

Proconvulsant Action of Two GABA_B Receptor Antagonists Is Age-Dependent

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Summary

Antagonists of GABA_B receptors are expected to have proconvulsant action also in developing brain. Two antagonists (CGP55845 and CGP46381) were tested in a model of cortical epileptic afterdischarges (ADs) in 12-, 18- and 25-day-old rat pups with implanted electrodes. CGP55845 was dissolved in dimethylsulfoxide and the results demonstrated marked proconvulsant action of this solvent which masked possible action of the antagonist. Water soluble antagonist CGP46381 led to marked potentiation of ADs in 12-day-old animals, its action decreased with age, it was negligible in 25-day-old rats. Our results demonstrated important inhibitory role of GABA_B receptors at very early stages of maturation.

Key words

GABA_B receptor antagonists • Cortical afterdischarges • Electroencephalogram • Immature rats

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Introduction

GABA_B receptors are metabotropic heterodimeric receptors localized post- as well as presynaptically (Bowery *et al.* 2002). Their role in generation and spread of epileptic seizures is not clear. Baclofen, a GABA_B receptor agonist, is for a long time used clinically in treatment of spasticity (Davidoff 1978, Kheder and Nair 2012) but its use in epileptic patients is at least questionable (Hansel *et al.* 2003, Terrence *et al.* 1983). Similarly, there are data demonstrating anticonvulsant or at least mixed anti- and proconvulsant

action of this agonist in laboratory animals (Chen *et al.* 2004, De Sarro *et al.* 2000, Gasior *et al.* 2004, Sokal and Large 2001, Van Rijn *et al.* 1987).

Situation is simpler with antagonists of GABA_B receptors. They suppress experimental absence seizures (Marescaux *et al.* 1992, Hosford *et al.* 1995) but potentiate convulsant seizures (Vergnes *et al.* 1997, Mareš 2010) and high doses can result in hippocampal seizures (Leung *et al.* 2005). Absence seizures are age-dependent; the anticonvulsant effect in this type of seizures may be related to brain maturation. To analyze the role of brain development in the effects of blockade of GABA_B receptors on epileptic seizures the action of two antagonists in cortical epileptic afterdischarges (ADs) in immature rats was studied. We hypothesized that the action of GABA_B receptor antagonists decreases with maturation.

Cortical ADs (Fig. 1) can be elicited reliably since the age of 12 postnatal days in rats (Mareš *et al.* 2002) and represent a model of myoclonic seizures. Experiments in freely moving rats with implanted electrodes offer a possibility to correlate epileptic EEG phenomena with their behavioral counterparts. In addition, repeated stimulations in immature rats during the first three weeks of postnatal life lead to progressive potentiation of afterdischarges (Szczurowska and Mareš 2012).

Methods

Experiments were performed in a total of 198 male 12-, 18- or 25-day-old rat pups of the Wistar strain from breeding of the Institute of Physiology. All experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy

