P42/44 MAP Kinase Inhibitor PD98059 Attenuates Multiple Forms of Synaptic Plasticity in Rat Dentate Gyrus In Vitro

ANDREW N. COOGAN, DEIRDRE M. O’LEARY, AND JOHN J. O’CONNOR

Department of Human Anatomy and Physiology, University College, Earlsfort Terrace, Dublin 2, Ireland

Coogan, Andrew N., Deirdre M. O'Leary, and John J. O'Connor. P42/44 MAP kinase inhibitor PD98059 attenuates multiple forms of synaptic plasticity in rat dentate gyrus in vitro. J. Neurophysiol. 81: 103–110, 1999. The effects of the specific p42/44 mitogen-activated protein (MAP) kinase cascade inhibitor, PD98059, were investigated on three types of long-term potentiation (LTP) in the medial perforant path of the rat dentate gyrus in vitro: LTP induced by 1 ) high-frequency stimulation (HFS-LTP), 2) application of 10 min of the K+ channel blocker, tetraethylammonium chloride (TEA-LTP), and 3) application of the metabotropic glutamate receptor agonist (S)-dihydrophenylglycine (S-DHPG) for 2 min (DHPG-LTP). Bath perfusion of PD98059 (50 &micro;m) for 1 h inhibited HFS-LTP (111 ± 5%, mean ± SE, at 90 min posttetanus in test slices compared with 144 ± 5% in control slices; n = 6–7). Concentrations of 10 and 20&micro;m PD98059 had no effect on HFS-LTP (n = 6). PD98059 (50 &micro;m) had no effect on the isolated N-methyl-D-aspartate excitatory postsynaptic potential (NMDA-EPSP) or on the maintenance phase of HFS-LTP. PD98059 (50 &micro;m) did not affect paired-pulse depression (PPD; interstimulus intervals of 10 and 100 ms) of synaptic transmission as is typically observed in the medial perforant path of the dentate gyrus. Bath application of (S)-DHPG (40 &micro;m) for 2 min gave rise to a potentiation of the EPSPs slope (148 ± 4% at 1 h post-DHPG wash out; n = 5). Pretreatment of slices with PD98059 (50 &micro;m) inhibited the DHPG-LTP (98 ± 3% at 1 h post-DHPG wash out; n = 5). The TEA-LTP (125 ± 4% at 1 h post-TEA wash out; n = 6) was found to be both &omega;-2-aminono-phosphopen-tanoic acid (MCPG; 100 &micro;m) and nifedipine (20 &micro;m) independent. However, the &omega; type voltage-dependent calcium-channel blocker, NiCl2 (50 &micro;m), completely inhibited the observed potentiation. The mGluR receptor antagonist &omega;-(S)-carboxy-phenyl gly-cine (MCPG; 100 &micro;m) and PD98059 (50 &micro;m) caused a complete block of the TEA-LTP. These data show for the first time an involvement of the p42/44 MAP kinase in the induction and expression of both an NMDA-dependent and two forms of NMDA-independent LTP in the dentate gyrus.

INTRODUCTION

Long-term potentiation (LTP) has been widely studied as a putative model of hebbian synaptic plasticity and is believed to form an important underlying neuronal mechanism of memory formation (Bliss and Collingridge 1993). The biochemical processes that are involved are not well understood. These include an elevation of intracellular calcium, N-methyl-D-aspartate (NMDA) receptor activation in area CA1 and dentate gyrus of the hippocampus, mobilization of retrograde messengers such as nitric oxide and arachidonic acid, and activation of several protein kinases and phosphatases (Collingridge et al. 1983; Medina and Izquierdo 1995; Nicoll and Malenka 1995; Roberson et al. 1996; Schulman 1995).

One of the protein kinase families that has been implicated in the expression of LTP are the mitogen-activated protein (MAP) kinases. These are a group of serine/threonine dual specificity kinases that have been shown to be activated by growth factors and are involved in cellular proliferation and differentiation (Seger and Krebs 1995). MAP kinases are also highly expressed in postmitotic neurons (Boulton et al. 1991) and recently have been shown to be activated in the hippocampus by glutamate and glutamatergic agonists (Kurino et al. 1995), by NMDA in cultured hippocampal neurons (Bading and Greenberg 1991; Xia et al. 1996), by membrane depolarization in the hippocampus and PC12 cells (Baron et al. 1996; Rosen et al. 1995), and by a high-frequency tetanic stimulation in the CA1 hippocampus (English and Sweatt 1996). For a recent review of the actions of MAP kinases in neurons, see Fukunaga and Miyamoto (1998).

