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Regulation of Amyloid Precursor Protein Catabolism Involves the Mitogen-Activated Protein Kinase Signal Transduction Pathway

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Catabolic processing of the amyloid precursor protein (APP) is subject to regulatory control by protein kinases. We hypothesized that this regulation involves sequential activation of the enzymes mitogen-activated protein kinase kinase (MEK) and extracellular signal-regulated protein kinase (ERK). In the present investigation, we provide evidence that MEK is critically involved in regulating APP processing by both nerve growth factor and phorbol esters. Western blot analysis of the soluble N-terminal APP derivative APP_s demonstrated that the synthetic MEK inhibitor PD 98059 antagonized nerve growth factor stimulation of both APP_s production and ERK activation in PC12 cells. Moreover, PD 98059 inhibited phorbol ester stimulation of APP_s production and activation of ERK in both human embryonic kidney cells and cortical neurons. Furthermore, overexpression of a kinase-inactive MEK mutant inhibited phorbol ester stimulation of APP secretion and activation of ERK in human embryonic kidney cell lines. Most important, PD 98059 antagonized phorbol ester-mediated inhibition of Aβ secretion from cells overexpressing human APP_{695} carrying the "Swedish mutation." Taken together, these data indicate that MEK and ERK may be critically involved in protein kinase C and nerve growth factor regulation of APP processing. The mitogen-activated protein kinase cascade may provide a novel target for altering catabolic processing of APP.

Key words: amyloid precursor protein; amyloid β-peptide; protein kinase C; nerve growth factor; mitogen-activated protein kinase; Alzheimer's disease

Amyloid β-peptide (Aβ), the principle constituent of senile plaques found in Alzheimer's disease (AD) brain (Hardy, 1997; Selkoe, 1997), is derived by proteolysis of an integral membrane protein known as the amyloid precursor protein (APP). Secretory processing of APP occurs via at least two pathways. One involves activation of an unidentified enzyme known as α-secretase, cleaving APP within the Aβ sequence (Sisodia et al., 1990; Anderson et al., 1991; Wang et al., 1991), precluding Aβ generation and releasing a soluble N-terminal APP fragment (APP_s) into the extracellular space. The alternative route involves two unidentified enzymes termed β- and γ-secretase, which cleave APP on the N and C termini of Aβ, respectively. The resultant Aβ is then released into the extracellular space (Haass et al., 1992b; Shoji et al., 1992).

Although catabolism of APP is constitutive, activation of signal transduction pathways can alter the relative amounts of APP_s and Aβ produced. Most studies of regulated APP processing have focused on stimulation of protein kinase C (PKC) and receptors linked to phospholipase-C that increase release of APP_s while inhibiting the release of soluble Aβ (Buxbaum et al., 1992, 1993; Caporaso et al., 1992; Nitsch et al., 1992; Gabuzda et al., 1993; Hung et al., 1993; Farber et al., 1995; Lee et al., 1995; Wolf et al., 1995; Mills and Reiner, 1996). Other signaling systems shown to stimulate APP_s release include calcium (Nitsch et al., 1992; Buxbaum et al., 1994), cAMP (Hu et al., 1996), growth factors (Schubert et al., 1989; Fukuyama et al., 1993), cytokines (Buxbaum et al., 1992, 1994), and estrogen (Jaffe et al., 1994).

The mechanism by which APP catabolism is regulated has not yet been elucidated. Phosphorylation of APP by PKC has been ruled out (Da Cruz et al., 1993; Hung and Selkoe, 1994; Jacobsen et al., 1994). Moreover, PKC-independent regulation of APP_s secretion exists (Buxbaum et al., 1994; Nitsch et al., 1996a) and may involve activation of protein–tyrosine kinases (Slack et al., 1995). These observations predict the existence of a pathway activated by multiple first and second messengers capable of regulating APP catabolism in both a PKC-dependent and a PKC-independent manner. These criteria are met by the mitogen-activated protein kinase (MAPK) signal transduction pathway (Cobb and Goldsmith, 1995; Graves et al., 1995; Malarkey et al., 1995; Pelech and Charest, 1996). MAPKs, also known as extracellular signal-regulated protein kinases (ERKs), are the terminal enzymes in a three-level kinase cascade involving the sequential activation of *raf*, mitogen-activated protein kinase kinase (MEK), and ERK (Pelech and Charest, 1996). Because MEKs are the only known physiological activators of ERKs (Bardwell and Thorner, 1996), MEKs provide a useful target for manipulating ERK activity. We used both PD 98059, a selective inhibitor of MEK1 (Alessi et al., 1995; Dudley et al., 1995; Lazar et al., 1995), and overexpresssion of a kinase-dead MEK1 mutant

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(Seger et al., 1994) to test the hypothesis that ERK activation is necessary for regulation of APP processing.

