# Effect of $GABA_B$ receptor agonist SKF97541 on cortical and hippocampal epileptic afterdischarges

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Running title:
Lack of anticonvulsant effect of SKF97541 in adult rats

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**Abstract:** Activation of $GABA_B$ receptors leads to longer inhibitory postsynaptic potentials than activation of $GABA_A$ receptors. Therefore $GABA_B$ receptors may be a target for anticonvulsant therapy. The present study examined possible effects of $GABA_B$ receptor agonist SKF97541 on cortical and hippocampal epileptic afterdischarges (ADs). Epileptic ADs elicited by electrical stimulation of sensorimotor cortex or dorsal hippocampus were studied in adult male Wistar rats. Stimulation series were applied 6 times with 10- or 20-minute interval. Either interval was efficient for reliable elicitation of cortical ADs but stimulation at 10-min intervals did not reliably elicit hippocampal ADs, many stimulations were without effect. SKF97541 in dose 1 mg/kg significantly prolonged cortical ADs. Duration of hippocampal ADs was not significantly changed by either dose of SKF97541 in spite of a marked myorelaxant effect of the higher dose. Our present data demonstrated that neither cortical nor hippocampal ADs in adult rats were suppressed by $GABA_B$ receptor agonist SKF97541. Proconvulsant effect on cortical ADs indicates a different role in these two brain structures. In addition, duration of refractory period for electrically-induced ADs in these two structures in adult rats is different.

**Key words:** $GABA_B$ receptor, agonist, hippocampus, cortex, epileptic afterdischarges, rat

## Introduction

GABA (gamma-aminobutyric acid) is the main inhibitory neurotransmitter in mammalian central nervous system. Its action is mediated by two classes of receptors: ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. Role of the two types of receptors in generation of epileptic afterdischarges is different as demonstrated in *in vitro* experiments (Higashima et al. 2000). $GABA_B$ receptors are localized post- as well as presynaptically (Bowery 1993). Action of GABA on postsynaptic $GABA_B$ receptors results in generation of inhibitory postsynaptic potentials (IPSPs); this hyperpolarization lasts longer and has higher amplitude than IPSPs elicited by $GABA_A$ receptors (Connors et al. 1988). Presynaptic $GABA_B$ receptors decrease release of transmitter from the presynaptic ending. They may be localized on GABAergic terminals as homoreceptors but also on glutamatergic endings as heteroreceptors. This localization is a reason for ambiguous effects of $GABA_B$ receptor agonists like baclofen (Bowery et al. 1979) on epileptic seizures – anticonvulsant, proconvulsant and direct convulsant effects were described in various animal models (Ault et al. 1986, van Rijn et al. 1987, Karlson et al. 1992, Mareš 2012).

Previously, we described effects of classical $GABA_B$ receptor agonist baclofen in adult rats (Mareš et al. 2007). Seabrook et al. (1990) demonstrated that another $GABA_B$ receptor agonist SKF97541 (γ-aminopropyl-methyl-phosphinic acid) is at least ten times more potent than baclofen. Comparing effects of these two $GABA_B$ receptor agonists – baclofen and SKF97541 – in developing 12- and 18-day-old rats we found that SKF97541 exhibits more anticonvulsant and less proconvulsant properties than baclofen but that both agonists are nearly ineffective in 25-day-old rats (Mareš 2008). To analyze if the failure at the age of 25 days is a transient developmental effect or if there is a permanent loss of anticonvulsant activity since the certain level of maturation is reached, we started the experiments in adult rats. Cortical epileptic afterdischarges (ADs) were used as the first model. This model allows evaluation of pattern and duration of ADs and motor phenomena accompanying stimulation and ADs. When the results indicated failure of SKF97541 administration we decided to extend the study to another model – hippocampal epileptic afterdischarges. We expected anticonvulsant effect of $GABA_B$ receptor agonist against hippocampal epileptic activity because $GABA_B$ receptor-elicited inhibitory postsynaptic potentials were demonstrated in hippocampal pyramidal cells as well (Dutar and Nicoll 1988).

**Methods**

Experiments were performed in 35 adult male Wistar rats with body weight 300-350 g. Experimental animals were bred under standard conditions (12/12 h light/dark period, temperature 22±1°C, humidity 50-60%) with free access to food and water. Experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic to be in agreement with Animal Protection Law of the Czech Republic and European Community Council directives 86/609/EEC.

