Evidence for NMDA and mGlu Receptor-Dependent Long-Term Potentiation of Mossy Fiber–Granule Cell Transmission in Rat Cerebellum

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D’Angelo, Egidio, Paola Rossi, Simona Armano, and Vanni Taglietti. Evidence for NMDA and mGlu receptor-dependent long-term potentiation of mossy fiber–granule cell transmission in rat cerebellum. J. Neurophysiol. 81: 277–287, 1999. Long-term potentiation (LTP) is a form of synaptic plasticity that can be revealed at numerous hippocampal and neocortical synapses following high-frequency activation of N-methyl-d-aspartate (NMDA) receptors. However, it was not known whether LTP could be induced at the mossy fiber–granule cell relay of cerebellum. This is a particularly interesting issue because theories of the cerebellum do not consider or even explicitly negate the existence of mossy fiber–granule cell synaptic plasticity. Here we show that high-frequency mossy fiber stimulation paired with granule cell membrane depolarization (~40 mV) leads to LTP of granule cell excitatory postsynaptic currents (EPSCs). Pairing with a relatively hyperpolarized potential (~60 mV) or in the presence of NMDA receptor blockers [5-amino-d-phosphonovaleric acid (APV) and 7-chloro-kyurenine acid (7-CI-Kyn)] prevented LTP, suggesting that the induction process involves a voltage-dependent NMDA receptor activation. Metabotropic glutamate receptors were also involved because blocking them with (+)-α-methyl-4-carboxyphenyl-glycine (MCPG) prevented potentiation. At the cytoplasmic level, EPSC potentiation required a Ca2+ increase and protein kinase C (PKC) activation. Potentiation was expressed through an increase in both the NMDA and non-NMDA receptor-mediated current and by an NMDA current slowdown, suggesting that complex mechanisms control synaptic efficacy during LTP. LTP at the mossy fiber–granule cell synapse provides the cerebellar network with a large reservoir for memory storage, which may be needed to optimize pattern recognition and, ultimately, cerebellar learning and computation.

INTRODUCTION

Long-term changes in synaptic transmission are thought to play an important role in brain learning and computation. Long-term potentiation (LTP) has been observed following high-frequency stimulation of glutamatergic synapses in the hippocampus and neocortex (Bliss and Collingridge 1993; Johnston et al. 1992; Kirkwood and Bear 1996). A common form of LTP is that involving N-methyl-d-aspartate (NMDA) receptors. Although several studies have shown that NMDA receptors are activated at the mossy fiber–granule cell (mf–GrC) relay in the cerebellum (D’Angelo et al. 1993; Ebralidize et al. 1996; Kadotani et al. 1996; Silver et al. 1992; Takahashi et al. 1996), it has not been known hitherto whether the mf–GrC relay could undergo LTP following high-frequency mossy fiber activity. This is indeed of interest because an influential theory of the cerebellum (Marr 1969) explicitly negates adjustable weights at the mf–GrC synapse, focusing interest on long-term depression (LTD) at the parallel fiber–Purkinje cell synapse (Linden 1995).

A hallmark of NMDA receptor-dependent LTP is its need for neuronal depolarization. Depolarization removes the Mg2+ block from NMDA channels, allowing Ca2+ permeation and consequent activation of intracellular Ca2+-dependent processes (Bliss and Collingridge 1993). Metabotropic glutamate (mGlu) receptors are probably also involved in NMDA receptor-dependent LTP (Bashir et al. 1993; Bortoletto et al. 1994; O’Connor et al. 1995). Among different subtypes, the most likely candidates are type-1/5 mGlu receptors, which activate the phosphatidylinositol diphosphate (PIP2) cascade, reinforcing intracellular Ca2+ signaling and activating protein kinase C (PKC) (Pin and Duvoisin 1995; Riedel and Reymann 1996). In cerebellar granule cells, it has been shown that membrane depolarization unblocks NMDA channels (D’Angelo et al. 1993, 1995) and that pharmacological activation of mGlu receptors increases Ca2+ (Irving et al. 1992), activates the PIP2 cascade (Aronica et al. 1993), and enhances synaptic transmission (Kinnamon and Slater 1993; Rossi et al. 1996). Cerebellar granule cells are therefore endowed with the basic cellular mechanisms needed for generating LTP.