MATERIALS AND METHODS

Cell lines and transfections. Human embryonic kidney (HEK) 293 cells were transiently transfected with pCMV695, an expression vector for APP_{695} (Selkoe et al., 1988), pCMVβ, an expression vector for bacterial β-galactosidase (Clontech Laboratories), and either pCDNAK97A, an expression vector for kinase-inactive MEK, or the expression vector alone using a high-efficiency calcium phosphate transfection protocol (Chen and Okayama, 1987) as described previously (Raymond et al., 1996). Transfection efficiency was assessed by staining for β-galactosidase and determining the percentage of positively stained cells according to the method of Raymond et al. (1996). HEK 293 cells stably transfected with a construct carrying the Alzheimer's disease-linked double ("Swedish") mutation (K695sw), known to secrete elevated levels of both $A\beta_{40}$ and $A\beta_{42}$ (Citron et al., 1996), were cultured in DMEM supplemented in 10% fetal calf serum. HEK 293 cells were cultured in MEM supplemented with 10% fetal calf serum as described previously (Raymond et al., 1996). Rat pheochromocytoma (PC12) cells were cultured in DMEM supplemented with 10% horse serum and 5% fetal calf serum. One day before stimulation, HEK 293 cells or PC12 cells were exposed to culture media containing charcoal-inactivated calf serum at the same percentage used previously for cell maintenance. All cell lines were exposed to drugs for 15 min. PC12 cells were exposed to drugs in DMEM according to the method of Buxbaum et al. (1990). HEK 293 cells were exposed to drugs in MEM supplemented with 1 mg/ml glucose, whereas K695sw cells were exposed to drugs in DMEM.

Cortical cell cultures and drug treatment. Timed pregnant Sprague Dawley rats were anesthetized with halothane at 18 d of gestation, and the cerebral cortex was removed from rat embryos and dissociated using a method described previously (Murphy et al., 1992). Culture maintenance and drug exposure were performed using the method of Fiore et al. (1993) with minor modifications. In brief, before drug treatment, cells were washed once with 1 ml HBSS and preexposed to PD 98059 or drug vehicle for 1 hr. Both PD 98059 and phorbol esters were diluted from 10 mM stocks, made up in dimethylsulfoxide.

Quantification of APP_s and Aβ in culture media. After drug exposure, the medium was centrifuged for 10 min at $16{,}000 \times g$ to remove cellular debris. For APP_s detection, the medium was subsequently desalted and concentrated by centrifugation in the presence of protease inhibitors (17 μg/ml phenylmethanesulfonyl fluoride, 2 μg/ml leupeptin, 10 μg/ml aprotinin, and 2 μg/ml pepstatin) according the method of Mills and Reiner (1996). APP was detected by Western blot analysis using an anti-APP N-terminal antibody (anti-PreA4 monoclonal antibody, Boehringer Mannheim, Laval, Quebec, Canada) or WO-2, a monoclonal antibody generated against the first 16 amino acids of the N-terminal region of Aβ (Ida et al., 1996; anti-1–16) as described previously (Mills and Reiner, 1996). All Western blots were probed first with the anti-PreA4 monoclonal antibody (22C11). In some experiments, membranes were subsequently stripped of antibodies and reprobed with the APP-selective antibody WO-2 to prevent detection of secreted APLP (Slunt et al., 1994). For Aβ detection, proteins were precipitated by trichloroacetic acid according to the method of Hames (1981). Aβ was separated by Tris/Tricene SDS-PAGE according to the method of Klafki et al. (1996) and detected by Western blot analysis according to the method of Ida et al. (1996) using the monoclonal antibody WO-2. After densitometric measurements, ANOVA followed by Fisher's *post hoc* analysis was used to determine the significance of observed differences. Data are expressed as mean ± SEM and, unless otherwise stated, are representative of three separate trials.

