[Frontiers in Bioscience 11, 356-367, January 1, 2006]

Regulation of vascular smooth muscle cell phenotype by cyclic GMP and cyclic GMP-dependent protein kinase

Thomas M. Lincoln [1], Xing Wu [2], Hassan Sellak [1], Nupur Dey [1] and Chung-Sik Choi [1]

[1] Department of Physiology, College of Medicine, University of South Alabama, Mobile, AL 36688, [2] Department of Pathology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294

TABLE OF CONTENTS

1. ABSTRACT

This basic science review examines the role of cGMP and cGMP-dependent protein kinase (PKG) in the regulation of vascular smooth muscle cell (VSMC) phenotype. The first such studies suggested a role for nitric oxide (NO) and atrial natriuretic peptides (ANP), and the downstream second messenger cGMP, in the inhibition of VSMC proliferation. Subsequently, many laboratories confirmed the anti-proliferative effects of the cGMP pathway in cultured cells and the anti-atherosclerotic effects of the pathway in *in vivo* animal models. Other studies suggested that the cGMP target, PKG, mediated the anti-proliferative effects of cGMP although other laboratories have not consistently observed these effects. On the other hand, PKG mediates cGMP-dependent increases in smooth muscle-specific gene expression, and *in vivo* studies suggest that PKG expression itself reduces vascular lesions. The mechanisms by which PKG regulates gene expression are addressed, but it still unknown how the cGMP-PKG pathway is involved in smooth muscle-specific gene expression and phenotype.

2. INTRODUCTION

With the emergence of nitric oxide (NO) as a major regulatory signal in smooth muscle relaxation, there has been increasing interest on the role of NO and its downstream signaling mediator, cGMP, in other aspects of smooth muscle function. The general acknowledgment that NO is involved in other cellular regulatory pathways besides those in smooth muscle relaxation (e.g., neuronal cell function) has bolstered this notion. Specifically, there is currently much interest in the effects of the NO-cGMP signaling pathway in vascular smooth muscle cell (VSMC) growth, differentiation and gene expression (1-3). In large part, these studies are being driven by investigations into the role of VSMC growth and differentiation in vascular diseases such as atherosclerosis, hypertension, and inflammatory disorders of the vasculature. If the NO-cGMP signaling pathway is involved in VSMC growth and differentiation, then the knowledge emerging from these studies will have impact regarding our understanding of and therapy for vascular disorders. The discussion below will focus primarily on findings from numerous

