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Biochemistry

Protein kinase A antagonizes platelet-derived growth factor-induced signaling by mitogen-activated protein kinase in human arterial smooth muscle cells

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ABSTRACT **Stimulation of aortic smooth muscle cells with platelet-derived growth factor BB homodimer (PDGF-BB) leads to the rapid activation of mitogen-activated protein kinase (MAPK) and MAPK kinase (MAPKK). Compounds that increase cAMP and activate protein kinase A (PKA)—prostaglandin E_2, isoproterenol, cholera toxin, and forskolin—were found to inhibit the PDGF-BB-induced activation of MAPKK and MAPK. Forskolin, but not the inactive analogue 1,9-dideoxyforskolin, inhibited PDGF-BB-stimulated MAPKK and MAPK activation in a dose-dependent manner. PKA antagonism of MAPK signaling was observed at all doses of PDGF-BB or PDGF-AA. PKA did not inhibit MAPKK and MAPK activity *in vitro*, and MAPKK and MAPK from extracts of forskolin-treated cells could be activated normally with purified Raf-1 and MAPKK, respectively, suggesting that PKA blocked signaling upstream of MAPKK. Neither PDGF-BB-stimulated tyrosine autophosphorylation of the PDGF receptor β subunit nor inositol monophosphate accumulation was affected by increased PKA activity, suggesting that PKA inhibits events downstream of the PDGF receptor. This study provides an example of cross talk between two important signaling systems activated by physiological stimuli in smooth muscle cells—namely, the PKA pathway and the growth factor-activated MAPK cascade.**

The p44 and p42 mitogen-activated protein kinases (MAPKs) (Erk1 and Erk2) are central components of a growth factor-stimulated protein kinase cascade found in organisms as diverse as mammals and yeast (reviewed in refs. 1–3). In addition to signals that act through receptor tyrosine kinases such as the platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin, numerous other effectors are also recognized as activators of the MAPK cascade. A kinase capable of phosphorylating and activating inactive MAPK was discovered in extracts of EGF-stimulated NIH 3T3 cells (4). This kinase, MAPK kinase (MAPKK), also referred to as MEK (5), is a dual-specificity threonine/tyrosine kinase (6, 7) and has been cloned from multiple sources (5, 8, 9).

A number of upstream activators of MAPKK have been reported, including Raf-1 (10–12), Mos (13), MEK kinase (14), and two enzymes from *Xenopus laevis* (15, 16). Activation of some of these enzymes may involve p21 Ras (16–18). Recently, a pathway leading to growth factor activation of p21 Ras has been described (ref. 19 and the references therein). The Src homology 2 (SH2) domain-containing protein Grb2 has been found to bind to the phosphorylated EGF receptor, forming a complex with the Ras GTP exchange protein, Sos, and catalyzing the formation of the active, GTP-bound form of Ras. Ras–GTP may then form a complex with and activate Raf-1, which can activate MAPKK (20).

The cAMP-dependent protein kinase (protein kinase A, PKA) is an integral constituent of a kinase cascade that links a number of extracellular signals to a variety of cellular functions (21, 22). Some of the cAMP and PKA effects are known to be in the opposite direction of the effects stimulated by growth factors whose receptors are protein-tyrosine kinases—e.g., the effect of cAMP on insulin-stimulated glycogen and triacylglycerol formation (23). It was thus of interest to examine the effect of cAMP on the stimulation of MAPK by growth factors.

We report here that PKA mediates inhibition of PDGF-BB-induced MAPK signaling in arterial smooth muscle cells (SMCs). The antagonism by PKA does not appear to be at the level of the PDGF receptor β subunit, MAPKK, or MAPK but is likely to occur between the receptor and MAPKK. To our knowledge, this is the first example of a physiological inhibition of MAPK signaling in cells.