Western blots of MAPK, MEK, and cellular APP. Cells were lysed in an extraction buffer containing 1% Nonidet P-40, 1% sodium deoxycholate, 4 mM *p*-nitrophenylphosphate, and 1 mM sodium vanadate, and the lysate was centrifuged to remove detergent-insoluble material. Twenty-five micrograms of cellular protein were separated by SDS-PAGE on 10% 20 cm gels or 12.5% low-bis (acrylamide/bis ratio of 118.5:1 instead of 37.5:1) mini gels for Western blots of either ERK or MEK. After gel electrophoresis, proteins were transferred electrophoretically to a nitrocellulose membrane and probed using a rabbit polyclonal antibody specific for ERK (Erk1-CT, Upstate Biotechnology, Lake Placid, NY), phosphorylated ERK (phospho-MAPK, New England Biolabs, Mississauga, Ontario, Canada), or MEK (Mek1-NT, Upstate Biotechnology). Five micrograms of cellular protein were separated on 10% mini gels for Western blots of APP, and membranes were probed subsequently with anti-PreA4 monoclonal antibody. Sequential Western blots are representative of three separate trials that may or may not have been taken from the exact same trial.

RESULTS

Pharmacological inhibition of MEK antagonizes nerve growth factor receptor stimulation of APP_s secretion and ERK activation

Activation of a wide variety of growth factor receptors having intrinsic or associated tyrosine kinase activity has been shown to stimulate ERK activation (Pelech and Sanghera, 1992; Pelech et al., 1993). Included among these are receptors for nerve growth factor (NGF), epidermal growth factor, and fibroblast growth factor, the stimulation of which has also been shown to increase APP_s release in cell lines (Refolo et al., 1989; Schubert et al., 1989; Fukuyama et al., 1993). These observations implicate the involvement of ERK in growth factor receptor-mediated regulation of APP_s release. To determine whether ERK activation is necessary for NGF receptor-dependent stimulation of APP_s release, we examined PC12 cells stimulated with NGF in the presence of the MEK1 inhibitor PD 98059. This pharmacological agent has been shown previously to antagonize tyrosine kinase receptor stimulation of ERK1 (Alessi et al., 1995; Dudley et al., 1995; Lazar et al., 1995; Pang et al., 1995) with an IC_{50} of ~10 μM (Dudley et al., 1995). APP_s production increased significantly when cells were incubated with 100 ng/ml NGF for 15 min, and this increase was antagonized in the presence of 10 μM PD 98059 (2.0 ± 0.3 and 1.0 ± 0.2, respectively; $n = 3$, $p < 0.05$) (Fig. 1*A*).

For ERK to become activated, it first must be phosphorylated by the dual-specificity kinase MEK on both a tyrosine and a threonine residue in the TEY motif (Anderson et al., 1990; Pague et al., 1991). Phosphorylated ERK can be detected either using Western blotting by a gel shift assay in which the electrophoretic mobility of phosphorylated ERK is retarded relative to its nonphosphorylated form (Posada and Cooper, 1992) or using antibodies raised against the phosphorylated TEY consensus sequence. The phosphorylation state of ERK was measured using these methods to ensure 10 μM PD 98059 antagonized NGF receptor stimulation of ERK activation. A 15 min exposure to NGF activated ERK1 and ERK2, and this activation was inhibited by PD 98059 (Fig. 1*B*).

Pharmacological inhibition of MEK antagonizes PKC stimulation of APP_s release and ERK activation

PKC stimulation by phorbol esters has been shown to increase dramatically the release of APP_s in a wide variety of cell lines (Buxbaum et al., 1992, 1993; Caporaso et al., 1992; Gabuzda et al., 1993; Hung et al., 1993). To determine whether ERKs are necessary for PKC-mediated regulation of APP catabolism, HEK 293 cells were exposed to 0.1 μM phorbol 12-myristate 13-acetate (PMA) with or without 10 μM PD 98059. Stimulation of APP_s release by PMA was inhibited by PD 98059 during a 15 min drug exposure as determined using the monoclonal antibody 22C11 (7.7 ± 1.5 and 4.4 ± 1.4, respectively; $n = 5$, $p < 0.05$) (Fig. 2*A*) or WO-2 (3.9 ± 0.5 and 2.0 ± 0.6, respectively; $n = 3$, $p < 0.05$) (Fig. 2*A*). To ensure that PD 98059 antagonized PMA-stimulated ERK activation in HEK 293 cells, the phosphorylation state and mobility of ERK were measured in Western blots. The experiments revealed that the PMA-induced electrophoretic shift was antagonized by PD 98059 (Fig. 2*B*, *top*; $n = 3$), as was the increase in phospho-ERK immunoreactivity induced by PMA (Fig. 2*B*, *bottom*; $n = 3$).

