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# Stimulation of $\alpha_{1A}$-Adrenoceptors in Rat-1 Cells Inhibits Extracellular Signal-Regulated Kinase by Activating p38 Mitogen-Activated Protein Kinase

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## ABSTRACT

In Rat-1 fibroblasts, endothelin-1 and a protein kinase C-stimulating phorbol ester stimulated extracellular signal-regulated kinase (ERK), whereas phenylephrine, acting at stably transfected human $\alpha_{1A}$-adrenoceptors, inhibited basal and endothelin-1- and phorbol ester-stimulated ERK. On the other hand, phenylephrine stimulated p38 mitogen-activated protein kinase (MAPK). Anisomycin caused p38 activation and ERK inhibition quantitatively similar to those produced by phenylephrine. SB 203,580, an inhibitor of p38, significantly attenuated phenylephrine- and anisomycin-induced ERK inhibition. The ERK inhibition by phenylephrine was not affected by the cytosolic phospholipase $A_2$ inhibitor arachidonyltrifluoromethyl ketone or the cyclooxygenase inhibitor indomethacin but was significantly attenuated by a combination of the phosphatase inhibitors $Na_3VO_4$ and okadaic acid. Neither SB 203,580 nor the phosphatase inhibitors significantly affected ERK inhibition by the adenylyl cyclase activator forskolin. We conclude that there is a previously unrecognized interaction between ERK and p38 MAPK, in which activation of p38 causes inhibition of ERK; this may at least partly involve MAPK phosphatases that inactivate ERK.

Receptors that are coupled to G proteins of the $G_q$ family can promote cellular growth in various tissues and cell types (Post and Brown, 1996). Stimulation of cellular hypertrophy and/or hyperplasia after activation of $G_q$-coupled $\alpha_1$-adrenoceptors has been observed in various cell types, including cardiomyocytes (Schlüter and Piper, 1992; Knowlton *et al.,* 1993), vascular smooth muscle cells (Chen *et al.,* 1995; Xin *et al.,* 1997), and renal tubular cells (Yang *et al.,* 1998). The role of $\alpha_1$-adrenoceptors as mediators of cellular growth is supported by the observation that the $\alpha_1$-adrenoceptor antagonist prazosin did not affect the blood pressure elevation observed upon chronic infusion of angiotensin II but inhibited angiotensin II-induced vascular hypertrophy, indicating that the growth-promoting effects of angiotensin II can occur indirectly via $\alpha_1$-adrenoceptor stimulation (van Kleef *et al.,* 1992).

MAPKs are a family of protein kinases that are thought to play a central role in the regulation of cellular growth; they can be divided into subfamilies, designated ERK, JNK (also known as stress-activated protein kinase), and p38 (Neary, 1997). MAPK can be activated in response to various stimuli, including the stimulation of receptors with intrinsic tyrosine kinase activity or G protein-coupled receptors (van Biesen *et al.,* 1996). Activation of ERK via $\alpha_1$-adrenoceptors has been shown in, for example, rat cardiomyocytes (Clerk *et al.,* 1994; Thorburn, 1994; Thorburn and Thorburn, 1994; Gillespie-Brown *et al.,* 1995; Yamazaki *et al.,* 1997), rat and human vascular smooth muscle cells (Hu *et al.,* 1996; Xu *et al.,* 1996; Xin *et al.,* 1997), rat hepatocytes (Spector *et al.,* 1997), canine renal tubular cells (Xing and Insel, 1996), and COS or PC-12 cells expressing cloned $\alpha_1$-adrenoceptors (Koch *et al.,* 1994; Hawes *et al.,* 1995; Zhong *et al.,* 1998). Much less is known about the effects of $\alpha_1$-adrenoceptor stimulation on JNK and p38 activation, but stimulation of JNK in aortic smooth muscle cells (Nishio *et al.,* 1996; Xu *et al.,* 1996) and of JNK and p38 in hepatocytes has also been reported (Spector *et al.,* 1997). Moreover, activation of ERK, JNK, and p38 has been found upon stimulation of $\alpha_{1A}$-adrenoceptors transfected into PC-12 cells (Zhong *et al.,* 1998). In this study, we have investigated the role of cloned human $\alpha_{1A}$-adrenoceptors, stably expressed in Rat-1 fibroblasts (Schwinn *et al.,* 1995), in the regulation of ERK and p38. We demonstrate that $\alpha_1$-adrenoceptor stimulation in these cells inhibits ERK and stimulates p38 and that the two effects are causally related.

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**ABBREVIATIONS:** MAPK, mitogen-activated protein kinase; AACOF3, arachidonyltrifluoromethyl ketone; ERK, extracellular signal-regulated kinase; JNK, c-Jun amino-terminal protein kinase; PMA, phorbol-12-myristate-13-acetate.

## Materials and Methods

**Cell cultures.** Rat-1 fibroblasts, which express endogenous endothelin receptors (Daub *et al.*, 1996) and which had been stably transfected with human $\alpha_{1A}$-adrenoceptors (Schwinn *et al.,* 1995), were obtained from Pfizer Central Research (Sandwich, Kent, UK). They were grown in an atmosphere of 5% $CO_2$/95% air at 37°, in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Subconfluent cells were subcultured every 3–4 days with a solution containing 0.5 g/liter trypsin and 0.2 g/liter $Na_4EDTA$. The antibiotic G418 (400 $\mu$g/ml) was added to all growing cells, to maintain selection pressure, but was not present during the experiments. For all experiments, the cells were cultured in the absence of serum for 24 hr before the experiments, to avoid interference of serum factors with MAPK stimulation.

