elicited by local application of bicuculline methiodide represent a model of interictal epileptic activity with a transition into ictal phases. We studied a role of GABA-B receptors in this model using GABA-B receptor antagonist CGP35348 in adult rats with implanted cortical electrodes and cannula. CGP35348 (100 or 200 mg/kg i.p.) did not affect interictal discharges but it augmented ictal activity. Latency to the first ictal episode was decreased by the lower dose of CGP35348, duration of episodes was increased by the higher dose. GABA-B receptor antagonist did not influence purely cortical epileptic phenomenon but it is proconvulsant in ictal activity generated with participation of subcortical structures.

Key words

Cerebral cortex • Bicuculline • CGP35348 • Epileptic focus • Rat

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Many types of epileptic seizures are due to a strong excitation dominating over inhibition. Experimentally, epileptic seizures can be elicited either by administration of drugs potentiating excitation (e.g. glutamate receptor agonists kainic acid and NMDA) or more frequently by drugs suppressing inhibition (e.g. GABA-A receptor antagonist bicuculline, glycine receptor antagonist strychnine – Velisek 2006). Role of

individual inhibitory systems can differ according to brain structures serving as generators of seizures. Recently we demonstrated an important role of inhibition mediated by GABA-B receptors in cortical epileptic afterdischarges in immature rats (Mareš 2010). It is in agreement with our older finding that administration of an antagonist of GABA-B receptors CGP35348 results in marked prolongation of cortical afterdischarges in adult rats (Mareš *et al.* 2007). Epileptic afterdischarges characterized by spike-and-wave rhythm in the EEG and clonic seizures are generated in the corticothalamic system (Pohl *et al.* 1986). It is in agreement with mechanisms of generation of spike-and-wave rhythm generally (Avanzini *et al.* 1992, Meeren *et al.* 2002). To know if GABA-B inhibitory system plays a role also in a purely cortical epileptic phenomena we extended our study to another model – cortical epileptic foci induced by local application of bicuculline methiodide (Soukupová *et al.* 1993, Eder *et al.* 1997, Bernášková and Mareš 2010). This model offers two different epileptic phenomena to be evaluated – in addition to purely cortical interictal focal discharges a transition into ictal activity characterized by spike-and-wave rhythm in the EEG accompanied by clonic seizures. Both EEG and motor ictal phenomena are identical with those characteristic for the cortical epileptic afterdischarges (Mareš *et al.* 2007). We hypothesized that antagonist of GABA-B receptors will augment epileptic activity also in a model of focal cortical seizures.

The experiments performed in male adult rats of the Wistar strain (body weight 240-260 g at the time of

surgery) were approved by Animal Care and Use Committee of the Institute of Physiology to be in agreement with Animal Protection Law of the Czech Republic as well as with European Community Council directives 86/609/EEC. Under ketamine-xylazine anesthesia recording electrodes were implanted epidurally over sensorimotor (frontal) and visual (occipital) cortical regions of both hemispheres, reference electrode was in the nasal bone, grounding electrode in occipital bone over cerebellum. Cannula made from an injection needle was implanted closely to the left frontal electrode. The whole assembly was fixed to the skull by means of miniature screws and fast curing dental acrylic. The animals were allowed to rest for one week and then the experiments started. Electroencephalographic activity was recorded on an EEG apparatus to have a possibility to compare without interruption individual parts of the recording. Three experiments were made in each of the 10 rats: two experimental sessions when animals were pretreated with an *in vivo* active GABA-B receptor antagonist CGP35348 (3-aminopropyl(diethoxymethyl)phosphinic acid, a generous gift of Novartis) in a dose of 100 or 200 mg/kg *i.p.* and a control one where saline was injected instead of CGP35348. Doses were chosen on a basis of data with cortical epileptic afterdischarges (Mareš 2010). Order of the experiments was pseudorandomly changed in individual animals to minimize possible effects of repeated exposure. All 10 rats had two sessions, one rat lost its connector before the last session, therefore the 200-mg group consisted from 9 animals. Recording started 20 minutes after administration of CGP35348 or saline. Spontaneous activity was recorded for 10 min, then bicuculline methiodide (Sigma, St. Louis; 2.5 μ l of 1 mMol freshly prepared solution) was applied epidurally on frontal cortical area and activity was recorded for 30 more minutes. Latency of focal discharges and their number between 10th and 15th min after bicuculline application were evaluated. In addition, incidence of transition of interictal into ictal activity was recorded and latency, total number and average duration of seizures were evaluated. Ictal episode was included into number of seizures if its duration was longer than 5 s, shorter episodes were registered but not statistically evaluated. Motor phenomena accompanying epileptic activities were coded directly into the recording. Data did not pass the test of normal distribution, therefore statistical evaluation was performed with ANOVA on Ranks (SigmaStat® Systat) with subsequent pairwise comparison by Holm-Sidak test.