We show here that high-frequency mossy fiber stimulation paired with granule cell membrane depolarization induces a stable NMDA receptor-dependent enhancement of transmission (mf–GrC LTP). mf–GrC LTP requires mGlu receptors and involves intracellular Ca2+ and PKC activation. mf–GrC LTP is expressed by both NMDA and non-NMDA receptor-mediated currents, as observed in the hippocampus (Clark and Collingridge 1995; Kulman et al. 1996; O’Connor et al. 1995). In addition, the NMDA current slowed down. The mechanisms of mf–GrC LTP and its implications for cerebellar functions are discussed.

METHODS

Acute 250-μm thick cerebellar slices were obtained from 19- to 22-day-old Wistar rats as reported previously (D’Angelo et al. 1993, 1995). Briefly, the rats were anaesthetized with halothane (Aldrich) and killed by decapitation. Slices were cut in the sagittal plane from the cerebellar vermis in cold Krebs solution and main-
Estimates of whole cell recording stability

The cerebellar granule cell has a compact electrototoxic structure and behaves like a lumped electrotonic compartment (D’Angele et al. 1993; Silver et al. 1992). It can therefore be treated as a simple resistance capacitance (RC) system, in which relevant parameters can be extracted by analyzing passive current relaxation induced by step voltage changes (D’Angele et al. 1995; Rossi et al. 1996). Monoexponential fitting to current transients elicited by 10-mV hyperpolarizing voltage steps was applied 10 ms before each mossy fiber stimulus, allowing granule cell series resistance to be monitored throughout the recordings (see below). The mossy fibers were stimulated with a bipolar tungsten electrode via a stimulus isolation unit at a test frequency of 0.1 Hz. Following a 10-min control period, eight bursts of 10 impulses at 100 Hz were repeated every 250 ms [theta-burst stimulation (TBS)]. During TBS, membrane potential was stepped from −70 mV to either −40 mV or −60 mV. Data are reported as means ± SD, and statistical comparisons were done by using Student’s t-test (a comparison was considered significant at P > 0.05).

Solutions and drugs

The patch-clamp pipette solution contained (in mM) 81 CsSO4, 2 KCl, 1.2 MgSO4, 0.02 CaCl2, 0.1 bis-(α-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA), 10 glucose, ATP-Mg 3, gulosine 5′-triphosphate (GTP) 10−4, and 15 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH adjusted to 7.2 with CsOH. This solution maintained resting free [Ca2+] at 100 nM (Irving et al. 1992). In some experiments BAPTA was increased to 10 mM. Krebs solution for slice cutting and recovery contained (in mM) 120 NaCl, 2 KCl, 1.2 MgSO4, 26 NaHCO3, 1.2 KH2PO4, 2 CaCl2, and 11 glucose and was equilibrated with 95% O2-5% CO2 (pH 7.4).

Bicuculline was obtained from Sigma, and BAPTA tetrapotassium salt from Molecular Probes (Eugene, OR). The glutamate receptor antagonists 5-amino-3-phosphonovaleric acid (APV), 7-chloro-kynurenic acid (7-Cl-Kyn) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and (+)-α-methyl-4-carboxyphenyl-glycine (MCPG) were obtained from Tocris Cookson (Bristol, UK).

RESULTS

In this paper we investigated the potentiation of mf–GrC transmission with the use of patch-clamp recordings in acute rat cerebellar slices. Because mf–GrC transmission and NMDA receptor subunit composition change during development (D’Angele et al. 1993; Ebralidize et al. 1996; Takahashi et al. 1996), recordings were carried out between postnatal day (P) 19 and P22, when most granule cells and mossy fiber synapses are functionally mature. mf–GrC EPSCs are generated by non-NMDA and NMDA currents. Whereas the non-NMDA current has fast and almost voltage-independent kinetics, the NMDA currents are slow and are blocked by Mg2+ at negative membrane potentials (D’Angele et al. 1993; Silver et al. 1992). Thus as reported previously (O’Connor et al. 1995; Rossi et al. 1996), the non-NMDA current was measured as the EPSC peak at negative membrane potentials (−70 mV), and the NMDA current as the average of 20 data points around the 25th ms after mossy fiber stimulation at positive potentials (+40/+60 mV).

Moreover, NMDA current duration was measured as the EPSC half-width at +40/+60 mV, excluding the first 15 ms to prevent any influence of the non-NMDA current. The reliability of these estimates was supported by experiments in which the NMDA and non-NMDA currents were blocked pharmacologically (cf. Figs. 3 and 6).

