Prolonged exposure of vascular smooth muscle cells (VSMC) to vasoconstrictors such as vasopressin or angiotensin II induces hypertrophy and increases expression of muscle-specific genes including smooth muscle α-actin (SM-α-actin). These vasoconstrictors signal through G-proteins, including members of the Gq family. To further investigate the role of Gq family members, VSMC were transfected with a constitutively active mutant of a Gq family member, Ga16 (Ga16Q212L). Stable expression of Ga16Q212L persistently stimulated phospholipase C, resulting in increased basal levels of inositol phosphates. These cells were hypertrophied and expressed elevated levels of SM-α-actin compared with wild-type VSMC or cells transfected with a control plasmid (Neo). SM-α-actin promoter activity was markedly increased in cells stably or transiently expressing Ga16Q212L. Basal c-Jun-NH2-terminal kinase (JNK) activity was increased 3–9-fold in cells stably expressing Ga16Q212L, while basal activity of the p42/44 mitogen-activated protein kinases (ERKs) was unaffected. Transient expression of a kinase inactive JNK kinase partially inhibited induction of SM-α-actin promoter activity in response to vasoconstrictors or expression of Ga16Q212L. These results indicate that expression of constitutively active Ga16 in VSMC mimics the effects of vasoconstrictors on hypertrophy and muscle-specific gene expression, and activation of JNK may play a role in these responses.

Vascular smooth muscle cells (VSMC) are capable of two distinct growth responses following agonist stimulation. Stimulation by platelet-derived growth factor (PDGF) or epidermal growth factor (EGF), which signal through tyrosine kinase receptors, results in cell proliferation (1) while stimulation by vasoconstrictors such as arginine vasopressin (AVP) or angiotensin II (AII) as sole agents results in hypertrophy of cells with no significant increase in cell number (2–4). These two classes of agents have also been shown to have opposing effects on the expression of muscle-specific genes including the smooth muscle isoform of α-actin (SM-α-actin). Vasoconstrictors increase both steady-state protein and mRNA levels for SM-α-actin, whereas PDGF decreases expression of SM-α-actin and suppresses the vasoconstrictor-induced increases (5). We have recently shown that these effects are mediated through regulation of the SM-α-actin promoter, involving two CArG elements (6).

AVP and AII signal through “seven-membrane spanning” receptors coupled to G-proteins. In VSMC, both AVP and AII, acting through a pertussis toxin-insensitive G-protein, probably Gq, cause activation of phosphatidylinositol-specific phospholipase Cβ (7), leading to the mobilization of intracellular Ca2+ and activation of protein kinase C (PKC). This increase in intracellular Ca2+ is likely to be responsible for the acute constrictor effects of these agents. The G-proteins and effector pathways involved in mediating the longer term effects of vasoconstrictors on induction of cellular hypertrophy and smooth muscle gene expression are much less well understood. Indeed, G-protein coupled receptors have been shown to couple to multiple G-proteins in the same cell type, and it is conceivable that the longer-term effects of AVP and AII involve G-proteins distinct from those involved in the acute contractile responses.

Vasoconstrictors have been shown to stimulate the extracellular signal-regulated kinase (ERK) members of the family of mitogen-activated protein (MAP) kinase family in VSMC (8). The ERK pathway has been implicated in mitogenesis in other cell types (9) and thus is an obvious candidate for mediating the growth-promoting actions of vasoconstrictors on VSMC. Other members of the MAP kinase family, the c-Jun-NH2-terminal kinases (JNKs)/stress-activated protein kinases (SAPKs) are strongly activated by G-protein-coupled receptors in other cell types (10); however, their role in VSMC is not well described. Both ERKs and JNKs are regulated in a parallel fashion involving distinct dual-specificity protein kinases, MEK for the ERKs, and JNKK or MKKs for the JNKs (11).

