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Participation of $GABA_B$ receptors in cortical postictal excitability in immature rats

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ABSTRACT

Arrest of seizures is due to an active inhibition and is followed in mature brain by period of refractoriness markedly present one min after the end of seizures. To study changes in cortical excitability after epileptic seizures we used electrical stimulation of sensorimotor cortical area in immature rats – 25-day-old ones with mature postictal refractoriness and 12-day-old where postictal potentiation of afterdischarges (ADs) is present instead of refractoriness at one minute after the end of the conditioning AD. $GABA_B$ receptor antagonist CGP35348 was found to partly suppress postictal refractoriness. In present study not only an antagonist CGP46381 (3 and 10 mg/kg i.p.) but also positive allosteric modulator of $GABA_B$ receptors CGP7930 (20 and 40 mg/kg i.p.) were used to study the role of $GABA_B$ receptors in both age groups. They were injected immediately after testing AD and 10 min later the two stimulations were repeated. CGP46381 partly antagonized postictal refractoriness in 25-day-old rats but did not significantly affect ADs in 12-day-old animals. CGP7930 did not significantly change ADs duration in either age group.

GABAB receptors participate in mechanism of postictal refractoriness but did not play an important role in 12-day-old rats where potentiation instead of refractoriness is present.

1. Introduction

Arrest of seizures is an active event due to activation of inhibitory systems during excessive electrical activity. These systems remain activated after the end of epileptic discharges, their activity progressively subsides and forms a background for postictal refractoriness – at first absolute, then relative. The mechanism of postictal refractoriness in the cerebral cortex is not so simple as in limbic system where opioid mu receptors are playing a dominant role (Frenk et al., 1979; Caldecott-Hazard and Engel, 1987). Different inhibitory receptors participate in this decrease of excitability in the cerebral cortex – adenosine A1 receptors (During and Spencer, 1992), opioid mu receptors (Velíšek and Mareš, 1992) and $GABA_B$ receptors (Mareš and Kubová, 2015a). $GABA_B$ receptors were demonstrated in the cerebral cortex of mature (Bowery et al., 1987) as well as immature rats (Princivalle et al., 2000) therefore we studied postictal period in a model of cortical epileptic afterdischarges (CxADs) in these rodents. Postictal refractoriness matures during the third postnatal week. In spite of the fact that predominantly inhibitory $GABA_B$ receptors are mature in 12-day-old rats this age group exhibits postictal potentiation instead of refractoriness (Fig. 1) (Mareš and Kubová, 2015b). It might be due to high representation of glutamatergic receptors in the rat brain at the end of the second postnatal week (Insel et al., 1990), role of $GABA_B$ system is not known.

The participation of $GABA_B$ receptors in postictal refractoriness was demonstrated in our laboratory with the very first antagonist of these receptors CGP35348 synthesized approximately 30 years ago in Ciba Geigy (now Novartis) company. This antagonist markedly prolonged CxAD (Mareš, 2010) and partly suppressed postictal refractoriness after CxAD in 25-day-old rats (Mareš and Kubová, 2015a). To be sure that the effect of CGP35348 (dissolved in dimethylsulfoxide) on postictal refractoriness is due to its $GABA_B$ antagonist action we started to check the effect of water soluble $GABA_B$ receptor antagonist CGP46381 on postictal refractoriness in this age group. The effect of increased activity of $GABA_B$ receptors on postictal excitability is not known. It might lead to an augmentation of postictal refractoriness therefore we used an allosteric positive modulator of $GABA_B$ receptors CGP 7930. Postictal potentiation in 12-day-old animals (a neglected phenomenon) might be suppressed by increased activity of $GABA_B$ receptors; a possible mirror effect if postictal potentiation could be affected by $GABA_B$ receptor activation was also studied in this age group.

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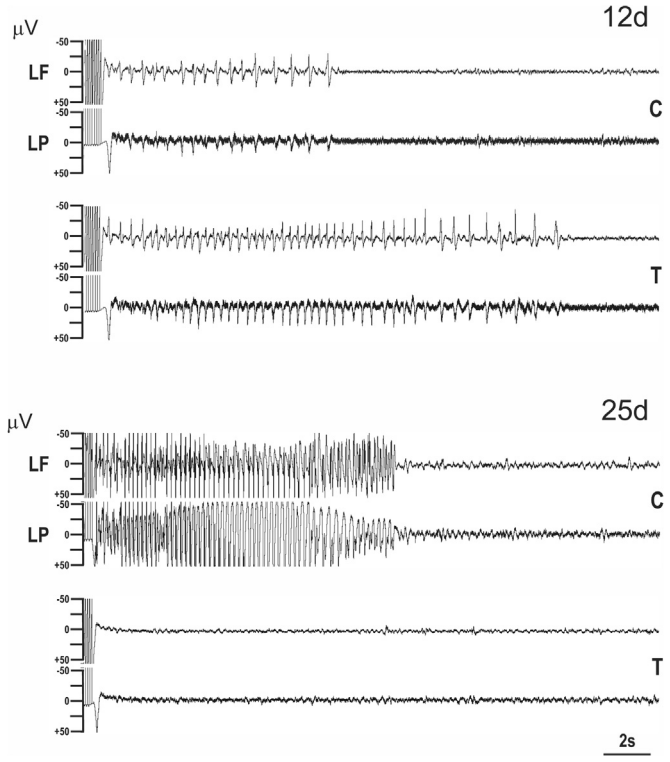

Fig. 1. EEG recording of paired afterdischarges from 12- (upper part) and 25-day-old (lower part) rat. The two channels are recordings from left frontal (LF) and left parietal (LP) regions. Conditioning (C) and testing (T) ADs are presented. Amplitude and time measures are in the figures.