MATERIALS AND METHODS

Materials. Forskolin and 1,9-dideoxyforskolin were dissolved in ethanol before use. Isoproterenol was dissolved in 5 mM ascorbic acid, prostaglandin E_2 (PGE_2) and 3-isobutyl-1-methylxanthine (IBMX) were dissolved in dimethyl sulfoxide, and cholera toxin (*Vibrio cholerae*, type Inaba 569B) was dissolved in sterile water. All chemicals were from Calbiochem. Recombinant human PDGF-BB was kindly provided by Zymogenetics, and recombinant human PDGF-AA was a gift from Hoffman–La Roche. PKI peptide (a specific peptide inhibitor of PKA) and Kemptide were synthesized at the Peptide Synthesis Facility, Howard Hughes Medical Institute, Seattle. MAPKK was purified from rabbit skeletal muscle, and recombinant Erk2 was activated by purified MAPKK (L.M.G. and E.G.K., unpublished data). Epitope-tagged, full-length Raf-1 was coexpressed in the baculovirus system with v-*src* (24). Raf-1 thus expressed was catalytically active and was immunoaffinity-purified from the Sf9 cells.

Cell Cultures. Human newborn (13 days) arterial SMCs were obtained from the thoracic aortas of infants following accidental death and cultured as described (25).

Cell Stimulation and Incubation with Agents That Increase cAMP. Confluent cell cultures in 100-mm dishes (≈5 million cells) were incubated in Dulbecco's modified Eagle's medium

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Abbreviations: EGF, epidermal growth factor; IBMX, 3-isobutyl-1-methylxanthine; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MBP, myelin basic protein; PDGF, platelet-derived growth factor; PGE_2, prostaglandin E_2; PKA, protein kinase A; SH2, Src homology 2; SMC, smooth muscle cell.

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(DMEM)/1% human plasma-derived serum for 2 days before experiments. The cells were washed twice in DMEM/0.25% bovine serum albumin and then were incubated for 30 min with forskolin as indicated or with 10 μM or 100 μM isoproterenol or 10 μM PGE_2 before stimulation with PDGF-BB. In some experiments, cells were incubated for 60 min with cholera toxin (2 μg/ml). With isoproterenol and PGE_2, the phosphodiesterase inhibitor IBMX (1 mM), was also added. IBMX or dimethyl sulfoxide alone had no effect on basal or PDGF-BB-stimulated MAPK activity. After stimulation with human PDGF-BB for 5 min, the cells were washed twice in ice-cold phosphate-buffered saline and cell extracts were prepared (26). None of the treatments described above was cytotoxic as measured by lactate dehydrogenase assay of the cell media (BioAnalytics, Palm City, FL).

MAPKK, MAPK, and PKA Assays. Cell extracts were assayed for MAPKK and MAPK activity as described (26). PKA was assayed by measuring phosphorylation of Kemptide (0.17 mM) in the presence or absence of PKI peptide (15 μM). PKA activity was calculated as the amount of Kemptide phosphorylated in the absence of PKI peptide minus that phosphorylated in the presence of PKI peptide. This method measures PKA activity stimulated by cellular cAMP carried over into reaction mixtures, which most likely reflects PKA activity in the cells.

Detection of MAPK Activity in SDS/Polyacrylamide Gels Containing Myelin Basic Protein (MBP). MAPK activity was measured in SDS/polyacrylamide gels containing MBP (27). Incubation was performed at room temperature for 1 hr in 10 ml of 40 mM Hepes, pH 8.0/2 mM dithiothreitol/100 μM EGTA/5 mM $MgCl_2$/25 μM ATP with a modification in the amount of [γ-^{32}P]ATP (250 μCi; 1 μCi = 37 kBq). The gel was washed extensively in 5% trichloroacetic acid/1% $NaPP_i$, dried, and exposed to x-ray film for 8–12 hr.

Activation of MAPKK by Raf-1 in Cell Extracts. The activation of MAPKK by Raf-1 was performed in 50 mM Hepes, pH 7.5/10 mM $Mg(OAc)_2$/1 mM dithiothreitol/80 mM NaCl/2 μM PKI peptide/100 μM ATP. Samples (15 μl) of Whatman DE-52-absorbed cell extracts were combined with active Raf-1 (≈60 ng) for 20 min at 30°C. A small sample was then removed for standard MAPKK assay.