*Experiment 1 – cortical afterdischarges*

Flat silver cortical epidural electrodes were implanted under ether anesthesia. Stimulation electrodes were placed over frontal, sensorimotor area of the right hemisphere at coordinates AP -1 and +1, ML 2.5 mm (Paxinos et al. 1997). Three recording electrodes were over the left hemisphere – frontal at coordinates AP 0, ML 2.5 mm; parietal at AP 3, ML 3 mm; occipital at AP 6, ML 4 mm. Occipital electrode was also over right hemisphere at AP 6, ML 4 mm. Reference and grounding electrodes were placed over cerebellum. Electrodes were connected to an 8-pin connector and fixed to the skull with fast curing dental acrylic. The animals were left to recover for one week and only then the stimulation sessions started. Low-frequency (8 Hz) 15-s series of 1-ms biphasic pulses of slightly suprathreshold intensity were applied six times with interval between series 10 or 20 min. These intervals were chosen on the basis of our previous data demonstrating that duration of ADs did not change with repeated stimulations in control adult animals.

*Experiment 2 – hippocampal afterdischarges*

Surgery was performed under isoflurane anesthesia. Deep hippocampal stimulation electrodes were implanted stereotaxically into right dorsal hippocampus, registration electrodes into left dorsal hippocampus at coordinates AP -3.5, ML 3.0 mm, DV 3.5 mm. Two recording electrodes were also placed over left hemisphere - sensorimotor cortex at coordinates AP -1, ML 2.5 mm; occipital at AP 6, ML 4 mm. Reference and grounding electrodes were placed over cerebellum. Electrodes were connected to an 8-pin connector and fixed to the skull with fast curing dental acrylic. One week after surgery threshold intensity for elicitation of hippocampal afterdischarges (ADs) was estimated using current intensities from 0.2mA to 2.0mA. Just suprathreshold intensity was used for all sessions in each experimental animal. Stimulation series of biphasic 1-ms pulses

applied for 1 s at 60-Hz frequency were repeated six times with interval between stimulations of 10 or 20 min.

*Recording and drug administration*

Three experiments were done in each animal with at least 3-day interval between two sessions. Experimental groups for either time schedule and all doses consisted from 9 to 14 animals. SKF97541 (in a dose of 0.1 or 1 mg/kg dissolved in saline in a concentration of 1mg/ml) or saline was administered intraperitoneally 5 min after the end of the first AD in series with 10-min interval or 10 min after the end of the first AD in 20-min interstimulation interval. EEG was amplified, digitalized at a 2-kHz rate and saved on a harddisc of the system. Activity was always registered for 20 s before stimulation, during stimulation and afterdischarge (if appeared) and at least 2 min after the end of the afterdischarge. Behavior of rats was coded directly into the recording. EEG pattern of afterdischarges and their duration were evaluated off-line.

*Statistics*

Duration of the six afterdischarges was statistically evaluated with One Way Repeated Measures ANOVA, corresponding afterdischarges in the three groups were compared with ANOVA and Mann-Whitney Rank Sum Test. Subsequent pairwise comparison was done with Holm-Sidak test. SigmaStat software (SYSTAT Inc., USA) used for all calculations started with a test of distribution and it recommends parametric or nonparametric test according to the result . $P<0.05$ was taken as statistically significant.

## Results

*Experiment 1 – cortical afterdischarges*

Epileptic afterdischarges were elicited in all animals in all six stimulations with 10- as well as 20-min intervals. Both interstimulation intervals resulted in a stable duration of ADs, there was no progressive change with repeated stimulations in control rats. In contrast, the 20-min interstimulation interval led to a tendency to longer ADs after the fourth to six stimulations in a group of animals injected with SKF97541 in the dose of 0.1 mg/kg. Higher dose of SKF97541 (1 mg/kg) resulted in significantly longer ADs after the fifth and sixth stimulations when compared with the first, predrug AD. Motor counterparts of both stimulation and spike-and-wave ADs remained unchanged by repeated stimulations in control as well as either treated group. The usual value of movements directly bound to stimulation as well as clonic seizures accompanying spike-

and-wave ADs was 3.0 with only a small variability in all groups with either 10- or 20-min interstimulation interval.

*Experiment 2 – hippocampal afterdischarges*

The first stimulation always induced a hippocampal AD, but in contrast to cortical epileptic afterdischarges repeated stimulations frequently failed if 10-min interval was used. Incidence of ADs elicited after repeated stimulation with 10-min interval varied from 42.9% to 71.3%. In contrast to 10 min interval the incidence of ADs elicited after repeated stimulation with 20-min intervals was more effective, it varied from 72.7% to 100% (Figure 4). Animals injected with higher dose of SKF97541 exhibited significantly longer ADs after the third stimulation in 20 min interval. After any other stimulation $GABA_B$ receptor agonist SKF97541 in either dose did not significantly influence the duration of hippocampal epileptic ADs. Hippocampal afterdischarges were accompanied by constant behavioral correlate – wet dog shakes (Lerner-Natoli et al. 1994). Intensity of these automatisms was markedly attenuated by myorelaxant effect of the $GABA_B$ receptor agonist.