**MAPK assays.** Because the activation of MAPK relies on tyrosine phosphorylation, we assessed the activity of ERK, JNK, and p38 with respect to the extent of tyrosine phosphorylation, using commercially available, epitope-specific, antiphosphotyrosine antibodies. For this purpose, Rat-1 fibroblasts grown in 60-mm dishes were incubated in the absence or presence of the indicated agents at 37° for 10 min, unless otherwise indicated. After aspiration of the culture medium, the cells were washed twice with ice-cold, $Ca^{2+}$-free, phosphate-buffered saline, and then 200 $\mu$l of sample buffer (2% sodium dodecyl sulfate, 50 mM dithiothreitol, 10% glycerol, 0.1% bromphenol blue, 62.5 mM Tris, pH 6.8 at 25°) was added. The cells were scraped off the dishes immediately and sonicated four times, for 10 sec each time, with continuous ice-cooling. The homogenates were boiled for 5 min and centrifuged at 14,000 $\times$ *g* for 5 min, and 20-$\mu$l aliquots of the supernatants from each experiment were loaded in parallel on two sodium dodecyl sulfate gels. The proteins were separated by electrophoresis (22 $\mu$A, for 2 hr), and the separated proteins were transferred to nitrocellulose membranes by electroblotting (40 V, overnight). The resulting blots were incubated for 2 hr (according to the instructions provided by the manufacturer) either with an antiserum recognizing total ERK, JNK, or p38 or with an antiserum specific for their tyrosine-phosphorylated forms. The blots were washed four times, for 10 min each time, with 80 ml of washing buffer (150 mM NaCl, 0.1% Tween-20, 50 mM Tris, pH 7.4 at 25°) and were then incubated for 1 hr with a secondary antibody (anti-rabbit immunoglobulin linked to horseradish peroxidase). After four more washes with buffer, detection was by enhanced chemiluminescence, according to the instructions provided by the manufacturer. The resulting autoradiographs were analyzed by quantitative two-dimensional densitometry, using commercially available software (Herolab, Wiesloch, Germany). The two-dimensional band intensity of tyrosine-phosphorylated MAPK was expressed relative to that of total MAPK, as assessed with a parallel blot prepared identically. The ratio for the control sample (i.e., no stimulator or inhibitor present) was set as 100%, and values for all other samples from the same blot were then expressed as percentages of control.

**Chemicals.** Kits for immunodetection of total and tyrosine-phosphorylated ERK, JNK, and p38 were obtained from New England Biolabs (Beverly, MA). The following reagents were purchased from the indicated sources: AACOF3 from Biomol (Plymouth Meeting, PA), endothelin-1 from Bachem (La Jolla, CA), forskolin from Calbiochem (La Jolla, CA), prazosin HCl from Tocris (Bristol, UK), SB 203,580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1*H*-imidazole] from Alexis (San Diego, CA), and anisomycin, indomethacin, okadaic acid, L-phenylephrine HCl, PMA, DL-propranolol HCl, and $Na_3VO_4$ from Sigma Chemical Co. (St. Louis, MO).

**Data analysis.** Data are shown as means ± standard errors of the indicated number of experiments. The statistical significance of differences between groups was tested by two-tailed, paired *t* tests or by repeated-measures analysis of variance followed by Dunnett's multiple-comparison tests, as indicated. All statistical calculations were performed with the Instat program (Graphpad Software, San Diego, CA), and $p < 0.05$ was considered significant.

## Results

Incubation of the Rat-1 cells with 100 nM endothelin-1 or 1 $\mu$M PMA for 3–10 min significantly enhanced ERK tyrosine phosphorylation, by 58 ± 22 and 33 ± 6% over basal values, respectively (Fig. 1). In contrast, incubation with 100 $\mu$M levels of the $\alpha_1$-adrenoceptor agonist phenylephrine did not stimulate ERK phosphorylation; rather, it significantly inhibited stimulation, by 69 ± 9% (Fig. 1). Phenylephrine also significantly inhibited ERK activation by endothelin-1 and PMA, by 58 ± 8 and 68 ± 8%, respectively (Fig. 1). The $\alpha_1$-adrenoceptor antagonist prazosin (300 nM) and the $\beta$-adrenoceptor antagonist propranolol (1 $\mu$M) did not affect ERK activation (Fig. 2). However, prazosin completely abolished the inhibition of ERK activation by phenylephrine; propranolol was without effect (Fig. 2).

The basal level of tyrosine phosphorylation of JNK in Rat-1 fibroblasts was too low for reliable quantitative detection by our methods, and phenylephrine, endothelin-1, and PMA did not cause consistent activation of JNK in these cells (data not shown). Although endothelin-1 and PMA did not cause significant activation of p38, phenylephrine enhanced p38 tyrosine phosphorylation approximately 6-fold; quantitatively similar activation of p38 was achieved with the positive control, anisomycin (50 $\mu$g/ml) (Fig. 3). The stimulatory effect of anisomycin was inhibited by 70 ± 12% by 20 $\mu$M levels of the p38 inhibitor SB 203,580 (four experiments, $p < 0.01$).

