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Effects of a GABA-B receptor agonist baclofen on cortical epileptic afterdischarges in rats

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ABSTRACT – Inhibition mediated by GABA-B receptors can play a role in epilepsy. We therefore studied its involvement in cortical epileptic afterdischarges in adult rats by means of a GABA-B receptor agonist baclofen. Three different experiments were performed with cortical epileptic afterdischarges and an additional experiment studied possible effect of baclofen on cortical interhemispheric (transcallosal) evoked potentials. Baclofen was administered intraperitoneally in doses of 3 or 6 mg/kg. In contrast to a marked proconvulsant action of a GABA-B receptor antagonist baclofen did not exhibit clear anticonvulsant action against EEG afterdischarges but a moderate effect on motor phenomena was observed. On the contrary, it tended to decrease threshold intensities for individual epileptic phenomena. Augmentation of postictal refractoriness by baclofen was found only with a short poststimulation interval (2 min). Cortical interhemispheric responses induced by single pulses were influenced only moderately by baclofen; paired-pulse potentiation induced by short intervals between stimuli was not changed but there was a depression of the second response induced 200 and 250 ms after the first one with the 6 mg/kg dose of baclofen. Failure of baclofen to exhibit an expected anticonvulsant activity might be due to a complexity of GABA-B inhibitory system (pre- as well as postsynaptic localization of GABA-B receptors).

Key words: cerebral cortex, electrical stimulation, epileptic afterdischarges, evoked potentials, baclofen

The main inhibitory neurotransmitter, GABA (gamma-aminobutyric acid) exhibits its action by means of ionotropic (A and C) and metabotropic (B) receptors. Inhibitory postsynaptic potentials mediated by GABA-B receptors last longer and have higher amplitude than those induced by activation of GABA-A receptors. Therefore their possible role as anticonvulsants should be expected (Sperk *et al.* 2004). Research in this field focused paradoxically on generalized absence seizures where GABA-B system plays a role in generation of spike-and-wave rhythm (Crunelli and Leresche 1991; Bernasconi *et al.* 1992). Antagonists of GABA-B receptors were shown to exhibit an excellent anti-absence action in GAERS rats, a generally accepted animal model of absence seizures (Marescaux *et al.* 1992). Intended clinical testing was prevented by data demonstrating proconvulsant effects of

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GABA-B receptor antagonists in models of other types of epileptic seizures (Vergnes *et al.* 1997). Recently not only proconvulsant effect (Mareš and Šlamberová 2006) but also direct convulsant action of GABA-B receptor antagonists (Leung *et al.* 2005) was proved. These data may lead to a revival of interest in the possible anticonvulsant action of GABA-B receptor agonists. Among this group baclofen has been known for a long time and is clinically used in treatment of spasticity (Bowery and Smart 2006).

Literary data on baclofen interaction with epileptic phenomena are contradictory: anti- as well as proconvulsant effects were demonstrated (*e.g.* Ault and Nadler 1983; Sokal and Large 2001); even baclofen applied intracortically could elicit epileptic foci (Van Rijn *et al.* 1987). We decided to test the possible anti- or proconvulsant action of baclofen in a model of cortical epileptic afterdischarges (ADs). These model seizures induced by rhythmic stimulation of sensorimotor cortical area allow evaluation of different phenomena – thresholds for movements directly elicited by stimulation, ADs of the spike-and-wave type, clonic seizures accompanying these ADs and transition of epileptic activity into limbic system (Mareš *et al.* 2002) as well as changes with repeated stimulations with stable intensity (Kubová *et al.* 1996). GABA-B systems may play a role in arrest in these ADs. Our published (Mareš and Šlamberová 2006) as well as unpublished (Mareš, in preparation) data in developing rats demonstrated that GABA-B receptor antagonist led to a marked prolongation of cortical ADs. Therefore we started with a comparison of effects of GABA-B antagonist CGP 35348 and agonist baclofen in adult rats in the same experimental paradigm as in our developmental study (Mareš and Šlamberová 2006). In the next part we studied only the presumed anticonvulsant action of baclofen.

