Mechanisms Underlying LTP of Inhibitory Synaptic Transmission in the Deep Cerebellar Nuclei

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Ouardouz, Mohamed and Bhagavatula R. Sastry. Mechanisms underlying LTP of inhibitory synaptic transmission in the deep cerebellar nuclei. J Neurophysiol 84: 1414–1421, 2000. Whole-cell recordings were used to investigate long-term potentiation of inhibitory synaptic currents (IPSCs) in neurons of deep cerebellar nuclei (DCN) in slices. IPSCs were evoked by electrical stimulation of the white matter surrounding the DCN in the presence of non-N-methyl-D-aspartate (non-NMDA) glutamate receptor antagonist 6,7-dinitroquin- oxaline-2,3-dione (20 μM). High-frequency stimulation induced a long-term potentiation (LTP) of the IPSC amplitude without changing its reversal potential, rise time, and decay-time constant. This LTP did not require the activation of postsynaptic γ-aminobutyric acid-A (GABA_A) receptors but depended on the activation of NMDA receptors. LTP of IPSCs in DCN neurons could also be induced by voltage-depolarizing pulses in postsynaptic neurons and appeared to depend on an increase in intracellular calcium as the LTP was blocked when the cells were loaded with a calcium chelator, 1,2-bis-(2-amino-phenoxy)-N,N,N',N'-tetraacetic acid (BAPTA, 10 mM). LTP of IPSCs was accompanied by an increase in the frequency of spontaneous IPSCs and miniature IPSCs (recorded in the presence of tetrodotoxin 1 μM), but there was no significant change in their amplitude. In addition, during the LTP, the amplitude of response to exogenously applied GABA_A receptor agonist 4,5,6,7-tetrahydroxyoxazolol[5,4-c]pyridin-3-ol hydrochloride was increased. Intracellular application of tetanus toxin, a powerful blocker of exocytosis, in DCN neuron prevented the induction of LTP of IPSCs. Our results suggest that the induction of LTP of IPSCs in the DCN neurons likely involves a postsynaptic locus. Plasticity of inhibitory synaptic transmission in DCN neurons may play a crucial role in cerebellar control of motor coordination and learning.

INTRODUCTION

Activity-dependent, long-term changes of synaptic transmission has been recognized as an important phenomenon in cellular learning and memory in the CNS. Plasticity of excitatory synapses has been extensively studied for the last two decades. The majority of the inhibitory synapses in the CNS use GABA as a neurotransmitter. Long-term changes in GABAAergic synaptic transmission should have significant implications for CNS function. Long-term potentiation (LTP) of GABAAergic synapses had been reported in CA1 pyramidal cells following tetanic stimulation (Morishita and Sastry 1991; Xie and Sastry 1991). This LTP does not require activation of N-methyl-D-aspartate (NMDA) receptors (Shew et al. 2000) or an increase of intracellular calcium (Morishita and Sastry 1991; Perez et al. 1999). However, LTP of inhibitory synapses reported on Purkinje cells (Kano et al. 1992), in visual cortex (Komatsu and Iwakiri 1993), in the CA3 area of hippocampus (McLean et al. 1996), and in deep cerebellar nuclei (DCN) (Aizenman et al. 1998) depends on an increase of intracellular calcium. The mechanisms underlying LTP of inhibitory synapses induced by intracellular calcium elevation are still unclear. Increase of intracellular-free calcium may lead to activation of certain protein phosphorylation, which has been reported to increase γ-aminobutyric acid-A (GABA_A) receptor function (Angelotti et al. 1993; Cheun and Yeh 1992; Feigenspan and Bornmann 1994; Kapur and Mcdonald 1996; Lin et al. 1994; Moss et al. 1995). Membrane fusion and insertion of new GABA_A receptors at synapses may also be involved in long-term changes observed during LTP of inhibitory synapses. Similar mechanisms have been described for excitatory synapses in CA1 pyramidal cells (Liao et al. 1999; Lledo et al. 1998; Petralia et al. 1999) where LTP has been extensively studied. In a recent work, Wan et al. (1997) showed that insulin causes GABA_A receptors to translocate rapidly from intracellular compartment to the plasma membrane in HEK 293 cells and in hippocampal slices.

