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# Stimulation parameters determine role of $GABA_B$ receptors in long-term potentiation

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*Abstract.* Blockade of $GABA_B$ receptors was reported to improve cognitive performance in mammals[1]. The physiological basis of this effect is poorly understood. We investigated the effect of the $GABA_B$ receptor antagonist CGP 35348 on long-term potentiation (LTP) in the CA1 area of the hippocampus in vitro and in vivo. In vitro the effect of CGP 35348 on LTP, induced either by two non-primed tetanic stimulations or by two primed bursts of stimuli, was investigated. In the presence of 1 mM CGP 35348 LTP was significantly facilitated following two non-primed tetanic trains, but was impaired following two primed burst stimulations. In vivo LTP was induced by applying non-primed trains of stimuli of increasing duration to the Schaffer collateral/commissural fibers. The potentiation of the population spike recorded in CA1 was significantly facilitated by CGP 35348 (100 mg/kg i.v.). In conclusion these findings demonstrate that the $GABA_B$ antagonist CGP 35348 facilitates LTP in vitro and in vivo if induced by non-primed tetanic stimulation. In vitro, the mode of stimulation determines the effect of the $GABA_B$ antagonist on LTP.
*Key words.* Long-term potentiation; hippocampus; $GABA_B$; synaptic plasticity.

Blockade of $GABA_B$ receptors has been shown to improve cognitive functions in mice, rats and monkeys[1]. The sequence of events leading from $GABA_B$ receptor blockade to facilitation of cognitive processes is unknown. The use-dependent strengthening of excitatory synaptic transmission in the hyppocampus is known as long-term potentiation (LTP). It is considered to be a cellular mechanism that may be linked to learning and memory in mammals. Recently, several laboratories reported that $GABA_B$ receptors may have an important role in modulating LTP. Initially it was found that the two $GABA_B$ receptor blockers phaclofen and CGP 35348 facilitate LTP in the hyppocampus induced in the CA1 area by two trains of 100 Hz stimuli applied to the Schaffer-commissural fibers[3] in vitro. Subsequently, however, the $GABA_B$ blockers 2-OH saclofen[4] and CGP 35348[5] were reported to block the induction of LTP in the CA1 area and dentate gyrus respectively. There was no obvious reason for the striking discrepancy between these studies. Since two different $GABA_B$ blockers suppressed the induction of LTP in both CA1 and the dentate gyrus it appears unlikely that the discrepancy is due to differences in the drug profile or to regional hippocampal differences. However, the studies vary significantly in the concentrations of the $GABA_B$ blockers used and in the mode of LTP induction. The drug concentration, and thus the extent of blockade of $GABA_B$ receptors, may be particularly crucial. The concentration is important because it may affect pre-and postsynaptic $GABA_B$ receptors differently. Facilitation of LTP in CA1[3] was observed at a concentration of CGP 35348 ten times lower than that producing suppression[5]. The concentrations used were 100 μM and 1 mM respectively. The three studies also differed with regard to the way LTP was induced. In the study in which LTP was facilitated, two trains (200 ms) of pulses applied at 100 Hz were used. The two studies reporting pronounced suppression of LTP following blockade of $GABA_B$ receptors used either primed bursts in the CA1 area[5] or 5 Hz trains in the case of the dentate gyrus[4]. We first repeated our previous experiments applying two 200 ms trains (30 and 100 Hz), raising the concentration of CGP 35348 from 100 μM to 1 mM and observing LTP for 20 instead of 5 min only. Under identical conditions we subsequently performed a second series of experiments in which we induced LTP by means of primed bursts and compared LTP in the presence or absence of 1 mM CGP 35348.

Given the observation that cognition is enhanced following $GABA_B$ receptor blockade[1], we were interested to determine whether the facilitation of LTP by CGP 35348 observed in vitro may also be seen in vivo. For this purpose, a series of experiments was performed in anaesthetized rats in which LTP was induced by non-primed trains of 100 Hz.