Methods

Experiments performed in adult male Wistar rats 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 (fully compatible with European Community Council directives 86/609/EEC). Cortical epidural electrodes were implanted under ketamine (100 mg/kg i.p.) – xylazine (20 mg/kg i.m.) anesthesia; two stimulation electrodes over right sensorimotor area (AP +1 and -1, L 2.5 mm), recording electrodes over left sensorimotor (AP 0, L 2.5) and parietal (AP 3, L 3) areas and over both occipital areas (AP 6, L 4). Rats were left to recover for at least one week after the surgery.

Stimulation was performed by means of biphasic pulses (duration = 1 ms) generated by a stimulator with constant current output. For elicitation of cortical epileptic afterdischarges (ADs) series of pulses with 8-Hz frequency were applied for 15 s, for evoked potentials single pulses or paired pulses with intervals from 20 ms up to 1 s were used. Electrical activity was preamplified, then digitalized at a rate of 500 Hz for ADs recording and 1 kHz for evoked potentials and saved on a harddisc (Kaminskij Biomedical Research Instruments, Prague).

Drugs: Baclofen was purchased by Sigma (St.Louis, MO), CGP 35348 was a gift from Novartis.

Experiment 1: threshold intensity for elicitation of cortical epileptic AD was established for each rat and this intensity was used throughout the experiment. Stimulation was repeated four times with 10-min intervals, immediately after the end of the first AD drugs influencing GABA-B receptors were applied – either an antagonist CGP 35 348 in doses of 100 or 200 mg/kg i.p. or an agonist baclofen in doses of 3 or 6 mg/kg i.p. In control sessions received animals a corresponding volume of saline (1 mL/kg i.p.).

The duration of ADs was measured; motor phenomena elicited by stimulation as well as accompanying ADs were classified according to the slightly modified Racine's 5-point scale (Racine 1972; Mareš *et al.* 2002; Lojková *et al.* 2006).

Experiment 2: rhythmic stimulation was repeated with increasing intensities from 0.2 to 15 mA to estimate thresholds for the four phenomena mentioned in the Introduction. Stimulation series were repeated with 10-min intervals. Only baclofen in a dose of 6 mg/kg i.p. was studied in this experiment.

Experiment 3: rhythmic stimulation was repeated twice with intervals 2, 3, 5, 10 or 20 min, then baclofen (6 mg/kg i.p.) was administered and 10 min later the paired stimulation series were repeated to examine the possible influence of baclofen on the postictal refractoriness.

Experiment 4: interhemispheric evoked potentials were used to study effect of baclofen (3 and 6 mg/kg i.p.) on transmission between the two hemispheres and on the paired-pulse potentiation. Single pulses were applied with intensities from 0.2 to 4 mA, paired pulses had always an intensity equal to two times threshold for single responses. Eight responses were always averaged and amplitude difference between peaks of the first positive and the first negative waves of the responses was measured. To avoid an influence of different threshold intensities, responses to threshold, two and three times threshold were compared. Paired-pulse responses were evaluated as a ratio between the amplitude of the second to the first response in the pair. Rats in experiment 2 could be exposed only once because series of stimulations with intensities increasing up to 15 mA changed the reactivity (Haugvicová *et al.* 2002), the remaining experiments (1, 3 and 4) were performed repeatedly in each animal starting always with a control session and then the other experiments were done in a random order.

Statistics: Duration of ADs as well as score for movements directly elicited by stimulation as well as for clonic seizures accompanying ADs were evaluated by means of RM

Anova on Ranks with subsequent pairwise comparison by Dunn's test. If effects of two doses were compared with controls, ANOVA on Ranks was used again with subsequent pairwise comparison. Comparison of one dose of baclofen with controls (experiments 2 and 3) was done by means of Kruskall-Wallis test. All statistics were calculated using SigmaStat® software (SPSS Inc.). The level of statistical significance was set at 5%.