PKC-dependent regulation of APP_s release has also been ob-

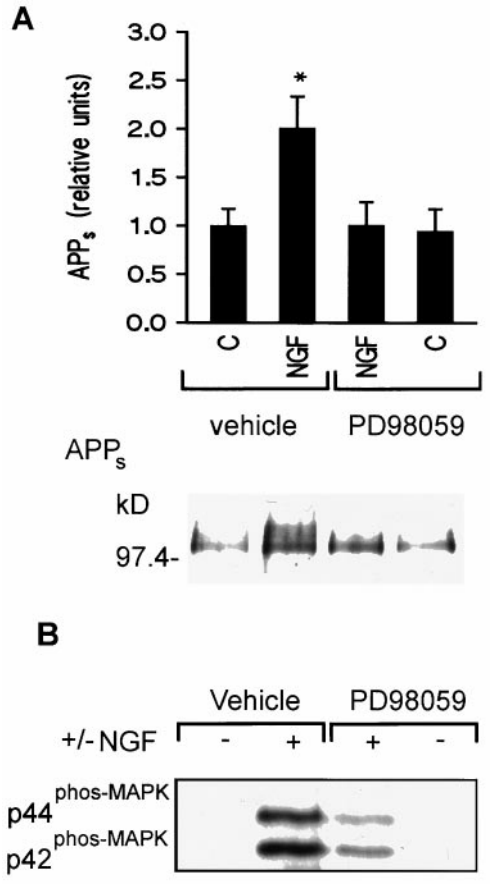

Figure 1. PD 98059 inhibits NGF receptor stimulation of APP_s secretion and ERK activation in PC12 cells. *A*, *Top*, Densitometric analysis of the effect of NGF (100 ng/ml) on basal APP_s release with or without PD 98059 (10 μM). Data are mean ± SEM of three experiments ($^*p < 0.05$, different from all other treatment groups). *Bottom*, Representative Western blot of APP_s fragments released in 15 min by PC12 cells alone or in the presence of NGF with or without PD 98059. *B*, Representative Western blot of phospho-ERK in PC12 cells after a 15 min drug exposure. The increase in immunoreactivity of the phospho-ERK-specific antibody in the presence of NGF was inhibited by PD 98059.

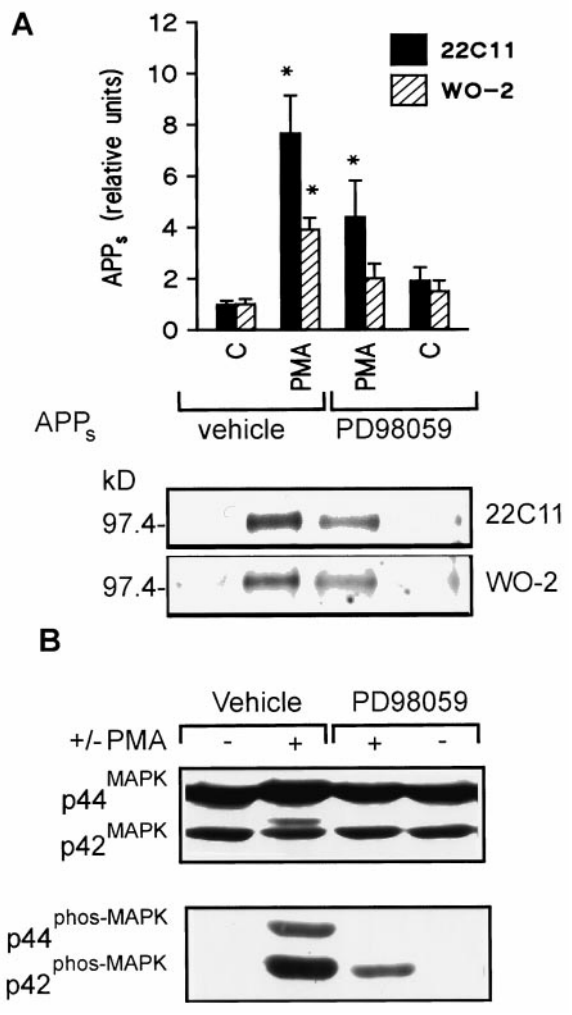

Figure 2. PD 98059 antagonizes phorbol ester stimulation of APP_s release in 15 min and ERK activation in HEK 293 cells. *A*, *Top*, Densitometric analysis of PMA (0.1 μM) stimulation of APP_s secretion with or without PD 98059 (10 μM). Data are mean ± SEM and represent five experiments for 22C11 (*filled columns*) or three experiments for WO-2 (*hatched columns*) ($^*p < 0.05$, different from all other treatment groups). *Bottom*, Representative Western blot of the effect of PMA on basal APP_s release alone or in the presence of PD 98059. *B*, Representative Western blot of ERK isoforms with ERK1 C terminus antibody (*top*) or phospho-ERK forms (*bottom*) in HEK 293 cells after a 15 min drug exposure. The PMA-induced "electrophoretic shift" was inhibited by PD 98059. Similarly, the increase in phospho-ERK immunoreactivity in the presence of PMA was antagonized by PD 98059.

