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$GABA_A$ receptor inhibition does not affect mGluR-dependent LTD at hippocampal Schaffer collateral-CA1 synapses

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ABSTRACT

Hippocampal synaptic plasticity between Schaffer collaterals and CA1 pyramidal neurons can be induced by activation of N-methyl-D-aspartate receptors (NMDARs) or of metabotropic glutamate receptors (mGluRs). Inhibitory GABAergic interneurons in this region abundantly terminate on pyramidal neurons and may thus influence synaptic plasticity. Although NMDAR-dependent synaptic plasticity is known to be influenced by inhibitory interneurons, little is known about the role of GABA on mGluR-dependent plasticity. Here, we used field potential recordings of the Schaffer collateral-CA1 synapses in rat hippocampal slices in order to study the effect of $GABA_A$ receptor ($GABA_AR$) inhibition on mGluR-dependent long-term depression (LTD). Without $GABA_AR$ blockade, mGluR-dependent LTD was induced pharmacologically by the group I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG, 100 μM, 10 min) as well as electrically by paired-pulse low-frequency stimulation (PP-LFS, 900 paired pulses at 1 Hz) resulting in a stable depression of the field response lasting at least 80 min after LTD induction. The $GABA_AR$ antagonist gabazine (5 μM) itself caused an increase of field responses suggesting an endogenous GABA release inhibiting CA1 field potentials. However, when either DHPG or PP-LFS was applied during $GABA_AR$ inhibition, the field responses were significantly reduced. Moreover, normalizing these responses to experiments without $GABA_AR$ blockade, there was no significant effect of gabazine on both DHPG- and PP-LFS-induced LTD. Thus, our results show that mGluR-dependent LTD at Schaffer collateral-CA1 synapses is unaffected by $GABA_AR$ mediated synaptic transmission.

GABAergic interneurons play a pivotal role in keeping the balance between excitation and inhibition of neuronal activity by releasing γ-aminobutyric acid (GABA), which is the main inhibitory transmitter of the adult brain. GABA-releasing synapses activate ionotropic $GABA_A$ receptors ($GABA_AR$) as well as metabotropic $GABA_B$ receptors. The probably most important inhibitory effects of GABAergic interneurons are membrane hyperpolarization (tonic inhibition) and shunting of glutamatergic excitatory input (phasic inhibition) in the consequence of Cl^--influx through $GABA_AR$ [1,16,18]. However, recent results provide evidence that inhibitory interneurons do not only influence basal synaptic transmission, but also have a crucial role in regulating synchronous network activity and mechanisms of learning and memory [10,28]. Especially in the hippocampus, inhibitory interneurons play a central role in modulating synaptic plasticity [16] which is widely accepted as a cellular model of learning and memory [5]. At hippocampal Schaffer collateral-CA1 synapses, GABAergic interneurons affect synaptic plasticity depending on activation of NMDA receptors (NMDAR). This is readily conceivable because $GABA_A$ receptor mediated hyperpolarization is thought to maintain the voltage-dependent Mg^{2+} block of NMDAR channels [8]. Indeed, GABAergic interneurons not only constrain the long-lasting enhancement of excitatory synaptic responses, termed long-term potentiation (LTP) [6], but also reduce NMDAR-dependent long-term depression (LTD) of synaptic efficacy [14,27].

In the hippocampal CA1 region a second, NMDAR-independent form of LTD can be observed that requires activation of metabotropic glutamate receptors (mGluR) [19]. This mGluR-LTD can be induced pharmacologically by the group I mGluR agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) [21], as well as electrically by paired-pulse low-frequency stimulation (PP-LFS) [12] and both PP-LFS-induced and DHPG-induced mGluR-dependent LTD are thought to share common mechanisms of expression [11]. In contrast to NMDAR-dependent LTD, the role of inhibitory interneurons is less clear in mGluR-LTD. Even though the $GABA_AR$ blocker picrotoxin appeared to enhance both DHPG-induced as well as PP-LFS-induced mGluR-LTD [20,21], other studies were unable to reproduce these results [19,22].