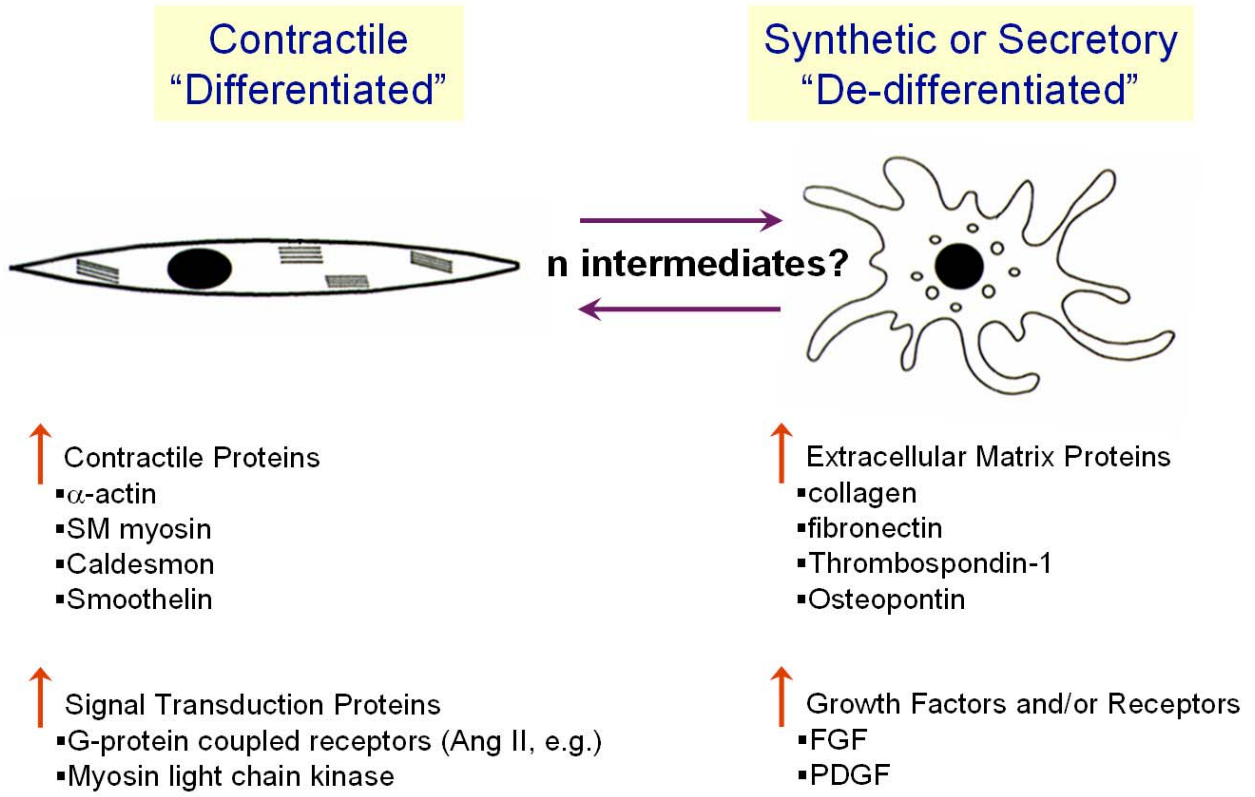

Figure 1. Phenotypic modulation of VSMC. When placed in a cultured environment, many types of VSMC alter their phenotype as an adaptation to the culture conditions. These adaptations include: loss of expression of contractile proteins, loss of expression of signaling proteins such as surface receptors and ion channels, increase in the expression of extracellular matrix proteins, increase in expression of growth factors/growth factor receptors, and increase in the expression of cell cycle regulatory kinases. These adaptations occur over several passages suggesting that intermediate phenotypes between the pure "contractile" and pure "synthetic" phenotype exist. Finally, the phenotypic changes in VSMC seen *in vitro* are similar to phenotypic changes in VSMC that occur *in vivo* in response to injury and atherosclerotic conditions.

laboratories over the past few years suggesting that the NO-cGMP pathway does indeed play a role in the regulation of VSMC proliferation, gene expression, and vascular diseases. Because of the brevity and focus of this review and the voluminous literature in the field, we apologize for not being able to cite all the literature that has contributed to our knowledge in the area.

3. BASIC MECHANISMS UNDERLYING VSMC GROWTH AND DIFFERENTIATION

More than three decades ago, investigators observed that the phenotype of VSMCs grown in culture resembles that which exists in atherosclerotic vascular lesions *in vivo* (4-7). As illustrated in Figure 1, cultured VSMCs not only proliferate at a higher rate characteristic of VSMC that exist in vascular lesions *in vivo*, but they also express lower levels of typical smooth muscle specific contractile proteins such as α-actin (8, 9). The hypothesis was put forward that VSMCs reversibly adapt their phenotype in culture to resemble a more fibroproliferative, "wound-healing" cell, similar to that which exists in vascular lesions (7, 10). To be sure, the pathophysiology of atherosclerosis and other inflammatory vascular diseases is far more complex than mere phenotype switching (see 10 for a review); however, this hypothesis provided a convenient framework with which to begin to understand the cellular and molecular mechanisms regulating VSMC growth and differentiation. Of further significance, this hypothesis identified the VSMC as the major cell type responsible for the generation of vascular lesions, a concept that remains unchallenged today.

When certain conduit arteries, such as those subject to the development of atherosclerotic lesions, are injured by means such as balloon catheter inflation, VSMC proliferation increases in the tunica media and the intima (11, 12). Migration of VSMC from media to intima increases, and changes in VSMC gene expression occur that result in increased deposition of extracellular matrix protein to form a "neointima." These cellular changes result in an expanded, thickened neointimal lesion, which can ultimately occlude the vessel and compromise blood flow. At the cellular and molecular levels, events such as increased expression of inflammatory adhesion molecules, oxidized LDL uptake, and growth factor receptor gene expression occur in VSMC in response to injury (13-16). The entire sequence of events is characteristic of the

generalized inflammatory, proliferative response to injury, a response that is necessary for subsequent wound-healing activity. An understanding of these mechanisms regulating VSMC proliferation and gene expression in the physiologic and pathophysiologic setting could have significant impact on our understanding of how vascular lesions develop.

