L-type Ca^{2+} channel-mediated short-term plasticity of GABAergic synapses

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In the cerebral cortex, the major inhibitory neurotransmitter GABA (γ-aminobutyric acid) is released by GABAergic neurons[1] onto $GABA_A$ and $GABA_B$ receptors, and regulates neuronal excitability, postsynaptic action potential firing, and dendritic and synaptic integration[2]. Various interneurons use either N- or P/Q-type Ca^{2+} channels for the Ca^{2+} influx into their terminals[3], whereas L-type Ca^{2+} channels are not normally associated with GABA release. In dual recordings from hippocampal basket cells and granule cells, we now report that short-term plasticity of GABA release is controlled by L-type Ca^{2+} channels at presynaptic firing rates in the gamma-frequency (40 Hz) range[4]; at these GABAergic synapses, L-type Ca^{2+} channel antagonists converted post-tetanic potentiation into depression, identifying L-type Ca^{2+} channels as important modulators of plasticity at GABAergic synapses.

Fast-spiking basket cells (**Fig. 1a**) were identified on the border between the granule cell layer and the hilus in the rat dentate gyrus in brain slices. In dual recordings from basket cells and granule cells in 3 mM kynurenic acid, stimulation of the basket cell to fire single action potentials (APs) evoked a $GABA_A$ receptor-mediated IPSC (eIPSC) in the granule cell (**Fig. 1b**). Baseline responses were obtained by presynaptic paired-pulse stimulation (50 ms interval) evoking two eIPSCs ($eIPSC_1$ and $eIPSC_2$) every 5 s. The amplitude of $eIPSC_1$ was 139 ± 32 pA (33°C, $n = 9$) and showed quantal-like variations, including failures (11.1 ± 5.4%). Perfusion of the L-type Ca^{2+} channel antagonist nifedipine (10 μM) did not affect the presynaptic AP (half-width, 0.53 ± 0.06 ms in control versus 0.60 ± 0.08 ms with nifedipine, $p > 0.05$, $n = 4$). Nifedipine also had no effect on the $eIPSC_1$ amplitude (96 ± 15% of control, $p > 0.05$, $n = 4$) or its variance, latency, 10–90% rise time or decay kinetics. Another L-type Ca^{2+} channel blocker, diltiazem (25 μM), also failed to affect $eIPSC_1$ (amplitude, 105 ± 16% of control, $p > 0.05$, $n = 4$) or the presynaptic AP. Miniature IPSCs recorded in granule cells in the presence of $CdCl_2$ (100 μM) were also unaffected by nifedipine or diltiazem.

We then examined the short-term plasticity of IPSCs evoked by high-frequency APs in the range of the normal firing behavior of basket cells[5]. We induced post-tetanic potentiation (PTP) of eIPSCs by stimulating the basket cells in the gamma-frequency range (40 Hz, 2 s). PTP is a reversible enhancement of transmitter release that lasts for minutes, and has been observed at many synapses[6,7]. In our experiments, PTP increased $eIPSC_1$ by 34.2 ± 9.2% (averaged at 5–45 s post-tetanus, $p < 0.05$, paired *t*-test) and lasted about 120 s (**Fig. 2a** and **d**, $n = 12$). During this enhancement, paired-pulse depression of the eIPSCs also increased, consistent with PTP being a presynaptic phenomenon. Hence, the $eIPSC_2$:$eIPSC_1$ ratio decreased from 0.95 ± 0.06 to 0.84 ± 0.07 ($p < 0.05$, paired *t*-test).

In sharp contrast to the lack of effect on single IPSCs, blockade of L-type Ca^{2+} channels eliminated PTP and turned it into a depression lasting for about 80 s (**Fig. 2b–d**). In nifedipine, the post-tetanus $eIPSC_1$ was reduced to 85.3 ± 11% of control, versus 129 ± 12% of control before nifedipine was perfused ($p < 0.05$, $n = 4$, paired *t*-test). In diltiazem, the post-tetanus $eIPSC_1$ was 76.3 ± 7.1% of control (**Fig. 3b**), versus 122 ± 6.4% of control before diltiazem ($p < 0.05$, $n = 4$). These findings are consistent with a critical role of L-type Ca^{2+} channels in the induction of post-tetanic potentiation of GABAergic IPSCs. In the presence of nifedipine, the post-tetanic IPSCs showed paired-pulse facilitation, so that the $eIPSC_2$:$eIPSC_1$ ratio increased from 0.69 ± 0.06 to 1.07 ± 0.15 (**Fig. 3c**, $p < 0.05$, paired *t*-test). In diltiazem, the tetanus increased the $eIPSC_2$:$eIPSC_1$ ratio from 0.86 ± 0.07 to 1.27 ± 0.25 ($p < 0.05$). The L-type channel antagonists had no effect on the progressive tetanic depression of the eIPSCs occurring during the 80-pulse stimulation at 40 Hz ($p > 0.05$). Nifedipine and diltiazem also failed to block the post-tetanus increase in spontaneous IPSCs. (See the supplementary information page of *Nature Neuroscience* online.)

