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RAPID COMMUNICATION

Action of GABA-B Antagonist on Cortical Epileptic Afterdischarges in Rats is Similar to that of GABA-A Antagonist

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Summary

Threshold intensities for epileptic phenomena induced by cortical stimulation were used for comparison of the action of GABA-B and GABA-A antagonists in rats with implanted electrodes. Both CGP 35348 (200 mg/kg i.p.) and bicuculline (4 mg/kg i.p.) significantly decreased thresholds for spike-and-wave afterdischarges and their motor counterpart (clonic seizures) whilst transition into the second, limbic type of afterdischarge as well as threshold for movements directly bound to stimulation remained uninfluenced by either drug.

Key words

Epileptic seizures • Cerebral cortex • Rat • CGP 35348 • Bicuculline

The role of GABA-B system in epileptic seizures is a matter of discussion. GABA-B antagonists synthesized in eighties were demonstrated to be very effective against absence seizures in GAERS rats (Genetic absence epilepsy rats from Strasbourg) (Marescaux *et al.* 1992) as well as in other models of absences (Hosford *et al.* 1995). On the other hand, CGP 36742 and CGP 56999 aggravated audiogenic seizures and – in high doses – induced convulsive seizures (Vergnes *et al.* 1997). In addition, another GABA-B antagonist SCH 50911 facilitated ethanol-withdrawal seizures (Carai *et al.* 2002). We decided to study the effect of a GABA-B antagonist CGP 35348 on thresholds for cortical epileptic afterdischarges (ADs) and to compare it with an action of a common GABA-A antagonist bicuculline (Curtis *et al.* 1970). Cortical epileptic afterdischarges were chosen because of four different phenomena elicited in this model: 1) movements induced by stimulation of sensorimotor cortical area, i.e. by direct activation of the motor system; 2) epileptic afterdischarge formed by spike-and-wave rhythm, probably of thalamocortical origin (Avanzini *et al.* 1992);

3) clonic seizures accompanying ADs, i.e. a spread of epileptic activity into the motor system; and 4) transition into a limbic type of ADs. Modification of the original model of estimation of threshold current values (Voskuyl *et al.* 1989) as described recently by us (Mareš *et al.* 2002) was used. Our data on the action of classical antiepileptics demonstrated the efficacy of phenobarbital and thus a possible role of GABA-A system in this model (Haugvicová *et al.* 2002).

The experiments were approved by Animal Use and Care Committee of the Institute of Physiology AS CR to be in agreement with Animal Protection Law of the Czech Republic. Animals (31 adult male Wistar rats) were prepared under pentobarbital anesthesia (Nembutal® Abbott, 40 mg/kg i.p.). Two stimulation electrodes were implanted over sensorimotor cortex at coordinates AP = -1 and +1, L = 2.5 mm (in relation to bregma) over the right hemisphere. Recording electrodes were located epidurally at coordinates AP = 0, L = 2.5 mm and AP = 3, L = 3 mm over the left hemisphere and AP = 6, L = 4 mm over both hemispheres. A reference electrode was put into the nasal bone, grounding electrode into the occipital bone. All electrodes were made from a silver wire, the end was flattened so that after a formatting into an L shape a surface of approximately 1 x 1 mm could be in contact with the brain. The electrodes were connected to a socket and fixed to the skull with fast curing dental acrylic. Recordings started after at least one-week resting period.