served in primary cultures of hippocampal and cortical neurons (Lee et al., 1995; Mills and Reiner, 1996). To determine whether ERK activation is necessary for PKC-mediated regulation of APP_s release in neurons, primary cultures of rat cortical neurons were incubated with PDBu (1 μM) with or without PD 98059 (10 μM) for 1 hr. Levels of APP_s in the culture media increased significantly in the presence of PDBu, and this increase was antagonized in the presence of PD 98059 (6.52 ± 1.51 and 3.01 ± 0.90, respectively; $n = 5$, $p < 0.05$) (Fig. 3*A*). Moreover, phorbol ester stimulation of ERK activation was also suppressed in the presence of PD 98059, as seen by Western blot analysis of ERK mobility (*top*) or phospho-ERK (*bottom*) (Fig. 3*B*).

Kinase-inactive MEK antagonizes PKC stimulation of APP_s release and ERK activation

Overexpression of mutant proteins has proven to be a powerful tool for studying the role of signaling pathways in various cellular processes. A kinase-inactive MEK mutant, K97A, was generated by mutating lysine 97 to alanine (D. Charest and S. Pelech, unpublished data). This lysine is critical to MEK's activity because it is found in the ATP-binding site (Seger et al., 1994). Previously, the K97A mutant has been shown to act in a "dominant negative" manner because its overexpression in NIH 3T3 cells inhibited phorbol ester stimulation of endogenous MEK and its downstream substrate ERK (Seger et al., 1994). Stimulation of APP_s by 0.1 μM PMA was measured in HEK 293 cells transiently overexpressing human APP_{695} together with the K97A mutant or vector alone. Densitometric analysis revealed that PMA stimulation of APP_s secretion was significantly inhibited in the presence of the kinase-inactive MEK compared with vector alone as determined using 22C11 (3.0 ± 0.3 and 1.9 ± 0.2, respectively; $n = 3$, $p < 0.05$) (Fig. 4*A*) or WO-2 (2.3 ± 0.2 and 1.4 ± 0.4, respectively; $n = 3$, $p < 0.05$) (Fig. 4*A*). Moreover, Western blots using the gel shift assay indicate that the PMA-induced increase in ERK phosphorylation was antagonized by expression of the

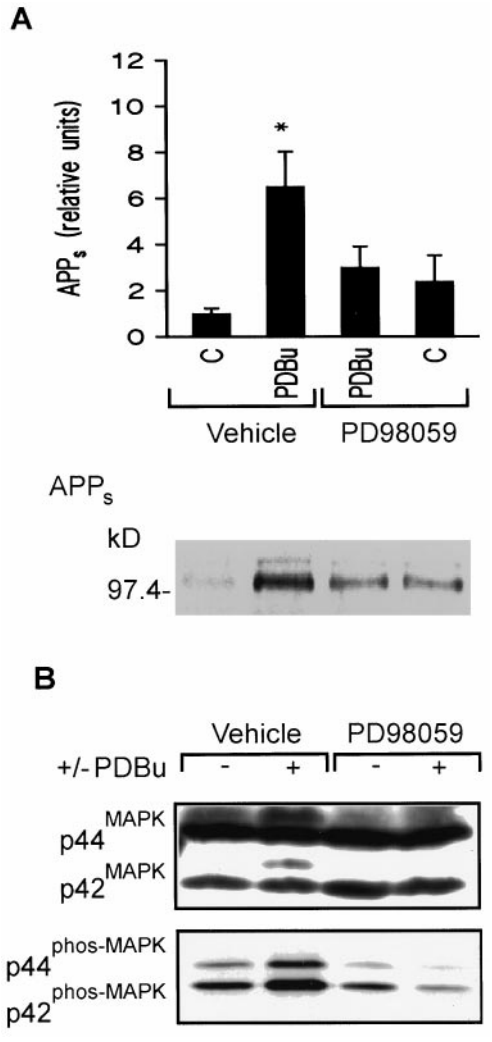

Figure 3. PD 98059 inhibits PKC stimulation of APP_s secretion and ERK activation in cortical neurons. *A*, *Top*, Densitometric analysis of PDBu (1 μM) stimulation of APP_s secretion in rat cortical cultures with or without PD 98059 (10 μM). Data are mean ± SEM of five experiments (*$p < 0.05$, different from all other treatment groups). *Bottom*, Representative Western blot of the effect of PD 98059 on PDBu stimulation of APP_s release in 15 min. *B*, Representative Western blot of ERK isoforms with ERK C terminus antibody (*top*) or phospho-ERK forms (*bottom*) in cortical cultures after a 1 hr drug exposure: phorbol ester-induced increase in the phosphorylation state of ERK was antagonized by pharmacological inhibition of MEK.