The p38 activator anisomycin inhibited ERK activation to

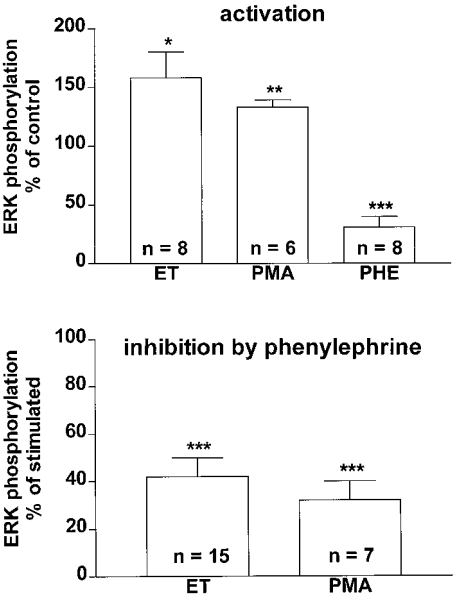


**Fig. 1.** Effects of the $\alpha_1$-adrenoceptor agonist phenylephrine (*PHE*) (100 $\mu$M), endothelin-1 (*ET*) (100 nM), and a protein kinase C-activating phorbol ester (PMA) (1 $\mu$M) on the tyrosine phosphorylation of ERK. Cells were incubated in the absence (control) or presence of the indicated agonists (*upper*) or in the presence of the indicated agonists plus phenylephrine (*lower*). Data are means ± standard errors from the indicated numbers of experiments (*n*) and are expressed as percentages of control values (*upper*) or as percentages of the values measured in the absence of phenylephrine (*lower*). Experiments with 3-min (three experiments) and 10-min (all others) incubations yielded similar results and were pooled for this analysis. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, compared with values measured in the absence of agonist, in a two-tailed, paired *t* test.

a similar extent as did phenylephrine, whereas the p38 inhibitor SB 203,580 significantly enhanced activation (Fig. 4). SB 203,580 also significantly attenuated the inhibition by phenylephrine and anisomycin (Fig. 4). Therefore, additional experiments were designed to identify pathways leading from p38 activation to inhibition of ERK. A 30-min pretreatment with the cyclooxygenase inhibitor indomethacin (10 $\mu$M) or with the cytosolic phospholipase $A_2$ inhibitor AACOF3 (10 $\mu$M) did not affect ERK phosphorylation (Fig. 5). Moreover, the two inhibitors did not affect the ability of phenylephrine to inhibit ERK activation (Fig. 5). On the other hand, a combination of the phosphatase inhibitors $Na_3VO_4$ (0.2 mM) and okadaic acid (1 $\mu$M) did not affect ERK activation but significantly attenuated the inhibitory effects of phenylephrine (Fig. 6). To assess whether SB 203,580 or the phosphatase inhibitors might have nonspecific effects in our cells, we investigated whether they affected the inhibition of ERK activation by forskolin. Forskolin (10 $\mu$M) markedly inhibited ERK activation, and this was not significantly affected by the presence of SB 203,580 or the phosphatase inhibitors (Fig. 7).

## Discussion

Natively expressed and transfected cloned $\alpha_1$-adrenoceptors can couple to activation of the ERK form of MAPK in a variety of cell systems (Clerk *et al.,* 1994; Koch *et al.,* 1994; Thorburn, 1994; Thorburn and Thorburn, 1994; Gillespie-Brown *et al.,* 1995; Hawes *et al.,* 1995; Hu *et al.,* 1996; Xing and Insel, 1996; Xu *et al.,* 1996; Spector *et al.,* 1997; Xin *et al.,* 1997; Yamazaki *et al.,* 1997), and only a few exceptions have been reported (Nishio *et al.,* 1996). In the present study, we observed that phenylephrine did not stimulate ERK but, rather, inhibited basal and endothelin-1- and PMA-stimulated ERK tyrosine phosphorylation in Rat-1 fibroblasts stably expressing cloned human $\alpha_{1A}$-adrenoceptors. Our experiments with prazosin and propranolol confirm that the ERK inhibition by phenylephrine is mediated by $\alpha_1$-adrenoceptors.

It is assumed that $G_q$-coupled receptors such as the $\alpha_{1A}$-adrenoceptor activate ERK via their stimulatory effects on phospholipase C/protein kinase C pathways (Post and Brown, 1996; van Biesen *et al.,* 1996). We previously demonstrated that phenylephrine stimulation of our human $\alpha_{1A}$-adrenoceptor-expressing Rat-1 fibroblasts causes $Ca^{2+}$ elevations similar to those produced by endothelin-1 stimulation and causes protein kinase C activation similar to that produced by PMA stimulation (Taguchi *et al.,* 1998). Thus, the lack of ERK activation by $\alpha_1$-adrenoceptor stimulation in the present study does not seem to be related to a lack of efficient phospholipase C/protein kinase C stimulation. In many cell types, $\alpha_1$-adrenoceptor stimulation can activate phospholipase $A_2$ to release arachidonic acid (Weiss and Insel, 1991; Perez *et al.,* 1993). Stimulation of cytosolic phospholipase $A_2$ may occur secondary to p38 activation for some receptors (Börsch-Haubold *et al.,* 1997), and cyclooxygenase- and lipoxygenase-derived arachidonic acid metabolites may inhibit cellular ERK activation and growth effects after $\alpha_1$-adrenoceptor stimulation (Li *et al.,* 1995; Nishio and Watanabe, 1997). However, our findings with the cytosolic phospholipase $A_2$ inhibitor AACOF3 and the cyclooxygenase inhibitor indomethacin do not support a role for either enzyme in the inhibition of ERK by phenylephrine.