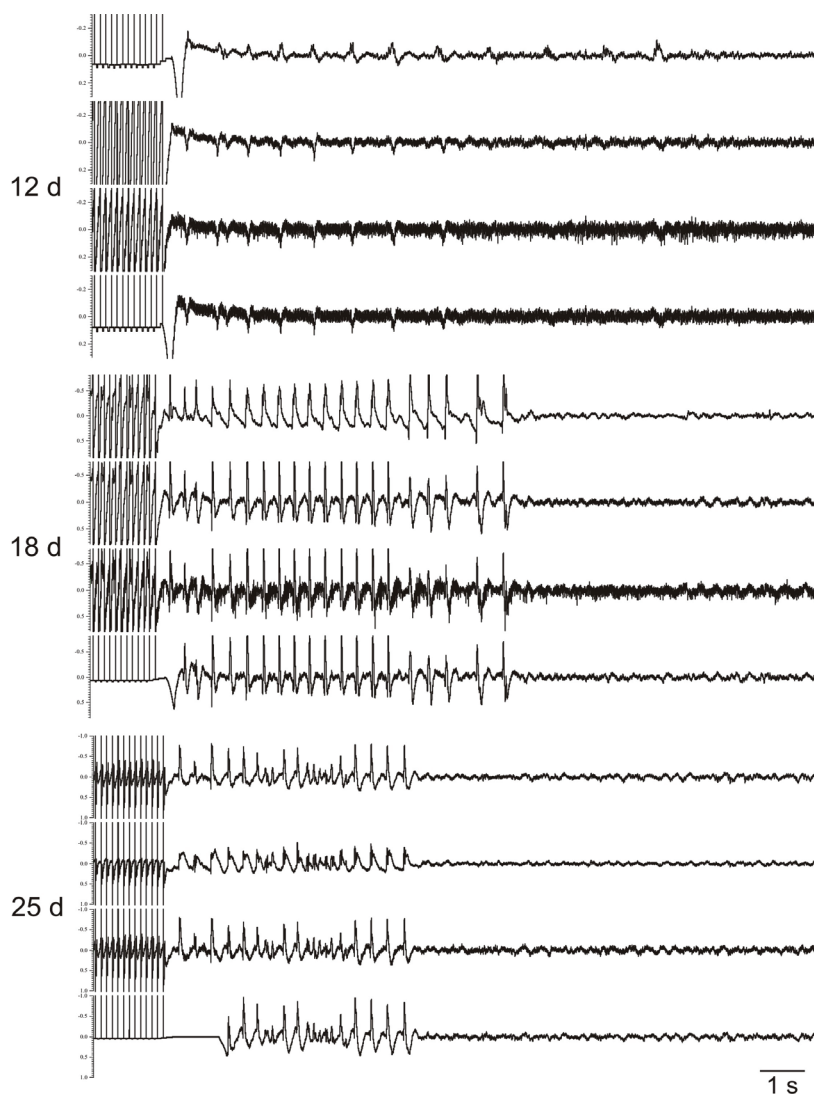


Fig. 1. Original EEG recordings of the first control afterdischarges from a 12-, 18- and 25-day old rat (from top to bottom). The four traces in each animal are from left frontal, left parietal, left occipital and right occipital regions (again from top to bottom). Time mark 1 s, amplitude calibration on the left. Note the different amplification in individual animals (± 0.3 mV in 12-, ± 0.75 mV in 18- and ± 1 mV in 25-day-old animals).

of Sciences to be in agreement with Animal Protection Law of the Czech Republic and European Community Council directives 86/609/EEC.

Cortical epidural electrodes were implanted under ether anesthesia. Flat silver electrodes were placed over right sensorimotor area (stimulation electrodes) and over left frontal sensorimotor, parietal association and occipital visual area and right occipital area. Reference and grounding electrodes were localized in the bone over cerebellum. The whole surgical preparation lasted less than 15 min, then the animals were allowed to recover for at least one hour and only then the experiments started. Epileptic afterdischarges (ADs) were elicited by series of biphasic pulses with an 8-Hz frequency, pulse duration of 1 ms and suprathreshold intensity. It was 3 mA for 18- and 25-day-old rats, the youngest group needed higher stimulation intensity (up to 6 mA). The first AD was always a control one, then the stimulation was repeated

five more times with 10-min intervals. EEG activity was digitalized at a frequency of 1 kHz and saved on the hard disc of the system. Recording started 10 s before stimulation and continued up to one min after the end of the AD. Five minutes after the first AD drugs or solvents were administered. CGP55845 (dissolved in dimethylsulfoxide in a concentration of 5 mg/2 ml; doses of 1, 2 or 5 mg/kg) or CGP46381 (dissolved in water in a concentration of 5 mg/1 ml; 3- or 10-mg/kg doses) were injected intraperitoneally. Control groups received solvents (dimethylsulfoxide or saline) in a volume of 2 ml/kg corresponding to the highest dose of antagonists. Each age and dose group consisted from 7 to 10 rats.

All motor phenomena were coded directly into the recording. Intensity of motor counterparts of both stimulation and ADs was quantified with a modified Racine's 5-point scale (Racine 1972, Szczurowska and Mareš 2012). Pattern and duration of ADs and the most