The molecular regulation of VSMC proliferation has been studied intensively by numerous laboratories, and will not be subsequently discussed. Suffice it to say that the mechanisms appear to be strikingly similar to general mechanisms controlling cellular division. Hence, mitogens – via activation of mitogen-activated protein kinases (MAP kinases) stimulate the expression of cyclins that activate cyclin-dependent protein kinases (Cdk). Cdks catalyze the phosphorylation of the retinoblastoma protein (Rb) causing it to dissociate from E2F, a transcription factor that induces S-phase gene expression. Cdks themselves are regulated by cyclin kinase inhibitors, p21 and p27, whose expression in turn, is controlled in part by p53.

Whereas the some of the details involving the mechanisms of VSMC DNA synthesis have been known for several years, the mechanisms of VSMC-specific gene expression are only beginning to come to light. As stated earlier, these studies have potential bearing on our understanding of the processes of atherosclerosis and restenosis because it is generally assumed that VSMCs "dedifferentiate" when modulating to a fibroproliferative phenotype and "differentiate" back to a quiescent, contractile VSMC phenotype as vascular lesions are resolved. Although several transcriptional mechanisms have been proposed for the regulation of VSMC-specific gene expression, it is now appreciated that the ubiquitous transcription factor, serum-response factor (SRF), may be the key regulator for VSMC-specific gene expression (17-19). SRF binds to DNA sequences, known as CArG boxes, in *cis* elements of VSMC-specific genes such as α-actin and SM22 (20, 21). The topology of SRF binding in smooth muscle-specific genes, however, is such that at least two or more SRF binding sites are spaced apart at specific distances along the DNA, and this spacing of DNA-bound SRF allows a multi-component complex to be formed with the muscle cell-specific co-transcriptional regulator, myocardin (22-25). The SRF-myocardin mechanism is perhaps the most thoroughly developed model to date, although other smooth muscle-specific gene expression mechanisms may exist, including the regulation by members of the GATA transcription factor family and the NFAT family (26-34). Currently, how these factors, alone or in combination with the SRF, result in a true pattern of VSMC differentiation is not known. Additionally, there are multiple isoforms of many of these factors; for example, SRF is composed of at least four different isoforms – three of which are positive regulators of gene expression while one isoform appears to be a negative regulator of gene expression (35, 36). Most of the transcription factors involved in VSMC-specific gene expression are cytosolic proteins and must be imported to the nucleus using the actin cytoskeleton and the small molecular weight GTPase family of proteins (37-40). In particular, the critical role for the RhoA/RhoA kinase (ROK) pathway to import SRF into the nucleus is the subject of intense investigation at the moment (40). And finally, changes in chromatin structure itself involving the acetylation of histones appears to be necessary for smooth muscle specific gene expression induced by SRF (41). The complexity of the mechanisms controlling VSMC-specific gene expression is acknowledged by all investigators in this area, and most seem to agree that we are only beginning to solve the riddles of VSMC-specific gene expression and differentiation.

4. ROLE OF THE CGMP PATHWAY IN REGULATING VSMC GROWTH AND PHENOTYPE

The first evidence that cGMP was involved in VSMC phenotypic modulation was generated by Hassid and co-workers (42-49). These investigators demonstrated that NO donor drugs, phosphodiesterase (PDE)-resistant cGMP analogs and natriuretic peptides inhibited DNA synthesis and proliferation of subcultured VSMCs and mesangial cells. Whereas the concentrations of natriuretic peptides necessary to inhibit VSMC proliferation fell within the physiologic ranges of the circulating hormones, possibly due to the effects of ANF to activate C-type receptors as well as the A or B type receptors, the concentrations of NO required to inhibit growth were considered to be higher than expected in the normal physiologic situation. Nevertheless, since these earlier studies, there are over 1000 citations too numerous to list individually describing an antiproliferative or pro-differentiation role for NO, cGMP, cGMP-dependent protein kinase (PKG), NO synthases, or cGMP PDE inhibitors both *in vitro* and *in vivo* (see 1, 45, 46 for more recent reviews). These include studies that have shown that adenovirally delivered NO synthase genes, guanylyl cyclase subunits and type I PKG constructs suppress neointimal growth and expansion in animal injury models. Specifically, in studies where animals were subjected to balloon catheter injury, components of the NO-cGMP-PKG signaling pathway suppressed VSMC proliferation, increased VSMC apoptosis, and decreased deposition of extracellular matrix protein in the lesion area where active repair was occurring (47-49). In these studies, only VSMCs that were undergoing the fibroproliferative response demonstrated decreased expression of sGC (48, 49), PKG (47), or both whereas in cells that were not undergoing a fibroproliferative response, there was no alteration in the expression of PKG (47). In autopsy tissues of atherosclerotic human coronary artery, there was dramatically less immunoreactive PKG staining in neointimal fibroproliferative VSMCs compared with normal medial VSMCs (47). Thus, a solid paradigm that has emerged that NO, acting through cGMP, inhibits the proliferation of and the dedifferentiation (phenotypic modulation) of different cultured VSMC model systems and neointimal VSMC growth in various animal models of arterial vascular injury *in vivo*.