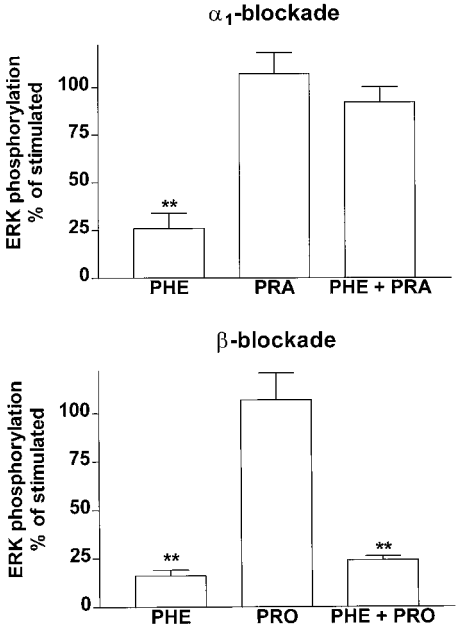


**Fig. 2.** Effects of adrenoceptor antagonists on the inhibition of ERK by phenylephrine (*PHE*). Cells were incubated in the absence (control) or presence of the $\alpha_1$-adrenoceptor antagonist prazosin (*PRA*) (300 nM) or the $\beta$-adrenoceptor antagonist propranolol (*PRO*) (1 $\mu$M). Data are means ± standard errors from four experiments and are expressed as percentages of the values measured in the absence of phenylephrine and the antagonists. **, $p < 0.01$, compared with control values, in a repeated-measures, one-way analysis of variance, followed by Dunnett's multiple-comparison test. Endothelin-1 (100 nM) or a protein kinase C-activating phorbol ester (1 $\mu$M) was used to stimulate ERK activation (two experiments each); results obtained with the two stimulators were similar and were pooled for this analysis.

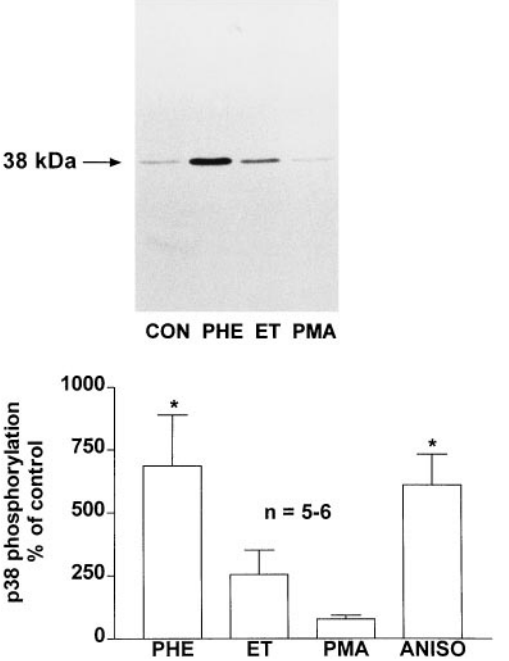


**Fig. 3.** Effects of phenylephrine (*PHE*) (100 $\mu$M), endothelin-1 (*ET*) (100 nM), a protein kinase C-activating phorbol ester (PMA) (1 $\mu$M), and anisomycin (*ANISO*) (50 $\mu$g/ml), relative to control (*CON*), on tyrosine phosphorylation of p38 MAPK. *Upper*, data are from a representative experiment; *lower*, data are means ± standard errors of five or six experiments and are shown as percentages of control values measured in the absence of the agonists. *, $p < 0.05$, compared with control values, in a two-tailed, paired *t* test.

Stimulation of p38, another member of the MAPK family, was previously demonstrated with endogenous $\alpha_1$-adrenoceptors in rat hepatocytes (Spector *et al.,* 1997) and with cloned $\alpha_{1A}$-adrenoceptors transfected into PC-12 cells (Zhong *et al.,* 1998). Our present findings with cloned $\alpha_{1A}$-adrenoceptors expressed in Rat-1 cells demonstrated marked stimulation of p38, which was quantitatively similar to that seen with anisomycin (i.e., approximately 6-fold). Interestingly, much less (if any) p38 activation was seen with endothelin-1 and PMA, although these two agonists produce similar activation of the phospholipase C/protein kinase C pathway in our cells (Taguchi *et al.,* 1998). Thus, p38 activation may occur via an additional signal generated by stimulation of $\alpha_1$-adrenoceptors that is not generated by endothelin receptors or direct protein kinase C activation.