Tyrosine Autophosphorylation of PDGF Receptor β Subunits. Cells in six-well plates were incubated in DMEM/1% human plasma-derived serum for 2 days and after 30 min with or without 10 μM forskolin, were stimulated with PDGF-BB as indicated for 7 min at 37°C. Stimulation was terminated by three washes with ice-cold phosphate-buffered saline and the cells were solubilized in 1% (vol/vol) Triton X-100/20 mM Tris, pH 8.0/137 mM NaCl/10% (vol/vol) glycerol/2 mM EDTA/1 mM phenylmethanesulfonyl fluoride/1 mM Na_3VO_4 with aprotinin (1 μg/ml) and leupeptin (1 μg/ml). The samples were electrophoresed in SDS/6% polyacrylamide gels and the separated proteins were blotted onto nitrocellulose membranes. The membranes were incubated with phosphotyrosine antibodies (PY20, 0.1 μg/ml; ICN ImmunoBiochemicals, Costa Mesa, CA) in 150 mM NaCl/50 mM Tris, pH 7.4/0.05% Tween 20/5% bovine serum albumin for 2 hr and the bands were visualized with the ECL kit (Amersham).

Inositol Monophosphate Accumulation. Cells in six-well plates were incubated with *myo*-[^{3}H]inositol (Amersham) at 2 μCi/ml for 24 hr at 37°C. The cells were incubated for 30 min in the presence of 20 mM LiCl and 10 μM forskolin or ethanol (vehicle) and then stimulated with PDGF-BB for 30 min at 37°C. Inositol monophosphate formation was measured after separation on BioRad 1-X8Ag columns.

RESULTS

Compounds That Increase Intracellular cAMP Inhibit the Activation of MAPKK and MAPK by PDGF-BB. The effect of compounds known to increase intracellular cAMP on PDGF-BB stimulation of MAPKK and MAPK activity in human aortic SMCs was investigated. PDGF-BB was a potent activator of both kinases (Fig. 1 *A* and *B*). Maximal activation was achieved 5 min after addition of the growth factor (data not shown). PGE_2 (10 μM) and the β-adrenergic receptor agonist isoproterenol (10 or 100 μM) inhibited PDGF-BB (0.3 nM)-induced activation of MAPKK and MAPK by 90% and 50–80%, respectively, and activated PKA 5- to 6-fold (Fig. 1*C*). Likewise, activation of adenylate cyclase with 10 μM forskolin inhibited PDGF-BB stimulation