In this study we have examined the effects of expressing constitutively active forms of members of the Gaq family in rat aortic VSMC. Our results indicate that activation of this family of G-proteins is sufficient to mimic the effects of vasoconstric-
tors on hypertrophy and induction of SM-α-actin expression and suggest that the JNK/SAPK pathway participates in this process.

**EXPERIMENTAL PROCEDURES**

**Materials**—Trypsin-EDTA and Eagle’s MEM were from Life Technologies, Inc. Hepes, AVP, phorbol 12-myristate 13-acetate (PMA), myosin basic protein (MBP), protein kinase inhibitor peptide, phenylmethylsulfonyl fluoride, pipes, and the formalin-fixed *Staphylococcus aureus* (protein A) were obtained from Sigma. Monoclonal antibody to smooth muscle α-actin was obtained from Boehringer Mannheim. Polyclonal antibody against αS was a gift of Dr. Gary Johnson (National Jewish Center for Immunology and Respiratory Medicine, Denver CO).

**PDGF-BB and polyclonal rabbit anti-rat MAP kinase R2/ERK1-CT** were from UBI (Lake Placid, NY). The catalytically inactive SERK-1 cDNA (JNKII/K116R) containing a Lys to Arg point mutation was a gift of Dr. Leonard Zon (Children’s Hospital, Boston, MA). Immobilon-P was purchased from Millipore Corp. (Bedford, MA). High molecular weight standards for SDS-PAGE and all other reagents for SDS-PAGE and Western blotting were obtained from Pharmacia Biotech Inc. and Life Technologies, Inc. [γ-32P]ATP and nuclease-free [3H]inositol were from Amersham Corp. (Arlington Heights, IL), and [35S]-protein A was from ICN (Irvine, CA).

**Rat Vascular Smooth Muscle Cell Culture and Retrovirus-mediated Gene Transfer**—Rat aortic VSMC were isolated and subcultured as described previously (12, 13). Packaging of LNCX-α16Q212L in GP + E-86 cells was performed as described previously (14). Constructs were transfected into GP + E-86 cells, which produce a retrovirus specific for cells of rodent origin. Media from the GP + E-86 cells containing retrovirus were incubated for 24 h in the presence of polybrene (8 μg/ml) with early passage VSMC. The infected cells were then plated in fresh media containing 500 μg/ml G-418 (Life Technologies, Inc.) for selection. Stable populations of G-418-resistant VSMC lines were then subcloned.

Three clones representative of more than 20 examined were chosen for further characterization (αS43, αS54, and αS77). The LNCX vector lacking DNA insert was packaged identically to pLNCX-α16Q212L and used as a control (Neo). Transfected cells up to passage 8 were used, and Neo controls of the same passage were employed in each experiment. For transient transfection experiments, cells were co-transfected with 5 μg of LNCX-α16Q212L or LNCX lacking an insert (Neo) as described below.

**Analysis of Inositol Phosphates**—Confluent αS43Q212L-transfected VSMC grown on 35-mm plates were incubated overnight with 1 μCi/ml of [3H]inositol in media containing 0.1% bovine serum albumin (8 μg/ml) with early passage VSMC. The labeled medium was removed, and cells were washed twice with media containing 0.1% bovine serum albumin and once with media containing 0.1% bovine serum albumin and 20 μM LiCl. Cells were then pre-incubated for 10 min at 37°C in 1 ml of media containing 0.1% bovine serum albumin and 20 μM LiCl. AVP (10−7 M) or AII (10−7 M) was added, and the incubation was continued for an additional 20 min. Treated cells were placed on ice, and 1 ml of ice-cold MeOH:HCl (100:1) was added followed by 2 ml of CHCl3. Samples were centrifuged for 15 min at 3,000 rpm, and total inositol phosphates in the aqueous phase were separated by anion-exchange chromatography (AG1-X8, Bio-Rad) as described previously (15).