## *Materials and methods*

In vitro experiments: Male Sprague-Dawley rats [180–250 g Tif:RAIf (SPF)] were decapitated under ether anaesthesia. The brain was rapidly dissected out and immersed in an artificial cerebrospinal fluid (ACSF) solution (pregassed with 95% $O_2$/5% $CO_2$). The hippocampi were carefully isolated and cut into 450 μm thick transverse slices using a McIlwain tissue chopper. The slices were placed on a nylon net in a superfusion

chamber and were maintained at room temperature for the first hour in a static condition with the ACSF level adjusted to their upper surface. Warm humidified gas (95% O2/5% CO2) was continuously circulated over the slices. The slices were subsequently superfused at 33 °C with gassed ACSF at a rate of 3 ml/min (1 chamber volume/min). The standard ACSF used in all experiments had the following composition in mM: NaCl 124.0, KCl 2.5, $KH_2PO_4$ 1.2, $MgSO_2$ 2.0, $CaCl_2$ 2.5, $NaHCO_3$ 26.0 and D-glucose 10.0. The $GABA_B$ receptor blocker CGP 35348 was dissolved in ACSF and added to the perfusion chamber at a concentration of 1 mM via the main perfusion line. Extracellular recordings of population spikes from the pyramidal cell layer of the hippocampal CA1 region were made with glass electrodes (2–5 MΩ) filled with 4 M NaCl. Synaptic input to the CA1 region was evoked by electrical stimulation (0.2 ms pulse width, 5-30 V) of Schaffer collateral/commissural fibres using bipolar stimulation electrodes located in striatum radiatum (orthodromic stimulation). In each hippocampal slice a population spike was evoked in CA1 with the voltage adjusted to evoke responses of half maximal amplitude.

Single pulse stimuli (0.2 ms duration) were given at a rate of 0.125 $s^{-1}$ for 5–10 min until responses of constant spike amplitude were obtained. The evoked responses were averaged (n = 4) by means of a Hitachi Digital oscilloscope VC-6041 and the averaged population spikes were plotted on a W + W 320 recorder. In the first series of experiments, LTP was induced by two consecutive trains of rectangular pulses (train interval 5 s) lasting 200 ms, delivered via the stimulation electrode placed on the Schaffer collateral/commisural fibre tract. Individual pulses lasted 0.2 ms. In each slice LTP was induced by two trains of either 30 or 100 Hz. Prior to the induction of LTP, the voltage was adjusted to evoke responses of half-maximal amplitude. In the second series of experiments stimuli were given at the same location in the form of 2 consecutive primed bursts. Each primed burst consisted of a single pulse followed 200 ms later by 4 pulses given at 100 Hz. Individual pulses lasted 0.2 ms. During control recordings the voltage was adjusted to evoke responses of half-maximal amplitude. In each experiment (1 rat, 4-8 slices) LTP was first induced in 2–4 slices in the absence of drugs. Subsequently, CGP 35348 was added via the main perfusion line to give a concentration of 1 mM in the perfusion chamber. CGP 35348 (P-(3-aminopropyl)-P-diethoxymethylphosphinic acid) was synthesized in the chemistry laboratories of Ciba-Geigy Ltd. Basel Switzerland.

All values are given as means ± SEM. Statistical significance was assessed by means of the Student's t-test.

In vivo experiments: The experiments were performed on 12 male Sprague-Dawley rats weighing 200–250 g anaesthetized with chloral hydrate (400 mg/kg i.p.; additional administrations were performed when necessary). The animals were mounted in a stereotaxic frame and body temperature was maintained between 37 and 38.5 °C. The stimulation electrode was introduced stereotactically into the Schaffer collateral/commissural fiber tract. The recording electrode, consisting of a glass pipette filled with 4 NaCl (2–5 MΩ), was placed stereotactically into the CA1 area. The jugular vein was cannulated for drug administration.

A population spike was evoked in the CA1 pyramidal cell layer by applying 0.2 ms pulses (8–15 V) to the Schaffer collateral/commissural fibres at a rate of 0.066 Hz. The voltage was adjusted to evoke population spikes of a third of the maximal amplitude. The signals were amplified and averaged on a digital oscilloscope (n = 4). Following a control period lasting 15 min, during which population spikes showed little variation in amplitude, NaCl (0.9%) or CGP 35348 was administered intravenously. Fifteen minutes later LTP was induced by a series of tetanic trains of stimulation of increasing duration. Tetanic trains were given at intervals of 30 min. In all experiments the following sequence of 100 Hz tetanic stimulations was given: one train, 100 ms; 2 trains, 100 ms; one train, 200 ms; two trains, 200 ms; 1 train 1000 ms; 2 trains, 1000 ms. Results are given as means ± SEM. The statistical significance was assessed by means of the Student's t-test.