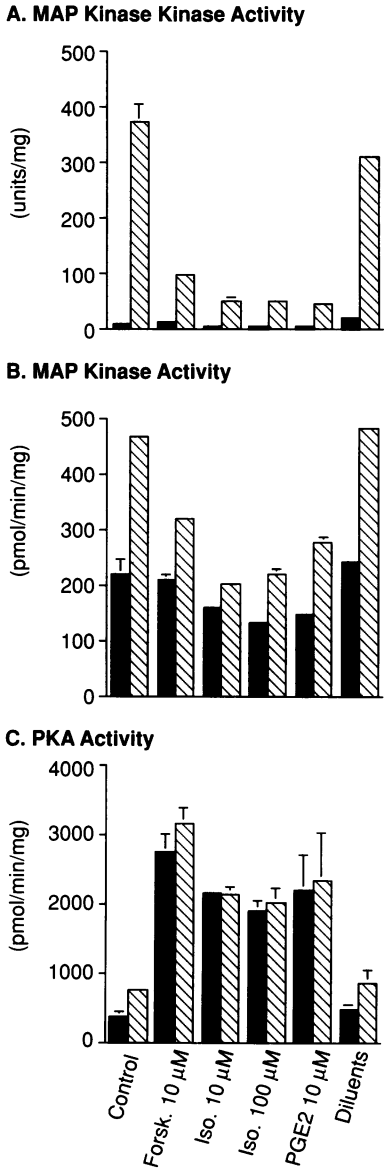

FIG. 1. Activation of PKA correlates with inhibition of PDGF-BB-induced activation of MAPKK and MAPK in SMCs. Cells were incubated in DMEM/1% human plasma-derived serum for 2 days, incubated with forskolin (forsk.), isoproterenol (iso.), PGE_2 or diluents (5 mM ascorbic acid or dimethyl sulfoxide) for 30 min, and then stimulated with 0.3 nM PDGF-BB for 5 min at 37°C. For MAPKK activity (*A*) cell lysates were mixed with DE-52, and the flow-through fraction was measured for phosphorylation of MBP by activated recombinant Erk2. MAPK activity (*B*) was measured by phosphorylation of MBP during 15 min at 30°C. PKA assays (*C*) measured phosphorylation of Kemptide in the presence or absence of the inhibitor peptide PKI. Results are expressed as mean ± SE of duplicate samples. Black bars, basal samples, hatched bars, PDGF-BB-stimulated samples.

of MAPKK and MAPK by 60–80%, with strong activation of PKA (Fig. 1). The inactive forskolin analogue, 1,9-dideoxyforskolin (10 μM), neither inhibited PDGF-BB (0.3 nM)-induced MAPKK or MAPK activity nor stimulated PKA activity (data not shown). Cholera toxin (2 μg/ml), an activator of the stimulatory GTP-binding protein G_s, inhibited PDGF-BB-activated MAPKK and MAPK by 32% and 23%, respectively, and activated PKA 4-fold. In the presence of IBMX, the inhibition of PDGF-BB-induced MAPKK and MAPK by cholera toxin at 2 μg/ml was comparable to the inhibition by 10 μM forskolin (data not shown). These results indicate a strong correlation between the activation of PKA and the inhibition of PDGF-BB-induced stimulation of MAPKK and MAPK.

Dose Dependency of Forskolin Inhibition of the PDGF-BB Stimulation of MAPKK and MAPK. SMCs were incubated with various concentrations of forskolin for 30 min prior to treatment with a suboptimal dose of PDGF-BB (0.3 nM). The cells were harvested after PDGF-BB stimulation for 5 min. Assays of the extracts for MAPKK, MAPK, and PKA activities demonstrated a dose-dependent activation of PKA by forskolin and a reciprocal inhibition of PDGF-BB-stimulated MAPKK and MAPK (Fig. 2). The lowest dose of forskolin found to activate PKA and inhibit MAPKK and MAPK was 0.1 μM. At 10 μM forskolin, MAPKK and MAPK were inhibited by ≈80% compared with their activities in untreated cells. Higher doses of forskolin (50–150 μM) did not result in further inhibition. In some experiments, the effect of PDGF-AA was studied. PDGF-AA (1 nM) activated MAPKK and MAPK by 66% and 88%, respectively, of the activation observed with 0.3 nM PDGF-BB. Forskolin (10 μM) inhibited PDGF-AA-stimulated MAPKK and MAPK by 65% and 44%, respectively. Addition of 25 μM cAMP to extracts from forskolin (10 μM)-treated cells did not further increase the incorporation of radioactivity into Kemptide, suggesting that PKA was already completely activated in extracts of cells incubated with 10 μM forskolin (data not shown).

Effect of Forskolin on MAPKK and MAPK Activity at Various Concentrations of PDGF-BB. The stimulation of MAPKK and MAPK by various concentrations of PDGF-BB was compared in SMCs incubated with or without 10 μM forskolin. The concentrations of PDGF-BB required to give half-maximal stimulation (EC_{50}) of MAPKK and MAPK in SMCs were similar in the presence and absence of forskolin (0.47 nM versus 0.40 nM for MAPKK and 0.34 nM versus 0.20 nM for MAPK). The maximal activation of both MAPKK and MAPK was inhibited by approximately 50% and 30%, respectively (Fig. 3). Further experiments showed that the time course of PDGF-BB-activation of these kinases was similar with or without 10 μM forskolin (data not shown).

