A Requirement for the Mitogen-activated Protein Kinase Cascade in Hippocampal Long Term Potentiation*

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The mitogen-activated protein kinase (MAPK) cascade has been intensely studied as a primary biochemical pathway through which a variety of extracellular stimuli initiate and regulate processes of cellular transformation. That MAPKs are abundantly expressed in postmitotic neurons, however, suggests different yet currently unknown functions for this cascade in the mature nervous system. Here we report that the MAPK cascade is required for hippocampal long term potentiation (LTP), a robust and widely studied form of synaptic plasticity. We observed that PD 098059, a selective inhibitor of the MAPK cascade, blocked MAPK activation in response to direct stimulation of the NMDA receptor as well as to LTP-inducing stimuli. Furthermore, inhibition of the MAPK cascade markedly attenuated the induction of LTP. PD 098059, however, had no effect on the expression of established LTP, and the MAPK cascade was not persistently activated during LTP expression. Our observations provide the first demonstration of a role for the MAPK cascade in the activity-dependent modification of synaptic connections between neurons in the adult mammalian nervous system.

The mitogen-activated protein kinase (MAPK) cascade has been classically studied as a critical biochemical pathway involved in cellular transformation events such as cell proliferation and determination. Such work has delineated a pathway by which growth factor receptor activation initiates a complex signaling cascade through which a variety of extracellular stimuli bind to activate the activation of Ras, Raf, and MEK, a dual-specific kinase that activates MAPKs via phosphorylations on both threonine and tyrosine residues (reviewed in Refs. 1 and 2). Although this cascade is typically studied in the context of mitotic cell regulation, its components are actually most abundantly expressed in postmitotic neurons of the developed nervous system (3, 4). At present, however, little is known about the physiologic roles of this cascade in mature neurons. We have begun to investigate the possible involvement of the MAPK cascade in the activity-dependent modulation of synaptic connections between neurons, a putative mechanism for the neural basis of learning and memory. In particular, we have examined the role of the MAPK cascade in hippocampal long term potentiation (LTP), a widely studied form of synaptic plasticity (reviewed in Refs. 5 and 6).

Recently, we reported that p42 MAPK (extracellular signal-regulated kinase 2) is activated during the induction of LTP in area CA1 of the hippocampus (7). Though this observation identifies the MAPK cascade as a potential component of the LTP induction cascades in area CA1, the physiologic necessity of MAPK activation during LTP induction remains to be established. To address this question, we have utilized the compound PD 098059 (8, 9), a recently described inhibitor of MEK, to block activation of the MAPK cascade during the delivery of LTP-inducing stimuli. Here we report that inhibition of the MAPK cascade greatly attenuates the induction but not expression of LTP in area CA1. Our observations provide an initial insight into a physiological role for the MAPK cascade in postmitotic neurons in the adult mammalian nervous system: the activity-dependent regulation of synaptic strength.

EXPERIMENTAL PROCEDURES

For hippocampal slice preparation, pharmacology, and electrophysiology, transverse hippocampal slices (400 μm) from 4–8 week old male Sprague-Dawley rats were prepared and maintained as described (7). Drug application, area CA1 subregion microdissection, tissue sonication, fractionation of soluble extracts, Western blotting, and densitometric analysis of phosphotyrosine immunoreactivity were conducted as described previously (7). All data are expressed as means ± S.E. In the present studies, we also utilized an antibody that selectively recognizes tyrosine phosphorylated extracellular signal-regulated kinases (New England Biolabs).

PD 098059 was dissolved in MeSO and diluted into artificial cerebrospinal fluid (ACSF; in mM 125 NaCl, 2.5 KCl, 1.25 NaHPO4, 25 NaHCO3, 10 d-glucose, 2.5 CaCl2, 1.25 MgCl2, saturated with 95% O2/5% CO2) to give the desired final concentration (with a final MeSO concentration of 0.3%). For drug application (either PD 098059 or 0.33% MeSO alone), ACSF solutions were maintained in a 32 °C water bath (to assure the complete solubility of PD 098059). For biochemical analysis of the effect of PD 098059 upon p42 MAPK activation, slices were preincubated in 50 μM PD 098059 or 0.33% MeSO for 45 min to 1 h prior to NMDA application (100 μM, 4) or HFS (strong induction paradigm, see below). 0.33% MeSO alone had no effect on NMDA- or HFS-mediated p42 MAPK activation (not shown).