Results

Experiment 1

GABA-B receptor antagonist CGP 35348 induced dose-dependent prolongation of ADs; intensity of movements directly induced by stimulation as well as of clonic seizures accompanying ADs was not changed *(figure 1)*. On the contrary, an increase of duration of ADs registered in

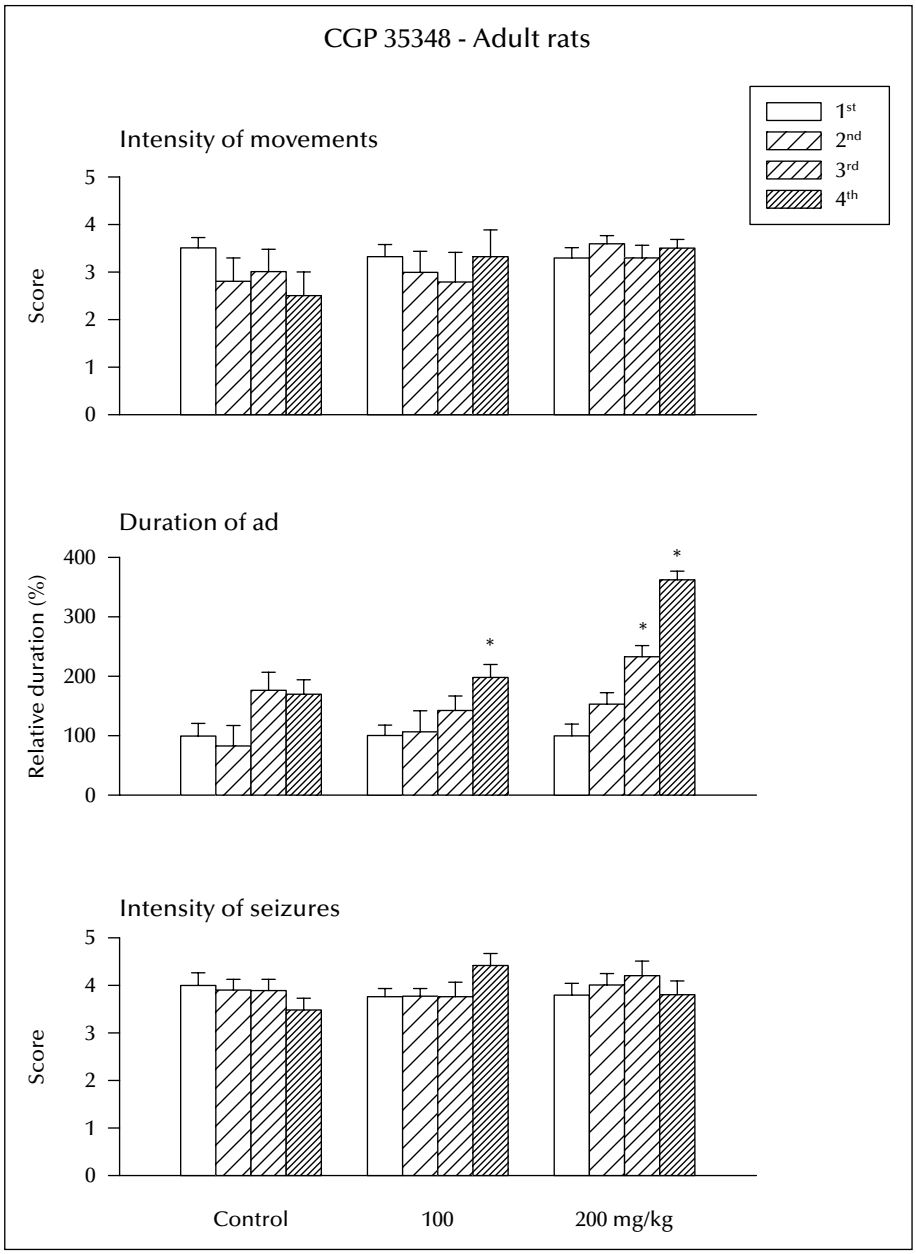

Figure 1. Effects of CGP 35348 on cortical epileptic afterdischarges. From top to bottom: intensity of movements elicited by stimulation, duration of ADs, intensity of clonic seizures accompanying ADs – always mean + S.E.M.
Abscissae: four stimulations (see inset) in control rats (control) and animals injected with the 100 and/or 200 mg/kg dose of CGP 35348 immediately after the first AD. Ordinates from top to bottom: score according to the five-point scale, duration in seconds, score.
* Significant difference in comparison with appropriate stimulation in the control group.

control sessions was not so marked after either dose of baclofen *(figure 2)*. Even the 6 mg/kg dose was not able to reverse the tendency to prolongation of ADs. Motor phenomena were attenuated by baclofen but the difference from the first stimulation reached the level of statistical significance only exceptionally *(figure 2)*.