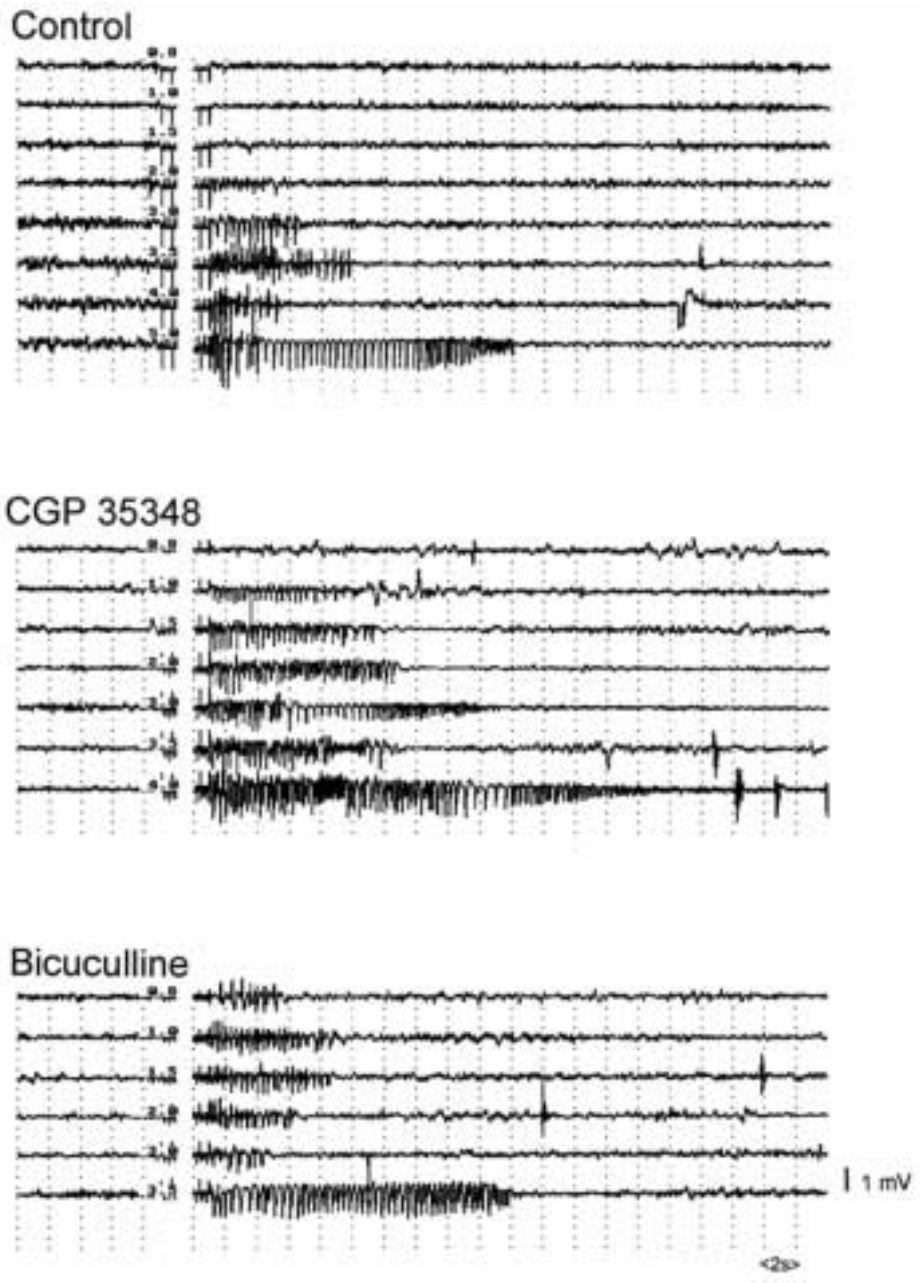

Fig. 1. *EEG recordings of afterdischarges from a control rat (top part), rat pretreated with 200 mg of CGP 35348 /kg i.p. (middle part) and an animal pretreated with 4 mg of bicuculline/kg i.p. (at bottom). Recordings in individual animals demonstrate responses to stimulations with increasing intensities from 0.8 mA to 5.0, 4.0 and 3.5 mA, respectively. After-discharges were elicited by 3.0-mA stimulation in the control rat and by 1.0- and 0.8-mA stimulations in the experimental animals. Time mark 2 s, amplitude callibration 1 mV.*

Bioelectric activity was amplified, digitized at a sampling rate of 500 Hz and then saved on a hard disc of a custom-made PC-based system. Stimulation (15-s series of 1-ms biphasic rectangular pulses with a frequency of 8 Hz) was repeated with at least 10-min intervals. Intensity was increased in following steps: 0.8; 1.0; 1.5; 2.0; 3.0; 3.5; 4.0; 5.0; 6.0; 8.0; 10.0; 12.0; 14.0 mA. EEG was recorded 40 s before stimulation and at least 2 min after the end of stimulation and/or afterdischarge. Behavior as well as motor seizures were coded directly into the recording. Ten control animals were injected with physiological saline, one experimental group (N=13) with CGP 35348 (a gift from Novartis AG, 200 mg/kg i.p.), the other (N=8) with bicuculline (Sigma, St. Louis, MO, 4 mg/kg i.p.) 15 min before the first stimulation. A dose of CGP 35348 was chosen on the basis of our unpublished data (Mareš *et al.* – in preparation), a subconvulsant dose of bicuculline according to our previous results (Zouhar *et al.* 1989).

Thresholds for all four different phenomena mentioned above were estimated. Statistical analysis was made by means of ANOVA with subsequent pairwise comparison (Bonferroni t-test, Sigma Stat® SPSS), the level of significance being set at 5%.