**Measurement of [Ca2+]i**—Cells grown on glass coverslips for 3 days and incubated in serum-free media overnight prior to the experiment. Changes in single cell intracellular Ca2+ ([Ca2+]i) were measured with the Ca2+ indicator Fura-2 using video imaging as described previously (8). The maximal ratio was measured after addition of 10 μM ionomycin, and the minimal ratio was measured after subsequent addition of 10 mM Tris-EGTA. [Ca2+]i was calculated using the formula of Grynkiewicz (16). Individual fields containing 10–20 cells were used for each determination.

**Quantification of αS2**—Cells were grown on glass coverslips for 3 days and incubated in serum-free media overnight prior to the experiment. Changes in single cell intracellular Ca2+ ([Ca2+]i) were measured with the Ca2+ indicator Fura-2 using video imaging as described previously (8). The maximal ratio was measured after addition of 10 μM ionomycin, and the minimal ratio was measured after subsequent addition of 10 mM Tris-EGTA. [Ca2+]i was calculated using the formula of Grynkiewicz (16). Individual fields containing 10–20 cells were used for each determination.

**Assay of Protein Kinases**—For determination of ERK activity, confluent cells on 35-mm plates were incubated in serum-free media for 1 day prior to experiments. Cells were stimulated by AVP (10−7 M), AII (10−7 M), PMA (10−5 M), EGF (10−5 M), or PDGF (20 ng/ml) for 5 min at 37°C. Following stimulation, cells were washed twice with bovine serum albumin (250 μg/ml) and centrifuged for 20 min at 1,500 rpm, and total inositol phosphates in the aqueous phase were separated by anion-exchange chromatography (AG1-X8, Bio-Rad) as described previously (15).

**Measurement of SM-α-actin Promoter Activity**—Cells were transiently co-transfected with 15 μg of the full-length pCAT-α-actin construct containing 713 base pairs of 5′ region previously described (6) (pCATACT−713/52), the pCAT-Basic vector (promoterless negative control), or the pCAT control vector (constitutively active positive control, Promega) together with 5 μg of a CMV-β-galactosidase vector (CLONTECH) by electroporation using a geneZAPPER (IBI). The cells were then plated overnight in culture medium containing 10% FCS for 18 h. Cells were then placed in Eagle’s MEM containing 0.2% FCS and exposed to AVP or PDGF for 72 h. Following exposure to agonists, cells were harvested by trypsinization and cell pellets frozen at −20°C. The CAT activity of the cell lysates was measured using a modification of the thin layer chromatographic method as described by Gorman et al. (19). CAT activity was normalized to the β-galactosidase activity (20) present in the same sample to correct for transfection efficiency and was calculated as pmol chloramphenical acetylated/ml/million of β-galactosidase. In experiments examining the effect of JNK, 10 μg of JNKK1(K116R) was added to the pCAT-α-actin and CMV-β-galactosidase vectors. For transient expression of αS5, 5 μg of LNCX-α16Q212L or empty vector was added to the transfection mix, and cells were incubated as described above.

**RESULTS**

**Stable Expression of αS43Q212L in Transfected VSMC**—To examine the role of members of the Gq family in the growth and differentiation of VSMC, rat aortic VSMC were initially transfected with constitutively active Gαq (αS43Q209L). Multiple transfections using selection in media containing G-418 failed to isolate cells expressing functional αS43Q209L, suggesting that expression of constitutively active Gαq may be cytoxic to these cells. We therefore repeated these experiments using another member of the Gq family, Gα16 (αS16Q212L). By examination of amino acid sequence, Gα16 has 57% identity with Gαq and also has been shown to couple to phospholipase C (22). G-418-resistant clones were screened by immunoblotting using an antibody specific for the αS16 polypeptide. Cells transfected with a plasmid lacking an insert (Neo) had undetectable levels of αS16 as examined by normal immunoblotting compared to cells of hematopoietic origin (23). By phase contrast microscopy, cells expressing αS16 Q212L appeared elongated and enlarged compared with Neo or wild-type (WT) VSMC and formed networks or aggregates. This effect appeared to be specific for αS16 since stable expression of constitutively active αS12 or αo results in cells that are indistinguishable in morphology
from Neo or wild-type cells (data not shown). Three representative clones (43, 54, and 77), having high levels of α16Q212L expression were selected for detailed study.