For extracellular field recordings of the Schaffer collateral synapses in area CA1, stimulus intensity was adjusted to elicit a population excitatory postsynaptic potential (pEPSP) that was approximately 25% of the maximum response (initial slope typically about 1 mV/ms), and responses (obtained at 0.05 Hz) were monitored for at least 15 min to ensure a stable baseline. Two LTP induction paradigms were employed: 1) modest: two trains of 1-s, 100-Hz stimulation at a stimulus intensity that generated 75% of the maximal pEPSP, with an intertrain interval of 20 s; and 2) strong: three sets of tetani, each set spaced 10 min apart and consisting of two trains of 1-s, 100-Hz stimulation at a stimulus intensity that generated 75% of the maximal pEPSP, with an intertrain interval of 20 s. PD 098059 (0.33% MeSO) was applied for 1 h prior to tetanization and was maintained throughout the recording period. In initial experiments testing the effect of PD 098059 on LTP elicited with the strong induction protocol, 30-ml solutions of normal ACSF, 0.33% MeSO, or 100 μM PD 098059 were prepared and recycled through the recording cham-

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The abbreviations used are: MAPK, mitogen-activated protein kinase; LTP, long term potentiation; NMDA, N-methyl-D-aspartate; PKC, protein kinase C; PKA, protein kinase A; CaMKII, calcium/calmodulin-dependent protein kinase II; pEPSP, population excitatory postsynaptic potential; ACSF, artificial cerebrospinal fluid; HFS, high frequency stimulation; APT, anti-phosphotyrosine; t,1APV, t,2-aminoo-5-phenyliminovaleate; MEK, MAPK/ERK kinase; CREB, cAMP response element binding protein.
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RESULTS

PD 098059 Blocks p42 MAPK Activation in Area CA1—To test the physiologic significance of MAPK activation during LTP induction, we employed a recently described inhibitor of MEK, the dual-specific protein kinase responsible for phosphorylating and activating MAPKs. We first determined whether this MEK inhibitor, PD 098059, blocks MAPK activation in area CA1 of the hippocampus. PD 098059 completely abolished basal p42 MAPK phosphorysinore activity as well as the NMDA- and HFS-mediated increase in p42 MAPK tyrosine phosphorylation (Fig. 1A, APT Western). Similar results were obtained using an antibody that selectively recognizes phosphorylated, activated MAPKs (Fig. 1A, a-PMAPK Western).

Quantitative analysis of p42 MAPK APT immunoreactivity is shown in Fig. 1B. Application of either NMDA or HFS led to a significant increase in p42 MAPK phosphorysine (NMDA application: Me2SO alone, 100 ± 13; n = 8; Me2SO/NMDA, 290 ± 38; n = 10 (p < 0.01, Student’s t test); HFS application: Me2SO alone, 100 ± 13; n = 6; Me2SO/HFS, 159 ± 20; n = 6 (p < 0.05, Student’s t test)). In the presence of the MEK inhibitor PD 098059, however, essentially all phosphorysine immunoreactivity (basal as well as the NMDA- and HFS-mediated increases) was eliminated (NMDA application: PD 098059 alone, 6 ± 12; n = 7; PD 098059/NMDA, 16 ± 6; n = 9; HFS application: PD 098059 alone, 9 ± 3; n = 3; PD 098059/HFS, 9 ± 3; n = 3). Together, these data demonstrate that NMDA receptor stimulation or LTP-inducing HFS elicits p42 MAPK activation in area CA1 and that PD 098059 blocks this activation.

PD 098059 Markedly Attenuates the Induction of LTP in Area CA1—Having established that PD 098059 blocks MAPK activation in area CA1, we next determined the effect of this inhibitor on LTP induction. LTP observed in control slices was not significantly different at any time point from LTP observed in slices treated with the drug vehicle, 0.33% Me2SO (pEPSP not significantly different at any time point from LTP observed in slices pretreated with either Me2SO (drug vehicle) or PD 098059). Right panels, control (CTL) and tetanized (HFS) slices pretreated with either Me2SO (DMSO) or PD 098059. B, normalized p42 MAPK phosphorysine immunoreactivity (% of Me2SO-treated control slices). C, control; N, NMDA. Analysis of variance comparisons: Me2SO/NMDA versus Me2SO alone (p < 0.001); PD 098059 alone versus Me2SO alone (p < 0.001); PD 098059 alone versus PD 098059/NMDA (not different). 0.001). Following 2.5 h of PD 098059 application of PD 098059 had no effect upon basal synaptic transmission (Fig. 3A). Following 2.5 h of PD 098059 application (50 μM), average initial slope of the pEPSPs were 87 ± 10% of base-line responses (n = 3, not significant). Similar results were also obtained with 100 μM PD 098059 applied for 1 h (not shown).