In control experiments (Fig. 1A), we observed that both the EPSC peak amplitude at −70 mV and the amplitude at 25 ms at +60 mV tended to decrease over time, leading to a 27.4 ± 15.3% (n = 5) and a 35.9 ± 16.3% decrease (n = 5), respectively, 30 min after having established the whole cell recording configuration. The average time course of changes in EPSC amplitude obtained from five different granule cells is shown in Fig. 1C. It should be noted that NMDA EPSC duration also tended to decrease over time (the change in EPSC half-width at +60 mV, the 1st 15 ms excluded, was −16.3 ± 16.5%, n = 5; Fig. 1D).

Three observations suggested that EPSC changes did not depend on EPSC filtering. First, EPSC reduction was greater...
FIG. 1. Control excitatory postsynaptic current (EPSC) recordings. A: EPSCs were recorded from a granule cell held at −70 mV or +60 mV, alternatively. Top traces: averaging of 12 consecutive EPSCs at +60 mV (scale bars: 20 pA, vertical bar; 50 ms, horizontal bar) and 4 EPSCs at −70 mV (scale bars: 50 pA, vertical bar; 5 ms, horizontal bar). Plot shows amplitude changes measured at −70 mV (EPSC peak; ○) or +60 mV (50th ms; ●) in individual EPSCs. EPSC amplitudes measured at −70 mV and +60 mV yielded an estimate of the non-N-methyl-D-aspartate (non-NMDA) and NMDA current amplitude, respectively. Note decrease in EPSC amplitude with time. During recording no obvious decrease in voltage-clamp rate was observed (voltage-clamp time constant $\tau_{VC}$ changed from 111 to 107 ms, accounting for a <1% change in EPSC amplitude). B: average EPSCs obtained after 2 and 30 min of recording are shown superimposed. Scaling EPSCs to their peak did not reveal any kinetic changes at either negative or positive membrane potentials. ○○○, 25th ms, at which the NMDA current amplitude was measured. C: average EPSC amplitude changes in control recordings (means ± SD) from 5 different granule cells. In each cell, parameters were measured from average EPSCs obtained at −70 mV (n = 5; ○) or +60 mV (n = 5; ●). D: average changes in EPSC half-width (1st 15 ms excluded) at positive membrane potentials (n = 5; ●). Half-width, which reflected NMDA current kinetics (see text), tended to decrease over time.

than expected from a time-dependent reduction in voltage-clamp rate (see METHODS). Second, reducing the voltage-clamp rate should cause a greater reduction in the peak current at −70 mV than in the slow current component at +60 mV, but in fact this did not occur. Finally, an EPSC slowdown at negative potentials, which would be expected following a reduction in voltage-clamp rate, was not observed (the change in EPSC half-width at −70 mV was $-19 \pm 24.7\%$, n = 5). As reported in cultured neurons, down-regulation of endogenous metabolic control of non-NMDA and NMDA membrane receptors may be responsible for the changes observed in control experiments (MacDonald et al. 1989; Wang et al. 1994).

**LTP of mf–GrC EPSCs**

To induce potentiation of mf–GrC transmission, following a 10-min control period the mossy fibers were stimulated with high-frequency impulse trains reproducing a TBS pattern (see METHODS) that effectively induces LTP in the hippocampus and neocortex (Bear and Malenka 1994; Bliss and Collingridge 1993; Kirkwood and Bear 1996). Because membrane depolarization is an important determinant of LTP induction at glutamatergic synapses (Bliss and Collingridge 1993), TBS was paired with granule cell depolarization to −40 mV. At this potential, Mg$^{2+}$ block is largely removed from granule cell NMDA receptors (see D’Angelo et al. 1993).

Following induction, the EPSCs showed a transient (<5 min) and a persistent phase of potentiation (Fig. 2A), lasting while the whole-cell recording configuration was maintained (typically 30 min, but 60 in 1 cell and 60 min in 2 cells). Both the NMDA and non-NMDA currents increased with a similar time course (Fig. 2A), as observed at other glutamatergic synapses (Clark and Collingridge 1995; Kullman et al. 1996; O’Connor et al. 1995). These results provide evidence that LTP of transmission can be induced at the mossy fiber–granule cell synapse of the cerebellum.