In animal models of hypercholesterolemic-induced atherosclerosis, activation of the NO-cGMP pathway not only inhibits, but also reverses, atherosclerotic lesion development (50-55). The mere supplementation of

dietary amino acids with L-arginine, the substrate for NO synthases, decreases lesion development in the atherosclerotic rabbit. In other animal studies, activation of inflammatory pathways associated with endothelial dysfunction and atherosclerosis were suppressed by NO donors, cGMP analogs and cGMP PDE inhibitors (56-59). In fact, the mechanism by which lesion development was attenuated in these studies was linked to the anti-inflammatory and anti-platelet effects of NO and cGMP, the anti-platelet effects of the NO-cGMP pathway. In sum, these data amassed over a period of approximately fifteen years seem to point to an important anti-inflammatory and anti-atherogenic role for the NO-cGMP pathway *in vivo*, and support the notion that the NO-cGMP pathway suppresses general inflammatory vascular disorders in animal models and, possibly, in the human disease.

Despite the body of evidence of an antiatherogenic, antiproliferative role for NO-cGMP signaling, there are several reports where the situation appears to be less settled. Hassid and co-workers demonstrated that in freshly isolated contractile rat aortic VSMC, NO donors and cGMP analogs do not inhibit cell proliferation but actually accentuate fibroblast growth factor (FGF)-induced VSMC proliferation (60). Once passaged, however, the cells respond to NO and cGMP treatment with inhibition of growth. These results suggest that perhaps the phenotype of the VSMC in culture may affect the proliferative response of the cells to NO-cGMP pathway activation. Similarly, transfected cultured rat aortic VSMC expressing PKG-I or the constitutively active catalytic domain display a number of features characteristic of the contractile phenotype, but the proliferation response of the cells was not greatly affected by the expression of an active PKG (61, 62). These findings led to the notion that the NO- and cGMP-dependent inhibition of VSMC proliferation may be due to the cross-activation of cAMP-dependent protein kinase (PKA) by cGMP, a finding that was verified by measuring the PKA activity ratio in the cells (62). The concentrations of cGMP that activate PKA are some 20-fold or more greater than exist in cells under basal conditions, but could in fact be realized in the pathophysiological condition under the influence of inflammatory cytokines that induce iNOS expression (62). In still other studies, NO and cGMP analogs activated rather than inhibited MAP kinase pathways in freshly isolated rat aortic VSMC, in contrast to the effects of cGMP in passaged cultures of VSMC (63). Because MAP kinase activation is usually associated with the stimulation of cell proliferation rather than the inhibition of proliferation, these studies have cast some question on the actual physiological role of cGMP in the regulation of VSMC proliferation.

The *in vitro* studies have recently been extended to an *in vivo* animal model recently. Wolfsgruber, *et al.* (64) constructed transgenic/knock-out mice where PKG-I was conditionally deleted in a smooth muscle cell-specific manner. When these mice were mated to ApoE deficient animals, atheromatous growth was attenuated in response to the inhibition of expression of PKG. These results led the authors to suggest that PKG plays a pro-atherogenic role *in vivo*. Additional studies in cultured aortic VSMC from wild-type mice suggested that high concentrations of cGMP analogs increased cell proliferation (rather than inhibited proliferation), whereas in PKG-deficient mice, high concentrations of cGMP analogs had no effect on proliferation. On the other hand, the effects of PKG on the expression of key VSMC-specific marker genes were not reported in this study. To date, this is the only report published where both a pro-proliferative and *pro-atherogenic* role for PKG has been proposed.