PD98059, a specific inhibitor of MAP kinase kinase (MEK) and p42/44 MAP kinase activation (Alessi et al. 1995; Pang et al. 1995), has been shown to block induction of LTP in area CA1 of the hippocampus (English and Sweatt 1997) and also blocks the related process of long-term facilitation in the mollusk Aplysia (Martin et al. 1997). At present, however, very little is known about the physiological role of this cascade in the activity-dependent modulation of synaptic connections between neurons.

In this study we have examined the effect of PD98059 on the expression of three forms of synaptic plasticity in the dentate gyrus. First, LTP was induced by high-frequency tetanic stimulation (HFS-LTP) (Bliss and Collingridge 1993; Bliss and Lomo 1973). We have also investigated two forms of NMDA receptor-independent synaptic potentiation: a plastic change produced by application of a K+ channel blocker, tetraethylammonium chloride (TEA), which has previously been shown to be partly NMDA receptor independent in the CA1 hippocampus (Ankszteln and Ben-Ari 1991), and a potentiation induced by application of the metabotropic glutamate receptor agonist (S)-dihydrophenylglycine (DHPG), which we have previously shown to be NMDA receptor independent (O’Leary and O’Connor 1997). Finally we have also studied the effect of PD98059 on baseline synaptic transmission, NMDA receptor-mediated synaptic transmission, and paired-pulse depression (PPD) (McNaughton 1980). Our results indicate a role for the p42/44 MAP kinase cascade in both NMDA receptor-dependent and independent forms of synaptic plasticity in the dentate gyrus in vitro.
METHODS

Transverse hippocampal slices were prepared from male Wistar rats (70–150 g) as previously described (O’Connor et al. 1994). Briefly, slices were equilibrated for 1–4 h at 19–21°C submerged in artificial cerebrospinal fluid (ACSF; composition in mM: 120 NaCl, 2.5 KCl, 2 MgSO₄, 2 CaCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, and 10 d-glucose) before being transferred to a recording chamber and being superfused at a flow rate of 5–7 ml/min with ACSF at 29–31°C.

Field excitatory postsynaptic potentials (EPSPs) were elicited by stimulation of the medial perforant path by a monopolar glass electrode at a frequency of 0.05 Hz. Responses were recorded by a glass electrode placed in the middle third of the molecular layer, and stimulus strength was adjusted to give a response 30–40% of maximal. All experiments were carried out in the presence of 100 μM picrotoxin (Sigma) to eliminate GABA<sub>B</sub> receptor–mediated responses. Stable baseline recordings were made for at least 30 min before application of drugs. LTP was induced by high-frequency tetanic stimulation (8 trains of 8 pulses at 200 Hz at 2-s intervals at a stimulus strength to give 75% maximal response). Recordings were analyzed off-line using the Strathclyde electrophysiology software (J. Dempster, University of Strathclyde).

NMDA receptor–mediated EPSPs (NMDA-EPSPs) were isolated by application of the non-NMDA receptor antagonist 6-nitro-7-sulphamoylbenzof[1]oxazine-2,3-dione (NBQX) (2 μM; Tocris Cookson, Bristol, UK) and by lowering extracellular Mg<sub>2+</sub> (Sigma) to 0.3 mM. PD98059 (Calbiochem-Novabiochem) was dissolved in dimethylsulfoxide to a final concentration of 0.4%. TEA (Sigma) was made up daily as a 10-mM stock in dimethyl sulfoxide and protected from light (lights off, tubings wrapped and microscope off). EPSP slope was measured from 1 ms post–presynaptic volley. LTP was expressed as a normalized average (5-min bin at 60 or 90 min postinduction) compared with a 5-min bin preinduction of LTP (baseline). Isolated NMDA-EPSPs are expressed as a percentage of the EPSP at 20 ms after the presynaptic volley.

All results are expressed as means ± SE. For statistical analysis, two-tailed paired Student’s t-tests were employed to test significance between baseline and posttreatment values, and two-tailed unpaired Student’s t-tests were employed for comparisons between different test and control slices. The differences were considered significant if P < 0.05.