Similarly, PD 098059 had no effect upon NMDA receptor-mediated pEPSPs elicited in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione, an antagonist of non-NMDA receptor
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**Fig. 2.** PD 098059 markedly attenuates the induction of LTP in area CA1. Average initial slope of the pEPSP, normalized to the average baseline response before tetanization, plotted over time. A, modest induction protocol. PD 098059 prevented the induction of stable LTP in six of six slices. Control (○, n = 10); 50 μM PD 098059 (■, n = 6). Inset, representative traces before and 90 min following tetanization in control slices and slices treated with PD 098059. B, strong induction protocol. PD 098059 prevented the induction of stable LTP in nine of ten slices. Control (○, n = 9); PD 098059 (■, n = 10). Inset, representative traces for average baseline responses and responses taken 60 min after the final tetanization in control slices and slices treated with PD 098059. Scale bars in this and subsequent figures are 2 mV and 3 ms except as noted.

subtypes of glutamate receptors (Fig. 3B). NMDA receptor-mediated responses following 1 h of 100 μM PD 098059 application were not different from baseline levels (amplitude: 99 ± 2% of baseline; initial slope: 94 ± 4% of baseline; n = 3; p > 0.05 for each). Application of a competitive antagonist of the NMDA receptor, 1,2-amino-5-phosphonovalerate (d, l-APV, 50 μM), completely and reversibly blocked the pEPSP.

Finally, PD 098059 did not alter the depolarization responses to high frequency stimulation (Fig. 3C). No differences were observed for either the total depolarization (integral over entire HFS response) or the steady-state depolarization (average over last 50 ms) observed during the initial tetanus for control, Me2SO- and PD 098059-treated slices (Integral: control: 100 ± 6.5%, n = 6; Me2SO: 88 ± 12%, n = 4; PD 098059: 100.5 ± 13.5%, n = 6). Steady-state depolarization: (control: 100 ± 7.8%, n = 6; Me2SO: 88 ± 14%, n = 4; PD 098059: 97.4 ± 11.7%, n = 6)). Overall, these results demonstrate that PD 098059 does not affect basal synaptic transmission, NMDA receptor-mediated responses, or HFS-mediated depolarization.

**Fig. 3.** PD 098059 does not affect basal synaptic transmission, NMDA receptor-mediated transmission, or HFS-mediated depolarization in area CA1. A, basal synaptic transmission. Following a period of baseline recordings (t = 20 to 0 min), 100 μM PD 098059 was applied for 2.5 h (at t = 0 min, solid horizontal bar). A shows pooled data of four 1-h applications and three 2.5-h applications. Inset, representative traces for base-line transmission (a) and responses following a 2.5 h PD 098059 application (b). B, NMDA receptor-mediated transmission. Average amplitude of the NMDA receptor-mediated pEPSP, normalized to the average baseline response before PD 098059 application (representative experiment). Following a period of baseline recordings (t = 20 to 0 min), 100 μM PD 098059 was applied for >2 h without effect. 50 μM d, l-APV completely and reversibly blocked the pEPSP (t = 60–90 min). Inset, representative pEPSP traces for responses prior to PD 098059 application (a), following 1 h of PD 098059 application (b), and following application of d,l-APV (c). CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione. C, Left, average HFS depolarization responses (normalized to control, CTL). Integral (total depolarization) and steady-state depolarization (SS Depol, average over last 50 ms) during the initial tetanus for control, Me2SO (DMSO)- and PD 098059-treated slices (modest induction protocol). Right, representative HFS-mediated depolarization responses. Scale bars, 2 mV and 90 ms.

and CaMKII. PD 098059 (used at concentrations ranging from 1 to 300 μM) did not affect the activity of these protein kinases (data not shown), suggesting that PD 098059 does not attenuate LTP induction by inhibiting any of these three protein kinases previously implicated in LTP induction.