We explored this question further and systematically studied the influence of $GABA_AR$ inhibition with gabazine on both pharmacologically and electrically induced mGluR-LTD in rat hippocampal slices (400 μm) obtained from male 2- to 3-month-old Wistar

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rats (Charles River Laboratories, Sulzfeld, Germany), following to the guidelines laid down by the Animal Care and Use Committee at the University of Rostock. After deep anesthesia with diethyl ether, the brain was dissected out rapidly. Horizontal hippocampal slices were prepared in ice-cold dissection fluid containing (in mM) 125 NaCl, 26 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 0.2 $CaCl_2$, 5 $MgCl_2$ and 13 D-glucose with a vibratome (Integraslice 7550 MM, Campden Instruments, Loughborough, UK) and then transferred into a holding chamber with artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 26 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.3 $MgCl_2$ and 13 D-glucose (gassed with 95% O_2, 5% CO_2; pH 7.4). Osmolality was adjusted to 306–314 mOsm/kg with sucrose. After 90 min of recovery, individual slices were placed in an interface recording chamber and superfused with ACSF using a peristaltic pump, at the rate of 2–3 ml/min. Drugs were added directly to the perfusion solution. Gabazine, (RS)-3,5-DHPG and D-(−)-2-amino-5-phosphonopentanoate (D-AP5) were purchased from Tocris (Bristol, UK). All other chemicals were purchased from Sigma (Taufkirchen, Germany).

Schaffer collateral fibers were stimulated via a bipolar platinum wire placed in the stratum radiatum of the CA1 region. Bipolar stimulation was delivered with a Master-8 stimulator (A.M.P.I., Jerusalem, Israel) at a rate of 0.033 Hz and baseline stimulation strength adjusted to 30–40% of the maximal field excitatory postsynaptic potential (fEPSP) amplitude. The PP-LFS was performed at double baseline stimulation strength, consisting of 900 paired stimuli (interstimulus interval 50 ms) at 1 Hz. Since NMDAR-dependent mechanisms of synaptic plasticity could not be excluded with this paradigm, all experiments with PP-LFS were conducted in the presence of 50 μM D-AP5. Field EPSPs were recorded at 32 °C (controlled by TC-10, npi electronic GmbH, Germany) in the CA1 stratum radiatum, using borosilicate glass pipettes (2–3 MΩ, pulled with PIP5 from HEKA Elektronik, Lambrecht, Germany) filled with ACSF. Amplified signals were filtered at 1 kHz (EXT-08, DPA-2F, npi), digitized (Power 1401, CED Cambridge Electronic design, Cambridge, UK) and analyzed (Signal 2.16, CED). The level of acute depression induced by DHPG or PP-LFS was measured as the mean of 10 consecutive fEPSP slopes (5 min) directly following LTD induction. The LTD level was assessed by averaging the fEPSP slopes within the last 5 min (10 sweeps) of the individual experiment. All data are expressed as means ± SEM. Statistical comparison was performed using Student's two-tailed *t*-test with a level of significance set to $p < 0.05$.

Since we wanted to study the role of $GABA_AR$ mediated synaptic transmission on mGluR-dependent LTD at Schaffer collateral-CA1 synapses, we first used DHPG, a selective agonist of group I mGluRs, to induce LTD pharmacologically. The activation of mGluRs by DHPG (100 μM, 10 min) caused an acute depression of the fEPSP slope to 51 ± 4% of baseline values (closed circles, $p < 0.001$ compared to pre-DHPG baseline, $n = 14$; Fig. 1A). After wash-out of