In the present work, we first show that high-frequency stimulation can induce LTP of inhibitory synaptic currents (IPSCs) in DCN neurons. This LTP appeared to depend on NMDA receptor activation and a rise of intracellular-free calcium levels and it did not seem to require activation of GABA_A receptors. Second, we analyzed the changes affecting spontaneous and miniature (recorded in the presence of tetrodotoxin) IPSCs and responses to exogenous application of a GABA_A receptor agonist, during LTP. Third, we examined the effects of an inhibitor of membrane fusion, tetanus toxin, on the expression of LTP.

METHODS

Slice preparation

Sagittal cerebellar slices were prepared as previously described (Morishita and Sastry 1996). The cerebellum was placed in cold artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 3
KCl, 1.8 NaH₂PO₄, 2 MgSO₄, 2 CaCl₂, 26 NaHCO₃, and 10 dextrose previously saturated with 95% O₂-5% CO₂. The pH was adjusted with NaOH to 7.3. Sagittal cerebellar slices (400-μm thick) were cut with a vibratome and transferred into a container filled with oxygenated ACSF at room temperature. After an incubation period of 1 h, a slice was placed into a recording chamber and maintained submerged using a U-shaped platinum wire. The slice was continuously perfused with oxygenated ACSF at room temperature.

**Recordings and stimulation**

The cells were visually identified using an upright microscope (Zeiss) equipped with Nomarski optics and an infrared camera. Patch electrodes were pulled from thin-wall, 1-mm outer diameter, borosilicate glass tubing with a filament (WPI Sarasota). Electrode resistance, measured in the bath solution, ranged from 5 to 10 MΩ. Recording electrode solutions contained (in mM) 135 K-glucinate, 10 HEPES, 10 KCl, 1 K₂-bis-(2-aminophenoxy)-N,N,N',N' -tetraacetic acid (BAPTA), 5 Mg-ATP, 0.1 CaCl₂, 10 Na₂-phosphocreatine, 0.4 Na₃-GTP, and 50 U/ml creatine phosphokinase (pH adjusted to 7.2–7.3 with KOH). In miniature IPSC experiments, K-glucinate was replaced with CsCl (135 mM). Tight seals (>2 GΩ) were obtained on DCN neuron cell bodies (Edwards et al. 1989) and whole-cell recordings were made after rupturing the membrane with negative pressure (Hamill et al. 1981). Voltage-clamp recordings were made using Axopatch 200A (Axon Instruments) with low-pass filtering at 2 kHz. Recordings were digitized and stored with a PC-DOS microcomputer-based data acquisition system (Digidata 1200 and pClamp6, Axon Instruments) at 5 kHz. Recordings were considered acceptable if the series resistance and capacitance could be properly compensated to 75% and if IPSCs and holding current were stable. If the series resistance changed by more than 15%, the cell was discarded from analysis.

**Stimulations and pharmacological manipulations**

IPSCs were evoked with a bipolar platinum microelectrode placed in the white matter surrounding the DCN. Control and test IPSCs were evoked by constant current pulses (0.1–0.2 ms duration, 0.2 Hz) using a stimulus isolator (Grass Instrument). Cells were accepted if IPSCs were stable during the initial 10-min control period. To elicit LTP, high-frequency stimulation (HFS) consisting of two trains of 20 stimuli at 100 Hz (20-s interval) were delivered at the control stimulus intensity under current-clamp conditions. Voltage-pulse-induced LTP was elicited by two trains of depolarizing pulses delivered into recorded neurons (18 pulses of 200 ms duration at 2 Hz, 20-s interval between trains) from a holding potential of ~50 to 0 mV. All experiments were carried out in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM). For statistical analysis of individual cells (t-tests and paired t-tests, level of significance, P < 0.05), the mean peak amplitude of IPSCs was compared between blocks of 60 consecutive responses: control (5 min before LTP induction) and 25–30 min after LTP induction. Group data were expressed as means ± SE.