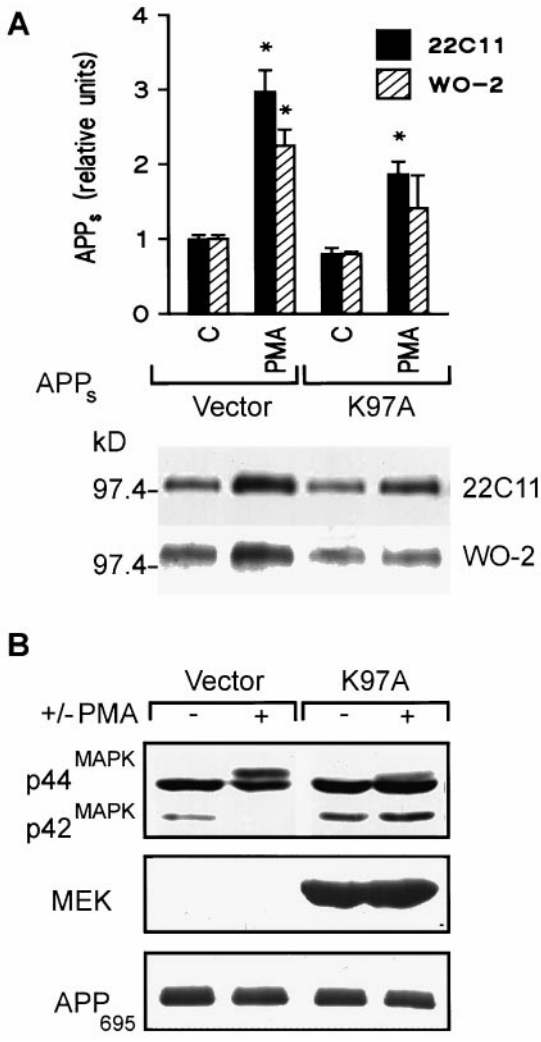

Figure 4. The kinase-dead mutant (*K97A*) inhibits phorbol ester stimulation of APP_s secretion and ERK activation in HEK 293 cells. *A*, *Top*, Densitometric analysis of PMA (0.1 μM) stimulation of APP_s secretion in cells expressing the MEK mutant (*K97A*) or vector alone (*Vector*). Data are mean ± SEM and represent three experiments for both 22C11 (*filled columns*) and WO-2 (*hatched columns*) (*$p < 0.05$, different from all other treatment groups). *Bottom*, Representative Western blot of the effect of PMA on basal APP_s release after transient transfection of vector alone or the K97A mutant. *B*, *Top*, Representative Western blot of ERK isoforms with an ERK C terminus antibody in HEK 293 cells transfected with the kinase-dead MEK mutant or vector alone. The "electrophoretic shift" induced by PMA treatment in cells expressing vector alone was inhibited in cells expressing the kinase-dead MEK mutant. *Middle*, Representative Western blot of MEK1 using a rabbit polyclonal antibody raised against the N terminus of MEK1. *Bottom*, Representative Western blot of cellular APP using a monoclonal antibody generated against the N terminus of APP.

K97A mutant (Fig. 4*B*). Incomplete antagonism of ERK activation may be attributed in part to transfection efficiency. β-Galactosidase staining indicated that the percentage of transfected cells was 81.4 ± 1.6% ($n = 3$). Overexpression of the K97A mutant was confirmed using a rabbit polyclonal antibody raised against the N terminus of MEK1 (Upstate Biotechnology) (Fig. 4*B*). Cellular levels of APP_{695} were not affected by overexpression of the dominant negative MEK (Fig. 4*B*).