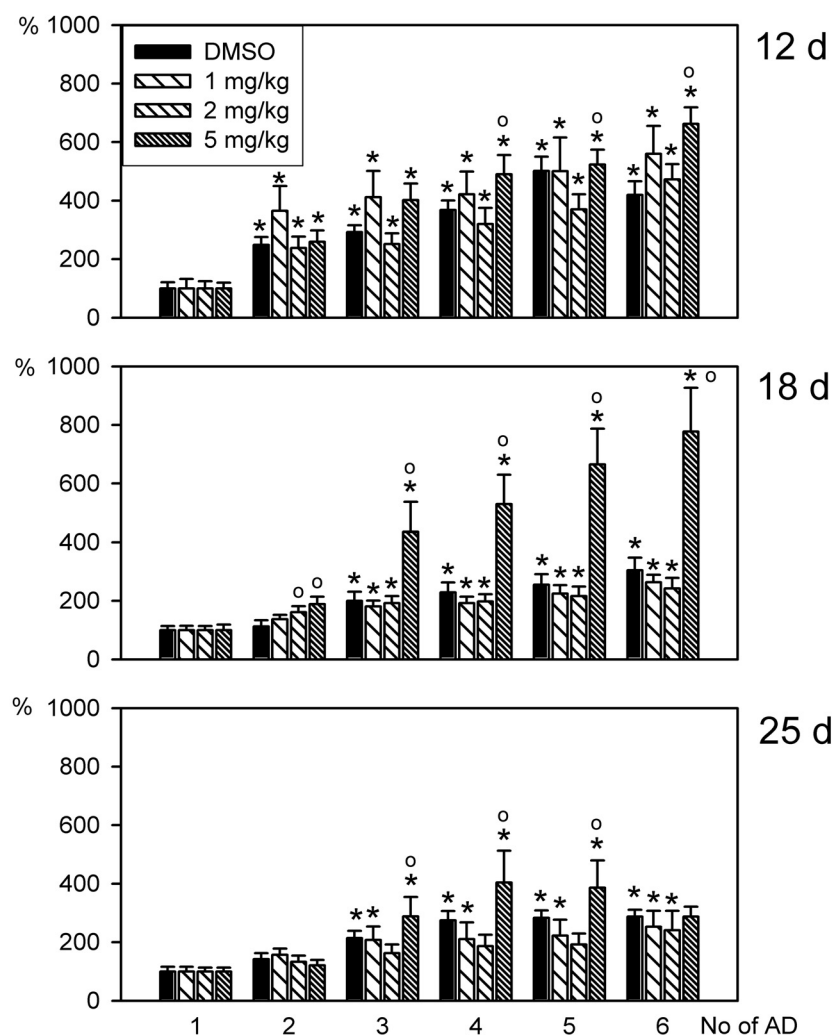


Fig. 2. Effects of CGP55845 on duration of cortical epileptic afterdischarges (mean + S.E.M.). From top to bottom – rats 12, 18 and 25 days old. Individual graphs – six subsequent ADs on abscissa, relative duration of ADs on ordinate (duration of the first AD in each dose group is taken as 100 %). Individual columns – see inset. Asterisks denote significant difference in comparison with the appropriate first (predrug) AD, circles – significant difference compared to the corresponding control AD.

severe motor phenomena were evaluated off-line. Comparison of the six series in each group was done with Repeated Measures ANOVA, comparison between groups with One Way ANOVA. Absolute values of duration were used for statistics but graphs were created with relative values – the first AD was taken as 100 % – to have a direct comparison of individual dose and age groups. Statistics was performed with SigmaStat software (SYSTAT Inc., USA), $p < 0.05$ was taken as significant.

Results

CGP55845 (Fig. 2)

Repeated stimulations resulted in progressive prolongation of ADs in all age and dose groups ($p < 0.001$ in all cases). Marked prolongation of ADs was present in all control groups injected with dimethylsulfoxide (DMSO). All postinjection ADs were significantly longer than the first control one in 12-day-old rats, prolongation of ADs reached the level of significance in the two older

groups since the third AD. CGP55845 did not augment this effect in the 12-day group. It was possible to demonstrate a marked effect of the highest dose of the antagonist in 18-day-old rats, where DMSO did not exhibit such strong action as in the youngest animals. Starting at the second stimulation ADs in the 5-mg/kg group of animals were significantly longer than the control ones, the 6th AD was more than eight times longer than the preinjection one. The oldest group was less influenced by CGP55845; only the 3rd, 4th and 5th ADs in the 5-mg/kg group were significantly longer than the corresponding control ADs.

Motor phenomena were not affected by DMSO or CGP55845 in any age group.

CGP46381 (Fig. 3)

Significant prolongation of ADs with repeated stimulations was demonstrated in control 12- and 18-day-old rats (starting at the 3rd AD) but only exceptionally in 25-day-old animals. Proconvulsant effect of this water-