Spontaneous IPSCs (sIPSCs) and miniature IPSCs (mIPSCs, recorded in the presence of TTX, 1 μM) were collected using Flechtere (Axon Instruments) and analyzed using MiniAnalysis Software (Jaejin Software, Leonia, NJ). The detection threshold was set at 5–10 pA. To calculate the frequency of mIPSCs or sIPSCs, the inter-event interval was quantitated for a 2-min bin and an average taken. Since mIPSCs were recorded in the presence of TTX, changes in the activity of Purkinje cells will not affect the number of mIPSCs observed. The cumulative probability distributions were constructed using a bin-width of 0.5 s for inter-event interval and 5 pA for the amplitude of sIPSCs (more than 500 events) and mIPSCs (more than 150 events) as illustrated in Fig. 4 and 5. Significant differences (P < 0.05 and <0.001) in the cumulative frequency and amplitude of sIPSC and mIPSC were analyzed using the Kolmogorov-Smirnov two-sample test using all the collected data (K-S test, Jaejin Software).

For iontophoretic application of drugs, patch electrodes were filled with 4,5,6,7-tetrahydroisoaxasol[5,4-c]pyridin-3-ol hydrochloride (THIP, 10 mM), a GABA_A-receptor agonist. The electrode was placed in the vicinity (50–100 μm) of the recorded cell. THIP was applied by an ejecting current of 100 nA for 2–3 s, at 3-min intervals.

Stock solutions of NMDA antagonist (±2-amino-5-phosphonovaleric acid (APV, 5 mM, Precision Biochemical), non-NMDA receptor antagonist DNQX (2 mM, Precision Biochemical) and bicuculline methiodide, a GABA_A-receptor antagonist (Bic, 2 mM, Sigma), were prepared in distilled water and stored frozen. On the day of the experiment, antagonists were diluted to their final concentration in ACSF and applied in the superfusion medium. For experiments with the calcium chelator BAPTA (Molecular Probes), the following changes were made to the pipet solution: 10 mM BAPTA was added and K-glucinate was reduced to 110 mM.

**RESULTS**

**LTP of IPSC in DCN neurons**

Whole-cell recordings in voltage-clamp mode were made from visually identified neurons in DCN. Electrical stimulation (at 0.2 Hz) of the white matter surrounding the DCN evoked outward synaptic currents (IPSCs, Fig. 1B, control) in the presence of DNQX (20 μM). This response was completely and reversibly blocked by bicuculline (25 μM; see Fig. 2, A1 and A2).

HFS (2 trains of 20 pulses at 100 Hz, 20 s interval), given in current-clamp mode when the cell was held at ~50 mV, resulted in an increase of the IPSC amplitude in DCN neurons. As illustrated in Fig. 1, the IPSC amplitude increased immediately after tetanization and remained potentiated for at least 30 min when the experiment was usually terminated. This change was statistically significant (141.7 ± 13.0% of IPSC control, paired t-test, P < 0.05, n = 11). When examined in individual cells, 8 of 11 cells displayed a significant LTP (t-test, P < 0.05). None of the cells displayed significant long-term depression (LTD) when tetanized at this frequency. The reversal potential of the IPSC was not significantly different after LTP induction (−67.1 ± 0.9 mV, 30 min after HFS) compared with control (−68.5 ± 0.9 mV, t-test, P > 0.05, n = 5). The rise time and decay time constants of potentiated IPSCs (3.4 ± 0.4 and 21.3 ± 1.3 ms, respectively, n = 8) were not significantly different from those of control IPSCs (3.0 ± 0.4 and 20.3 ± 1.8 ms, respectively, t-test, P > 0.05), suggesting that the IPSC kinetics remained unchanged after LTP induction.

**Induction of LTP does not depend on the activation of GABA_A receptors but requires the activation of NMDA receptors**

In previous work from this laboratory, it was shown that the induction of LTD of the IPSCs in DCN neurons does not require activation of GABA_A receptors (Morishita and Sastry 1996). To test whether the induction of LTP depends on GABA_A receptor activation, HFS stimulation was given during bath application of bicuculline methiodide (20 μM for 5 min). The IPSC was completely blocked after 3 min perfusion in the drug (Fig. 2, A1 and A2). In control experiments (without HFS), the IPSC recovered to 90.4 ± 2.5% of control after 30
min \((n = 6)\). When HFS was given at the end of bicuculline application while the IPSC was completely blocked, a depolarizing potential and in some cases a series of action potentials were recorded (see Fig. 2B, HFS). In this case, the IPSC recovered faster than in controls and the amplitude of the IPSC was significantly increased compared with control \((132.6 \pm 12.0\% \text{ of control IPSC amplitude, paired } t \text{-test, } P < 0.05, n = 7)\). These results suggest that LTP of the IPSC, as in the case of LTD (Morishita and Sastry 1996), does not require the activation of GABA\(_A\) receptors. However, during HFS, a depolarizing potential was revealed, which in some cases was accompanied by a series of action potentials. This excitatory potential may be due to the activation of NMDA receptors as the non-NMDA receptors were blocked by DNQX (20 mM).