Thirty minutes after induction, the non-NMDA current had increased by $32.2 \pm 13.6\%$ (n = 10; P < 0.02), whereas the NMDA current increased by $45.1 \pm 14.7\%$ (n = 10; NMDA $P < 0.01$), revealing a greater NMDA than non-NMDA current potentiation. The amplitude loss caused by a decrease in voltage-clamp rate ($-3.5 \pm 2.1\%; n = 10$; see METHODS) could not account for the difference between non-NMDA and NMDA current amplitude changes. Instead, we noted that the potentiated EPSCs slowed down at positive potentials (Fig. 2B, top traces), mimicking the NMDA cur-
FIG. 2. Long-term potentiation (LTP) in composite EPSCs. A: EPSCs were recorded from a granule cell held at −70 mV or +60 mV, alternatively. Top traces: averages of 12 consecutive EPSCs at −70 mV (scale bars: 50 pA, vertical bar; 50 ms, horizontal bar) and 4–5 EPSCs at +60 mV (scale bars: 50 pA, vertical bar; 5 ms, horizontal bar). Amplitude changes measured at −70 mV (peak amplitude; ○) and +60 mV (25th ms; ●) in individual EPSCs. Theta-burst stimulation (TBS) was applied at \( t = 0 \) while holding membrane potential at −40 mV. Note the persistent increase in EPSC amplitude following TBS. During recording no obvious decrease in voltage-clamp rate was observed (\( \tau_{VC} \) changed from 117 to 125 \( \mu s \), accounting for a <2% change in EPSC amplitude). B: average EPSCs obtained during control (con) and 20 min after LTP induction (ind) are shown superimposed. Scaling EPSCs to their peak shows a broadening of the EPSC at positive membrane potentials, whereas no apparent kinetic changes occurred in the EPSC at negative potentials. ⋯ 25th ms, at which the NMDA current amplitude was measured. Note that at the 25th ms the potentiated current is greater than the control current in peak-scaled EPSCs (inset: tracings enlarged). C: average EPSC amplitude changes in 10 different granule cells in which LTP was induced. In each cell, parameters were measured from average EPSCs obtained at −70 mV (\( n = 10; \Delta \)) or +60 mV (\( n = 10; \bullet \)). Note the persistent increase in EPSC amplitude following TBS. D: average plot of changes in EPSC half-width (1st 15 ms excluded; ●; \( n = 8 \)) at positive potentials. Half-width increased following TBS.

Current slowdown induced by metabotropic receptor agonists in this same preparation (Rossi et al. 1996). By scaling control to potentiated EPSCs, it turned out that potentiation at 25 ms after mossy fiber stimulation was enhanced by 11.2 ± 6.9% relative to peak. Thus together with a slight underestimate of non-NMDA current changes, NMDA current slowdown accounted for the difference between NMDA and non-NMDA current potentiation.

That EPSC broadening at positive membrane potentials was related to the NMDA current was confirmed by the observation that no comparable broadening occurred in the EPSC measured at −70 mV in which the non-NMDA current is dominant (Fig. 2B, bottom traces). At 20 min after induction, EPSC duration (half-width at positive potentials, the 1st 15 ms excluded) increased by 67.7 ± 32.5% (\( n = 10; P < 0.04 \)). Conversely, EPSC half-width at negative potentials increased by only 17.8 ± 27.6% (\( n = 10; P = 0.08 \)). It should be noted that this result does not mean that NMDA current changes are absent at negative potentials, but simply reflects the greater contribution of non-NMDA than NMDA current to half-width. A direct demonstration that EPSC broadening was related to changes in the NMDA current is provided in the following section.

The average time course of changes in EPSC amplitude and kinetics obtained from 10 granule cells in which TBS was paired with a membrane potential of −40 mV is shown in Fig. 2, C and D. Both EPSC amplitude and half-width showed a progressive increase during the 30 min following TBS.

**LTP of the NMDA current (with non-NMDA receptors blocked)**

In recordings performed in the presence of the non-NMDA receptor blocker, 10 \( \mu M \) CNQX (\( n = 5 \)), pairing TBS with a depolarization to −40 mV increased and slowed
down the NMDA–EPSC (Fig. 3, A and B). At +60 mV (30 min after induction), NMDA–EPSC amplitude increased by 48.5 ± 15.3% (n = 5; P < 0.01) and half-width by 75.6 ± 29.2% (n = 5; P < 0.05). These changes did not differ statistically from those of the NMDA current in composite EPSCs at the same potential. Also, the time course of changes in NMDA EPSC amplitude and duration was similar to that measured in composite EPSCs (Fig. 3, C and D). At −70 mV the NMDA current was too small to carry out an extensive analysis; however, a 68.5 ± 32% amplitude increase (n = 5; P < 0.05) indicated that NMDA current potentiation occurred at negative as well as at positive potentials. Thus NMDA current potentiation explained the half-width increase observed at positive (and to a lesser extent negative) membrane potentials.