RESULTS

Bath application of PD 98059 inhibits tetanically induced LTP (HFS-LTP)

Perfusion of PD98059 (50 μM) had no significant effect on baseline synaptic transmission. Pooled results from experiments shown in Figs. 1B, 3C, and 6B show that application of PD98059 for 1 h had no effect on baseline transmission (96.0 ± 0.02% of baseline after 1 h treatment; mean ± SE, n = 17). In control slices, application of a high-frequency tetanus gave rise to a robust potentiation of the EPSP slope that persisted for as long as recording was continued (145 ± 5% of baseline slope at 120 min posttetanus; n = 7; P < 0.01, paired Student’s t-test; Fig. 1, A and B). Bath application of PD98059 at concentrations of 10 and 20 μM for 1 h before application of the tetanus did not produce a significant reduction in the magnitude of HFS-LTP (Fig. 1D). However, application of 50 μM PD98059 for 1 h before tetanus produced a significant reduction in HFS-LTP (111 ± 5% at 90 min posttetanus; n = 6; P < 0.01 compared with control slices; Fig. 1, A and B). Figure 1C shows the results from each of the six slices treated with PD98059 (50 μM). There is a degree of variability in the amount of potentiation produced in the period of 30 min post-HFS (the short-term potentiation phase), whereas all slices show an inhibition of LTP in the period of 30–180 min after HFS.

We then examined the effect of PD98059 on the maintenance phase of HFS-LTP. PD98059 (50 μM) was applied 30 min after application of the tetanus, and no significant effect on the maintenance phase of HFS-LTP was seen during the 1-h treatment with PD98059 (141 ± 5% at 90 min posttetanus, 60 min PD98059 treatment; n = 4; Fig. 2A).

PD98059 does not affect NMDA receptor–mediated synaptic transmission and PPD

Because tetanically induced LTP in the dentate gyrus has been shown to be dependent on calcium influx via the NMDA receptor (Bliss and Collingridge 1993; Errington et al. 1987), we examined the possibility that PD98059 may inhibit the induction of LTP by attenuating NMDA receptor–mediated synaptic transmission and thus preventing sufficient calcium influx to trigger the biochemical cascades involved in the expression of LTP. The NMDA-receptor field EPSP (NMDA-EPSP) was isolated, and PD98059 (50 μM) was applied for 1 h, during which time there was no significant change in the magnitude of the NMDA-EPSP (96 ± 4% at 1 h; n = 5; Fig. 2, B and C). It is therefore likely that PD98059 is not serving to inhibit LTP via inhibition of NMDA receptor function.

Bath application of PD98059 (50 μM) for 1 h did not affect the magnitude of PPD. For interstimulus intervals of 10 and 100 ms, the depression of the second EPSP in PD98059-treated slices was 42.9 ± 3.1% and 19.8 ± 0.9% compared with controls of 42.8 ± 3.2% and 19.7 ± 1.8%, respectively (n = 5; P > 0.1 for PD98059 vs. controls at both intervals; Fig. 2D).

PD98059 inhibits (S)-DHPG–induced potentiation

As previously reported (O’Leary and O’Connor 1997) bath application of the specific mGluR group I agonist (S)-DHPG (40 μM) produced a depression of the EPSP slope during perfusion (89 ± 3% of baseline; n = 5; P < 0.05) followed by a robust synaptic potentiation upon its wash out (148 ± 4% at 1 h after DHPG wash out; n = 5; P < 0.01; Fig. 3, A and B). Pretreatment of slices with PD98059 (50 μM) reduced both the (S)-DHPG–induced depression and the subsequent potentiation upon wash out [98 ± 3% during (S)-DHPG application; 95 ± 3% at 1 h after DHPG wash out; n = 5; P < 0.01 from slices treated solely with (S)-DHPG; Fig. 3C]. Subsequent HFS led to a robust LTP.

These results suggest that the p42/44 MAP kinases cascade is involved in both the inhibition and excitation of synaptic transmission by (S)-DHPG.