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**FIG. 1.** Long-term potentiation (LTP) of inhibitory postsynaptic currents (IPSCs) in deep cerebellar nuclear (DCN) neurons. A: time course of IPSC amplitudes (expressed as % of control) following high-frequency stimulation (HFS, filled circles, \(n = 11\)) and in the absence of HFS (control experiments, open triangles, \(n = 5\)). HFS (2 trains of 20 stimuli at 100 Hz, with 20-s inter-train interval) was given when the recorded cells were held in current-clamp mode (see arrow). Note that there was a long-lasting increase in IPSC amplitude after HFS. B: evoked GABA\(_A\) IPSCs (averages of 12 consecutive sweeps) recorded for the periods indicated in A (1: before HFS, 2: 30 min after HFS); the trace in the middle shows the record taken during HFS. 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 \(\mu M\)) was present throughout the experiment in A and B.

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**FIG. 2.** Induction of LTP of the IPSC in DCN neurons does not depend on GABA\(_A\)-receptor activation but requires the activation of NMDA receptors. A1: time course of the effect of bicuculline (bar, Bic 20 \(\mu M\), applied for 5 min) on IPSC amplitude expressed as % of control \((n = 6)\). A2: evoked IPSCs (average of 12 consecutive sweeps) 1: before, 2: during, and 3: after 30 min washout of bicuculline, as indicated in graph A1. B1: same protocol as in A1 but high-frequency stimulation (HFS as in Fig. 1, \(n = 7\)) was given at the end of bicuculline application. B2: evoked IPSCs 1: before and 2: at 30 min after HFS (see Recovery) in the middle response to HFS recorded in current clamp conditions. C1: time course of IPSC amplitude expressed as % of control. Note that HFS given in the presence of APV (50 \(\mu M\), \(n = 5\)) did not induce LTP. C2: evoked IPSCs recorded 1: before and 2: 30 min after HFS.
To address this issue, HFS was given in the presence of NMDA receptor antagonist APV (50 μM). Under these conditions, HFS induced a hyperpolarizing potential followed in some cells by a series of action potentials (Fig. 2C2). The IPSC was, however, not potentiated (92.8 ± 18.3% of control IPSC amplitude 30 min after HFS, paired t-test, \( P > 0.05, n = 5 \)), suggesting that NMDA-receptor activation and probable increase of intracellular calcium were needed for LTP induction.

**Voltage-depolarizing pulse-induced LTP of the IPSC in DCN neurons**

Intracellular calcium can be increased by activation of voltage-gated calcium channels in DCN neurons (Muri and Knopfel 1994). In recent studies, it has been shown that rebound action potentials following a tetanic stimulation of inhibitory synapses in the presence of kynurenate (an excitatory amino acid receptor antagonist) can induce LTP in DCN neurons (Aizenman et al. 1998). We tested if depolarizing voltage pulses given at high frequency into the recorded DCN neurons can induce a sustained potentiation of IPSCs. In these experiments, IPSCs were evoked in the presence of APV (50 μM) and DNQX (20 μM) before and after a series of voltage-depolarizing pulses. The cells were voltage clamped at −50 mV; two trains of 18 depolarizing voltage pulses to 0 mV for 200 ms at 2 Hz were delivered through the recording pipette with an inter-train interval of 20 s. Thirty minutes after the depolarizing voltage pulses, the IPSC amplitude was significantly increased to 126.4 ± 6.2% of control (paired t-test, \( P < 0.05, n = 11 \)). The LTP induced by the depolarizing pulse protocol developed slowly and is weaker than the one induced by HFS involving activation of NMDA receptors. One of 11 cells showed a significant LTD of the IPSC (85.2 ± 3.4% of control, t-test, \( P < 0.05 \)) and two cells did not exhibit any significant change in IPSC amplitude. To check if the potentiation observed with depolarizing voltage pulses, or following HFS of the inputs, was due to an increase of postsynaptic calcium, cells were loaded with a calcium chelator BAPTA (10 mM) to prevent a rise in intracellular-free calcium level. After establishing whole-cell configuration, a 10-min period was given to allow BAPTA to diffuse into the cell. Under these conditions, the IPSC amplitude was not potentiated after the depolarizing pulse protocol (\( n = 5 \)) or after HFS (\( n = 3 \)) (90.23 ± 11.2% of IPSC amplitude control, see Fig. 3B, pooled data, paired t-test, \( P > 0.05 \)). These results suggest that the induction of LTP, by depolarizing pulses or HFS, may depend on an increase in intracellular-free calcium.