Electrocorticographic activity of control rats recorded before stimulation was characterized by beta waves with low amplitude, i.e. the animals were in the state of active wakefulness. The animals in the control group exhibited movements bound to individual stimuli at an average current intensity of 0.87±0.04 mA (mean ± S.E.M.), spike-and-wave ADs as well as clonic seizures were elicited by 1.58±0.15 mA on the average and the mean intensity necessary to induce transition into the second type of ADs was 4.38±0.69 mA (Figs 1 and 2).

CGP 35348 pretreatment did not change spontaneous electrocorticogram, whereas animals pretreated with bicuculline exhibited a few short (2-5 s) sections of spike-and-wave activity. The two antagonists did not influence all phenomena evaluated but their action was always similar. Threshold for movements elicited by cortical stimulation was not significantly changed by either drug. In contrast, spike-and-wave type of cortical epileptic afterdischarges was induced by significantly lower current intensities in both experimental groups in comparison with controls (Fig. 2). Clonic seizures accompanied each AD of this type, i.e. the values were identical with thresholds for spike-and-wave ADs and therefore these data are not shown. Unexpectedly the thresholds for the transition into the other type of ADs remained uninfluenced by both drugs.

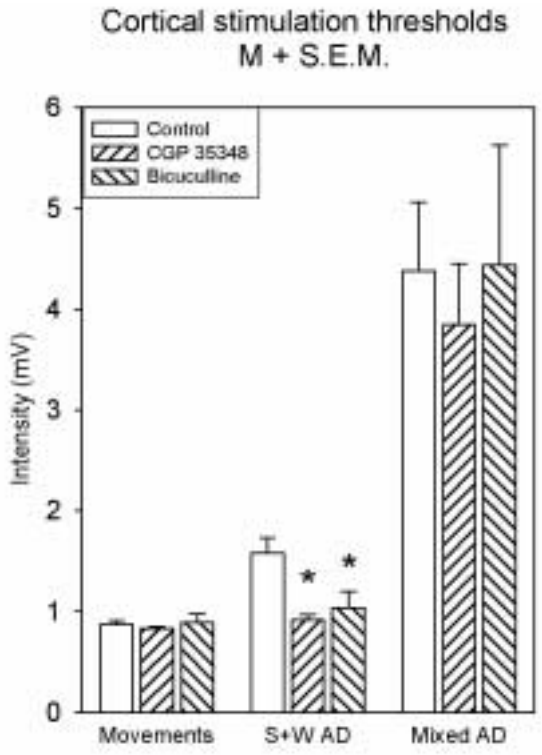

Fig. 2. *Thresholds for movements elicited by stimulation, spike-and-wave afterdischarges and mixed type of afterdischarges (mean ±S.E.M.). Abscissa – three phenomena evaluated; ordinate – intensity in mA. Individual columns – see inset. Asterisks denote statistical significance in comparison with the control group.*

Our results did not show a difference in the action of the two antagonists. Failure of both drugs to decrease the threshold for movements accompanying stimulation indicates their inability to influence directly motor system or at least motor cortical area. This result was unexpected because GABA-B as well as GABA-A receptors were demonstrated in the frontal cortex of rats (Bowery *et al.* 1987, Princivalle *et al.* 2001). It is necessary to take into account another possibility that our test was not sufficiently sensitive and much lower current intensities had to be used. The decrease of the threshold for spike-and-wave type of ADs is in agreement with our hypothesis as well as with literary data. Thalamocortical system responsible for generation of spike-and-wave rhythm involves thalamic nuclei (ventrobasal complex and reticular nucleus – Avanzini *et al.* 1992) known to contain both types of GABA receptors (Bowery *et al.* 1987). The fact that bicuculline induced episodes of

spike-and-wave rhythm before stimulation, whereas CGP 35348 did not, supports our hypothesis that electrographically similar EEG spike-and-wave rhythms may have different pharmacological sensitivity (data with ethosuximide – Mareš 1998) and probably also different significance.

Neither drug affected the transition into the second, limbic type of ADs. This finding cannot be due to technical reasons as in the case of stimulation-bound movements mentioned above. It means that in spite of the presence of both GABA-A and GABA-B inhibition in the hippocampus (Alger and Nicoll 1982) systemic administration of antagonists did not influence the spread of epileptic activity from the thalamocortical and motor systems into limbic structures. Either the limbic type of cortically elicited ADs is not generated in the hippocampus or GABAergic inhibition does not modulate the pathway to the limbic structures (probably thalamic nuclei known to have limbic projections – anterior group or mediodorsal nucleus – Paxinos 1995).

The role of the GABA-A and GABA-B systems in our model is indistinguishable and further analysis of their participation in cortical pathophysiology is necessary.

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