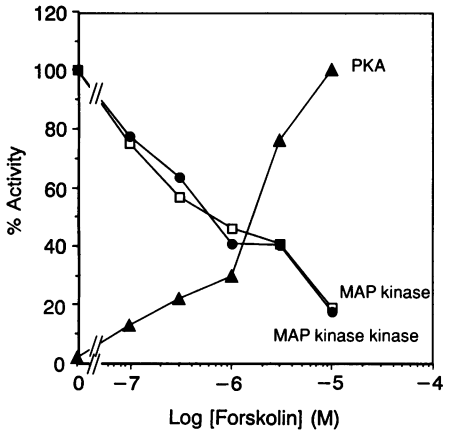

FIG. 2. Forskolin inhibits PDGF-BB-induced activation of MAPKK and MAPK and activates PKA in a dose-dependent manner. Human arterial SMCs were prepared as for Fig. 1 and incubated with the indicated concentrations of forskolin or ethanol (vehicle) for 30 min. Cells then were stimulated with 0.3 nM PDGF-BB for 5 min at 37°C, and cell extracts were assayed for MAPK (□), MAPKK (●), and PKA (▲) activities as for Fig. 1. MAPKK and MAPK activities are expressed as percent of the activity in extracts from cells stimulated with 0.3 nM PDGF-BB in the absence of forskolin; PKA activity is expressed as percent of the activity in extracts from cells stimulated with an optimal concentration of forskolin. Values are means of duplicate samples.

MAPK from Extracts of Forskolin-Treated Cells Can Be Activated by Purified MAPKK *in Vitro*. Cytosolic extracts from control, PDGF-BB plus 10 μM forskolin (PDGF-BB/forskolin)-, or PDGF-BB-stimulated cells were incubated with purified MAPKK plus Mg^{2+}/ATP and analyzed for Erk1 and Erk2 activity by the SDS/PAGE "in-gel" phosphorylation of MBP. Two MBP-phosphorylating activities corresponding to Erk1 and Erk2 were found in the PDGF-BB-stimulated cell extracts, whereas minor amounts of Erk1 and Erk2 activity were found in both the control and PDGF-BB/forskolin extracts (Fig. 4*A*). Although extracts contained additional MBP-phosphorylating activities, only Erk1 and Erk2 were significantly stimulated by PDGF-BB as determined by the in-gel assay. Incubation of control and PDGF-BB/forskolin extracts with MAPKK resulted in >2-fold activation of Erk1 and Erk2 as determined by densitometry (Fig. 4*A*). Some additional activation of Erk1 and Erk2 was observed in the PDGF-BB-stimulated cell extracts by MAPKK, suggesting that PDGF-BB incubation did not completely activate Erk1 and Erk2 (Fig. 4*A*). Thus, MAPK activity from PDGF-BB/forskolin-treated cells could be activated *in vitro* by MAPKK to an extent equivalent to enzyme from control cells, suggesting that MAPK from forskolin-treated cells is not directly inhibited by PKA. Furthermore, incubation of MAPK (Erk2) with PKA (catalytic subunit at 0.7 μg/ml) did not inhibit the phosphorylation of MBP by MAPK *in vitro* (data not shown).

MAPKK in Extracts from Forskolin-Treated Cells Can Be Activated by Raf-1. Cell extracts from control, PDGF-BB/forskolin (10 μM)-, and PDGF-BB-incubated cells were mixed with DE-52 ion exchanger to yield a partially purified fraction of MAPKK. Incubation of MAPKK-containing frac-