Here we confirm that L-type Ca^{2+} channels are not involved in low-frequency synaptic transmission at GABAergic synapses[8]. However, the contribution of L-type channels to synaptic transmission became evident when GABAergic synapses were driven by high-frequency firing in the gamma-frequency range[4], which led to PTP. The rapid onset of PTP suggests that the presynaptic L-type Ca^{2+} channels are located near the GABAergic terminals. This is surprising because L-type channels have yet to be anatomically detected at these sights. Given that L-type Ca^{2+} channel antagonists block PTP but not the 'late release' (above and ref. 8), whereas EGTA-AM blocks 'late release' but not PTP[7,9], we propose that the L-type Ca^{2+} channels are coupled to second messenger cascades regulating the availability of transmitter vesicle pools. L-type Ca^{2+} channel activity could activate calmodulin-mediated processes[10,11], which in

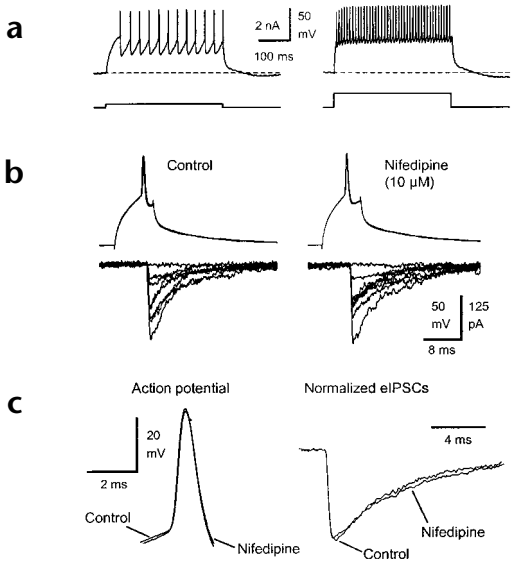

Fig 1. Nifedipine did not affect GABAergic IPSCs evoked by low-frequency basket cell firing. (**a**) Basket cell firing in the dentate gyrus in rat brain slices. (**b**) Dual whole-cell recording from a basket cell and a granule cell (bottom) in 3 mM kynurenic acid. Stimulation of the basket cell to fire single action potentials evoked short-latency eIPSCs in the granule cell. Consecutive eIPSCs were evoked every 5 s and superimposed. The L-type Ca^{2+} channel antagonist nifedipine (10 μM) did not affect the eIPSCs. (**c**) The presynaptic action potential and the eIPSCs shown on an expanded time scale. Their time course was unaffected by nifedipine.