Since members of the Gq family couple to phospholipase C, production of inositol phosphates was examined in the transfected cells. WT and Neo control VSMC had low basal levels of inositol phosphates, which were stimulated 12–20-fold by exposure of the cells to AVP or AII (Fig. 1). In three independent clones expressing α16Q212L, basal inositol phosphates were markedly increased and were comparable to agonist-stimulated levels in WT and Neo cells. Stimulation of the transfected cells by vasoconstrictors caused no further increase in inositol phosphate production. These results indicate that α16Q212L protein stably expressed in VSMC is functionally active. Resting intracellular Ca2+ ([Ca2+]i) levels were not significantly higher in cells expressing α16Q212L than in Neo or WT cells, averaging 150–200 nM. Increases in [Ca2+]i, in response to either AVP (data not shown) or AII were blunted compared with Neo controls (Fig. 2). Similar desensitization of agonist-induced Ca2+ mobilization has been reported in Swiss-3T3 cells and small lung cancer cells expressing α16Q212L (14, 21).

Cells expressing α16Q212L grew more slowly than Neo or WT-VSMC (data not shown), consistent with previous reports showing growth inhibition by α16Q212L in Swiss-3T3 cells (21), PC12 cells (24), and small cell lung cancer cells (14). At 7 days after plating, the protein content/cell, a measure of hypertrophy was determined. In four individual clones expressing α16Q212L, protein content/cell was increased 75% compared with Neo control cells (Neo, 20.4 ± 2.1 μg/105 cells; α16Q212L, 35.1 ± 0.5 μg/105 cells; p < 0.01). These results indicate that expression of α16Q212L in VSMC is sufficient to induce hypertrophy. Again, this effect was specific for α16 since stable expression of constitutively active forms of α2, αo, or αi did not induce hypertrophy (data not shown).

Expression of α16Q212L resulted in a marked elevation of steady-state SM-α-actin protein levels compared with Neo control cells, which was not significantly increased by exposure to AVP (Fig. 3A). To determine whether expression of α16Q212L affected promoter activity, VSMC stably expressing α16Q212L were transiently transfected with a plasmid encoding the SM-α-actin promoter ligated to the chloramphenicol acetyltransferase gene (pCATACT−713/52)). Cells were then exposed to AVP or PDGF for 72 h. AVP stimulated CAT activity approximately 5–6-fold in Neo cells similarly to what has previously been observed in wild-type cells (Fig. 3B). In two independent clones expressing α16Q212L, basal CAT activity was dramatically enhanced (>50-fold), and stimulation of these clones with AVP only slightly increased CAT activity in one of the clones.
Exposure to PDGF suppressed CAT activity in cells expressing $\alpha_{16} Q212L$ although activity was still much higher than in Neo control cells. Three additional $\alpha_{16} Q212L$-expressing clones have been examined, all of which exhibited elevated levels of SM-$\alpha$-actin protein and promoter activity (data not shown). To eliminate the effects of compensatory mechanisms on the regulation of SM-$\alpha$-actin expression, transient co-transfection of $\alpha_{16} Q212L$ together with pCATACT was also carried out. Transient expression of $\alpha_{16} Q212L$ also markedly increased SM-$\alpha$-actin promoter activity (see Fig. 6B), indicating that the action of $\alpha_{16} Q212L$ represents a direct effect of the GTPase-deficient $\alpha$ subunit on the promoter.