*Results*

*The effects of CGP 35348 on LTP in vitro.* Long-term potentiation of the population spike amplitude was obtained following stimulation with two trains of tetanic stimuli. Twenty minutes following LTP induction the mean population spike increase was 20% with 30 Hz and 45% with 100 Hz tetanic stimulations. In both cases, slices exposed to 1 mM CGP 35348 showed significantly stronger LTP (fig. 1). The differences were statistically significant 5, 10, 15 and 20 min following LTP induction (fig. 1). The enhancement of LTP induced by CGP 35348 was more pronounced in slices stimulated by 30 than by 100 Hz trains. In slices stimulated at the lower frequency the potentiation was doubled 20 min after the trains were applied and in slices stimulated at 100 Hz LTP was increased by approximately one third only.

However, different results were obtained in slices in which LTP was evoked by primed bursts. Stimulation with two primed bursts applied at an interval of 5 sec resulted in stable potentiation of the population spike, similar in magnitude to the potentiation observed with two trains of non-primed tetanic stimuli (fig. 1 and 2). In the presence of 1 mM CGP 35348 there was still an immediate transient potentiation lasting some five to ten

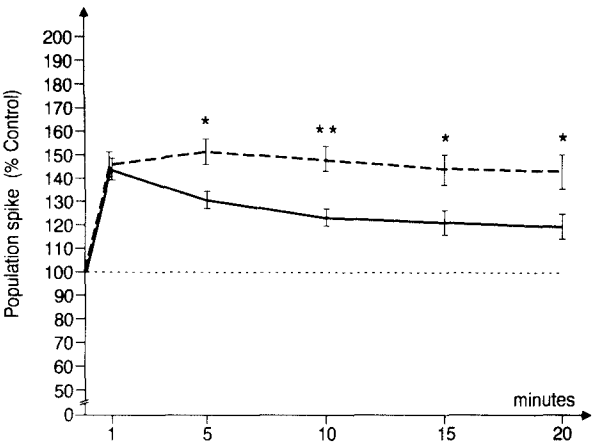


Figure 1. The facilitatory action of CGP 35348 on LTP induced by 30 Hz (left panel) or 100 Hz (right panel) is depicted. The data obtained in the absence or presence of CGP 35348 (1 mM) are represented by the solid or dashed lines respectively. The time course of the changes in population spike amplitude are shown. The data is normalized for the mean amplitudes recorded prior to tetanic stimulation ( = 100%). LTP was facilitated by CGP 35348 if induced at 30 (left panel, n = 14) or 100 Hz (right panel, n = 15). *p < 0.005, **p < 0.001, $\bar{x} \pm$ SEM.

min (fig. 2), but there was no LTP. We interpret this transient potentiation to represent posttetanic potentiation. At longer intervals the population spike even appeared to be weakly depressed. The data obtained in the presence or absence of CGP 35348 was statistically significant at all five time points (fig. 2). Typical recordings of population spikes in the presence of 1 mM CGP 35348 before and after the induction of LTP are depicted in figure 3. Whereas pronounced potentiation occurs following two trains of 100 Hz stimuli, no potentiation occurred following two primed bursts.

*The effect of CGP 35348 on LTP in vivo.* CGP 35348 had no effect on amplitude of the population spikes recorded prior to the first tetanic stimulation. The series of tetanic stimulations of increasing duration elicited pronounced LTP which increased in magnitude with each additional stimulation (fig. 4). In the animals treated with CGP 35348, LTP was facilitated and it reached a plateau much earlier than the control animals (fig. 4). Following the last tetanic stimulation the difference between the two groups was not significant.