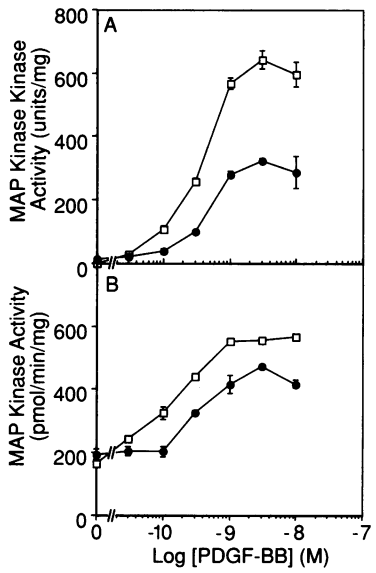

FIG. 3. Dose-dependent stimulation of MAPKK and MAPK by PDGF-BB in forskolin-treated cells. SMCs were incubated with 10 μM forskolin (●) or vehicle (□), stimulated with PDGF-BB as indicated, and assayed for MAPKK (*A*) and MAPK (*B*) activities as in Fig. 1. Results are expressed as mean ± SE of duplicate samples.

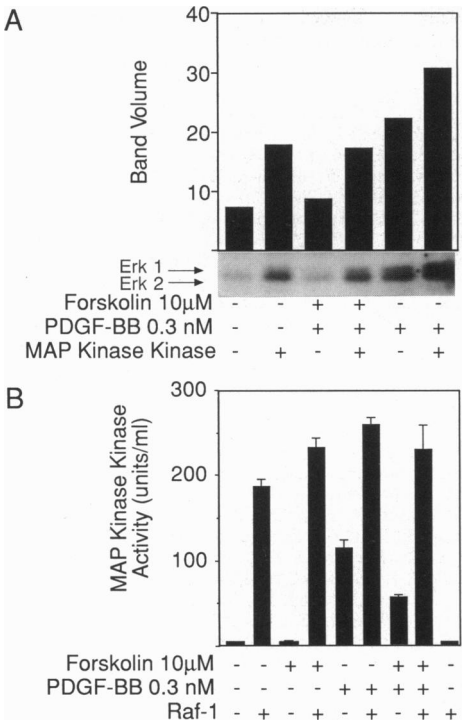

FIG. 4. MAPKK and MAPK from extracts of forskolin-treated cells can be activated with Raf-1 and MAPKK. (*A*) Extracts of cells were incubated with purified MAPKK, after which time MAPK activity was measured in SDS/polyacrylamide gels containing MBP. The intensities of the bands representing the MAPKs (Erk1 and Erk2) were estimated by scanning the exposed film with a Bio-Rad GS-670 densitometer. Activated recombinant Erk2 was used as standard. Band volume was determined as band intensity/area according to the manufacturer's instructions. (*B*) Extracts (15 μl) from control, PDGF-BB-treated, control/forskolin-treated and PDGF-BB/forskolin-treated cells were incubated with Whatman DE-52 ion-exchange resin to prepare a partially purified fraction of MAPKK. The DE-52 samples were incubated with activated Raf-1 (≈60 ng) and Mg^{2+}/ATP at 30°C for 30 min, after which 5 μl was removed and for MAPKK assay as in Fig. 1. The rightmost represents activated Raf-1 in the absence of cell extract.

tions with Mg^{2+}/ATP and activated Raf-1 resulted in a significant activation of MAPKK activity from both control and PDGF-BB/forskolin-treated cells (Fig. 4*B*). Following Raf-1-incubation, the amount of MAPKK activity exceeded that found in extracts from cells treated with 0.3 nM PDGF-BB, probably because 0.3 nM PDGF-BB is a suboptimal dose (Fig. 3), whereas MAPKK activation of Raf-1 *in vitro* corresponds to a maximal activation by PDGF-BB. These results show that MAPKK from either control or PDGF-BB/forskolin-incubated cells could be activated to a similar degree by Raf-1, suggesting that MAPKK is not likely to be directly inhibited by PKA in forskolin-treated cells. PKA (0.7 μg/ml) did not phosphorylate purified skeletal muscle MAPKK *in vitro* or inhibit the ability of MAPKK to activate Erk2 to a significant level (data not shown).

