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$GABA_B$, not $GABA_A$ receptors play a role in cortical postictal refractoriness

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ABSTRACT

Postictal refractoriness may be taken as an expression of lasting activity of inhibitory systems arresting seizures. We tested drugs interfering with GABAergic inhibitory system in pairs of cortical epileptic afterdischarges induced with 1-min interval in rats. Under control conditions the second stimulation failed to elicit an afterdischarge. This postictal refractoriness was not affected by antagonists of $GABA_A$ receptors acting at three binding sites (bicuculline, picrotoxin, benzodiazepine inverse agonist Ro 19-4603) as well as by a less specific antagonist pentetrazol. In contrast, antagonist of $GABA_B$ receptors CGP35348 partially blocked the refractoriness. Cooperation of different inhibitory systems is probably necessary to abolish postictal refractoriness in neocortex.

This article is part of the Special Issue entitled 'GABAergic Signaling in Health and Disease'.

1. Introduction

An arrest of epileptic seizures is an active process. Targets of excessive activity of epileptic neurons are not only excitatory but also inhibitory elements in the brain. At certain moment inhibition prevails over excitation and seizures stop. Activation of inhibitory systems arresting seizures overlasts for some time after the end of seizures and is responsible for postictal period. Among phenomena characteristic for postictal period are depression of EEG activity, behavioral depression, postictal analgesia, and postictal refractoriness. Postictal refractoriness is a phenomenon which may be exactly measured using experimental seizures elicited by electrical stimulation. Postictal refractoriness was demonstrated in models of limbic seizures. Naloxone abolished the refractoriness after amygdala (Frenk et al., 1979) as well as hippocampal ADs (Caldecott-Hazard and Engel, 1987). Velíšek and Mareš (1992) confirmed that refractoriness after hippocampal ADs is blocked by naloxone but that this drug only partly suppressed refractoriness after maximal electroshock seizures. This result indicates that various systems might participate in postictal refractoriness in seizures generated in different brain structures. Therefore we started to analyze postictal refractoriness in epileptic afterdischarges elicited by stimulation of sensorimotor cortical area. These afterdischarges are characterized by spike-and-wave rhythm in the EEG generated in corticothalamocortical circuits (Avanzini et al., 1992; Steriade, 2006). This activity spreads into the motor system and is accompanied by clonic seizures of head and forelimb muscles.

The first choice for testing postictal refractoriness was the main inhibitory system in the brain using GABA as a mediator. Both basic types of GABA receptors are present in the cerebral cortex – $GABA_A$ and $GABA_B$. We hypothesized that $GABA_A$ receptors are more important, therefore we started with drugs influencing this supramolecular complex. Different antagonists were used – competitive antagonist bicuculline, an antagonist binding into the chloride channel picrotoxin, an inverse benzodiazepine agonist Ro 19-4603 and convulsant drug binding probably on more than one site mentioned for the three preceding drugs – pentetrazol. With the first negative results with drugs acting at $GABA_A$ receptor we decided to use also a $GABA_B$ receptor antagonist CGP35348.

Recently we demonstrated that postictal refractoriness develops during the third postnatal week in rats and 25-day-old animals exhibit the same refractoriness after CxAD as adult rats. Therefore to economize 25-day-old not adult animals were used in the present series of experiments.

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2. Material and methods

2.1. Animals

The experiments were approved by Animal Care and Use of the Institute of Physiology to be in agreement with Animal Protection Law of the Czech Republic and European Community Council directives 86/609/EEC.

Male albino Wistar rats 25 days old were used. Flat silver epidural electrodes were implanted under ether anesthesia: two stimulation electrodes over right sensorimotor area (AP −1 and +1; L 2 mm), recording electrodes over left sensorimotor (AP 0; L 2 mm) and visual (AP 5.5; L 3.5 mm) regions and over right visual area (AP 5.5; L 3.5 mm). The whole surgery lasted 10 min, then the animals were allowed to recover for one hour, righting and placing reflexes were checked and only then the experiment started.

2.2. Stimulation

Stimulation was performed by a stimulator with constant current output. Series of 1-ms biphasic pulses with an 8-Hz frequency were 15 s long. Intensity was 3 mA, i.e. suprathreshold for elicitation of ADs in all animals. One min after the end of the AD the second stimulation started. Immediately after the end of the second AD (or of the second stimulation if AD fails to appear) drugs were injected intraperitoneally and 10 min later the paired stimulation was repeated.