The MAPK Cascade Is Not Persistently Activated during LTP Expression—Ongoing kinase activity is thought to underlie LTP expression (13, 14), and both PKC and CaMKII are persistently activated during LTP expression (15–17). To determine whether ongoing MEK activation is required for LTP expression, we examined the effect of PD 098059 on established LTP in area CA1. In these experiments, stable LTP was elicited in six of six slices using the modest induction protocol, and PD 098059 was applied for 1 h beginning 30 min after tetanization. PD 098059 had no effect on LTP expression (Fig. 4A). Consistent with these observations, PD 098059 also had no effect upon the expression of LTP elicited with the stronger induction paradigm (not shown). Note that a 1-h application of PD 098059 is sufficient for complete reversal of basal p42 MAPK tyrosine phosphorylation (Fig. 1). These results suggest that ongoing activation of the MAPK cascade is not necessary for LTP expression.
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REFERENCES


One important caveat to this conclusion, however, is that MAPKs, like PKC and CaMKII, can be persistently activated via phosphorylations (18). Because MEK inhibition would have no effect on persistently activated MAPKs, it remains a possibility that persistently activated MAPKs contribute to LTP expression. We tested this hypothesis by examining p42 MAPK activation levels during LTP expression. No difference in either phosphotyrosine or phospho-MAPK immunoreactivity for p42 MAPK was observed between control and LTP slices taken 45 min following delivery of the strong HFS paradigm, indicating that p42 MAPK is not persistently activated during LTP expression (Fig. 4, B and C; normalized p42 MAPK phosphotyrosine immunoreactivity: 114 ± 20% of control; n = 6; not significant).

DISCUSSION

The abundant expression of MAPKs in mature neurons of the developed nervous system suggests a specialized function for the MAPK cascade in these postmitotic cells. At present, however, little is known regarding either the regulatory mechanisms or physiologic roles of MAPKs in neurons. Using both electrophysiology and biochemistry, we have found that the MAPK cascade is involved in the activity-dependent modulation of synaptic strength, the leading candidate for the neuronal basis of information storage. In particular, we have identified p42 MAPK as a critical component of the biochemical cascades that underlie the induction of hippocampal long term potentiation.

Finally, it is well established that important early events in LTP induction include NMDA receptor stimulation, subsequent postsynaptic calcium influx, and the initiation of several protein kinase cascades. MAPKs appear to be localized in both presynaptic and postsynaptic structures (4, 19) and are thus well positioned to participate in these early events. Interestingly, consideration of several MAPK substrates suggests possible functions of MAPKs in these induction cascades: generation of putative retrograde messengers (e.g. cytosolic phospholipase A2 and the formation of arachadonic acid/platelet-activating factor) (20–22), synaptic vesicle regulation (e.g. synapsin) (19, 23), cytoskeletal modulation (e.g. MAP2 protein) (24), and regulation of gene expression (e.g. transcription factors such as Elk-1) (reviewed in Ref. 25), and CREB (26). In short, the necessity of MAPK activation for LTP induction and the known localization of MAPKs in postmitotic neurons strongly suggest that MAPKs play a critical role in LTP induction cascades. An important step in understanding the function of MAPKs in the strengthening of synaptic connections will be the determination of which MAPK substrates are utilized during LTP.

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FIG. 4. The MAPK cascade is not persistently activated during LTP expression. A, PD 098059 does not affect the expression of established LTP. Average initial slope of the pEPSP, normalized to the average response before tetanization. Stable LTP was induced in six of six slices, and 50 μM PD 098059 was applied for 1 h starting 30 min following tetanization (at t = 30 min, solid horizontal bar). Inset, representative traces for base-line transmission (a), responses 30 min following tetanization (last time point before drug application) (b), and responses 90 min following tetanization (i.e. after one h of PD 098059 application) (c). B, p42 MAPK is not persistently activated during LTP expression. Representative anti-MAPK (α-MAPK), APT, and anti-phospho-MAPK (α-PMAPK) Western blots of soluble extracts from individual area CA1 subregions from control (CTL) and 45′ LTP slices. C, normalized p42 MAPK phosphotyrosine immunoreactivity from control (CTL) and 45′ LTP slices (n = 6; not significantly different).