Bath application of TEA causes a synaptic potentiation that is NMDA receptor independent

Numerous studies have shown that bath application of the K⁺ channel blocker TEA causes a long-lasting potentiation...
PD98059 INHIBITS LTP IN THE DENTATE GYRUS

FIG. 1. Effect of PD98059 on tetanically induced high-frequency stimulation–long-term potentiation (HFS-LTP) in the rat dentate gyrus. A: average of 10 sweeps taken 1) 60 min before and 2) 90 min after the application of the high-frequency tetanus in a control slice (see methods for protocol); 3) 60 min before and 4) 90 min after the application of the tetanus in a slice treated with PD98059 (50 μM). B: time course for the effect of PD98059 (50 μM) on the expression of HFS-LTP. PD98059 was applied for 1 h before application of the tetanus and left in the bath for the duration of the experiment. Application of the tetanus is denoted by the arrow. There was a significant reduction in the magnitude of potentiation produced in PD98059-treated slices (●) compared with control slices (■) (P < 0.01 at 60 min posttetanus, Student’s t-test; n = 6 for both PD98059-treated and control slices). C: results from each of the 6 slices shown in B plotted separately. There is a degree of variability in the amount of potentiation produced in the period of 30 min post-HFS (the short-term potentiation phase), while all slices show an inhibition of LTP in the period of 30–180 min after HFS. D: dose response for the effect of PD98059 on LTP in the dentate gyrus. LTP is expressed as the slope normalized to baseline at 90 min posttetanus. PD98059 does not become significantly effective until treatment with a concentration of 50 μM.

in the area CA1 of the hippocampus (e.g., Aniksztejn and Ben-Ari 1991; Hanse and Gustaffson 1994; Huang and Malenka 1993; Huber et al. 1995). Bath application of TEA (25 mM) for 10 min gave rise to a depression of the EPSP slope (65 ± 6% at 10 min TEA; n = 6; P < 0.01), and upon wash out of TEA a potentiation of the EPSP was seen (125 ± 4% 1 h after wash out; n = 6; P < 0.05; Fig. 4, A and B). Pretreatment of slices with the NMDA receptor antagonist D-AP5 (100 μM) from 30 min before application of TEA, failed to antagonize the observed potentiation (122 ± 5% at 1 h after TEA wash out; n = 5; Fig. 4C). These results suggest that the synaptic potentiation produced by TEA in the dentate gyrus may be NMDA receptor independent.

**TEA-induced potentiation is Ni²⁺ and (s)-MCPG sensitive**

Pretreatment of slices with the L-type voltage-dependent calcium channel (VDCC) blocker nifedipine (20 μM) failed to antagonize the TEA-LTP (132 ± 5% at 1 h post-TEA wash out; n = 5; P > 0.05 compared with control TEA-LTP; Fig. 5A). The low-voltage–activated (LVA) calcium channel blocker Ni²⁺ (50 μM) caused an initial depression of the EPSP slope (82 ± 3% at 30 min Ni²⁺ treatment; n = 6; P < 0.05 compared with baseline; Fig. 5B). When stimulation voltage was adjusted to restore original baseline responses, application of TEA and its subsequent wash out failed to produce any potentiation. Indeed there was a persistent depression of the EPSP slope (83 ± 4% at 1 h after TEA wash out; n = 6; P < 0.05 compared with control baseline values and P < 0.01 compared with control TEA-treated slices).

Pretreatment of slices with the nonspecific mGluR antagonist (s)-MCPG (100 μM) from 30 min before TEA application and during the rest of the experiment inhibited the potentiation caused by TEA wash out (93 ± 4% at 1 h after TEA wash out; n = 6; P < 0.05 from control TEA slices; Fig. 5C). These results suggest that the synaptic potentiation produced by TEA may be dependent on LVA calcium channels and also the activation of mGluRs.

**PD98059 attenuates the TEA-induced potentiation**

Application of PD98059 (50 μM) for 1 h before TEA application had no effect on its own but significantly reduced
control slices; Fig. 6, A and B). These results may suggest that the TEA-LTP requires activation of the p42/44 MAP kinase cascade for its expression.