2.3. Drugs

Doses of all drugs were subthreshold for convulsions on the basis of our older experiments. Antagonists of $GABA_A$ supramolecular receptor complex: bicuculline (1 mg/kg; Zouhar et al., 1989) as a specific competitive antagonist, picrotoxin (1 mg/kg; Stanková et al., 1988) as an antagonist binding into the chloride channel, Ro 19-4603 (50 mg/kg; Kubová and Mareš, 1994) as an antagonist at the benzodiazepine binding site, pentetrazol (20 mg/kg; Velíšek et al., 1992) as an antagonist probably affecting more than one binding site. $GABA_B$ receptor antagonist CGP35348 in high doses used in previous and present experiments did not elicit seizures (Mareš, 2010), therefore the 100- and 200-mg/kg doses were administered. All drugs with the exception of bicuculline were dissolved in water, to prepare solution of bicuculline few drops of HCl should be used, water was added and then pH was corrected with NaOH. Control group received physiological saline, six animals were injected with acidified saline to have appropriate control for bicuculline. Data from the two control subgroups did not differ, therefore they are presented as one control group. Each drug group consisted from 9 to 11 rats, control group was bigger ($N = 16$). One way ANOVA did not show and statistical difference among duration of the first predrug AD in individual groups (it varied from 4.5 ± 0.7 to 7.8 ± 0.7 s).

2.4. Statistics

All statistics was calculated with original data, relative duration was used only in Figures to make the differences clear. If AD was not elicited by the second stimulation in the pair, zero was taken for calculation. Duration of the second AD after drug administration was compared with duration of the corresponding second AD in the control group. Statistical program SigmaStat (SPSS) started always with testing the distribution of data. If normal Gaussian distribution was confirmed, *t*-test was used for comparison, in the negative result nonparametric Mann Whitney test was applied. Level of statistical significance was put on 5%.

3. Results

3.1. Controls (Figs. 1 and 2)

Duration of the first AD in the first and second pair did not significantly differ. The second AD in the first pair was elicited only in two animals and the duration was only 4.6% of the duration of the first AD in this pair. The second pair showed different results, testing (second) AD was registered in four rats and average duration was 20.4 ± 5.4% of the first in this pair Fig. 2.

3.2. Experimental groups (Figs. 2 and 3)

One way ANOVA did not show and statistical difference among duration of the first predrug AD in individual groups (it varied from 4.5 ± 0.7 to 7.8 ± 0.7 s). The first pair demonstrated either none (bicuculline, Ro 19-4603, pentetrazol) or a short second AD (picrotoxin 17.2% of mean duration of the first AD). First AD in all postdrug pairs was significantly longer than the first AD in the predrug pair after all four drugs acting on $GABA_A$ receptor. No significant change of the first AD in the pair was induced by either dose of $GABA_B$ receptor antagonist CGP35348.

Bicuculline: Bicuculline administration resulted in the second AD with duration of 23.8 ± 8.4% of the first one. Comparison with the corresponding AD in the control group did not show significant difference.

Picrotoxin: The results under the influence of picrotoxin were practically identical with the predrug data (five out of ten rats, i.e.the same percentage as in the predrug pairs, exhibited the second AD with an average duration of 16.6% of the first AD). Again, there was no significant difference from data of control group.

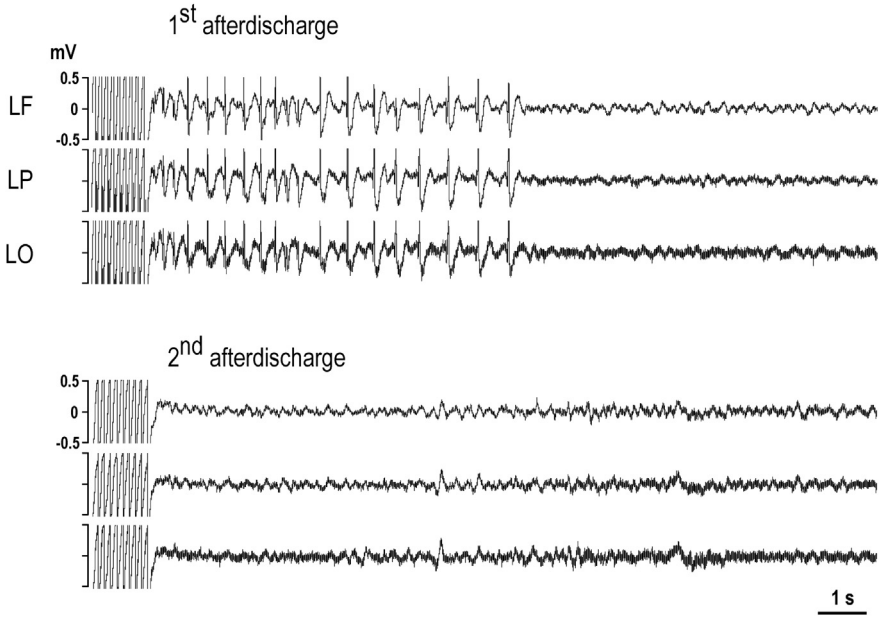

Fig. 1. Electrocorticographic recording of epileptic afterdischarges ina 25-day-old rat. Upper part – the first afterdischarge, lower part – the second stimulation starting 1 min after the end of the first afterdischarges. Recordings from the left hemisphere are presented in both parts (LF – frontal, LP – parietal and LO – occipital cortical area in reference connection). Time mark 1 s, amplitude calibration ±0.5 mV.