**Activation of Kinase Pathways**—Since expression of $\alpha_{16} Q212L$ mimicked the effects of vasoconstrictors on growth and induction of SM-$\alpha$-actin, signaling pathways known to be activated by vasoconstrictors were examined. Fig. 4 shows ERK activity in Neo and three independent clones expressing $\alpha_{16} Q212L$. No significant difference in basal ERK activity was observed among the cell lines. However, stimulation of ERKs by AVP, AII, or PMA was decreased in all of the clones expressing $\alpha_{16} Q212L$. This was not a result of a generalized desensitization of the ERK pathway since EGF and PDGF stimulation was comparable with that observed in Neo cells. These data were confirmed by immunoprecipitation with anti-ERK antibodies and assay of kinase activity in the immunoprecipitates (data not shown).

In contrast to stimulation by growth factor receptor tyrosine kinases, vasoconstrictor stimulation of ERKs in VSMC has been shown to require activation of protein kinase C (8). To determine if the inability of vasoconstrictors to stimulate ERKs was due to impairment in PKC activation, PKC activation in response to PMA was measured using a permeabilized cell assay. PMA stimulated PKC 2.3 ± 0.1-fold in Neo cells, comparable with what has been seen in other cell types (18). In three clones expressing $\alpha_{16} Q212L$, PMA stimulated PKC activity 2.5 ± 0.2 (clone 54), 2.5 ± 0.2 (clone 43), and 1.9 ± 0.4 (clone 77), respectively. Thus, the inability of vasoconstrictors and phorbol esters to stimulate ERKs in these cells must be due to changes in steps distal to PKC. Increased cAMP levels have been shown to inhibit activation of ERKs in several cell types including VSMC (25). However, basal cAMP levels in cells expressing $\alpha_{16} Q212L$ were not significantly different from Neo or WT cells (data not shown).

Activation of the JNK/SAPK pathway has been shown in response to cytokines, UV light (26, 27), as well as factors that signal through receptor tyrosine kinases and G-protein coupled receptors (28). The ability of vasoconstrictors to stimulate JNK activity in VSMC was therefore examined. JNK activity was assayed by binding and phosphorylation of GST-c-Jun-(1–79) coupled to glutathione-agarose. After washing, phosphorylation reactions were performed as described under “Experimental Procedures.” The GST-c-Jun-(1–79) polypeptides were identified in Coomassie-stained SDS gels, excised, and counted in a scintillation counter. *, $p < 0.05$ versus basal. B, Neo controls and three independent clones expressing $\alpha_{16} Q212L$ were incubated overnight in serum-free media and then stimulated for 15 min with AII. Extracts were prepared as in panel A and assayed for JNK/SAPK activity. For both panels, results represent the mean ± S.E. of three independent experiments. *, $p < 0.05$; **, $p < 0.01$ versus basal Neo.

**Fig. 4.** ERK activity in Neo and $\alpha_{16} Q212L$-transfected VSMC. Confluent cells were incubated in serum-free media for 1 day. Cells were exposed to $10^{-7}$ M AVP, $10^{-7}$ M AII, $10^{-6}$ M PMA, $10^{-6}$ M EGF, 20 ng/ml PDGF, or vehicle for 5 min. Cell lysates were assayed for ERK activity using MBP and normalized by protein. Data are expressed mean ± S.E. of three to six individual experiments.