Pharmacological inhibition of MEK antagonizes PKC regulation of Aβ release

Activation of PKC is also known to regulate Aβ secretion. Specifically, a reduction of Aβ secretion has been observed after phorbol ester treatment (Buxbaum et al., 1993; Gabuzda et al., 1993; Hung et al., 1993; Jacobsen et al., 1994; Querfurth et al., 1994), direct activation of phospholipase C (Buxbaum et al., 1993), and first messengers (Hung et al., 1993) known to activate the PLC/PKC pathway. However, the cellular mechanisms underlying this regulation are poorly understood. To determine whether ERKs are involved in PKC regulation of Aβ secretion, HEK 293 cells overexpressing human APP_{695} carrying the Swedish mutation were exposed to 1 μM PMA for 15 min with or without 10 μM PD 98059. Densitometric analysis revealed that PMA inhibition of Aβ secretion was antagonized by PD 98059 (0.48 ± 0.05 and 0.83 ± 0.07, respectively; $n = 7$, $p < 0.05$) (Fig. 5).

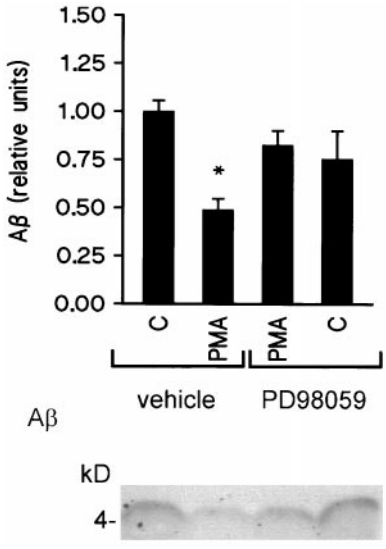

Figure 5. PD 98059 antagonizes phorbol ester inhibition of Aβ secretion in K695sw cells. *Top*, Densitometric analysis of PMA (1 μM) inhibition of Aβ secretion in K695sw cells with or without PD 98059 (10 μM). Data are mean ± SEM of seven experiments [$*p < 0.05$, different from control (vehicle alone)]. *Bottom*, Representative Western blot of the effects of the MEK antagonist PD 98059 on PMA inhibition of Aβ release.

DISCUSSION

The major finding of the present study is that activation of the MAPK pathway is necessary for regulation of the secretory processing of APP. Antagonism of MEK inhibits phorbol ester and NGF receptor stimulation of APP_s release as well as phorbol ester-mediated inhibition of Aβ release. The strength of the current study derives from the use of two distinct approaches for inhibiting the MAPK cascade, the pharmacological agent PD 98059 and gene transfer with a kinase-dead MEK mutant, both of which provided mutually supportive results. Moreover, the effects that we have observed are manifest in several different cell lines including neurons, suggesting that they are likely to be general rather than cell-specific.

These data also have broader implications for the function of ERKs. Correlative evidence has suggested that secretory stimuli activate ERKs in a variety of cells (Stratton et al., 1991; Frodin et al., 1995; Cox et al., 1996), but evidence demonstrating a requirement for ERK activation in secretion has not been obtained. Moreover, it has been shown that activation of the MAPK pathway is not required in some instances (Khoo and Cobb, 1997). The present experiments clearly implicate ERKs in regulation of APP secretory processing and, therefore, provide the first direct evidence for the necessity of the MAPK pathway in secretory events.

These results are relevant to our understanding of the molecular mechanisms by which APP catabolism is regulated in cells. A strong case has been made for the role of PKC activation in the regulation of APP catabolism (Nitsch and Growdon, 1994). PKC regulation of APP processing has been characterized extensively and has been shown to occur in a wide variety of cell lines (Buxbaum et al., 1990; Caporaso et al., 1992; Buxbaum et al., 1993; Gabuzda et al., 1993) and in central neurons (Lee et al., 1995; Mills and Reiner, 1996). However, the downstream effectors remain unknown. Our studies using both pharmacological and gene transfer approaches imply that MEK and/or ERK are necessary effectors for PKC-mediated stimulation of APP_s release in both cell lines and neurons. Antagonism of PKC-mediated inhibition of Aβ secretion with the MEK inhibitor PD 98059 indicates that the MAPK pathway is also downstream of PKC regulation of Aβ production and that activation of MEK and/or ERK may reduce Aβ secretion.