**Changes in spontaneous IPSCs during LTP**

DCN neurons recorded in voltage-clamp mode at −55 mV displayed outward sIPSCs (an example is illustrated in Fig. 4A) with an average frequency of 8 ± 2.3 Hz (\( n = 4 \)). Their amplitudes varied from 10 to 130 pA (average amplitude 31.2 ± 1.7 pA) and the sIPSCs were blocked in the presence of bicuculline (data not shown). After 15 min of establishing whole-cell recording, two trains of depolarizing pulses were applied to the DCN neurons as in the protocol used in Fig. 3 or HFS was given to the inputs. Thirty minutes after the LTP induction either by HFS (\( n = 3 \)) or depolarizing current pulse protocol (in one cell), the frequency of sIPSCs was significantly increased to 144.3 ± 21.8% of control (paired t-test, \( P < 0.05, n = 4 \)). The inter-event interval cumulative probability distribution of sIPSCs was significantly shifted to the left in three of four cells tested (Fig. 4D, K-S test, \( P < 0.001 \)). However, no significant change was observed in the mean sIPSC amplitude (106.5 ± 3.8 of control, paired t-test, \( P > 0.05, n = 4 \)). The amplitude cumulative probability distribution was not different from controls (an example is shown in Fig. 4C, K-S test, \( P > 0.05 \)).

**Changes in miniature IPSCs during LTP**

mIPSCs were recorded in chloride-loaded neurons at a holding potential of −60 mV in the presence of TTX (1 μM), DNQX (20 μM), and APV (50 μM) (Fig. 5A shows an example of mIPSCs). IPSCs were considered miniature IPSCs after abolition of the evoked synaptic response and of sodium action potentials by TTX. The frequency of mIPSCs (3.3 ± 1.1 Hz, \( n = 6 \)) was 2.6-fold lower than the sIPSC frequency indicating that some of sIPSCs were action-potential dependent. Depolarizing pulse protocol induced a significant increase of the frequency of mIPSCs (163.8 ± 23.5% of control, paired t-test, \( n = 6 \); an example is shown in Fig. 5, A and B). Figure 5D illustrates change in cumulative probability distribution of inter-event intervals observed after LTP induction. This shift to the left was significant in three of six cells tested at a level of \( P < 0.001 \) (K-S test) and in five of six cells at a level of \( P < 0.05 \) (K-S test). As in the case of sIPSCs, the mean amplitude
of mIPSC (39.2 ± 6.9 pA, n = 6) was not affected (97.8 ± 2.7% of control mIPSCs amplitude, paired t-test, \( P < 0.05, n = 6 \)) and there was no significant change in cumulative probability distribution of the amplitude after LTP induction (K-S test; \( P > 0.05 \)). Figure 6D shows an example of mIPSC amplitude cumulative distribution before and after LTP induction. These results suggest that LTP of IPSCs can be either a presynaptic phenomenon caused by an increase in the probability of release of GABA or a postsynaptic one induced possibly by recruitment of quantal responses of comparable size at previously silent synapses.

**Responses to exogenously applied GABA\(_A\) agonist, THIP, during LTP**

One way to test if GABA\(_A\) receptors on the postsynaptic cells are affected during LTP induction is by determining if the action of exogenous application of GABA\(_A\)-receptor agonist THIP (10 mM) is changed. This agent was, therefore, iontophoretically applied (100 nA, 2–3 s) in the vicinity of the recorded cell at 3-min intervals. Depolarizing pulse protocol induced an increase in the amplitude of the THIP response in DCN neurons (see Fig. 6). This increase was statistically significant and occurred in a manner consistent with an increase in the number of active synapses.