Experiment 2

Baclofen in a dose of 6 mg/kg exhibited a tendency to decrease the threshold intensities of stimulation current necessary for elicitation of all four phenomena measured (movements directly elicited by stimulation, spike-and-wave ADs and accompanying clonic seizures, transition into mixed type of AD *[figure 3]*). The change of the threshold for mixed type of AD stayed just below the 5%-level due to a high variability of results in the control group.

Experiment 3

The first pair of ADs exhibited shorter second AD with interstimulation interval of 2 min; similar tendency was observed with 3-min interval. On the contrary, 5 and

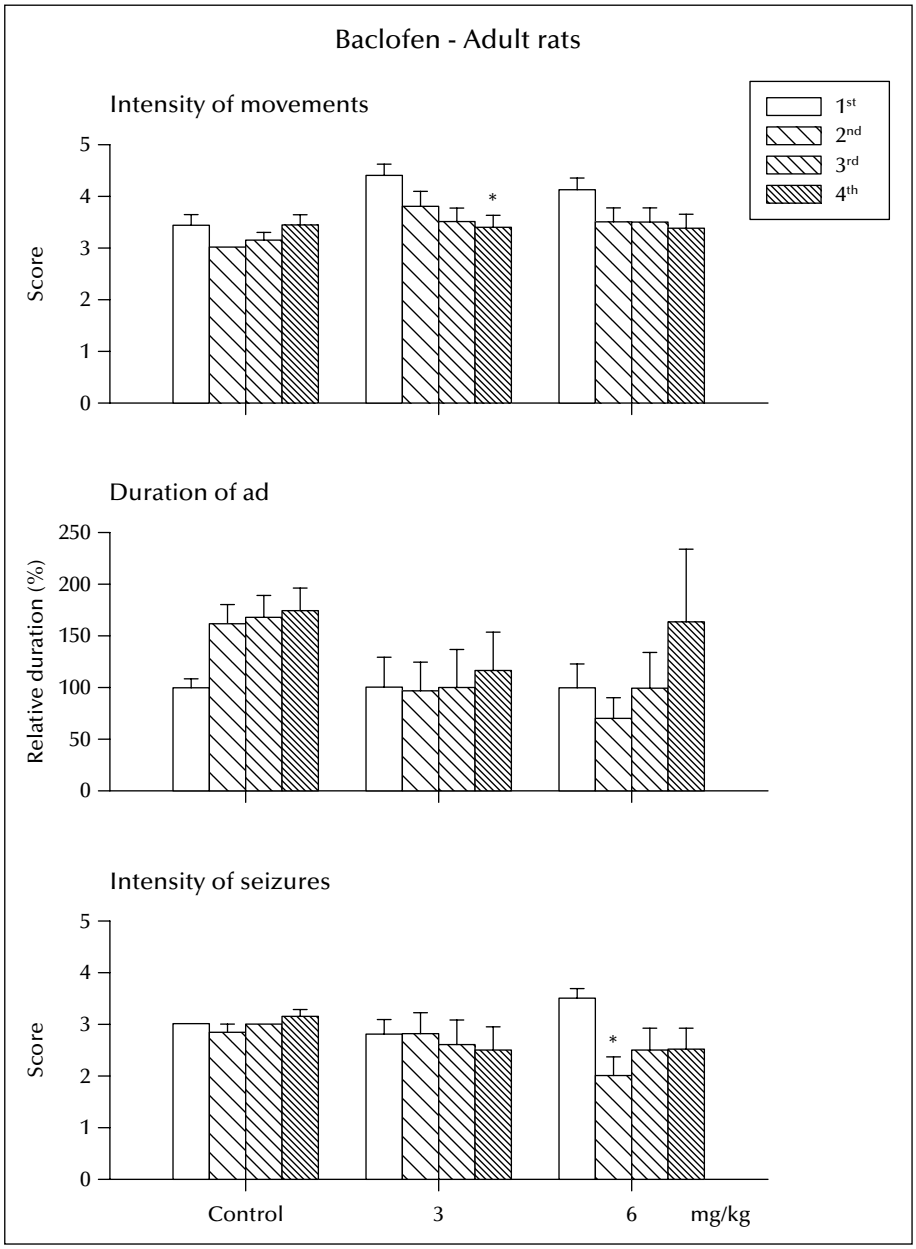

Figure 2. Effects of baclofen on cortical epileptic afterdischarges.
Abscissae: baclofen 3 and 6 mg/kg. Other details as in *figure 1*.