## Discussion

Our present study demonstrated a failure of anticonvulsant effect of $GABA_B$ receptor agonist SKF97541 on electrically-induced epileptic aftedischarges in adult rats. On the contrary, the high dose of SKF97541 exhibited a proconvulsant effect if the intervals between stimulations were 20 min. It corresponds with effects observed in our previous study in 25-day-old animals (Mareš et al. 2008). Mixed anti- and proconvulsant effects of SKF97541 in a model of cortical afterdischarges were demonstrated in immature rats. SKF97541 exhibited anticonvulsant effect – shortening of duration of ADs - in 12-day old rats, whereas administration in 18- and 25-day old rats resulted in an increased duration of ADs. Our present data indicate that neither cortical nor hippocampal ADs in adult rats were suppressed by $GABA_B$ receptor agonist SKF97541. Indeed, SKF97541 in a high dose of 1-mg/kg was able to potentiate progressive prolongation of cortical ADs with repeated stimulations. Duration of hippocampal ADs was changed by the 1-mg/kg dose of SKF97541 only exceptionally. Difference between effects on cortical and hippocampal ADs might be due to different distribution of interneurons and their relationship with principal neurons in the two structures. Neocortex has mixed population of projecting neurons and interneurons in layers II-VI whereas only

pyramidal layer has similar composition. The other two layers (stratum radiatum and lacunosum molecular) contain only interneurons. This distribution suggests more tight connections between the two basic types of neurons in neocortex. Previous studies demonstrated the proconvulsant action of $GABA_B$ receptor agonist after intracortical application (Brailowski et al. 1995) as well as mixed anti- and proconvulsant action of $GABA_B$ receptor agonist in hippocampus (Motalli et al. 1999; Avoli et al. 2004). These results are similar to our findings in cortical ADs in adult rats and support the fact that changes in anticonvulsant and proconvulsant action of $GABA_B$ agonists may be due to the level of maturation. Possible role of maturation of hematoencephalic barrier can be excluded because of repeatedly demonstrated effects of SKF97541 in behavioral experiments in adult rats (Frankowska et al. 2009) and mice (Carter et al. 2005). Presynaptic $GABA_B$ receptors suppressing release of GABA and glutamate could also modulate the effects on excitatory or inhibitory presynaptic terminals (Roth et al. 2012). In addition, quantitative differences in subcellular location of $GABA_B$ receptor subunits in the hippocampus and neocortex in adult rats (Kulik et al. 2003) could result in various affinity to $GABA_B$ agonist SKF97541. In our study, 10-min interval between stimulations was usually inadequate to elicit the hippocampal afterdischarge, but was sufficient to elicit cortical afterdischarge in all experimental animals. These data suggest that there is a longer postictal refractoriness after hippocampal ADs than after cortical ADs. Similar difference was found in our old acute experiments (Mareš and Marešová 1989).

To make a conclusion from our results, different postnatal development and distribution of $GABA_B$ receptors, their subunits as well as modulation their affinity to GABA could be possible explanation for ambiguous effect of $GABA_B$ agonist SKF97541 on cortical and hippocampal afterdischarges and postictal refractoriness. Anyway, a further analysis of the role of the $GABA_B$ receptors in these two brain structures is necessary.

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**Text to Figures**

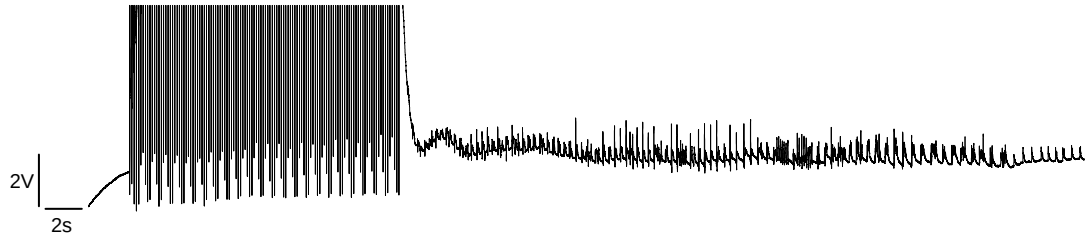
**Fig.1**
EEG recording of cortical afterdischarge (AD) from sensorimotor area opposite to stimulation electrodes (upper part).
EEG recording of hippocampal AD from dorsal hippocampus opposite to stimulation site (lower part).
Activity was recorded in reference connection, amplitude and time mark - see inset.