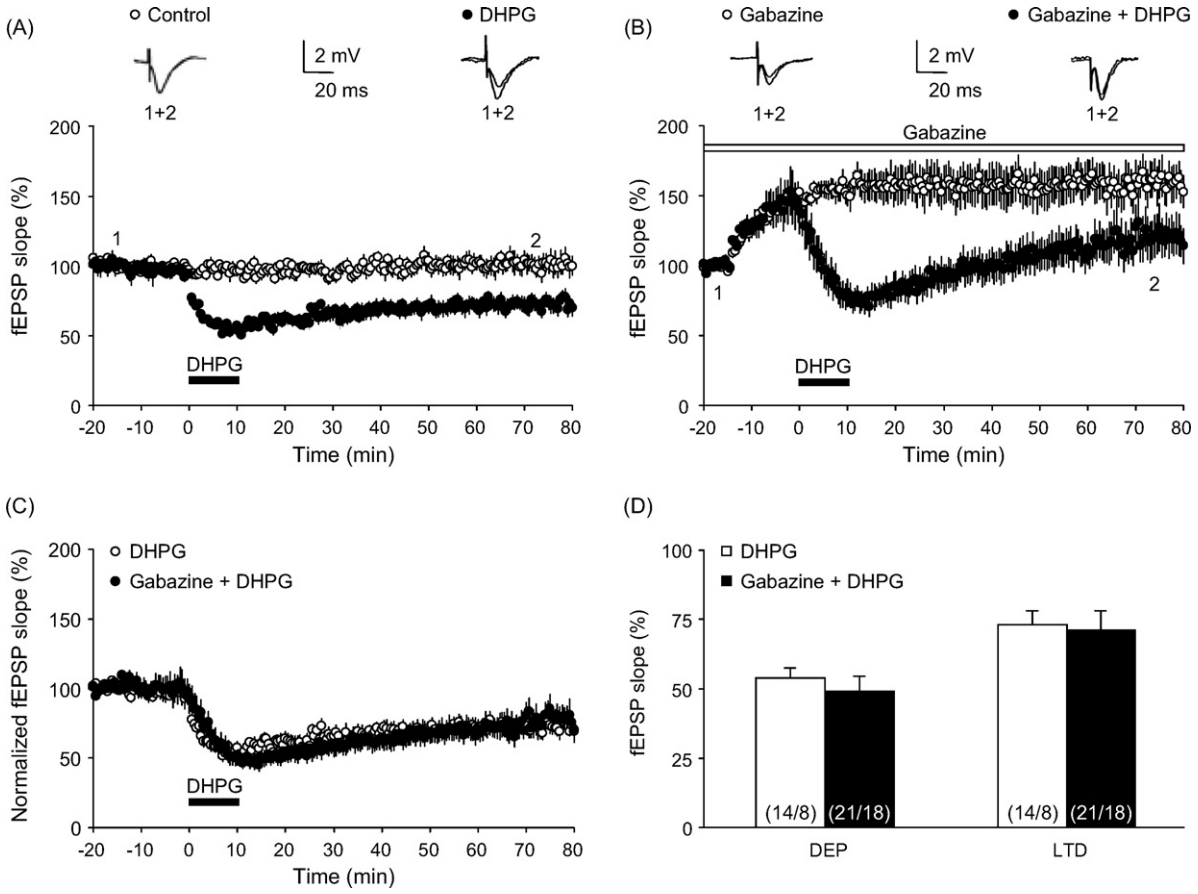

Fig. 1. $GABA_AR$ inhibition by gabazine does not affect DHPG-induced LTD. (A) Time course of the fEPSP slope following DHPG treatment (100 μM, indicated by the black bar, closed symbols) shows a significant LTD compared to slices without any treatment (open symbols). Representative sample traces were taken from the timepoints indicated by numbers. (B) Under $GABA_AR$ blocking conditions with gabazine (5 μM, indicated by the open bar), the fEPSP slope following DHPG treatment (indicated by the black bar, closed symbols) shows a marked reduction compared to experiments without DHPG treatment (open symbols) that show a strong fEPSP enhancement above baseline values. (C) Time course of the fEPSP slope following DHPG treatment normalized to slices without DHPG application. Note that there is no difference in DHPG effect between control (open symbols) and $GABA_AR$ blocking conditions (closed symbols). (D) Bar graph comparing the acute depression following DHPG treatment (DEP) and DHPG-induced LTD in control (open bars) and in $GABA_AR$ blocking conditions (closed bars). The number of slices is given in parenthesis (number of DHPG-treated slices/number of interleaved slices without DHPG application).