Several lines of evidence in our study suggest that p38 activation by $\alpha_1$-adrenoceptor stimulation is the cause of ERK inhibition. First, quantitatively similar p38 activation, independent of $\alpha_1$-adrenoceptors, by anisomycin caused quantitatively similar ERK inhibition. Second, SB 203,580, at a concentration that inhibits anisomycin-induced p38 activation by 70%, at least partly prevented ERK inhibition by phenylephrine and anisomycin. On the other hand, SB 203,580 did not affect ERK inhibition by forskolin, which is known to inhibit ERK via Raf inhibition (van Biesen *et al.,* 1996). Third, SB 203,580 alone activated ERK. Finally, a recent study of baboon smooth muscle cells also suggested ERK inhibition secondary to p38 activation (Daum *et al.,* 1998). Taken together, these data clearly demonstrate cross-

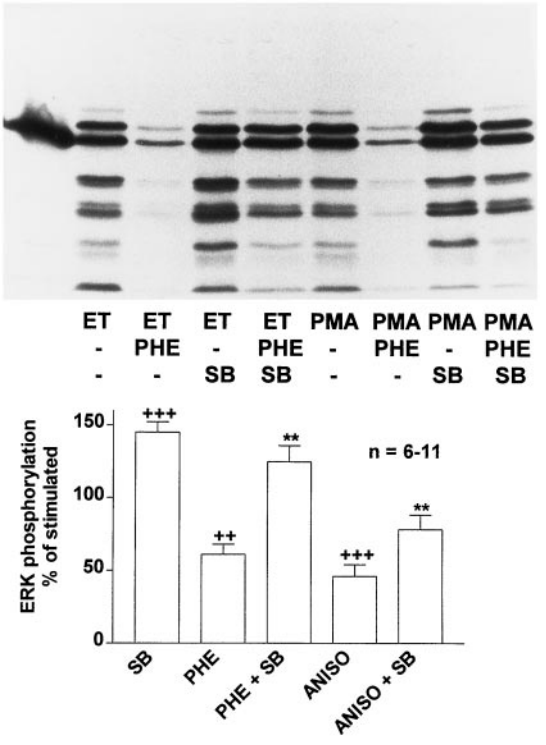


**Fig. 4.** Effects of the p38 inhibitor SB 203,580 (*SB*) (20 $\mu$M) on the inhibition of ERK by phenylephrine (*PHE*) (100 $\mu$M) and anisomycin (*ANISO*) (50 $\mu$g/ml). A protein kinase C-activating phorbol ester (PMA) (1 $\mu$M, two or three experiments) or endothelin-1 (*ET*) (100 nM, all other experiments) was used to stimulate ERK activation. Data are from a representative experiment (*upper*) or are means ± standard errors of 6–11 experiments (*lower*). Because results obtained with the two stimulators were similar (*upper*), they were pooled for quantitative analysis and are shown as percentages of values measured in the absence of phenylephrine, anisomycin, and SB 203,580 (control). ++, $p < 0.01$; +++, $p < 0.001$, compared with control values; **, $p < 0.01$, compared with data obtained in the absence of SB 203,580, in a two-tailed, paired *t* test. *Upper*, *left lane*, tyrosine-phosphorylated ERK standard (42-kDa form).

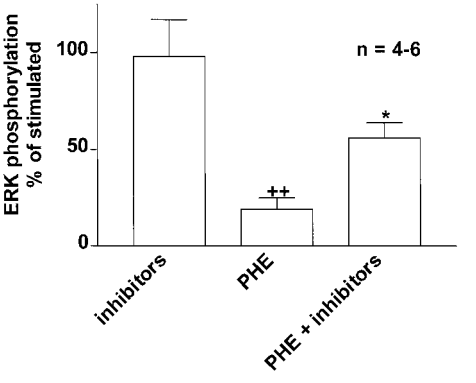


**Fig. 6.** Effects of the phosphatase inhibitors (*inhibitors*) $Na_3VO_4$ (0.2 mM) and okadaic acid (1 $\mu$M) on the inhibition of ERK by phenylephrine (*PHE*) (100 $\mu$M). Endothelin-1 (100 nM) was used to stimulate ERK activation. Data are means ± standard errors of four to six experiments and are shown as percentages of values measured in the absence of phenylephrine and the inhibitors (control). ++, $p < 0.01$, compared with control values; *, $p < 0.05$, compared with data obtained in the absence of the inhibitors, in a two-tailed, paired *t* test.

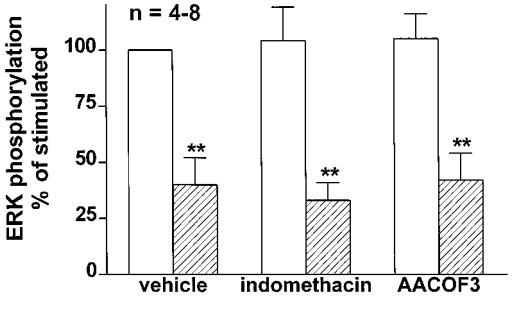


**Fig. 5.** Effects of the cyclooxygenase inhibitor indomethacin (10 $\mu$M) and the cytosolic phospholipase $A_2$ inhibitor AACOF3 (10 $\mu$M) on tyrosine phosphorylation of ERK. Cells were incubated with 100 nM endothelin-1 in the absence (*vehicle*) or presence of the inhibitors and in the absence (control) (□) and presence of 100 $\mu$M phenylephrine (▨). Data are means ± standard errors of four to eight experiments and are shown as percentages of values measured in the presence of endothelin-1 alone (vehicle control). **, $p < 0.01$, compared with data obtained in the absence of phenylephrine, in a two-tailed, paired *t* test.