**Fig. 5.** JNK activity in $\alpha_{16} Q212L$-transfected VSMC. A, cells were incubated in serum-free media overnight and stimulated for 15 min with the indicated agents. Cells were then lysed in MAP kinase lysis buffer. Soluble extracts were matched for protein and incubated for 2 h at 4 °C with GST-c-Jun-(1–79) coupled to glutathione-agarose. After washing, phosphorylation reactions were performed as described under “Experimental Procedures.” The GST-c-Jun-(1–79) polypeptides were identified in Coomassie-stained SDS gels, excised, and counted in a scintillation counter. *, $p < 0.05$ versus basal. B, Neo controls and three independent clones expressing $\alpha_{16} Q212L$ were incubated overnight in serum-free media and then stimulated for 15 min with AII. Extracts were prepared as in panel A and assayed for JNK/SAPK activity. For both panels, results represent the mean ± S.E. of three independent experiments. *, $p < 0.05$; **, $p < 0.01$ versus basal Neo.
cells were transiently transfected as above, with the addition of 5 μM vers (ments performed in duplicate. *, JNKK(K116R) inhibited the induction of SM-a-actin gene expression. As shown in Fig. 6A, WT-VSMC were transiently transfected by electroporation with full-length pCAT-α-actin, a CMV-β-galactosidase vector, and 5 μg of pCDNA-3 or vector encoding JNKK(K116R). After incubation overnight in full media, cells were exposed for three days to 0.2% FCS (Basal) or 0.2% FCS + AVP. Extracts were prepared and assayed for CAT activity as in Fig. 3B. Results represent the mean of three experiments performed in duplicate with the S.E. shown. *, p < 0.05 versus basal. **, p < 0.05 versus pCDNA-3 exposed to AVP. B, identical cells were transiently transfected as above, with the addition of 5 μg of LNCK-α16Q212L or pCDNA-3 (Mock) in the presence or absence of JNKK(K116R). After incubation overnight in full media, cells were incubated for three days in 0.2% FCS. Extracts were prepared and assayed for CAT activity. Results represent the mean of four experiments performed in duplicate. *, p < 0.05 versus pCDNA; **, p < 0.05 versus α16Q212L alone.

were co-transfected with or without a catalytically inactive JNK kinase, JNKK(K116R), along with the SM-α-actin promoter construct and then exposed to media containing or lacking AVP for 3 days. As shown in Fig. 6A, co-transfection with JNKK(K116R) inhibited the induction of SM-α-actin promoter activity by AVP by approximately 50% while co-transfection with a control plasmid (pCDNA-3) had no effect. In three independent α16Q212L clones, expression of JNKK(K116R) inhibited basal CAT activity by 49 ± 6% (clone 43), 41 ± 11% (clone 54), and 79 ± 5% (clone 77), respectively. Finally, stimulation of SM-α-actin promoter activity induced by transient expression of α16Q212L was also blunted by co-transfection with JNKK(K116R) (Fig. 6B), indicating that the JNK pathway is responsible at least in part for vasoconstrictor and α16-induced SM-α-actin gene expression.

**DISCUSSION**

The rapid mobilization of intracellular Ca2+ in VSMC by vasoconstrictors has been shown to be mediated through activation of members of the Gq family. However, it has not been established whether these G-proteins mediate the longer term effects of AVP and AII on hypertrophy and induction of smooth muscle gene expression. Transfection of VSMC with constitutively active Goq failed to generate clones expressing this protein, suggesting that it may be cytotoxic to VSMC. We therefore employed a constitutively active mutant of another member of the Gq family, Goqα16Q212L. Stable expression of α16Q212L reproduced the physiologic effects of chronic exposure to AVP or AII, as the stably transfected cells were hypertrophied, had similar morphology, and contained elevated levels of SM-α-actin protein compared with Neo controls or wild-type cells. Induction of SM-α-actin expression was mediated through constitutive activation of the SM-α-actin promoter. In fact, both steady-state protein levels and promoter activity were more strongly activated by constitutively active Goqα16Q212L than by the transient G-protein activation achieved by vasoconstrictors. Correspondingly, PDGF, which completely suppressed SM-α-actin expression in WT and Neo cells, only partially inhibited promoter activity in cells expressing α16Q212L, resulting in no significant decreases in steady-state protein. Both effects were specific for Gq family members and were not observed with cells expressing other G-protein α-chains (α12, αo, or αo) or H-Ras.2

To begin to identify the downstream effectors of α16, we examined alterations in signaling pathways in cells expressing α16Q212L. Basal inositol phosphate production was elevated in the transfected cells, indicating that α16Q212L was functionally mimicking the effects of Gq. However, as in other cell types (24), this did not result in elevated levels of [Ca2+]i, compared with Neo controls, and in fact the ability of vasoconstrictors to mobilize [Ca2+]i was inhibited, suggesting activation of compensatory mechanisms desensitizing the Ca2+ response. It therefore appears unlikely that activation of phospholipase Cβ plays an important role in mediating the effects of α16Q212L on hypertrophy and SM-α-actin induction.