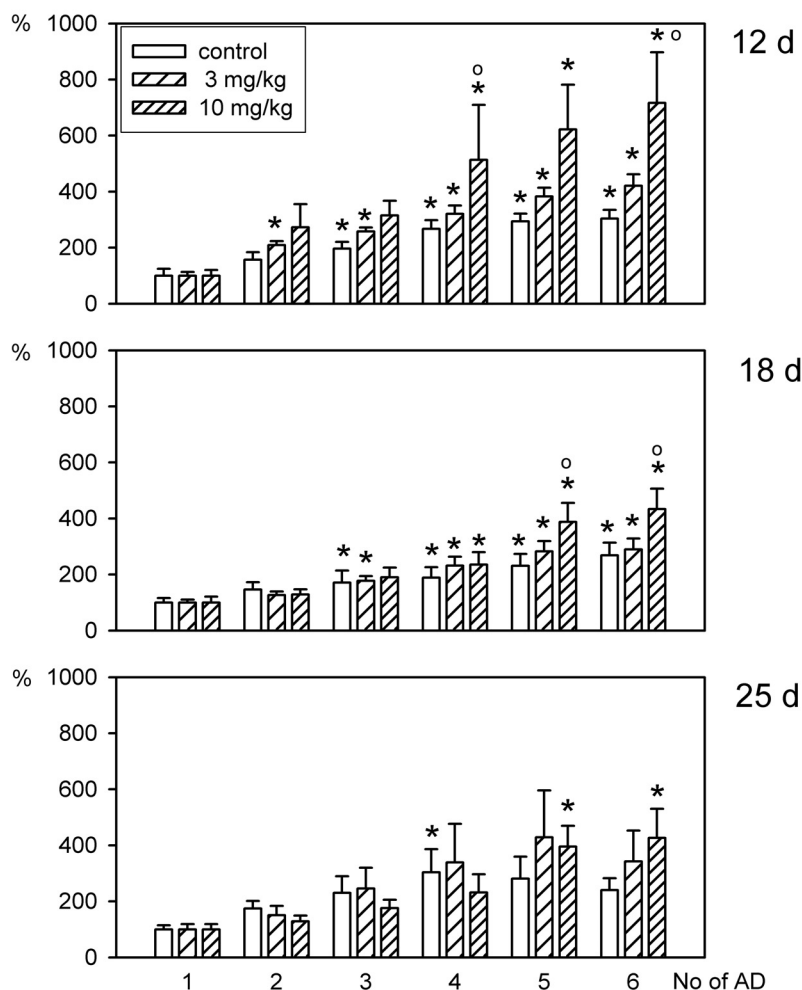


Fig. 3. Effects of CGP46381 on duration of cortical epileptic afterdischarges (mean + S.E.M.). All details as in Fig. 2.

soluble antagonist was found in the two younger groups. The 10-mg/kg dose resulted in significantly longer 4th and 6th AD in 12- and 5th and 6th AD in 18-day-old group when compared to the corresponding control ADs. Similar effect failed to appear in 25-day-old rats, ADs were only marginally affected by this antagonist.

Motor phenomena connected with stimulation or ADs were again not affected by this antagonist.

Discussion

The results with CGP55845 were compromised by the action of DMSO. Administration of this solvent resulted in significant prolongation of ADs in all three age groups whereas control animals in the series with CGP46381 injected with saline exhibited prolongation in 12- and 18-day-old rats and no changes in the oldest group. In spite of this tendency comparison of the corresponding ADs in the DMSO and saline control groups did not reveal any significant difference. An unexpected result was summation of effects of DMSO

and the highest dose of CGP55845 in 18-day-old rats. The extreme potentiation suggests a possible supraadditive effect but further analysis is necessary.

Data with water-soluble CGP46381 are more relevant for studies of a possible proconvulsant action of GABA_B receptor antagonists. The experiments with CGP46381 demonstrated strong proconvulsant action in 12-day-old rats and a decrease of this effect with age.

This developmental change may be put in connection with data for GABA_B receptor agonist SKF97541 exhibiting mixed anti- and proconvulsant effects (Mareš 2008, Mareš and Tabashidze 2008) and a positive allosteric modulator CGP7930 with nearly pure anticonvulsant action (Mareš *et al.* 2013). All these drugs influencing GABA_B receptors exhibit strongest action in the 12-day-old and younger rat pups. It is in agreement with data on development of GABA_B receptors in the cerebral cortex – they are present at early developmental stages (Princivalle *et al.* 2000) and peak at postnatal day 14 (Snead 1994, Turgeon and Albin 1994). An uneven maturation of pre- and postsynaptic GABA_B receptors

(Fukuda *et al.* 1993) may play an important role but other possibilities (e.g. maturation of G-protein mechanisms – Kagan *et al.* 2012) should be taken into account. The development of the blood-brain barrier probably does not play a role in spite of the fact that it matures during the third postnatal week in rats because action of these drugs is reported also in adult rodents (Olpe *et al.* 1993).

Our data demonstrated an important role of inhibitory GABA_B receptors at early stages of postnatal development.

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Conflict of Interest

There is no conflict of interest.

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