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![Figure 4](http://jn.physiology.org/)

**Fig. 4.** Changes in spontaneous IPSCs during LTP. Spontaneous IPSCs (sIPSCs) were recorded from DCN neuron voltage clamped at \(-55\) mV, in the presence of DNQX (20 \(\mu\)M). A: examples of consecutive traces of sIPSCs before (control) HFS (2 trains of 20 stimuli at 100 Hz, at 20-s interval). B: consecutive traces of sIPSCs recorded 30 min after HFS. C: sIPSC amplitude cumulative probability distribution before (control) and after HFS (LTP) derived from 2-min recordings; note that the amplitudes of sIPSCs in the two are not significantly different (K-S test, \( P > 0.05 \)). D: inter-event interval cumulative probability of sIPSCs before (control) and 30 min after HFS (LTP) derived from 2-min recordings. Note that the frequency of sIPSCs was significantly increased after LTP induction (K-S test, \( P < 0.001 \)).

![Figure 5](http://jn.physiology.org/)

**Fig. 5.** Changes in miniature IPSCs during LTP. Miniature IPSCs (mIPSCs) were recorded from chloride-loaded DCN neuron voltage clamped at \(-60\) mV, in the presence of TTX (1 \(\mu\)M), DNQX (20 \(\mu\)M), and APV (50 \(\mu\)M). A: example of consecutive traces of mIPSCs before (control) a series of voltage-depolarizing pulses (18 pulses at 2 Hz, 2 times at 20-s interval) applied into the recorded cells. B: consecutive traces of mIPSCs recorded 30 min after the voltage-depolarizing pulse protocol (LTP) applied into the recorded cells. C: mIPSC amplitude cumulative probability distribution plots before (control) and 30 min after voltage-depolarizing pulse protocol (LTP) derived from 2-min recordings; note that the two are not significantly different from each other (K-S test, \( P > 0.05 \)). D: inter-event interval cumulative probability of mIPSCs, before (control) and 30 min after voltage-depolarizing pulse protocol (LTP) derived from 2-min recordings. Note that the frequency of mIPSCs was significantly increased after LTP induction (K-S test, \( P < 0.001 \)).
significant and persisted for at least 30 min (134.6 ± 14.4% of control THIP response, paired t-test, \( P < 0.05, n = 6 \)). The potentiation of the THIP response is more robust than the potentiation of evoked IPSCs induced by depolarizing pulse protocol.

**Blockade of LTP in DCN neurons injected with tetanus toxin**

We tested if LTP of IPSCs is dependent on exocytosis using tetanus toxin, a powerful inhibitor of exocytosis (Montecucco and Schiavo 1994). Tetanus toxin (20 nM) was, therefore, included in the recording solution. Under these conditions, the rise-time and decay-time constants of IPSCs (3.1 ± 1.1 and 20.2 ± 2.2 ms, respectively, \( n = 8 \)) were not significantly changed compared with cells recorded with the standard solution (t-test, \( P > 0.05 \)). Voltage-depolarizing pulse protocol (\( n = 5 \)) or HFS (\( n = 3 \)) given after 15 min of establishing whole-cell recordings induced no significant change in IPSCs amplitude in DCN neurons. In all cells, the IPSC amplitude 25–30 min after LTP induction protocol was 91.6 ± 5.7% of control (paired t-test, \( P < 0.05, n = 8, \) Fig. 7A), raising the possibility that exocytosis from postsynaptic cells might play a role in LTP of IPSCs in DCN neurons. To test whether the effect was due to the toxin, control experiments were conducted with heat-inactivated toxin in the recording solution. In these experiments, HFS induced a long-lasting increase of the IPSC amplitude (139.2 ± 12.5, paired t-test, \( P < 0.05, \) Fig. 7B). While our studies were in progress, similar studies on excitatory synapses in CA1 pyramidal cells had shown that LTP was reduced or blocked by N-ethylmaleimide and clodtridal toxin, agents that were known to block synaptosomal associated proteins (SNAP) and membrane trafficking (Lledo et al. 1998).