Expression of α16Q212L had differential effects on members of the MAP kinase family. Basal ERK activity was not affected by expression of α16Q212L. However, ERK stimulation in response to vasoconstrictors was blunted, while PDGF stimulation was unaffected. The inability of vasoconstrictors to activate these kinases is likely due in part to down-regulation of the receptors for both AVP and AII. Scatchard analysis of radiolabeled hormone binding indicated that both AVP and AII receptor number was decreased by approximately 60% in α16Q212L clones compared with Neo or WT cells, without significant changes in Kd (data not shown).3 However, PMA-induced stimulation of ERK activity was also blunted in cells expressing α16Q212L, suggesting effects of α16Q212L distal to PKC activation. This selective inhibition of ERK activation reflects the multiple pathways operative in VSMC. PDGF and EGF signal through the well described pathway involving Ras and Raf (28–30). Vasoconstrictors, however, signal through a PKC-dependent pathway that only minimally activates Ras and Raf (8).

Our results support a role for JNK as a mediator of SM-α-actin induction. In contrast to ERKs, basal JNK activity was elevated in cells expressing α16Q212L, and the level of JNK activation in individual clones correlated well with the ability to induce SM-α-actin expression (compare Fig. 5B with Fig. 3B). In particular, clone 77 expressed the highest level of JNK activity and the greatest induction of SM-α-actin. This was not due to increased levels of α16Q212L expression compared with the other clones and may reflect differences in the activation of compensatory mechanisms. Importantly, expression of a dominant-negative JNKK construct partially blocked the induction of SM-α-actin promoter activity by AVP in WT cells, as well as that induced by either stable or transient expression of α16Q212L. Two JNK kinases, designated MKK3 and MKK4, have recently been described (11). MKK4 appears to be the

2 X. Li, V. van Putten, F. Zarinetchi, L. E. Heasley, and R. A. Nemenoff, submitted for publication.

3 X. Li, L. E. Heasley, and R. A. Nemenoff, unpublished observations.
human homologue of SEK1(31). The partial inhibition of SM-
α-actin promoter activity may therefore be a result of only
partially inhibiting JNK activity or of inhibiting one of multiple
JNKKs activated by vasoconstrictors. These results also do not
preclude the involvement of other signaling pathways that
converge on the SM-α-actin promoter.

The region of the SM-α-actin promoter required for vasocon-
strictor stimulation contains two CArG elements (6), which are
homologous to the core region of the serum-response element
(SRE) found in the promoter of intermediate early response
genes such as c-fos (32). Transcriptional regulation at the SRE
has been shown to involve binding of two proteins: serum
response factor and members of the ets family including elk-1
(33). Significantly, this latter class of proteins has been shown
to involve binding of two proteins: serum
response factor and members of the ets family including elk-1
(33). This latter class of proteins has been shown to be
phosphorylated by the ERKs (33), as well as the JNKS
(34). While the proteins binding to the SM-α-actin promoter
have not been well defined, it is conceivable that constitutive
activation of JNKS will result in persistent phosphorylation of
specific transcriptional factors leading to enhanced promoter
activity.

Expression of constitutively active G_{o16} has recently been
described in PC12 cells (24) and was associated with neurite
outgrowth and differentiation of these cells into neuronal phe-
notype. As observed in our study, these cells demonstrated
constitutive activation of JNK activity, with no increase in
ERK activity. Since elevated expression of SM-α-actin has been
used as a marker for the contractile phenotype of VSMC (35,
36), our data support an emerging role for the JNK family of
protein kinases in cell differentiation.

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