**DISCUSSION**

We have demonstrated that an inhibitor of the p42/44 MAP kinase cascade, PD98059 (Alessi et al. 1995; Pang et al. 1995), prevents the induction of both NMDA receptor–dependent and –independent forms of synaptic potentiation in the medial perforant path of the dentate gyrus. We have shown that application of PD98059 at 50 μM for 1 h to hippocampal slices inhibits the induction of HFS-, TEA-, and DHPG-induced LTP while having no effect on baseline transmission, the maintenance phase of HFS-LTP, PPD, or NMDA receptor–mediated synaptic transmission. We suggest that PD98059 at the concentrations used in this study has a specific inhibitory effect on MAP kinase kinase (MEK) because previous studies have found no effect of similar

FIG. 2. PD98059 does not affect the maintenance phase of HFS-LTP, the isolated N-methyl-D-aspartate–excitatory postsynaptic potential (NMDA-EPSP) and paired-pulse depression (PPD) in the dentate gyrus.

A: time course of the effect of PD98059 (50 μM) on the maintenance phase of HFS-LTP in the dentate gyrus. HFS-LTP was induced with a high-frequency tetanus (indicated by the arrow). Thirty minutes after induction of HFS-LTP, PD98059 was added. No significant effect was seen on the maintenance phase of HFS-LTP. B: average of 10 sweeps of the isolated NMDA-EPSP taken 1) 5 min before application and 2) 1 h after application of PD98059 (50 μM). C: time course of the effect of PD98059 (50 μM) on the isolated NMDA-EPSP amplitude. Treatment with PD98059 for 1 h had no significant effect on the isolated NMDA-EPSP amplitude. D: PD98059 has no effect on PPD in the medial perforant path of the dentate gyrus. Insets: typical control PPD of the EPSP. Interstimulus intervals (ISIs) were 1) 10 ms and 2) 100 ms. Open bars shown the summary data for control PPD at both 10 and 100 ms ISI. Gray bars indicate the observed PPD 1 h after treatment with PD98059 (50 μM). No significant effects were seen (n = 5 for all bars).

the TEA-LTP. In fact, as was the case for Ni2+, a persistent depression of the EPSP slope was observed (81 ± 4% at 1 h TEA wash out in PD98059; n = 6; P < 0.05 compared with control baseline and P < 0.01 compared with TEA

FIG. 3. (S)-dihydrophenylglycine [(S)-DHPG] induces a long-lasting enhancement of the field EPSP slope that is inhibited by PD98059. A: average of 10 sweeps taken 1) 5 min before, 2) during, and 3) 60 min after wash out of DHPG (40 μM). B: graph of the slope of the EPSP plotted against time before, during, and after application of 40 μM (S)-DHPG for 10 min. There was a significant inhibition of EPSP slope during perfusion (91 ± 2% compared with controls; n = 5; P < 0.01; black bar). After wash out, there was a fast onset increase in EPSP slope reaching a maximum at 12–14 min. During the plateau phase, tetanic stimulation did not give rise to further potentiation (not shown). C: graph of the slope of the EPSP plotted against time before, during, and after application of 40 μM (S)-DHPG for 10 min. There was a significant inhibition of EPSP slope during perfusion (91 ± 2% compared with controls; n = 5; P < 0.01; black bar). After wash out, there was a fast onset increase in EPSP slope reaching a maximum at 12–14 min. During the plateau phase, tetanic stimulation did not give rise to further potentiation (not shown).
HFS-LTP in the dentate gyrus has previously been shown to be NMDA receptor dependent (for example, Errington et al. 1987), and p42/44 MAP kinase has been shown to be activated by calcium influx via the NMDA receptor in cultured hippocampal neurons (Bading and Greenberg 1991; Kurino et al. 1995; Xia et al. 1996). It is not surprising then that we see a role for the p42/44 MAP kinase in tetanically induced LTP in the dentate gyrus. However, it has also been shown that membrane depolarization produced by applica-

![Graph A](image1.png)

**FIG. 4.** Tetraethylammonium (TEA) induces a NMDA-independent synaptic potentiation in the dentate gyrus. A: average of 10 sweeps taken 1) 20 min before, 2) 5 min after TEA application, and 3) 60 min after TEA wash out in a control TEA-treated slice. B: graph of the time course of the TEA-induced potentiation (TEA-LTP). Application of TEA (25 mM) for 10 min leads to a depression of the EPSP slope (65 ± 6% of baseline) followed by a potentiation of the synaptic response upon wash out of TEA (125 ± 4% of baseline 1 h post-wash out; n = 6; P < 0.05 compared with baseline). C: time course of the effect of D-AP5 (100 µM) on TEA-LTP. Application of D-AP5 for 30 min before application of TEA and for the duration of the rest of the experiments had no effect on the TEA-LTP (122 ± 5% at 1 h post-TEA wash out; n = 6).

doses of PD98059 (50 µM) on a number of other protein kinases (Alessi et al. 1995).