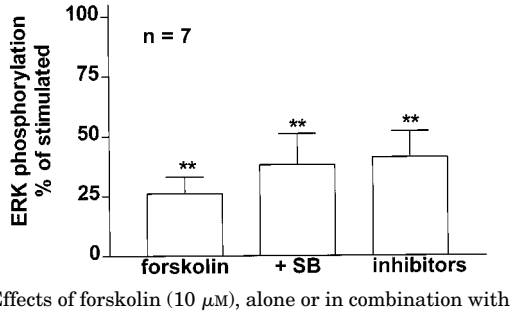


**Fig. 7.** Effects of forskolin (10 $\mu$M), alone or in combination with the p38 MAPK inhibitor SB 203,580 (*SB*) (20 $\mu$M) or the phosphatase inhibitors (*inhibitors*) $Na_3VO_4$ (0.2 mM) and okadaic acid (1 $\mu$M), on endothelin-1 (100 nM)-stimulated phosphorylation of ERK. Data are means ± standard errors of seven experiments and are shown as percentages of values measured in the presence of endothelin-1 alone (control). **, $p < 0.01$, compared with control values, in a two-tailed, paired *t* test. Data obtained with forskolin in the presence of the inhibitors were not significantly different from data measured in their absence, in a repeated-measures analysis of variance ($p = 0.3378$).

talk between the p38 and ERK forms of MAPK, in which the former causes inhibition of the latter. In Rat-1 fibroblasts this inhibition may be tonically active, because the p38 inhibitor alone significantly enhanced ERK activity.

Inhibition of ERK could result from reduced activation and/or accelerated inactivation. Inactivation of ERK and other MAPKs occurs via specific phosphatases, several of which have been identified (Chu *et al.,* 1996; Groom *et al.,* 1996; Muda *et al.,* 1996). To test the involvement of MAPK phosphatases in ERK inhibition, we used a combination of the general phosphatase inhibitors $Na_3VO_4$ and okadaic acid, which blocks MAPK phosphatase activity in Madin-Darby canine kidney cells (Itoh *et al.,* 1995). These phosphatase inhibitors significantly attenuated ERK inhibition by $\alpha_1$-adrenoceptor stimulation but did not affect endothelin-1-induced ERK activation, indicating the possible involvement of MAPK phosphatases in phenylephrine-induced ERK inhibition. Although the phosphatase inhibitors were specific antagonists of the inhibitory effects of $\alpha_{1A}$-adrenoceptor stimulation, relative to those of forskolin, in the present study, it should be noted that the inhibitors act on many types of protein phosphatases and are not specific for MAPK phosphatases. Previous reports on the activation of MAPK phosphatases have largely focused on their transcriptional regulation (Bokemeyer *et al.,* 1996; Brondello *et al.,* 1997). However, this is unlikely to account for ERK inhibition in our experiments, because we detected ERK inhibition after only 3 min. Therefore, nontranscriptional activation of MAPK phosphatases may be involved in phenylephrine-induced ERK inhibition in Rat-1 cells. Unfortunately, the mechanisms of nontranscriptional control of MAPK phosphatase activity are largely unknown.

ERK inhibition by phenylephrine and other p38 activators could also occur via reduced ERK activation. The activation of ERK by $G_q$-coupled receptors involves parallel $p21^{ras}$/raf-dependent and -independent pathways (Post and Brown, 1996; van Biesen *et al.,* 1996). The adenylyl cyclase stimulator forskolin can block ERK activation downstream of $p21^{ras}$, at the level of Raf (van Biesen *et al.,* 1996). Whereas forskolin effectively reduced ERK activation in the present study, neither the p38 inhibitor SB 203,580 nor the phosphatase inhibitors $Na_3VO_4$ and okadaic acid significantly altered the forskolin effects. Thus, inhibitors that were effective against phenylephrine-induced ERK inhibition did not affect inhibition based on interference with the ERK-activating pathways, at least not those that are Raf-dependent. Although these data cannot exclude the possibility of reduced activation of ERK upon phenylephrine-induced p38 activation, they are consistent with our hypothesis that ERK inhibition by $\alpha_1$-adrenoceptor stimulation involves accelerated inactivation by phosphatases.

In summary, our data show that stimulation of human $\alpha_{1A}$-adrenoceptors expressed in Rat-1 fibroblasts, in contrast to endothelin-1 and the protein kinase C activator PMA, activates p38. The p38 activation results in inhibition of ERK activation, revealing previously unrecognized cross-talk between these two members of the MAPK family. We propose that the ERK inhibition may involve accelerated ERK inactivation by MAPK phosphatases, but the exact links between p38, the MAPK phosphatase, and ERK remain to be elucidated. Therefore, the effects of $\alpha_1$-adrenoceptor stimulation on cellular growth processes may depend on the balance between ERK and p38 activation and their roles in growth regulation in a given cell type. Whether such effects also occur with $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptors is not known, but studies of other signal transduction pathways of $\alpha_1$-adrenoceptors suggest that differences between the subtypes are largely quantitative, rather than qualitative (Theroux *et al.,* 1996; Taguchi *et al.,* 1998).