**Fig. 4.** Absence of potentiation in granule cells in which TBS was paired with a membrane potential of −60 mV (t = 0). A: averages of 10 EPSCs at −70 mV and 5 EPSCs at +40 mV obtained in control (con) and 20 min after induction (ind). B–C: average time course of EPSC amplitude and half-width changes in the 4 granule cells. In each cell, NMDA (n = 4 ▲) and non-NMDA (n = 4; ●) currents were measured from average EPSCs obtained at +60 mV or −70 mV, respectively.
amplitude and half-width changed by $-14.1 \pm 15\%$ and $-26.3 \pm 20.1\%$, respectively; non-NMDA current amplitude changed by $-13.5 \pm 9.1\%$; $P > 0.05$). At $-60 \text{ mV}$ the NMDA channel is blocked by $\text{Mg}^2\text{+}$, and this may explain the lack of LTP.

However, the other two granule cells covered in this section showed an enhancement in slow EPSC components, consistent with a selective NMDA current potentiation (Fig. 5). Because LTP was not prevented at mf–Gr C synapses other than those connected to the recorded granule cell, a selective NMDA receptor–mediated potentiation may depend on glutamate spillover from neighboring potentiated synapses (see DISCUSSION) (Kullmann et al. 1996; Rusakov and Kullmann 1998).

**Effects of NMDA receptor block**

Direct evidence for NMDA receptor involvement in LTP induction was obtained by perfusing the NMDA receptor blockers APV (100 $\mu$M) and 7-Cl-Kyn acid (50 $\mu$M) shortly before and during the pairing of TBS with a depolarization to $-40 \text{ mV}$ ($n = 5$; Fig. 6, A and B). These blockers strongly inhibited the NMDA current while leaving the non-NMDA current intact (Fig. 6C). During the subsequent wash, potentiation was prevented in both the NMDA and non-NMDA current (30 min after induction, NMDA current amplitude and half-width changed by $-23.6 \pm 15.6\%$ and $-28.6 \pm 19.1\%$, respectively; non-NMDA current amplitude changed by $3.5 \pm 11.5\%$; $P > 0.05$). However, it should be noted that the non-NMDA current tended to increase compared with the NMDA current measured in the same experiments, as well as compared with the non-NMDA current measured in control recordings (cf. Fig. 1). These effects may depend on weak NMDA receptor activation during induction at negative) membrane potentials. These results showed that non-NMDA receptors are not needed to induce potentiation, at least as far as the NMDA current is concerned.

**Effects of a relatively hyperpolarized membrane potential**

To test the role of membrane potential during induction, we paired TBS with a relatively hyperpolarized membrane potential ($-60 \text{ mV}$). In four of six granule cells (Fig. 4, A–C), potentiation was prevented in both the NMDA and non-NMDA current (30 min after induction NMDA current £42-8/9k30$$ja08 12-29-98 23:21:41 neupa LP-Neurophys by 10.220.33.2 on October 29, 2017 http://jn.physiology.org/ Downloaded from
(≈20% of the NMDA current persisted during pharmacological block; Fig. 6C; see Aniksztejn et al. 1995) and incomplete washout of NMDA receptor blockers.

In conjunction with results reported in Figs. 4–5, these results indicate that both NMDA receptor activation and membrane depolarization are needed to induce mf–Gr C LTP.

Effects of mGlu receptor block

The mGluR agonist, trans-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD), has been shown to potentiate mf–Gr C transmission (Kinney and Slater 1993; Rossi et al. 1996). To test whether mGlu receptors are involved in LTP induced by high-frequency mossy fiber stimulation, we used the phenylglycine derivative, MCPG. MCPG has been reported to prevent LTP induced by high-frequency transmission at hippocampal synapses (Bashir et al. 1993; O’Connor et al. 1992; Vickery et al. 1997; but see Selig et al. 1995), possibly by preventing covered mGlu receptor–dependent changes occurring prior to induction (Bortolotto et al. 1994; Cohen and Abraham 1996). We therefore applied 500 μM MCPG from the outset of the recordings (n = 5). MCPG did not significantly alter the control EPSC before TBS. However, in the presence of MCPG, TBS paired with depolarization to –40 mV did not generate any LTP (Fig. 7), indicating that mGlu receptors are involved (30 min after induction NMDA current amplitude and half-width changed by –2.6 ± 21.1% and 6.7 ± 17.1%, respectively; non-NMDA current amplitude changed by –19.2 ± 14.1%, P > 0.05). It should be noted that the preventative effect of MCPG was less marked than that of other inhibitors.