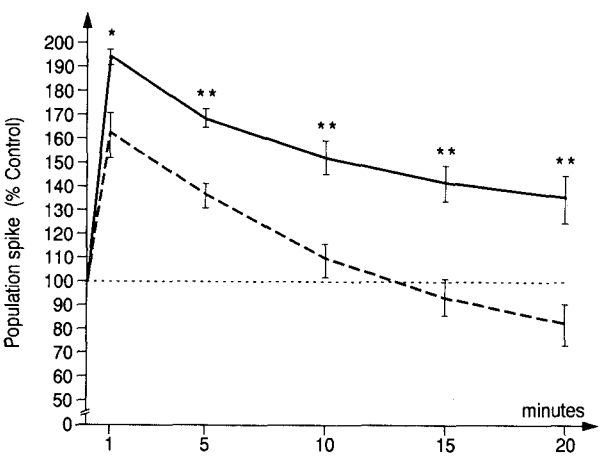


Figure 2. CGP 35348 applied at a concentration of 1 mM impaired the development of LTP induced by two primed bursts. The primed burst consisted of a single pulse followed by 4 pulses given at 100 Hz. Solid line, without CGP 35348; dashed line, in the presence of 1 mM CGP 35348 (N = 18). $\bar{x} \pm$ SEM. *p < 0.005; **p < 0.001.

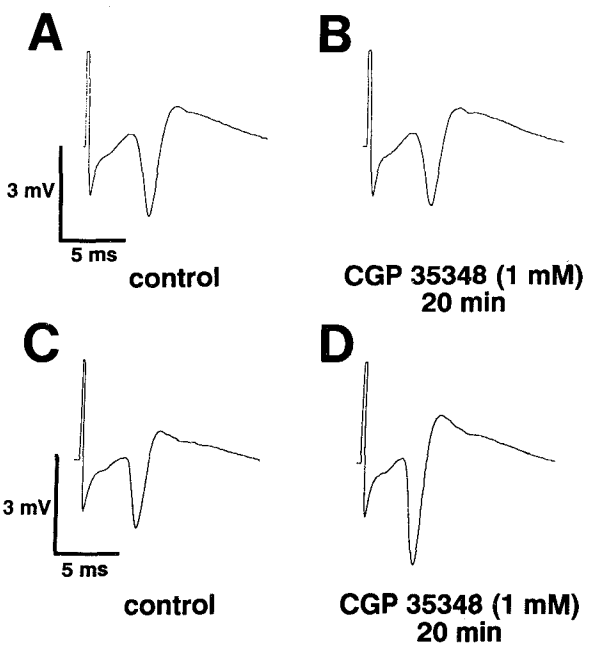


Figure 3. CGP 35348 impaired LTP if induced by two primed bursts (upper panel) or facilitated it if induced by two non-primed tetanic stimulations (lower panel). The recordings were taken from two separate experiments. The population spikes were evoked by stimulation of the Schaffer collateral/commissural fibres and recorded in CA1. A, control; B, 20 min after primed bursts; C, control; D, 20 min after non-primed bursts.