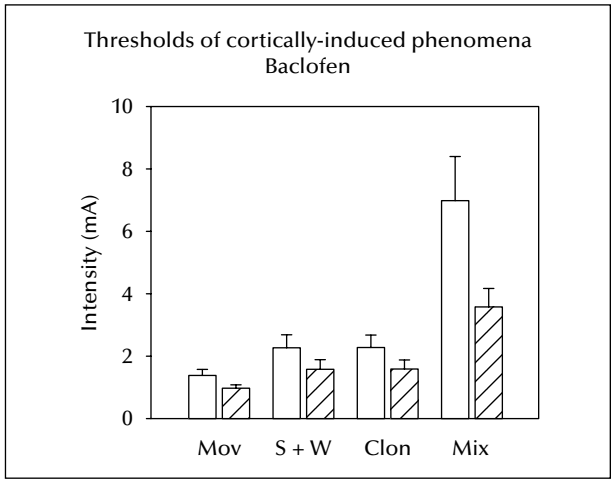

Figure 3. Threshold intensities of stimulation current (mean + S.E.M.) for elicitation of movements induced by stimulation, ADs of the spike-and-wave type, clonic seizures, transition into ADs of limbic type.

10 min intervals (but not 20 min one) tended to prolong the second AD *(figure 4)*. Control sessions demonstrated that the second pair of ADs was not significantly different from the first one, only the shortening of the second AD after the 2 min interval was no longer significant. Baclofen augmented significantly the first AD in the series with 2 min interval, shortening of the second AD in this series was preserved. There were no significant changes induced by baclofen in stimulations with other intervals *(figure 4)*.

Experiment 4

Amplitude of evoked interhemispheric responses increased with increasing intensity of stimulation current. An average threshold intensity in the control group was 1.0 ± 0.2 mA and it was not changed by either dose of baclofen. Comparison of amplitudes of responses evoked by threshold, 2 and 3 times threshold intensities did not reveal any difference among the control and two baclofen groups. Latencies measured to the first positive as well as the first negative peak of the response exhibited a tendency to prolongation after the 6 mg/kg dose of baclofen, statistical significance was reached only exceptionally (latency of the first positive peak at stimulation intensity equal to two times threshold).

Paired-pulse stimulation demonstrated potentiation of responses especially at short intervals between the two stimuli (50 and 70 ms). Baclofen did not change this potentiation at short intervals but it diminished the second response at longer intervals (200 ms and longer *[figure 5]*). The level of statistical significance was reached only with the 6 mg/kg dose of baclofen at intervals of 200 and 250 ms and with both doses at an interval of 1 s.