**DISCUSSION**

In the present work, our results indicate that activation of NMDA receptors by HFS of inputs to DCN neurons can induce LTP of the IPSC and that activation of postsynaptic GABA\(_A\) receptors is not required for this induction. LTP of evoked IPSCs was accompanied by an increase in the frequency of mIPSCs but not of their amplitude. Furthermore, responses of the postsynaptic neurons to exogenous application of GABA agonist, THIP, were increased during the LTP. The tetanus-induced LTP was not induced in the presence of APV, or in neurons recorded with BAPTA-containing electrodes, suggesting the involvement of NMDA receptor activation and intracellular calcium. Depolarizing pulses could induce LTP in control, but not in BAPTA-injected cells, supporting the involvement of activation of voltage-gated calcium channels as had been also suggested by Aizenman et al. 1998. Induction of the LTP was blocked by intracellular injection into postsynaptic cells of tetanus toxin, an agent that was known to prevent exocytosis.

**Plasticity of IPSC in DCN neurons**

IPSCs in DCN neurons can undergo either LTP following HFS at 100 Hz (2 trains of 20 pulses, with inter-train interval
of 20 s) or LTD after a 10-Hz stimulation for 5 min. The tetanus-induced LTP or LTD appears to depend on NMDA receptor activation and an increase in intracellular calcium, as they are both blocked by application of NMDA-receptor antagonist APV or the calcium chelator BAPTA. Activation of voltage-gated calcium channels in DCN neurons by depolarizing pulses can also induce LTP when given at 2 Hz or LTD when given at 0.1 Hz (Morishita and Sastry 1996).

LTP induced by HFS and activation of NMDA receptors is stronger than the one induced by voltage-depolarizing pulse or by rebound action potentials (Aizenman et al. 1998). The induction of LTP appears to depend on the number of synapses affected by the elevation of intracellular calcium concentration in postsynaptic cells. In fact, morphological data have shown that axon terminals of Purkinje cells make synaptic contacts on the soma as well as the proximal and distal dendrites and the initial segment of DCN neurons (Chan-Palay 1973, 1977; Palkovits et al. 1977). Excitatory synapses made by collaterals of climbing fibers (Iked and Matsushita 1974) and mossy fibers (Iked and Matsushita 1973) are also distributed on the soma and the dendritic tree. Activation of these terminals evoked excitatory synaptic responses in DCN neurons (Gardette et al. 1985; Ito et al. 1970; Llinas and Muhlhaler 1988). Stimulation of excitatory synapses and activation of NMDA receptors, which can occur during HFS, may induce a larger increase of intracellular calcium in the soma and the dendritic tree leading to a stronger LTP of IPSCs. In contrast, LTP induced by depolarizing pulses in postsynaptic cells was smaller, presumably because only the synapses located in the soma and proximal dendrites were affected. In fact, depolarizing pulse and rebound action potentials induce an increase of calcium mainly in the soma and proximal dendrites (Aizenman et al. 1998; Muri and Knopfel 1994). It is interesting that responses to THIP, applied near the soma, showed a robust potentiation after the depolarizing pulse protocol, suggesting that intracellular depolarizing voltage pulses affect only GABAA receptors in this area.

Mechanisms of LTP of IPSCs

Efficacy of inhibitory synaptic transmission can be modulated presynaptically by changes in the probability of release or the quantum content, or postsynaptically by changing the properties or the number of postsynaptic GABAA receptors. Analyses of spontaneous and miniature IPSCs show that during LTP, their frequency increases without any change in their amplitude, suggesting an increase in the probability of release at the presynaptic level as a mechanism. However, we cannot exclude changes at the postsynaptic level contributing to this change, especially since responses to ionophoretic applications of THIP near the soma are increased during the LTP of the IPSC. These results suggest that LTP of IPSCs, like LTD (Morishita and Sastry 1996) in DCN neurons, might occur at the postsynaptic level.