Forskolin Does Not Inhibit PDGF-BB-Induced Receptor β-Subunit Autophosphorylation or Inositol Monophosphate Accumulation. Tyrosine autophosphorylation of PDGF receptor β subunits was measured in cells incubated in the presence or absence of forskolin. PDGF-BB induced a dose-dependent tyrosine autophosphorylation of the PDGF receptor (Fig. 5). The 185-kDa band represents mainly the PDGF receptor β subunit, since the level of PDGF receptor α-subunit expression is about 10-fold lower in these cells (K.E.B., unpublished observation). No difference in tyrosine autophosphorylation between forskolin-treated and untreated

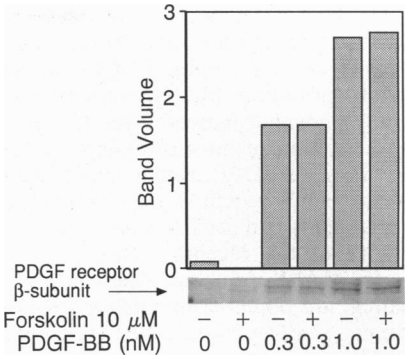

FIG. 5. PDGF-BB-induced receptor tyrosine autophosphorylation is not inhibited by forskolin. SMCs were incubated in the absence or presence of 10 μM forskolin, stimulated with PDGF-BB as indicated for 7 min at 37°C, and solubilized for SDS/6% PAGE followed by immunoblot analysis with phosphotyrosine antibodies (PY20, 0.1 μg/ml); bands were visualized with an ECL kit (Amersham). The intensity of the 185-kDa band representing the PDGF receptor β subunit was estimated as in Fig. 4*A*.

cells was demonstrated by densitometric scanning of the band representing the PDGF receptor (Fig. 5).

PDGF-BB-induced formation of inositol monophosphate was examined in control and forskolin-treated cells. PDGF-BB (0.3 nM) increased inositol monophosphate formation 3.5 ± 0.7-fold in the absence of forskolin and 3.1 ± 0.7-fold in the presence of 10 μM forskolin (mean ± SEM, n = 4). Thus, forskolin did not inhibit PDGF-BB-induced formation of inositol monophosphate.

DISCUSSION

This study describes an antagonism between PKA and the MAPK cascade. Rapid activation of MAPKK and MAPK follows binding of PDGF-BB to its receptors on human SMCs. Our results demonstrate that various compounds that increase cAMP and PKA activity in these cells can significantly inhibit the stimulation of MAPKK and MAPK by PDGF-BB. A reciprocal relationship was found between the activation of PKA and the inhibition of MAPKK and MAPK. This phenomenon occurred at all concentrations of PDGF-BB, suggesting that in SMCs, PKA may act to attenuate growth factor signaling.

In this study, the PDGF receptor β subunit probably contributed predominantly to MAPK signaling, since the number of PDGF receptor α subunits is much lower in SMCs (K.E.B., unpublished observation). However, our results are not specific to the PDGF receptor β subunit, as forskolin also inhibited MAPKK and MAPK activation in PDGF-AA-treated cells.

We have investigated some of the points at which PKA might inhibit signaling in the MAPK cascade. Neither MAPKK nor MAPK is a direct substrate for PKA in intact cells, since MAPKK and MAPK obtained from extracts of forskolin-treated cells were activated with Raf-1 and MAPKK, respectively, *in vitro*. While previous reports have shown that PKA can phosphorylate and partially inhibit the autophosphorylation of the insulin and EGF receptors (28–30), no such inhibition of the PDGF receptor β subunit was found here. Our findings are consistent with those of Heldin *et al.* (31), who reported that forskolin treatment of human fibroblasts inhibited PDGF-stimulated c-*myc* expression without decreasing the tyrosine autophosphorylation of PDGF receptor (31).

It is conceivable that one or more of the PDGF receptor-associated molecules is a target for PKA *in vivo*. PDGF receptors can bind several SH2-containing proteins, such as

phospholipase C-γ (PLC-γ), GTPase-activating protein (GAP), and phosphatidylinositol 3-kinase. In our experiments, forskolin did not inhibit PDGF-stimulated inositol monophosphate formation. PLC-γ1 is a substrate for PKA in some cells, and phosphorylation at Ser-1248 has been implicated in the inhibition of inositol phosphate signaling (32). However, in SMCs, PLC-γ2, rather than PLC-γ1, seems to interact with the PDGF receptors (33). This isozyme does not contain an analogous site for PKA phosphorylation.