5. ROLE OF CGMP AND PKG IN VSMC-SPECIFIC GENE EXPRESSION IN CULTURED CELLS

The role of the NO-cGMP pathway in regulating gene expression in general has been addressed recently (65). With regard to VSMC-specific gene expression, however, there is very little information available. As noted earlier, VSMC in response to injury or culture conditions, change phenotype to a more proliferative, secretory state. These changes involve alterations in the expression of genes that encode contractile proteins, cytoskeletal elements, cell cycle regulators, adhesion molecules and extracellular matrix proteins. The rat aortic smooth muscle cell system has been used extensively to study phenotypic changes of VSMC. After only a limited time in culture, usually within a few passages, contractile protein expression is down regulated whereas extracellular matrix protein expression is increased. Several laboratories have also observed changes in the expression of enzymes of the cGMP signaling pathway as well. Adult rat aortic VSMC or rat pulmonary arterial VSMC that have been passaged several times express lower levels of PKG-I mRNA and protein than freshly isolated cells, whereas in other types of VSMC models, sGC expression is down regulated (48, 66-69). Thus, parallel changes in the expression of genes encoding both the NO-cGMP-PKG pathway, and the phenotype of VSMC have been reported.

Our laboratory reported that passaged, PKG-I deficient rat aortic VSMCs when transfected with cDNAs encoding PKG-I or transduced with adenoviruses containing the cDNA for PKG-I to restore physiologic levels of the kinase, change their phenotype to resemble a more contractile state (61, 69). As shown in Figure 2, VSMCs deficient in PKG expression transform from a fibroproliferative morphology characterized by cell spreading to a more contractile morphology characterized by spindle-shaped cellular structures – the so-called “hill and valley” morphology when transfected with PKG or its active catalytic domain. These results indicate that PKG most likely regulates VSMC-specific gene expression in some manner. We recently conducted a gene microarray study of smooth muscle cell-specific gene expression by PKG. Using cultured rat aortic VSMC made deficient in PKG expression by multiple passaging, we expressed PKG-I under the control of a tetracycline-“on” promoter system to restore physiologic levels of PKG. The mRNAs were isolated and the expression of genes identified on Affymetrix chips. As shown in Table 1, of over 300 genes that were found to be regulated by two-fold or more following PKG expression in the cells, the expression of

Table 1. Microarray analysis of pkg-dependent gene expression in VSMC

GENES INCREASED BY PKG EXPRESSION		
Gene	**Acc. #**	**Fold-change**
Vascular α-actin	X06801	8.9
Aortic caldesmon	AI180288	6.1
Myogenic regulatory factor	M84176	5.5
Angiotensin receptor (AT1)	M86912	4.0
Tissue factor protein	U07619	3.9
Matrix metalloproteinase inhibitor (TIMP)	AI169327	3.1
High mobility group protein 1 (HMG1)	X62875	2.1
SM22	M83107	1.9
GENES DECREASED BY PKG EXPRESSION		
Gene	**Acc. #**	**Fold-change**
c-myc	Z38067	-3.9
Cdk-activating kinase	X83579	-3.8
Alpha1-VIII collagen	S63458	-3.7
Collagen alpha 1 type V	AJ005394	-3.3
Plasminogen activator inhibitor (PAI-2A)	X64563	-3.3
Annexin V-binding protein (ABP-7)	D64061	-3.1
Integrin alpha-1	X52140	-2.2
Myosin light chain isoform C	S77900	-2.1

VSMC: vascular smooth muscle cell

several genes that are involved in VSMC contractility, cytoskeletal function, or VSMC signaling were modulated by PKG expression. For example, SMC-specific α-actin was unregulated whereas collagen was down regulated by PKG expression. Other interesting patterns were observed. The expression of several growth-related genes (e.g., FGF, angiotensin II-Type 1 receptor) in response to increased PKG was suppressed, which might possibly explain the observed effects of NO and cGMP to decrease VSMC proliferation. On the other hand, the expression of the angiotensin II- Type 1 receptor was increased by PKG. The angiotensin II receptors are generally down regulated by passage in culture and the modulation of VSMCs to the synthetic phenotype. These results suggest that PKG not only increases structural and contractile protein expression to VSMCs, but also increases the expression of signal transduction systems characteristic of the differentiated, contractile VSMC.

Perhaps the effects of PKG on the expression of SMC-specific contractile proteins are the most clearly observed effects, however. As shown in the western blots in Figure 3, several proteins generally considered to be reliable markers for the contractile phenotype of VSMC were unregulated in cultured VSMC following PKG expression in the cells. These include the "gold-standard" smooth muscle myosin heavy chain, smoothelin (a cytoskeletal proteins whose expression is specifically increased in adult, contractile VSMC), calponin, and heavy caldesmon. Thus, PKG is capable of affecting SMC-specific gene expression. Yet, despite such an increase in contractile phenotype marker protein expression following PKG transfection, there was not a full restoration of the expression of these gene products when compared with intact aortic tissue (61, and unpublished observations). This suggests that the NO-cGMP-PKG pathway likely participates in phenotypic modulation of VSMC, but is clearly not the sole signaling pathway regulating the process. This is perhaps obvious since phenotypic modulation is a highly complex process involving multiple signaling pathways such as integrin-adhesion molecule-matrix signaling and inhibition of growth factor signaling.