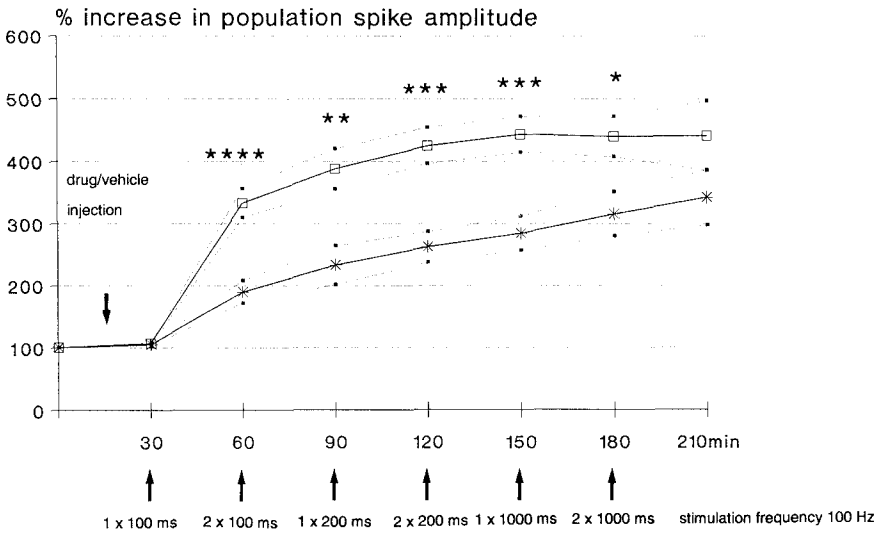


Figure 4. The facilitation of long-term potentiation (LTP) by CGP 35348 in vivo is shown. The population spike recorded in CA1 was evoked by stimulation of the Schaffer collateral/commissural fibre tract in chloral hydrate anaesthetized rats. LTP was induced by tetanic stimulations of increasing duration applied at intervals of 30 min. CGP 35348 or the vehicle were applied 15 min prior to the first tetanic stimulation, $*p < 0.05$, $**p < 0.01$, $***p < 0.005$, $****p < 0.001$.

Throughout all experiments the population spikes displayed no signs of epileptic-like hyperexcitability in the form of multiple population spikes.

*Discussion*

The in vitro findings strongly suggest that the blockade of $GABA_B$ receptors has opposite effects on LTP depending on the stimulation parameters used to induce LTP. Since the experiments were performed with slices kept under identical conditions using the same $GABA_B$ blocker at the same concentration it appears safe to conclude that the manner of inducing LTP accounts for the existing discrepancies[3–5]. Following stimulation with two trains of 100 Hz tetanic stimulation LTP was facilitated in the presence of 1 mM CGP 35348 to a similar extent as previously reported when the drug was administered at 100 μM[3]. The present results extend our previous data by showing that the facilitation of LTP lasts at least 20 min. However, in accordance with a previous study also conducted in the hippocampal CA1 area, no LTP was observed following primed burst stimulation.

The question arises as to how these in vitro findings may be reconciled with our current notions on the role of hippocampal $GABA_B$ receptors. Blockade of the GABA-autoreceptor facilitates GABA-release[6] in vitro and this may result in reduced pyramidal cell excitability. On the other hand, blocking the postsynaptic receptor located on pyramidal neurons is likely to induce an increased pyramidal cell excitability since the $K^+$-conductance underlying the late inhibitory potential is removed[7]. At a concentration of 1 mM CGP 35348 blocks pre-and postsynaptic $GABA_B$ receptors[5]. It has been proposed that LTP induced by primed bursts develops as a result of the autoreceptor-mediated reduction of GABA-release. Blocking this receptor thus results in suppression of LTP induction. The suppression of LTP observed with CGP 35348 in the primed burst paradigm suggests also that the presynaptic site of action of the blocker is dominating the postsynaptic disinhibitory one. On the other hand, the blockade of the postsynaptic $GABA_B$ receptor may become more important in the case of non-primed tetanic stimulations since the tetanic trains were longer in experiments involving non-primed than primed stimulations. Alternatively it is conceivable that the autoinhibition of GABA-release may not be fully operational or may even be abolished at high stimulation frequencies. In keeping with this latter hypothesis is the observation that the autoinhibition of GABA release from brain slices operates optimally in the frequency range of 0.25–4 Hz. At higher frequencies the autoinhibition is diminished[6] and more GABA is being released per stimulation[6].

The present data demonstrate for the first time that a $GABA_B$ receptor antagonist strongly facilitates LTP in vivo if LTP is induced in the non-primed manner. This effect occurs in the absence of any signs of increased basal pyramidal cell excitability, since the population spike was not increased by CGP 35348 prior to tetanic stimulation and no multiple spikes were noticed. Multiple population spikes might have indicated proconvulsive properties of the drug. The facilitation of LTP by CGP 35348 is in keeping with the observation that $GABA_B$ receptor blockade facilitates cognitive processes in vivo[1]. However, a strict comparison of the two sets of data is somewhat hampered by the fact that

another $GABA_B$ blocker has been used in the behavioral study[1]. Nonetheless the present findings raise the interesting question of which mode of LTP induction is more relevant to cognition.

In conclusion, the present in vitro results suggest that $GABA_B$ receptors differentially affect LTP depending on the mode of LTP induction, but the reasons for this phenomenon remain to be elucidated. LTP is facilitated by CGP 35348 both in vitro and in vivo if induced by non-primed tetanic stimulation. The possible link between the drug's positive effect on LTP and cognitive functions is a challenging task that remains to be solved.

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