These results are in accordance with those of English and Sweatt (1996, 1997), who reported that in the CA1 a high-frequency tetanus such as that used in this study produced a marked activation of the p42 MAP kinases. Also the induction of LTP was attenuated by prior treatment with a similar dose of PD98059 as used in our study. Our effects would seem to be dose dependent because lower doses of PD98059 did not inhibit HFS-LTP. The fact that the maintenance of LTP is not affected by PD98059 suggests that the p42/44 MAP kinase cascade is not persistently activated during LTP. However, we cannot rule out an autonomous activation system for persistent MAP kinase activation, as has been suggested for both PKC and calcium/calmodulin kinase II (Lisman 1994; Roberson et al. 1996). PD98059 is an inhibitor of the activation of the MAP kinases, via MEK, which may be transient and not an inhibitor of the catalytic activity that may be persistent.

![Graph A](image2.png)

**FIG. 5.** Effect of nifedipine, NiCl₂, and α-methyl-4-carboxy-phenyl glycine (MCPG) on the TEA-LTP. A: graph of the effects of the L-type voltage-dependent calcium channel (VDCC) blocker, nifedipine (20 µM), on the time course of the TEA-LTP. Nifedipine was applied for 30 min before the TEA perfusion and left in the bath for the duration of the experiment. No effect on the TEA-LTP was observed (132 ± 5% 1 h post-TEA wash out; n = 6). B: graph of the effects of the low-voltage-activated VDCC blocker NiCl₂ (50 µM) on the TEA-LTP. Bath application of NiCl₂ gave rise to a reduction in the baseline EPSP slope during perfusion (82 ± 3% at 30 min after NiCl₂ treatment; n = 6; P < 0.05 compared with baseline). Before TEA application, stimulus strength was adjusted to restore initial baseline responses. TEA-application and wash out did not lead to a potentiation. Instead a persistent depression of the responses was observed (83 ± 4% at 1 h post-TEA wash out; n = 6; P < 0.05 compared with baseline). C: graph of the effect of the mGluR antagonist (s)-MCPG on the TEA-LTP. Bath application of (s)-MCPG (100 µM) for 30 min before treatment with TEA and for the rest of the experiments caused an inhibition of the TEA-induced potentiation (93 ± 4% at 1 h after TEA wash out; n = 6).
Boxylic acid (trans-trans the mGluR agonist hippocampal neurons where MAP kinases are activated by (Peavy and Conn 1997). The reason why a persistent depression of the effect of PD98059 (50% et al. 1998) remains obscure, although it may be due to a shift in the protein kinase cascade that is not sensitive to the L-type VDCC blocker nifedipine. These findings, coupled with the demonstration that calcium entry via the (S)-DHPG-induced reduction and the subsequent potentiation of EPSPs upon DHPG wash out. This indicates that the P42/44 MAP kinase cascade may involve signaling by group I mGluRs in the dentate gyrus.

We have previously described a potentiation induced by bath application of the group I mGluR agonist (S)-DHPG and have shown this to be NMDA receptor independent (O’Leary and O’Connor 1997). In the present study we have shown that prior treatment with PD98059 prevents both the (S)-DHPG-induced reduction and the subsequent potentiation of EPSP slope upon DHPG wash out. This indicates that the P42/44 MAP kinase cascade may be involved in signaling by group I mGluRs in the dentate gyrus. This is in accordance with findings in cultured glial cells, which show that treatment with DHPG activates the p42/44 MAP kinase and the subsequent potentiation of EPSPs in the dentate gyrus. This might trigger the Ca^2+ entry via VDCCs.

However, more recent studies have indicated both a NMDA receptor–dependent and – independent component of the TEA-induced potentiation in the CA1 (Hanse and Gustaffson 1994; Huber et al. 1995). Also the TEA-LTP in the CA1 has previously been shown by Platt et al. (1995) to be attenuated by the mGluR antagonist MCPG. In the present study we have demonstrated for the first time a TEA-induced potentiation in the dentate gyrus that is wholly unaffected by treatment with D-AP5 and is also not antagonized by nifedipine, in contrast to the TEA-induced potentiation in the CA1.