2. Materials and methods

2.1. Animals and surgery

All experiments 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 and with European Community Council directives 86/609/EEC. Experiments were performed in a total of 117 male Wistar rats 12 and 25 days old. Individual age and dose groups consisted from 8 to 12 (mostly 10) animals. Flat silver cortical electrodes were implanted epidurally under ether anesthesia – two stimulation electrodes over right sensorimotor area (AP −1 and +1, L 2 mm), four recording electrodes over left sensorimotor, parietal and occipital area and over right occipital area. Reference electrode as well as grounding electrode were implanted over cerebellum (for details see Mareš and Stehlíková (2010)). The whole surgical implantation lasted less than 15 min, then the animals were allowed to rest for one h, basic reflexes were checked and rats were taken into experiment. Twelve-day-old rats were on a pad electrically heated to 34 °C (i.e.to the temperature in the nest) during the whole experiment.

2.2. Stimulation and recording

Stimulator with constant current output of our own construction was used, 15-s series of biphasic pulses (d = 1 ms, f = 8 Hz) with a suprathreshold intensity for elicitation of epileptic afterdischarges (3 mA for 25-day-old rats and 6 mA for 12-day-old ones) were applied. The first stimulation series always elicited epileptic afterdischarge which was taken as a conditioning one. One minute after the end of the conditioning AD, the second testing stimulation series using the same stimulation parameters started. Immediately after the end of the testing AD (or stimulation if the AD was not elicited) the drug (either $GABA_B$ antagonist CGP46381 or positive allosteric modulator CGP7930) was administered intraperitoneally and 10 min later the two stimulation series were repeated. EEG activity was digitalized at a 500-Hz frequency and saved into the harddisk of the system.

2.3. Drugs

An antagonist of $GABA_B$ receptors CGP46381 was dissolved in physiological saline in a concentration of 5 mg/1 ml and administered in doses of 3 and 10 mg/kg. $GABA_B$ receptor positive allosteric modulator CGP7930 was dissolved in dimethylsulfoxide (DMSO, 5 mg/1 ml) and administered in doses of 20 and 40 mg/kg. Doses were chosen on the basis of our previous experiments, 10 min is the time of marked effect of either drug (Mareš, 2013; Mareš et al., 2013). Two groups of control animals were formed – one with physiological saline (2 ml/kg) for CGP46381, the other with DMSO (8 ml/kg) for CGP7930.

2.4. Statistics

Evaluation of ADs was performed off-line. Duration of ADs was measured and original (absolute) values were statistically evaluated. SigmaStat® software was used for analysis of data. The distribution of data was checked and then parametric or nonparametric tests – Two way Analysis of Variance or Analysis of Variance on Ranks - were applied. Group factors were treatment (control and two doses of drugs) and session (ADs elicited by stimulations one to four, see para 2.2.). Subsequent pairwise comparison with Holm-Sidak test was performed. If only two values were compared, *t*-test or Mann-Whitney test were used. Level of statistical significance was set at 5%, all statistically significant data are presented in Table 1. Relative duration of ADs is presented in Figures taking the first (predrug) conditioning AD as 100% for better demonstration of changes.

3. Results

3.1. $GABA_B$ receptors antagonist CGP46381

3.1.1. 12-day-old rats

The duration of the pre-drug conditioning ADs was 6.25 ± 2.2, 8.67 ± 1.22, and 5.25 ± 0.89 s in the control, 3-mg/kg and 10-mg/kg groups, respectively. The differences were not statistically significant (p = 0.11). Two way ANOVA results in significant main effect of treatment $F_{(2,88)} = 5.39$, $p = 0.006$, and session $F_{(3.88)} = 21.74$ $p < 0.001$. Multiple comparison tests revealed that saline as well as both doses of CGP46381 increased the duration of the first testing ADs (i.e.the second

Table 1
Statistically significant differences of durations of afterdischarges (p values).

	GROUP	ANOVA	C1T1	C1C2	C2T2	T1T2
CGP46381						
12 days	Control	0.027		***0.016***		
	3 mg/kg	< 0.001	0.00278	0.0013		
	10 mg/kg	< 0.001	0.0106	0.00186		0.016
25 days	Control	0.002	0.0139		0.009	
	3 mg/kg	< 0.001	< 0.001	***0.035***	0.0102	***0.025***
	10 mg/kg	< 0.001	< 0.001		< 0.001	
CGP7930						
12 days	DMSO	0.034	***0.05***	0.00377	***0.05***	
	3 mg/kg	0.015	0.00226	***0.027***		
	10 mg/kg		***0.005***	***0.05***		
25 days	DMSO	< 0.001	< 0.001		0.00182	***0.048***
	3 mg/kg	0.001	0.00502		0.00335	
	10 mg/kg	< 0.001	< 0.001		< 0.001	

Lines demonstrate individual age and dose groups of the two drug groups, columns present (from left to right) result of ANOVA, comparison of the first conditioning and testing afterdischarges (C1T1), comparison of the two conditioning ADs (C1C2), of the second conditioning and testing ADs (C2T2) and the two testing ADs (T1T2).