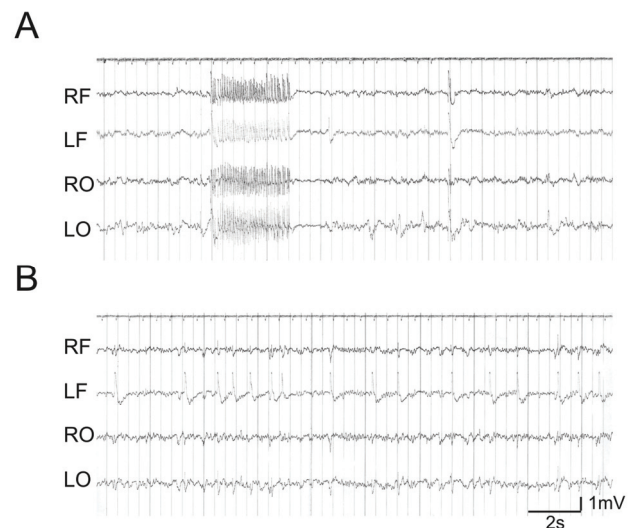


Fig. 1. Electroencephalographic recording of ictal episode (**A**) and focal activity (**B**). Individual leads in both parts from top to bottom: RF – right frontal; LF – left frontal; RO – right occipital; LO – left occipital cortical region. Time mark 2 s, amplitude calibration 1 mV.

Application of bicuculline methiodide elicited focal discharges (Fig. 1) in all rats in each session. Latency of the first focal discharge was not changed by either dose of CGP 35348, the outlined tendency did not reach the level of statistical significance (Fig. 2). Focal discharges were accompanied by jerks of contralateral forelimb; only exceptionally a few initial discharges failed to elicit jerks. Number of focal discharges counted between the 10th and 15th min after bicuculline application was compromised by early appearance of ictal activity in either group of CGP 35348-pretreated animals therefore it was lower especially in the 100-mg/kg group (Fig. 2).

Ictal activity (Fig. 1) was registered in 6 out of 10 control sessions, CGP 35348 pretreatment resulted in 10 and 9 out of 10 recordings in the lower and higher dose group, respectively (Fig. 2). All EEG ictal episodes characterized by spike-and-wave rhythm were accompanied by clonic seizures of head and forelimb muscles. Ictal activity interfered with interictal discharges – in addition to seizures there was always a postictal period when the number of focal discharges was low. Latency to the first seizure was significantly decreased by the 100-mg/kg dose of CGP 35348 but not by the higher dose. Opposite effect of the two doses was found in average duration of seizures (Fig. 2); they were significantly prolonged by the 200-mg/kg dose, the change in the 100-mg/kg group remained below the level