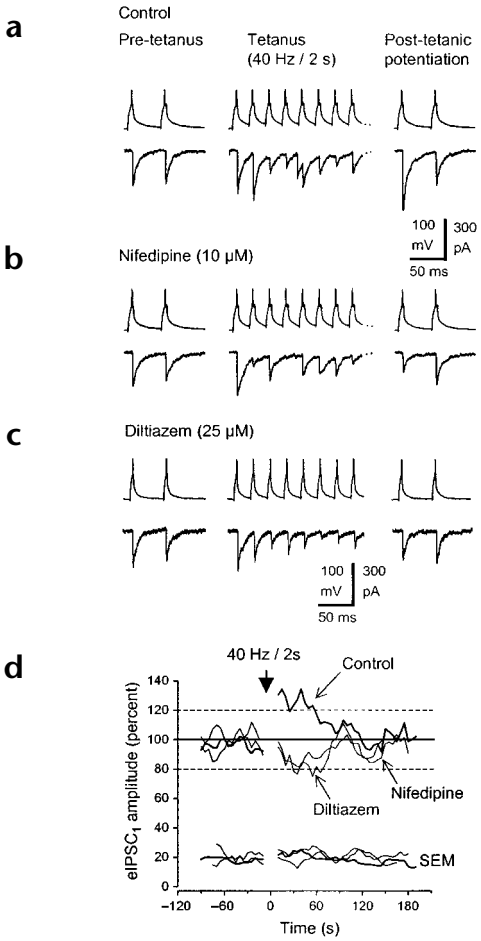

Fig. 2. Post-tetanic potentiation of IPSCs was blocked by L-type Ca^{2+} channel antagonists. (**a**) Paired-pulse stimulation of the basket cell to fire APs evoked two IPSCs ($eIPSC_1$ and $eIPSC_2$) every 5 s in the granule cell. After tetanic stimulation of the basket cell (40 Hz, 2 s), $eIPSC_1$ displayed post-tetanic potentiation by 75% and the paired-pulse depression of $eIPSC_2$ increased. (**b**) Following perfusion of nifedipine, the pre-tetanic $eIPSC_1$ was unaffected, whereas the post-tetanic $eIPSC_1$ was depressed to 63% of control and $eIPSC_2$ showed paired-pulse facilitation. (**c**) The L-type channel antagonist diltiazem (25 μM) also caused post-tetanic depression. (**d**) Post-tetanic potentiation of $eIPSC_1$ in control (n = 12) compared with post-tetanic depression in nifedipine (n = 4) or diltiazem (n = 4). Tetanic stimulation of the basket cell was delivered at 0 s. For each trial, $eIPSC_1$ amplitudes were normalized to the pre-tetanic baseline (100%), plotted and averaged across cells. The plot was smoothed by a running average of six responses. The standard error for each group (also smoothed) is shown below.

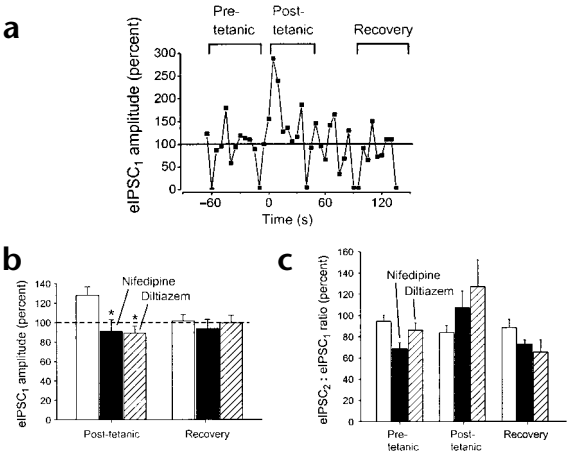

Fig. 3. The post-tetanic eIPSC amplitude and paired-pulse modulation. (**a**) PTP in a single cell pair. Data were analyzed as indicated by brackets. Pre-tetanic, average of 12 eIPSCs before the tetanus; post-tetanic, average of 9 eIPSC after the tetanus; recovery, average of 12 eIPSCs when the responses had recovered. (**b**) Post-tetanic $eIPSC_1$ in control (white bars, n = 12), in nifedipine (n = 4) and in diltiazem (n = 4). The normalized $eIPSC_1$ amplitudes showed PTP in control, and depression in nifedipine and diltiazem ($p < 0.05$). (**c**) The $eIPSC_2$:$eIPSC_1$ ratios illustrating the paired-pulse behavior of the IPSCs. In control, post-tetanic IPSCs showed a small increase in paired-pulse depression (white bars, $p < 0.05$). In the presence of the L-type Ca^{2+} channel blockers, post-tetanic eIPSCs displayed paired-pulse facilitation ($p < 0.05$), indicating that the release probability had been lowered.

turn could activate kinases, such as myosin light-chain kinase (MLCK), controlling vesicle recycling and the mobilization of a vesicle reserve pool[12]. Ca^{2+} entry through L-type Ca^{2+} channels may thus mobilize vesicles into the releasable pool to ensure steady transmission following high-frequency bursts. Accordingly, we observed a transient post-tetanic depression when L-type channels were blocked, resembling the long-lasting (>20 s) recovery from synaptic depression induced by extensive presynaptic trains[13]. With the potentiating L-type Ca^{2+} channel-dependent mechanism engaged, the terminals would be protected against synaptic depression by the increase of the releasable pool. *In vivo*, GABAergic basket cells[14] fire at frequencies in the 40 Hz (gamma) range[5]. Thus, it is likely that hippocampal gamma activity activates the L-type channel-mediated potentiation of GABA release, which may be critical for frequency shifts[15] in the cerebral cortex. Therefore, L-type Ca^{2+} channels could be important in controlling the population activity in highly interconnected hippocampal neurons.

Note: Supplementary methods are available on the Nature Neuroscience web site (http://neuroscience.nature.com/web_specials.)

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