**Fig.2** Relative duration of six successive cortical ADs (mean + S.E.M.). Upper graph – stimulation with 10-min intervals, lower graph – stimulation with 20-min intervals. Duration of the first AD in each dose group was taken as 100%. Individual columns – see inset. Asterisks denote significant difference in comparison with appropriate first AD, circles – in comparison with corresponding control AD.

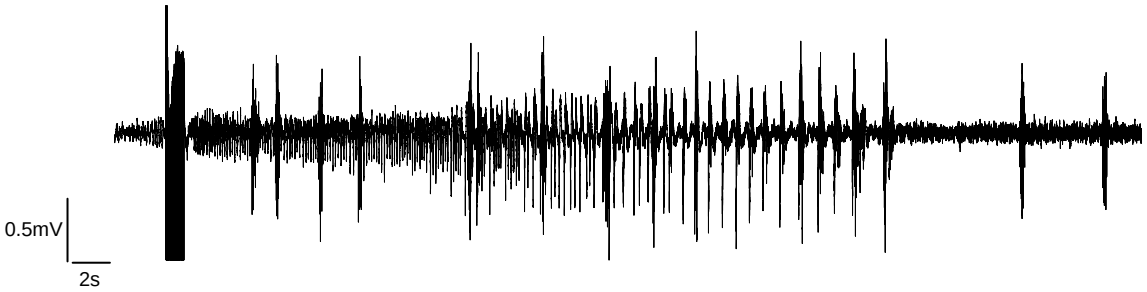
**Fig.3** Relative duration of six successive hippocampal ADs elicited with 20-min intervals (mean + S.E.M.). Details as in Fig.2.

**Fig.4**
Upper graph: Incidence of elicited hippocampal afterdischarges after each stimulation – incidence of stimulation with 10-min intervals. Details - see inset.
Lower graph: Incidence of elicited hippocampal afterdischarges after each stimulation – incidence of stimulation with 20-min intervals. Details - see inset.