Our observations that the response to applied THIP, but not the mean amplitude of mIPSCs, is increased during LTP are not easy to reconcile. If LTP is associated with an increase in the extrasynaptic but not subsynaptic GABA-receptor activity, the results can be explained. If this really happens, then perhaps the LTP of IPSC is maintained through presynaptic mechanisms and our results that postsynaptic BAPTA and tetanus toxin interfere with LTP induction suggest that a retrograde message is involved. However, at present we really do not know whether only extrasynaptic GABA receptors are changed during LTP. Moreover, it is possible that a recruitment of silent synapses into active ones contribute to LTP. Since our mIPSC samples were from multiple synapses, as long as the mean amplitude of the mIPSCs from the newly recruited synapses fall within the range of the control mIPSCs, we may not detect any change in the overall amplitude even if the change is postsynaptic. If this were to happen, then an increase in the THIP response might well be due to an increase in subsynaptic receptors. Further studies are needed to investigate this problem.

Molecular studies of GABAα-receptor sub-units have demonstrated the presence of consensus sequences for different protein kinases (Browning et al. 1990, 1993; Tehrani and Barnes 1994; Wafford et al. 1991; Whiting et al. 1990). Increased protein phosphorylation may increase GABAA-receptor function (Angelotti et al. 1993; Cheun and Yeh 1992; Feigenspan and Bormann 1994; Kapur and Mcdonald 1996; Lin et al. 1994; Moss et al. 1995). The long-lasting increase of IPSC amplitude observed in the present study is unlikely to be due to changes in the phosphorylation state of the GABAA receptors, since such a change in the GABAA-receptor channel function could conceivably affect the amplitude of miniature IPSCs, which was not associated with LTP.

Another explanation for LTP of the evoked IPSC accompanied by an increase in the frequency of mIPSCs is an increase in active GABAergic synapses involving a recruitment either of very low probability release sites into high-probability sites or of subsynaptic receptor clusters that were previously non-functional at silent synapses. Changes in silent synapses had been described for plasticity of excitatory synapses in CA1 pyramidal cells (Durant et al. 1996; Isaac et al. 1995; Liao et al. 1999; Petralia et al. 1999). In a recent work, Wan et al. (1997) showed that activation of insulin receptors could cause GABAα receptors to translocate rapidly from an intracellular compartment to the plasma membrane. In fact, in the present study, tetanus toxin, a powerful inhibitor of exocytosis, blocked the induction of LTP of IPSCs when loaded into the postsynaptic cells, suggesting the involvement of postsynaptic neuronal exocytotic machinery in LTP. It is, however, not clear whether the relevant substances act as a retrograde messenger on presynaptic terminals and/or are involved in inducing postsynaptic changes. Similarly, LTP at excitatory synapses in CA1 pyramidal cells was suggested to involve postsynaptic membrane fusion as a mechanism (Lledo et al. 1998).

Insulin-induced increase in the response of CA1 neurons to applied GABA is accompanied by an increase in the amplitude as well as frequency of mIPSCs (Wan et al. 1997). However, in the present study on DCN neurons, no significant changes in the mean amplitudes of either sIPSCs or mIPSCs were observed during LTP. As mentioned previously, GABAergic synapses on DCN neurons are present on wide areas of the soma-dendritic tree. Therefore, our records of sIPSCs or mIPSCs are very likely from a number of these synapses. If the numbers of mIPSCs and sIPSCs of different amplitudes in our control samples, presumably from wide areas of the soma-dendritic tree, were increased during LTP, we may not detect any change in the mean amplitude of our sIPSCs and mIPSCs, even if the change is postsynaptic. Further studies are required.
to determine if the increase in the frequency of sIPSCs and mIPSCs is due to a presynaptic change.

Neuronal network behavior in the CNS is determined by a balance between inhibition and excitation. GABA is the major inhibitory transmitter in the mammalian CNS and changes in the efficacy of this transmission could significantly modulate the network behavior and, therefore, CNS function. In the cerebellum, DCN can influence motor behavior through various premotor centers in the brain stem such as the red nucleus, the thalamus, and superior colliculus. DCN neurons are under the control of an important inhibitory drive from Purkinje cells. Depending on the pattern of activation of the excitatory inputs from climbing and mossy fiber collaterals, either LTP (present work) or LTD (Morishita and Sastry 1996) can be induced, suggesting a complex modulatory role for these excitatory inputs. Plasticity in the inhibitory drive to DCN neurons can have significant implications for cerebellar control of motor coordination and learning.

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REFERENCES