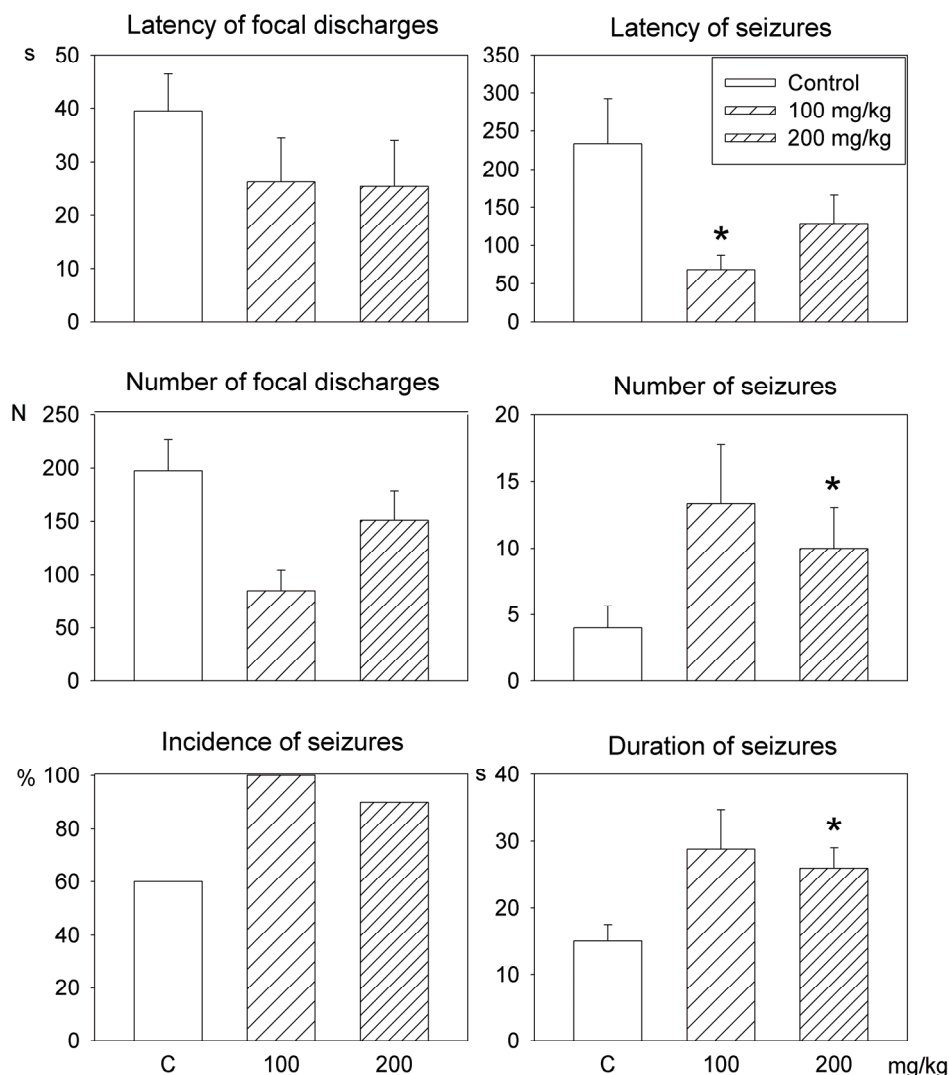


Fig. 2. Effects of CGP35348 on latency of focal discharges (upper left graph), their number (middle left), incidence of ictal episodes (lower left), their latency (upper right), number (middle right) and duration (lower right). Abscissae in all graphs – controls (C), 100-mg/kg and 200-mg/kg doses of CGP35348. Ordinates in the upper row – latencies in seconds; in the middle row – number of discharges and/or episodes; in the lower left graph – percent of rats exhibiting ictal activity; in the lower right graph – average duration of ictal episodes. Asterisks denote significant difference in comparison with controls.

of significance ($p=0.062$). Tendency to a higher number of seizures in the two CGP 35348 groups did not reach the level of significance due to very high variability – there were 2 to 38 seizures in individual rats (Fig. 2). In addition to ictal episodes longer than 5 s there were short sections of rhythmic spikes after a focal discharge, especially in sessions with application of CGP35348. They could represent an unsuccessful attempt to start ictal activity and their number was closely related to the number of ictal episodes longer than 5 s.

Our study demonstrated proconvulsant effects of CGP 35348 – tendency to shorter latency of focal discharges, shorter latency to the first ictal episode and longer duration of ictal episodes. Focal interictal discharges represent a purely cortical phenomenon, they

can be elicited in cortical slices *in vitro* (Borbely *et al.* 2006). CGP35348 did not markedly influence this phenomenon. It is in agreement with failure of CGP35348 administration to suppress GABA-withdrawal syndrome, a model of simple partial (i.e. cortical) epileptic activity (Brailowsky *et al.* 1995). On the other hand, it is in contrast with *in vitro* data of Sutor and Luhmann (1998) demonstrating potentiating effect of CGP35348 on intracellularly recorded bicuculline-induced epileptiform phenomena. CGP35348 was demonstrated to abolish focal activity elicited by intracortical injection of baclofen but in this case it is a direct interaction of agonist and antagonist of GABA-B receptors (Brailowsky *et al.* 1995).

Ictal activity was characterized by EEG spike-

and-wave rhythm and clonic seizures of forelimbs. To generate such activities, epileptic activity should spread into thalamocortical system (spike-and-wave rhythm) and into motor system (clonic seizures). Whereas generation of spike-and-wave rhythm in the cortico-thalamo-cortical circuits with a leading role of cerebral cortex was repeatedly demonstrated (Avanzini *et al.* 1992, Meeren *et al.* 2002, Timofeev and Steriade 2004, Steriade 2005), the way how epileptic activity invades motor system is unknown. The most plausible explanation is involvement of motor cortex (bicuculline application was relatively diffuse on both somatosensory and somatomotor areas, focal discharges were accompanied by jerks of contralateral forelimb from the very beginning), another possibility is a transmission of activity into basal ganglia by corticostriatal pathway. Marked prolongation of ictal activity is in agreement with the fact that GABA-B receptors play an important role in arresting physiological as well as pathological (seizure) activity (Mann *et al.* 2009, Kohl and Paulsen 2010). This conclusion is

supported also by our data on cortical afterdischarges in adult (Mareš *et al.* 2007) as well as immature rats (Mareš 2010). In conclusion, GABA-B receptors did not play a decisive role in cortically generated interictal focal discharges but their involvement in corticothalamic seizures, especially in their arrest, is important.

Conflict of Interest

There is no conflict of interest.

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