Effects of high Ca²⁺ buffering and PKC inhibition

Downstream of membrane receptor activation, intracellular Ca²⁺ elevation is thought to link high-frequency impulse trains to intracellular modulatory systems (Bear and Malenka 1994; Bliss and Collingridge 1993). A high Ca²⁺ buffer concentration in the intracellular solution (10 mM BAPTA; n = 5) was not accompanied by significant EPSC changes in EPSCs recorded before TBS. TBS paired with depolarization to –40 mV did not thereafter induce any LTP (Fig. 8). Instead, after a transient phase of potentiation, the EPSCs showed a marked depression comparable to the EPSC rundown measured in control experiments (30 min after induction NMDA current amplitude and half-width changed by –48.4 ± 12.8% and –33.2 ± 9.8%, respectively; non-NMDA current amplitude changed by –26.7 ± 8.3%; P < 0.05).

Among the numerous Ca²⁺-sensitive enzymes taking part in LTP induction, PKC is particularly interesting because it requires both Ca²⁺- and diacylglycerol (DAG) for activation, being well suited to detect the coincidence of NMDA and metabotropic receptor signaling (Ben-Ari et al. 1993; Otani et al. 1992). When the pseudosubstrate PKC inhibitor 19-36 (5 μM PKC-I) was introduced into the cell through the patch-pipette (n = 5) (Wang et al. 1994), no significant

FIG. 7. Metabotropic glutamate (mGlu) receptor blockage prevented mf–Gr C LTP. Application of 500 μM (+)-α-methyl-4-carboxyphenyl-glycine (MCPG) prevented LTP induction (at t = 0) by TBS paired with a membrane potential of –40 mV. A: averages of 10 EPSCs at –70 mV and 5 EPSCs at +40 mV obtained in control (con) and 20 min after induction (ind). B: average time course of EPSC amplitude changes in recordings from 5 different granule cells. In each cell, NMDA (n = 5; •) and non-NMDA (n = 5; □) currents were measured from average EPSCs obtained at +60 mV or –70 mV, respectively.
EPSC changes were observed before TBS. TBS paired with depolarization to −40 mV (Fig. 9) did not thereafter induce any LTP of the NMDA or non-NMDA current, although the preventative effect was not as dramatic as with high intracellular BAPTA (30 min after induction, NMDA current amplitude and half-width changed by −14.7 ± 20.8% and −19.7 ± 13.5%, respectively; non-NMDA current amplitude changed by −7.2 ± 9.9%; P > 0.05).

Possible mechanisms involving intracellular Ca\(^{2+}\) signaling and PKC activation in mf–GrC LTP are discussed in the next section.

**Discussion**

Cerebellar granule cells are traditionally considered as a presynaptic element in LTD of parallel fiber–Purkinje cell transmission (Linden 1997; Linden and Connor 1995). In this paper we show that high-frequency mossy fiber activity can induce LTP of synaptic transmission at the mf–GrC relay of the cerebellum.

mf–GrC LTP depended on an NMDA receptor–dependent mechanism, in which membrane depolarization was probably needed to remove the Mg\(^{2+}\) block from the NMDA channel, allowing Ca\(^{2+}\) permeation and PKC activation. mGlu receptors were also needed for LTP to be induced. LTP was manifest as a stable enhancement in both the NMDA and non-NMDA receptor–mediated currents and as a slowdown in NMDA current. Potentiation could usually be observed for as long as 30 min, accounting for an early phase of LTP. LTP persistence over longer times remains to be assessed.