**Cortical afterdischarge - SKF97541 1.0mg/kg**

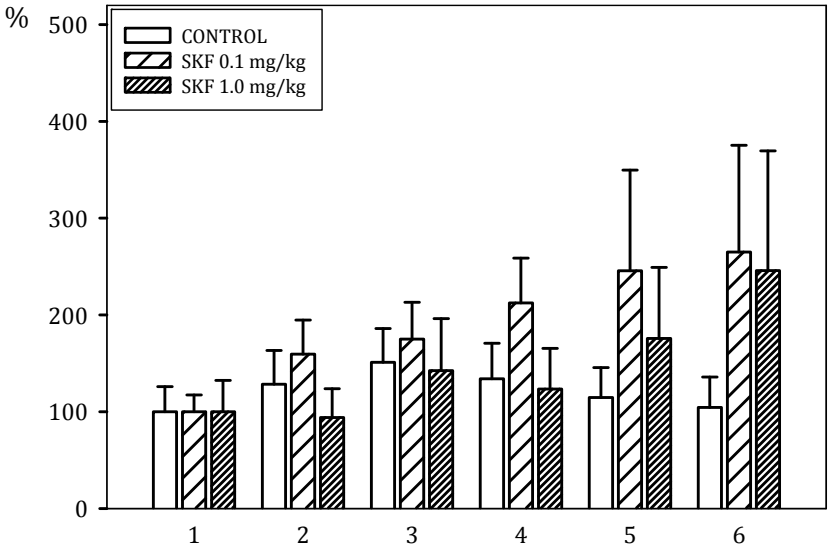



**Hippocampal afterdischarge - SKF97541 1.0mg/kg**

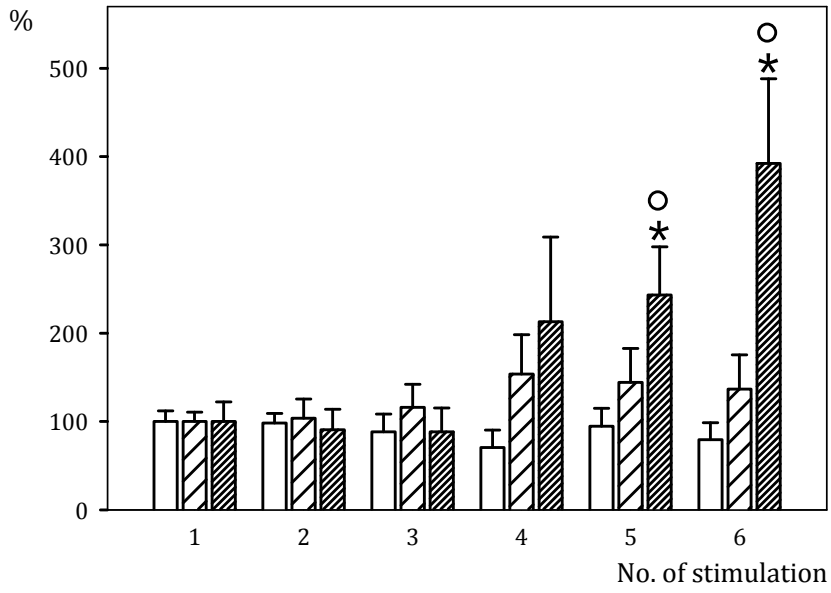


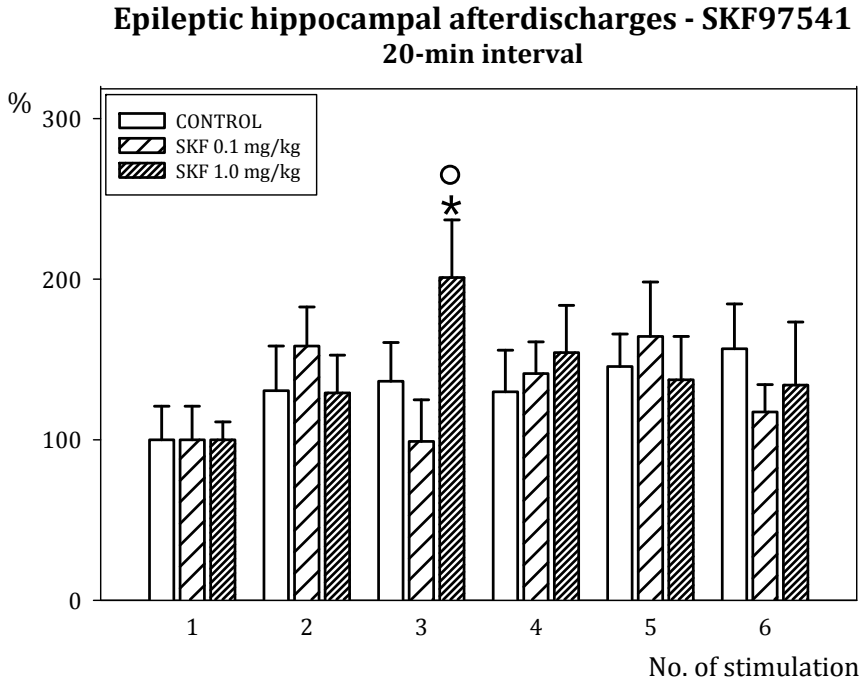
**Epileptic cortical afterdischarges - SKF97541**

**10-min interval**



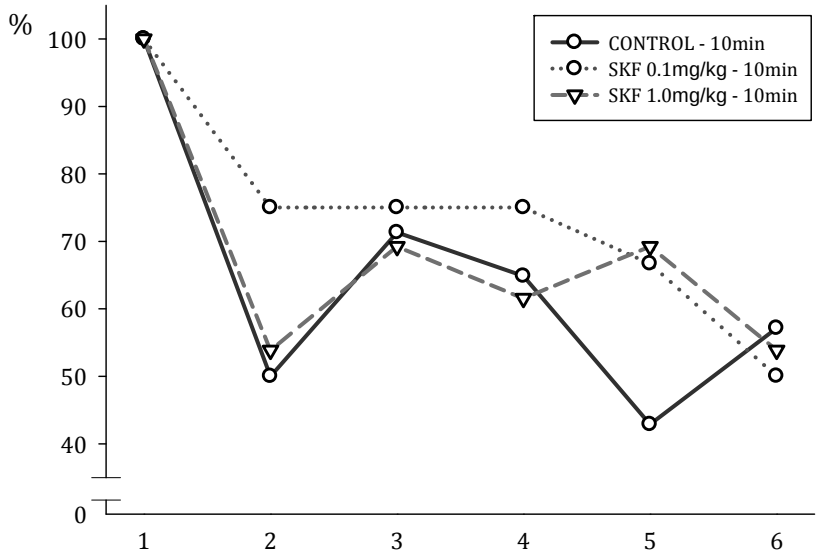

**20-min interval**




Epileptic hippocampal afterdischarges - SKF97541
20-min interval
%
300
200
100
0
CONTROL
SKF 0.1 mg/kg
SKF 1.0 mg/kg
1
2
3
4
5
6
No. of stimulation

**Incidence of hippocampal ADs after stimulation**

**10-min interval**




**20-min interval**