We also show that the potentiation in the dentate gyrus is not attenuated by treatment with the LVA calcium channel blocker Ni^2+ (Avery and Johnston 1996; Fox et al. 1987) and the mGluR antagonist MCPG. Interestingly, Wang et al. (1997a,b) have shown that NMDA receptor–independent forms of LTD and LTP, induced by afferent stimulation paired to postsynaptic depolarization, were insensitive to nifedipine but were sensitive to Ni^2+. Also their LTP was shown to be sensitive to MCPG. Our results may suggest that TEA induces a form of synaptic potentiation that is triggered by activation of mGluR either in the induction or in the expression phase of the potentiation and calcium entry via LVA calcium channels, possibly R-type channels regulating transmitter release (Wu et al. 1998) or T-type VDCCs regulating dendritic amplification of EPSPs (Gilessen and Alzheimer 1997).
cation may activate insufficient Ca$^{2+}$ influx to trigger the kinase activation needed for synaptic potentiation but may activate phosphatase cascades with a higher Ca$^{2+}$ affinity that could lead to the expression of a type of LTD. Indeed in the CA1 it has recently been shown that LTD induction actually leads to a decrease in p42/p44 MAP kinase activity compared with baseline controls (Thiels et al. 1997), indicating that the balance between phosphorylation/activation and dephosphorylation/deactivation of the p42/p44 MAP kinases may play a role in specifying whether LTP or LTD is induced. Because PD98059 is an inhibitor of the activation (via MEK) but not of the catalytic activity of the MAP kinases, a balance may be shifted to favor the dephosphorylation/deactivation of the MAP kinases, thus favoring the expression of a type of LTD. The fact that no such LTD was observed in slices treated with PD98059 before HFS may indicate a significant difference in the biochemical pathways involved in HFS- and TEA-induced LTP.

In the present study we have not been able to resolve whether or not the induction of LTP by the three methods described produces a specific activation of the p42 or the p44 MAP kinase as has been reported in the CA1 after tetanic stimulation (English and Sweatt 1996) and KCl pulse (Baron et al. 1996). It has been demonstrated that exogenous glutamate and glutamate receptor agonist application show equal activation of both the p42 and p44 isoforms (Kurino et al. 1995). It is not clear whether MAP kinase activation in tetanically induced LTD is due to activation of upstream second messenger such as Ras (Rosen et al. 1995) by Ca$^{2+}$ entry either via NMDA-receptors or VDCCs. It may also be due to activation of mGluR-linked second-messenger systems, because mGluR activation has been shown to be required for LTP in the dentate gyrus (Ben-Ari and Ankszttein 1995).

It also remains unresolved what the downstream targets of the p42/p44 MAP kinases may be in the expression and induction of LTP. A number of putative targets have been suggested that may be also involved in the normal expression of HFS-LTP: cytosolic phospholipase A2 (Lin et al. 1993), which is involved in arachidonic acid metabolism and in turn acts as a retrograde messenger in LTP (Medina and Izquierdo 1995); microtubule-associated proteins (Walton et al. 1997), which may be involved in ultrastructural changes underlying LTP expression (Bailey and Kandel 1993); and synapsin (Matsubara et al. 1996), which is involved in increased glutamate release associated with LTP (Lynch et al. 1994). MAP kinases are known to affect gene expression (Xia et al. 1996), but due to the inhibition of the early phase of LTP ($<$3 h) (reviewed in Roberson et al. 1996), it is transcription independent, a cytosolic target for MAP kinases seems likely.

In conclusion, the results presented in the present study indicate a role for the p42/p44 MAP kinase cascade in NMDA receptor–dependent HFS-LTP, NMDA receptor–independent DHPG-induced potentiation and a newly characterized TEA-induced potentiation in the dentate gyrus in vitro.

We thank Dr. Luke O’Neill for advice on the experiments and manuscript. This work was supported by grants from the Health Research Board (HRB), Ireland, to A. N. Coogan, Forbairt, Ireland, to D. O’Leary and the Presidents Prize, University College, Dublin, to J. J. O’Connor.

Address reprint requests to J. J. O’Connor.

Received 11 May 1998; accepted in final form 25 September 1998.

REFERENCES