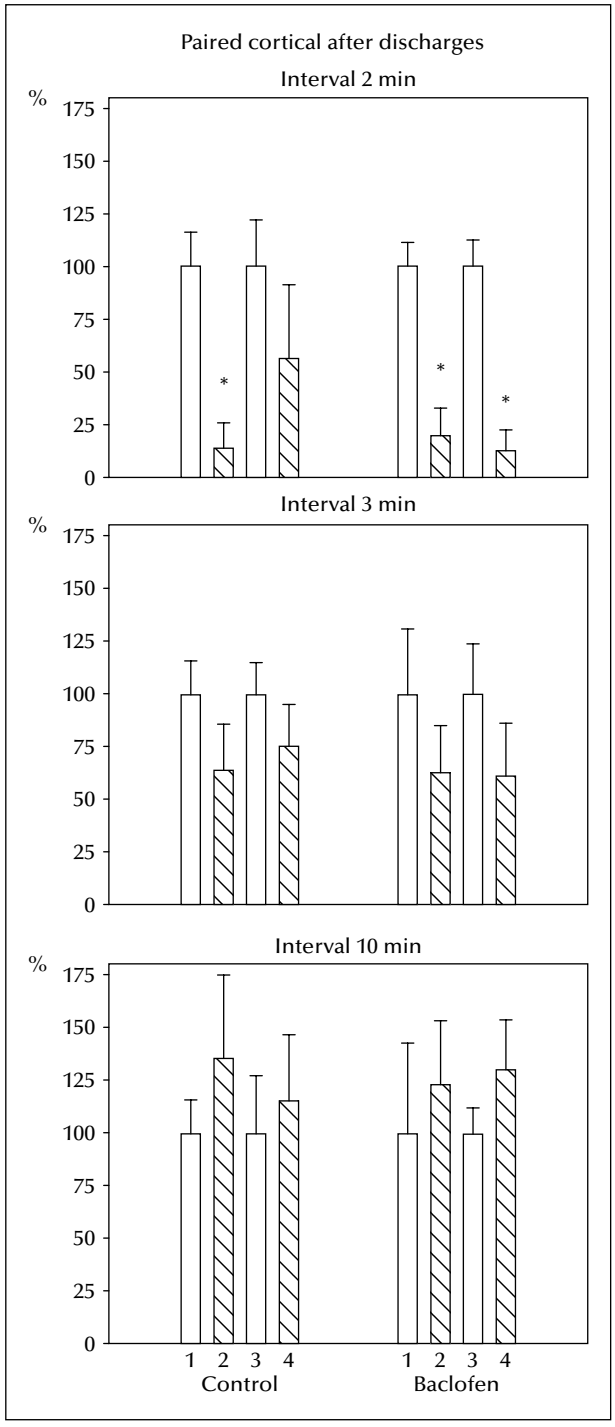

Figure 4. Duration of ADs elicited by paired stimulations (mean + S.E.M.) with intervals 2, 3 and 10 min (from top to bottom). White columns always demonstrate duration of the first AD in the pair; stripped columns the second AD.
Abscissae: two pairs of ADs in control (left) and baclofen-treated rats (right), between the first (1, 2) and the second (3, 4) pair is always a 10 min interval, saline or baclofen (6 mg/kg i.p.) were injected immediately after the end of the first pair. Ordinates: relative duration of ADs, the first one in the pair is always taken as 100%. * Significant difference in comparison with the first, predrug AD.

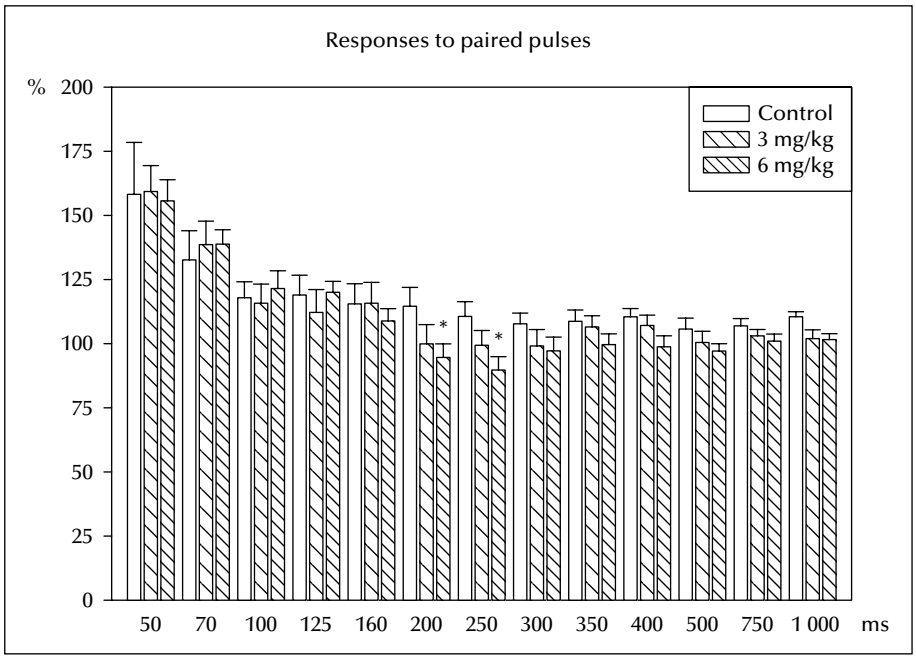

Figure 5. Effects of baclofen (3 and 6 mg/kg i.p.) on paired-pulse potentiation of cortical interhemispheric responses.
Abscissa: interpulse intervals; ordinate: ratio of amplitude of the second to amplitude of the first response expressed in percents. Individual columns (see inset).
* Significant difference in comparison with corresponding interval in the control group.