## References


Bokemeyer D, Sorokin A, Yan M, Ahn NG, Templeton DJ, and Dunn MJ (1996) Induction of mitogen-activated protein kinase phosphatase 1 by the stress-activated protein kinase signaling pathway but not by extracellular signal-regulated kinase in fibroblasts. *J Biol Chem* **271:**639–642.

Börsch-Haubold AG, Kramer RM, and Watson SP (1997) Phosphorylation and activation of cytosolic phospholipase $A_2$ by 38-kDa mitogen-activated protein kinase in collagen-stimulated human platelets. *Eur J Biochem* **245:**751–759.

Brondello J-M, Brunet A, Pouyssegur J, and McKenzie FR (1997) The dual specificity mitogen-activated protein kinase phosphatase-1 and -2 are induced by the p42/$p44^{MAPK}$ cascade. *J Biol Chem* **272:**1368–1376.

Chen L, Xin X, Eckhart AD, Yang N, and Faber JE (1995) Regulation of vascular smooth muscle growth by $\alpha_1$-adrenoreceptor subtypes *in vitro* and *in situ*. *J Biol Chem* **270:**30980–30988.

Chu Y, Solski PA, Khosravi-Far R, Der CJ, and Kelly K (1996) The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity *in vivo* toward the ERK2 sevenmaker mutation. *J Biol Chem* **271:**6497–6501.

Clerk A, Bogoyevitch MA, Andersson MB, and Sugden PH (1994) Differential activation of protein kinase C isoforms by endothelin and phenylephrine and subsequent stimulation of p42 and p44 mitogen-activated protein kinases in ventricular myocytes cultured from neonatal rat hearts. *J Biol Chem* **269:**32848–32857.

Daub H, Weiss FU, Wallasch C, and Ullrich A (1996) Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature (Lond)* **379:**557–560.

Daum G, Kalmes A, Levkau B, Wang Y, Davies MG, and Clowes AW (1998) Pervanadate inhibits mitogen-activated protein kinase kinase-1 in a $p38^{MAPK}$-dependent manner. *FEBS Lett* **427:**271–274.

Gillespie-Brown J, Fuller SJ, Bogoyevitch MA, Cowley S, and Sugden PH (1995) The mitogen-activated protein kinase kinase MEK1 stimulates a pattern of gene expression typical of the hypertrophic phenotype in rat ventricular cardiomyocytes. *J Biol Chem* **270:**28092–28096.

Groom LA, Sneddon AA, Alessi DR, Dowd S, and Keyse SM (1996) Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specificity phosphatase. *EMBO (Eur Mol Biol Organ) J* **15:**3621–3632.

Hawes BE, van Biesen T, Koch WJ, Luttrell LM, and Lefkowitz RJ (1995) Distinct pathways of $G_i$- and $G_q$-mediated mitogen-activated protein kinase activation. *J Biol Chem* **270:**17148–17153.

Hu Z-W, Shi X-Y, Lin RZ, and Hoffman BB (1996) $\alpha_1$-Adrenergic receptors activate phosphatidylinositol 3-kinase in human vascular smooth muscle cells: role in mitogenesis. *J Biol Chem* **271:**8977–8982.

Itoh T, Yamauchi A, Imai E, Ueda N, and Kamada T (1995) Phosphatase toward MAP kinase is regulated by osmolarity in Madin-Darby canine kidney (MDCK) cells. *FEBS Lett* **373:**123–126.

Knowlton KU, Michel MC, Itani M, Shubeita HE, Ishihara K, Brown JH, and Chien KR (1993) The $\alpha_{1A}$-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. *J Biol Chem* **268:**15374–15380.

Koch WJ, Hawes BE, Allen LF, and Lefkowitz RJ (1994) Direct evidence that $G_i$-coupled receptor stimulation of mitogen-activated protein kinase is mediated by $G_{\beta\gamma}$ activation of $p21^{ras}$. *Proc Natl Acad Sci USA* **91:**12706–12710.

Li X, Zarinetchi F, Schrier RW, and Nemenoff RA (1995) Inhibition of MAP kinase by prostaglandin $E_2$ and forskolin in rat renal mesangial cells. *Am J Physiol* **269:**C986–C991.

Muda M, Theodosiou A, Rodrigues N, Boschert U, Camps M, Gillieron C, Davies K, Ashworth A, and Arkinstall S (1996) The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *J Biol Chem* **271:**27205–27208.

Neary JT (1997) MAPK cascades in cell growth and death. *News Physiol Sci* **12:**286–293.

Nishio E, Nakata H, Arimura S, and Watanabe Y (1996) $\alpha_1$-Adrenergic receptor stimulation causes arachidonic acid release through pertussis toxin-sensitive GTP-binding protein and JNK activation in rabbit aortic smooth muscle cells. *Biochem Biophys Res Commun* **219:**277–282.