Based on its NMDA receptor–dependence, LTP at the mf–GrC relay of the cerebellum resembles LTP at the perforant path-dentate granule cell synapses and Schaffer collateral/commissural path–CA1 synapses in the hippocampus (Bliss and Collingridge 1993) as well as LTP at certain neocortical synapses (Kirkwood and Bear 1996), but differs from LTP of mossy fiber–CA3 synapses in the hippocampus (Johnston et al. 1992; Nicoll and Malenka 1995). Voltage-dependent Ca\(^{2+}\) channels probably played a secondary role in mf–GrC LTP, as suggested by their marginal activation at the membrane potential used for pairing (−40 mV) (see Rossi et al. 1994). Non-NMDA channels were unlikely to contribute to mf–GrC LTP because LTP of the NMDA current was not prevented by blocking non-NMDA receptors and because non-NMDA channels do not show any appreciable Ca\(^{2+}\) permeability in granule cells (Silver et al. 1996).

**Observations on LTP recordings**

Neuromodulatory processes may be influenced by cytoplasmic wash out caused by the pipette solution in the whole cell recording configuration. Wash out might cause EPSC rundown in control recordings (MacDonald et al. 1989). However, wash out did not prevent LTP, suggesting that no critical changes in the induction mechanism had occurred. Membrane depolarization prior to induction is also known to prevent LTP (Clark and Collingridge 1995). However, in our experiments LTP induction was not impaired by s-journeys at positive potentials, probably because these were brief and transitory.

Following induction, NMDA and non-NMDA current amplitude as well as NMDA current duration showed a progressive increase. This trend would be accentuated by taking control recordings for reference (i.e., if EPSC rundown is subtracted), leading to EPSC changes 20–30% greater than reported in our measurements. Moreover, when control recordings are taken as reference, in some experiments LTP inhibition appears incomplete. In different cases this may depend on incomplete preventative actions of glutamate receptor antagonists (APV and 7-Cl-Kyn or MCPG), on the contribution of kinases different from PKC, or on cross talk between neighboring synapses (see below).

In the experiments in which LTP was prevented, a transient potentiation was nonetheless observed following high-frequency stimulation. This may reflect posttetanic potentiation (PTP), a presynaptic process independent from LTP, or short-term potentiation (STP), a transient potentiation phase whose conversion into LTP may involve mGlu receptor and PKC activation (Bear and Malenka 1994; Ben-Ari et al. 1992; Bliss and Collingridge 1993). At glutamatergic synapses, LTD was also reported following specific induction patterns different from those used for inducing LTP. No evidence for LTD is currently available at the mf–GrC synapse. It should be noted that the synaptic depression that was observed especially with enhanced intracellular Ca\(^{2+}\) buffering was not statistically different from that occurring in control experiments and could not be taken as evidence for LTD (Bear and Malenka 1994).

**Membrane receptors and intracellular processes in LTP induction**

The observation that NMDA and mGlu receptor stimulation induces LTP through a Ca\(^{2+}\)- and PKC-dependent mechanism
integrates previous results obtained in granule cells into a functional framework. NMDA receptor–mediated Ca\(^{2\+}\) influx potentiates the effect of mGlu receptor stimulation in releasing Ca\(^{2\+}\) from intracellular stores (Irving et al. 1992), and phospholipase C (PLC)–coupled mGlu receptors cause PIP\(_2\) hydrolysis, releasing inositol tris-phosphate (IP\(_3\)) and DAG (Aronica et al. 1993). It follows that PKC, which is activated by elevations in DAG and Ca\(^{2\+}\), is well suited to detect whether both mGlu and NMDA receptors have been activated. LTP induction may follow the simultaneous activation of NMDA and mGlu receptors (cf. Rossi et al. 1996). However, we cannot rule out that covert mGlu receptor–dependent changes prior to induction subsequently facilitate LTP, as was proposed for the Schaffer collateral–CA1 synapse of the hippocampus (Bortolotto et al. 1994; Cohen and Abraham 1997). A conditioning role may also be played by PKC (Ben-Ari et al. 1992) because Ca\(^{2\+}\) influx through the NMDA receptor can potentiate its own response depending on previous PKC phosphorylation (Zheng et al. 1997).

The induction mechanism outlined above would specifically implicate postsynaptic type-1/5 mGlu receptors (Pin and Duvoisin 1995; Riedel and Reymann 1996), which are expressed in cerebellar granule cell axons at the developmental stage of our recordings (Catania et al. 1993). Type-2 mGlu receptors, which are linked to the cyclic AMP (cAMP) pathway, are also expressed in granule cells (Catania et al. 1993; Ohishi et al. 1994), and might play a role in LTP. However, type-4 mGlu receptors are unlikely to be involved because they are located presynaptically and inhibit parallel fiber–Purkinje cell transmission (Glaum and Miller 1994). Likewise, type-4 mGlu receptors, whose mRNA is expressed in neurons projecting to the cerebellum (Glaum and Miller 1994), are unlikely to contribute to mf–GrC LTP. No evidence for type-2/3 mGlu receptors in the mossy fiber terminals has so far emerged (Ohishi et al. 1994).