One or more of the recently identified components in EGF-stimulated MAPK signaling, which may be conserved in PDGF signaling, are potential targets for PKA-mediated antagonism. Some examples include the SH2-containing protein Grb2, the GTP-exchange protein Sos, Ras, or small GTP-binding proteins such as Rap1A (also known as Krev-1) and Raf-1. Neither Grb2 nor Ras has sites considered optimal for PKA phosphorylation, whereas potential PKA sites are present in human Sos and Raf-1 (34). Rap1A has been shown to be phosphorylated by PKA in human platelets (35). In *Saccharomyces cerevisiae*, the mouse Sos homologue CDC25 is phosphorylated by PKA, resulting in a partial relocalization of CDC25 from the membrane to the cytosol, thereby reducing its accessibility to membrane-bound Ras (36). Phosphorylation of Sos or Rap1A by PKA may result in a decreased activation of membrane-bound Ras, or inhibit Ras activation of Raf-1, with subsequent inhibition of the MAPK cascade.

cAMP has been shown to be both inhibitory and stimulatory to cell proliferation, depending on the cell type studied (37–39). In thyroid cells, cAMP is mitogenic without an apparent activation of MAPK (40). In SMCs, increased cAMP inhibits PDGF-stimulated DNA synthesis and cell proliferation (37, 41, 42). However, inhibition of DNA synthesis by forskolin cannot be attributed solely to antagonism of the initial MAPK signaling, since DNA synthesis was blocked when forskolin was added 1–6 hr after PDGF-BB (data not shown).

It is pertinent to consider what consequences PKA inhibition of MAPK signaling might have on cell regulation. In addition to affecting transcriptional events brought about through the regulation of c-Myc, c-Jun, c-Fos, NF-IL6, $p62^{TCF}$, and ATF-2, MAPK is involved in the activation of S6 kinase II ($pp90^{RSK}$), MAPKAP-2, and phospholipase A_2 (refs. 1 and 2 and the references therein). Thus, a cAMP effect on these targets might result in a change in glycogen metabolism, heat shock protein phosphorylation, or arachidonate production. Effects on the cytoskeleton might also be anticipated.

The data demonstrate a functional cross talk between two distinct protein kinase cascades in aortic SMCs. It will be particularly interesting to investigate antagonism between PKA and MAPK signaling in other cell types. During the completion of this study we learned that a similar antagonism of insulin-induced MAPK signaling by PKA was found in adipocytes (J. Lawrence, personal communication; see ref. 43, which immediately follows this paper).

L.M.G. and K.E.B. contributed equally to this study. We acknowledge Dr. John Lawrence (Washington University, St. Louis) for sharing unpublished results while our manuscript was in preparation. We thank Dr. Joe Beavo, Dr. Dan Storm, Dr. Eui Choi, Scott Wong, and William Schiemann (Department of Pharmacology, University of Washington, Seattle) for useful technical suggestions. The excellent technical assistance of Li-Chuan Huang is gratefully acknowledged. We are grateful to Dr. Melanie Cobb (University of Texas) for providing recombinant Erk2. The work was supported by grants from the National Institutes of Health (HL18645 and HL03174 to R.R. and E.W.R.; DK42528 and GM42508 to E.G.K.), a grant from the Muscular Dystrophy Association (to E.G.K.) and a Pilot Project Grant from the W. M. Keck Center for Advanced Studies of Neural Signaling at the University of Washington (to E.G.K.). L.M.G. is supported in part by a mentor-based postdoctoral grant from the American Diabetes Association. K.E.B. is supported by a postdoctoral scholarship from the Swedish Society for Medical Research and the Swedish Medical Research Council.

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