Nishio E and Watanabe Y (1997) The regulation of mitogenesis and apoptosis in response to the persistent stimulation of $\alpha_1$-adrenoceptors: a possible role of 15-lipoxygenase. *Br J Pharmacol* **122:**1516–1522.

Perez DM, DeYoung MB, and Graham RM (1993) Coupling of expressed $\alpha_{1B}$- and $\alpha_{1D}$-adrenergic receptors to multiple signaling pathways is both G protein and cell type specific. *Mol Pharmacol* **44:**784–795.

Post GR and Brown JH (1996) G protein-coupled receptors and signaling pathways regulating growth responses. *FASEB J* **10:**741–749.

Schlüter K-D and Piper HM (1992) Trophic effects of catecholamines and parathyroid hormone on adult ventricular cardiomyocytes. *Am J Physiol* **263:**H1739–H1746.

Schwinn DA, Johnston GL, Page SO, Mosley MJ, Wilson KH, Worman NP, Campbell S, Roock MO, Furness LM, Parry-Smith DJ, Peter B, and Beiley DS (1995) Cloning and pharmacological characterization of human *alpha*-1 adrenergic receptors:

sequence corrections and direct comparison with other species. *J Pharmacol Exp Ther* **272:**134–142.
Spector MS, Auer KL, Jarvis WD, Ishac EJN, Gao B, Kunos G, and Dent P (1997) Differential regulation of the mitogen-activated protein and stress-activated protein kinase cascades by adrenergic agonists in quiescent and regenerating adult rat hepatocytes. *Mol Cell Biol* **17:**3556–3565.
Taguchi K, Yang M, Goepel M, and Michel MC (1998) Comparison of human $\alpha_1$-adrenoceptor subtype coupling to protein kinase C activation and related signalling pathways. *Naunyn-Schmiedeberg's Arch Pharmacol* **358:**100–110.
Theroux TL, Esbenshade TA, Peavy RD, and Minneman KP (1996) Coupling efficiencies of human $\alpha_1$-adrenergic receptor subtypes: titration of receptor density and responsiveness with inducible and repressible expression vectors. *Mol Pharmacol* **50:**1376–1387.
Thorburn A (1994) Ras activity is required for phenylephrine-induced activation of mitogen-activated protein kinase in cardiac muscle cells. *Biochem Biophys Res Commun* **205:**1417–1422.
Thorburn J and Thorburn A (1994) The tyrosine kinase inhibitor, genistein, prevents $\alpha$-adrenergic-induced cardiac muscle cell hypertrophy by inhibiting activation of the Ras-MAP kinase signaling pathway. *Biochem Biophys Res Commun* **202:** 1586–1591.
van Biesen T, Luttrell LM, Hawes BE, and Lefkowitz RJ (1996) Mitogenic signaling via G protein-coupled receptors. *Endocr Rev* **17:**698–714.
van Kleef EM, Smits JFM, De Mey JGR, Cleutjens JPM, Lombardi EM, Schwartz SM, and Daemen MJAP (1992) $\alpha_1$-Adrenoceptor blockade reduces the angiotensin II-induced vascular smooth muscle cell DNA synthesis in the rat thoracic aorta and carotid artery. *Circ Res* **70:**1122–1127.
Weiss BA and Insel PA (1991) Intracellular $Ca^{2+}$ and protein kinase C interact to regulate $\alpha_1$-adrenergic- and bradykinin receptor-stimulated phospholipase $A_2$ activation in Madin-Darby canine kidney cells. *J Biol Chem* **266:**2126–2133.
Xin X, Yang N, Eckhart AD, and Faber JE (1997) $\alpha_{1D}$-Adrenergic receptors and mitogen-activated protein kinase mediate increased protein synthesis by arterial smooth muscle. *Mol Pharmacol* **51:**764–775.
Xing M and Insel PA (1996) Protein kinase C-dependent activation of cytosolic phospholipase $A_2$ and mitogen-activated protein kinase by *alpha*$_1$-adrenergic receptors in Madin-Darby canine kidney cells. *J Clin Invest* **97:**1302–1310.
Xu Q, Liu Y, Gorospe M, Udelsman R, and Holbrook NJ (1996) Acute hypertension activates mitogen-activated protein kinases in arterial wall. *J Clin Invest* **97:**508–514.
Yamazaki T, Komuro I, Zou Y, Kudoh S, Shiojima I, Hiroi Y, Mizuno T, Aikawa R, Takano H, and Yazaki Y (1997) Norepinephrine induces the Raf-1 kinase/mitogen-activated protein kinase cascade through both $\alpha_1$- and $\beta$-adrenoceptors. *Circulation* **95:**1260–1268.
Yang M, Büscher R, Taguchi K, Grübbel B, Insel PA, and Michel MC (1998) Protein kinase C does not mediate phenylephrine-induced down-regulation of MDCK cell $\alpha_{1B}$-adrenoceptors. *J Pharmacol Exp Ther* **286:**36–43.
Zhong H, Williams NG, and Minneman KP (1998) Effects of $\alpha_1$-, $\alpha_2$- and $\beta$-adrenergic receptors on MAP kinase activation and differentiation in transfected PC12 cells. *FASEB J* **12:**A452.

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