Possible mechanisms in LTP expression

Potentiation of the NMDA and non-NMDA EPSC components had similar time courses, intensities, and sensitivities to inhibitors of membrane receptors and intracellular transduction systems. This observation suggests that both EPSC components share a common mechanism of potentiation that, as proposed for hippocampal synapses, may involve an increased glutamate release (Clark and Collingridge 1995; O’Connor et al. 1995). This may cause glutamate to spill over the synaptic cleft, resulting in delayed activation of extrasynaptic NMDA receptors and NMDA current slowdown. A selective effect on the NMDA current is expected from the 100-fold higher affinity of NMDA than non-NMDA receptors for glutamate (Kullman et al. 1996; Rusakov and Kullman 1998). Glutamate spill over may affect the same granule cell whose synapses have been potentiated, as well as neighboring granule cells (cross talk). Similar to GABA, glutamate cross talk may take advantage of the restricted diffusion space and high density of synaptic contacts within the cerebellar glomerulus (Rossi and Hamann 1998). The spill over/cross talk hypothesis correctly predicts the NMDA current slowdown observed in granule cells in which LTP should otherwise have been prevented by pairing TBS with a relatively hyperpolarized membrane potential. Thus cross talk may contribute to NMDA current potentiation, at least at certain mf–GrC synapses.

Although mf–GrC LTP expression is compatible with a pre-synaptic mechanism, postsynaptic mechanisms cannot be ruled out at the present state (Nicoll and Malenka 1995). Rundown in control responses suggests that postsynaptic mechanisms can indeed modulate synaptic efficacy (Chen and Huang 1992; MacDonald 1997; Wang et al. 1994). Postsynaptic receptor modulation may account for the dis-association of non-NMDA from NMDA current potentiation observed with partial block of NMDA receptors during induction (Ankisztejn et al. 1995). The results reported in this paper are thus compatible with both the pre- and postsynaptic mechanisms, whose effective contribution to LTP expression remains to be established.

Implications for mf–GrC information processing

The effects of LTP on mf–GrC information processing can be predicted considering that the non-NMDA current determines EPSP amplitude, whereas the NMDA current protracts EPSP duration (D’Angelo et al. 1995). Non-NMDA and NMDA current increases, together with NMDA current slowdown, are therefore expected to enhance detection of coinciding mossy fiber impulses by both increasing EPSP amplitude and extending the time window for EPSP temporal summation. Moreover, increasing and slowing down the NMDA current would enhance repetitive granule cell discharge (D’Angelo et al. 1995). The critical role of NMDA receptors in regulating mf–GrC synaptic efficacy and signal coding may have important functional consequences, as suggested by the impairment in motor coordination and learning in mice lacking the NR2A and NR2C NMDA receptor subunits (Kadotani et al. 1996).

Implications for cerebellar function

In mf–GrC LTP, NMDA receptor activation and postsynaptic membrane depolarization provide the substrate for an associative mechanism of coincidence detection (Kelso et al. 1986). Critical regulatory factors will be the pattern (number, frequency, duration, and rhythmicity) of mossy fiber activity and the regulation of granule cell excitation through the inhibitory Golgi cell circuit. In addition to network factors, further mf–GrC LTP regulation will depend on mGlu receptors and the associative properties of PKC. Multiple mechanisms of coincidence detection should ensure LTP localization at specific mf–GrC contacts (Bliss and Collingridge 1993). By favoring selected combinations of mossy fiber inputs, mf-GrC LTP would then improve pattern recognition, the primary function attributed to the cerebellar mf–GrC relay (for a recent review see Arbib et al. 1998).

The present evidence for mf-GrC LTP is in contrast with Marr’s (1969) assumption that the mf-GrC synapse is not modifiable, extending the number of the possible learning sites in the cerebellar cortex (Linden 1997; Linden and
Connor 1995). As far as synaptic plasticity is a way to store information in neuronal networks, mGluR-C LTP represents a large potential for cerebellar memory because there are as many as 10^{11} granule cells and four times as many mGluR-C synapses (see, e.g., Arbib et al. 1998). mGluR-C LTP is therefore, potentially, an important determinant of the cerebellar function.

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