Discussion

GABA-B receptor antagonist CGP 35348 influenced cortical epileptic afterdischarges in adult rats in the same way as in younger rats (Mareš and Šlamberová 2006). Its proconvulsant action was expected because it is in agreement also with data from other laboratories (Badran *et al.* 1997; Vergnes *et al.* 1997; Leung *et al.* 2005). Effects of GABA-B receptor agonist baclofen were far from expected ones: baclofen was able only to attenuate prolongation of ADs with repeated stimulations. In contrast to an antagonist it influenced motor phenomena. It did not exhibit an anticonvulsant effect on threshold intensities for elicitation of cortical epileptic phenomena. On the contrary, it tended to decrease the thresholds. Baclofen also failed to augment postictal refractoriness, only a moderate effect was observed with the shortest (2 min) interval between the two ADs. Mixed anti- and proconvulsant effects reflect controversies described in literature. It is possible to find papers demonstrating anticonvulsant action of baclofen *in vivo* (Ault *et al.* 1986; Veliskova *et al.* 1996, De Sarro *et al.* 2000; Sokal and Large 2001) and *in vitro* (Ault and Nadler 1983; Swartzwelder *et al.* 1986; Lewis *et al.* 1989; Higashima *et al.* 2000) as well as proconvulsant action *in vivo* (Hoskinson *et al.* 2004) and *in vitro* (Mott *et al.* 1989; Watts and Jefferys 1993). The convulsant action of baclofen was demonstrated after intracortical application (Van Rijn *et al.* 1987; Brailowski *et al.* 1995). There is also previous literature describing mixed anti- and proconvulsant effects in hippocampal slices (Motalli *et al.* 1999; Avoli *et al.* 2004) – these results are similar to our data. An explanation of the variability of effects might be in the fact that GABA-B receptors are localized pre- as well as postsynaptically (Bowery 2006) and, in addition presynaptic receptors can function as autoreceptors diminishing release of GABA from presynaptic terminals and/or as heteroreceptors restricting release of glutamate. Different effects of baclofen on pre- and postsynaptic GABA-B receptors were found in vitro experiments (Deisz *et al.* 1993; Gloveli *et al.* 2003) but discrimination between presynaptic auto- and heteroreceptors is not yet reliably possible.

There is a contrast between negligible action of baclofen against EEG afterdischarges and a clear action against motor phenomena. This difference indicates an action of baclofen in other brain structures than those involved in generation of an afterdischarge (thalamocortical system). Not only the spinal cord but also basal ganglia have to be taken into account. Chen *et al.* (2004) demonstrated an anticonvulsant effect of baclofen injected into globus pallidus against PTZ-induced tonic seizures.

Potentiation of spike-and-wave rhythm induced by low doses of pentylenetetrazol as well as of rhythmic spikes characteristic for genetic absence models in rats by baclofen has been described (Vergnes *et al.* 1984; Marescaux *et al.* 1992; Snead 1996) and clinical and experimental

data are in agreement (Gloor and Fariello 1988). The spike and wave rhythm is taken as a thalamocortical phenomenon (Avanzini *et al.* 1992; Steriade 2005). Therefore we expected in paired-pulse stimulation paradigm of cortical interhemispheric responses an augmentation of the second response at interpulse intervals corresponding to the frequency of spike-and-wave rhythm in adult rats. In contrast, we found a depression of the second interhemispheric response at intervals corresponding to frequency of 4-5 Hz (*i.e.* the frequency of spike-and-wave rhythm induced by low doses of pentylenetetrazol Schickerová *et al.* 1984).

Our results lead to a conclusion that application of baclofen into different brain structures will be necessary to analyze its complex action in experimental epilepsy. □

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