The best characterized means of stimulating the MAPK pathway is by activation of receptor tyrosine kinases (Pelech and Sanghera, 1992; Cobb and Goldsmith, 1995). After ligand binding, these receptors autophosphorylate, promoting the association of *ras* with GTP leading to the sequential activation of *raf1*, MEK, and ERK. Autophosphorylation also promotes interaction of the receptor with a number of alternative target proteins including PLC-γ (Meisenhelder et al., 1989; Ronnstrand et al., 1992; Middlemas et al., 1994; Eriksson et al., 1995). Because of the abundant evidence that PKC activation regulates APP catabolism (Nitsch and Growdon, 1994), it is tempting to hypothesize that regulation of APP catabolism via receptor tyrosine kinases might be mediated by activation of PLC-γ. However, it is equally plausible that the "direct route" of ERK activation by receptor tyrosine kinases may be sufficient for regulation of APP catabolism by growth factor receptors. Regardless of the detailed molecular circuitry involved, our data demonstrate that ERK activation is necessary for growth factor stimulation of APP_s secretion.

The mechanism by which the MAPK pathway regulates APP catabolism is unknown. Because of the time course involved in the present experiments, ERKs are unlikely to increase APP_s secretion by increasing overall expression of cellular APP. Rather, it seems likely that ERKs are acting to phosphorylate one or more targets within the cell to modify APP catabolism. Direct phosphorylation of the APP holoprotein is unlikely because activated ERK does not phosphorylate the cytoplasmic domain of APP under conditions in which it is able to hyperphosphorylate tau (Alplin et al., 1996). Alternatively, ERKs may regulate APP processing indirectly by phosphorylating proteins involved in intracellular trafficking. For example, like PKC, ERK may increase APP_s secretion by phosphorylating a tightly associated *trans*-Golgi network protein, thereby altering the formation of constitutive secretory vesicles containing mature APP (Xu et al., 1995). Also, presenilin-1, another protein thought to alter APP processing via its effects on protein trafficking (Borchelt et al., 1996; Lemere et al., 1996; Citron et al., 1997; Weidemann et al., 1997), has a consensus sequence for ERK-dependent phosphorylation and has been shown recently to be a substrate for PKC (Seeger et al., 1997; Walter et al., 1997a). Of course, the yet to be identified secretases that cleave APP remain potential candidates for phosphorylation by the MAPK cascade, either directly or indirectly.

A number of structurally unrelated membrane proteins undergo cleavage and subsequent release of their ectodomains into the extracellular medium, much like APP (Echlers and Riordon, 1991; Mattson et al., 1997); many of these share a common mechanism of regulation (Arribas and Massague, 1995). In addition to APP, PKC regulation of membrane protein processing has been observed for proTGF-α (Pandiella and Massague, 1991) colony-stimulating factor-1 (Stein and Rettenmier, 1991), colony-stimulating factor-1 receptor (Downing et al., 1989), and LAR transmembrane protein tyrosine phosphatase (Mullberg et al., 1992). Our data implicating the MAPK cascade in regulation of APP catabolism suggest that this mechanism of regulation may also be relevant to these membrane proteins.

Juxtamembrane cleavage serves to liberate APP_s, which may act as a paracrine signaling factor. For example, APP_s has been

shown to stimulate a cGMP-dependent protein kinase (Furukawa et al., 1996) as well as ERKs (Greenberg et al., 1994, 1995), and this function may be altered by phosphorylation of the ectodomain (Walter et al., 1997b). ERK activation by APP_s is intriguing in light of the present findings, because it suggests that there may be a positive-feedback pathway whereby activation of ERK stimulates APP_s release, which in turn activates the MAPK pathway.

Cell surface receptors known to regulate APP processing include heterotrimeric G-protein-coupled receptors and tyrosine kinase-coupled receptors (for review, see Beyreuther et al., 1996; Buxbaum and Greengard, 1996; Nitsch et al., 1996b). The effector system responsible can be regulated in either a PKC-dependent or a PKC-independent manner (Buxbaum et al., 1994; Slack et al., 1995; Nitsch et al., 1996a) and may involve activation of tyrosine kinases (Slack et al., 1995). All of these criteria are met by the MAPK signal transduction pathway. Our data for the first time implicate MEK and/or ERK in both PKC and tyrosine kinase receptor regulation of APP catabolism. Indeed, preliminary evidence from our laboratory suggests that MEK and/or ERK are critically involved in NMDA receptor stimulation of APP_s secretion (J. Mills and P. Reiner, unpublished data), suggesting that the MAPK pathway may be critical for regulation of APP catabolism by a number of first messengers.

It is widely hypothesized that production and deposition of amyloid are early events in AD and may be the key pathological event that triggers the disease process (Hardy, 1997; Selkoe, 1997). As such, any manipulation that diminishes the production of Aβ is of potential therapeutic utility. The results presented in this study suggest that strategies aimed at activating the MAPK cascade may be a viable approach in this respect.

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