As mentioned earlier, one aspect of VSMC phenotypic modulation is an increase in the expression of extracellular matrix proteins as the cells become more phenotypically fibroproliferative or "synthetic." PKG-I inhibited the production of extracellular matrix proteins such as collagen I, thrombospondin-1 (TSP-1) and osteopontin in VSMCs (69) and in rat mesangial cells (70). In the latter cell type, endogenous PKG expression does not decline measurably in culture. When exposed to high concentrations of glucose, however, mesangial cells express and secrete TSP-1, which in turn, activates latent TGF-β. Activated TGF-β induces the expression of a number of extracellular matrix proteins such as fibronectin and collagen. Using the tetracycline – "on" system to induce the expression of the active PKG-I catalytic domain in the mesangial cells, there was a suppression of glucose-induced increases of TSP-1 expression and matrix deposition. These finding could be important from two standpoints: first, a pathophysiological one, and second from a mechanistic point of view. With regard to the pathophysiology, a major component of vascular wound healing activity and wall remodeling is the elaboration of extracellular matrix proteins and matrix metalloproteinases (MMPs). The suppression of matrix protein expression by activating PKG could be one component, at least, in the reduction of intimal thickening that occurs in response to either NO or PKG transfection (49). Likewise, there have been studies demonstrating that NO decreases MMP expression in VSMCs (71-73), and recent findings from our laboratory has shown that PKG transfection into cultured VSMC inhibits MMP-2 expression while increasing tissue inhibitor of metalloproteinase (TIMP) expression (see Table 1). Decreased matrix protein elaboration coupled

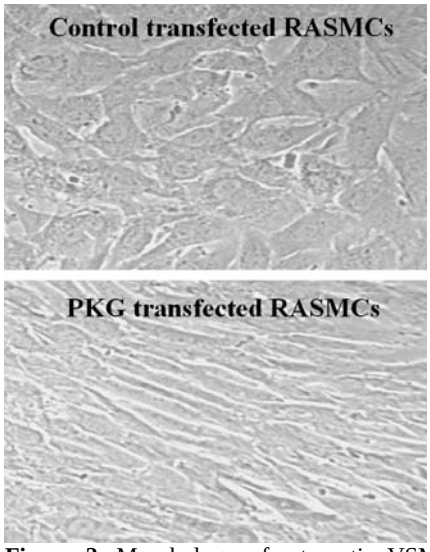

Figure 2. Morphology of rat aortic VSMC after stable transfection of cells with control vectors or vectors harboring the PKG-Iα cDNA. These cells were transfected at the third passage when endogenous PKG-I expression begins to decline and the cells transform into a synthetic phenotype. Control transfected cells morphologically resemble the synthetic phenotype with a flattened, fibroblastic appearance whereas PKG-transfected cells morphologically resemble the contractile phenotype with spindle-shaped cells that grow in the "hill and valley" fashion.

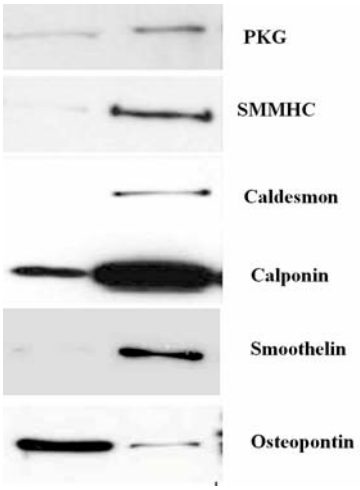

Figure 3. Western blot analysis of protein expression in control-transfected and PKG-transfected rat aortic VSMC. These cells were transfected as described in the Materials and Methods and grown for an additional two passages before western blotting was performed. PKG expression at the top is increased in the PKG-transfected cells compared with control-transfected cells. The other proteins examined using western blotting are smooth muscle myosin-specific heavy chain (SMMHC), heavy caldesmon, calponin, high molecular weight smoothelin, and osteopontin. PKG increases the expression of SMMHC, caldesmon, calponin, and smoothelin and decreases the expression of osteopontin relative to control-transfected cells.

with decreased enzymatic activity responsible for the turnover of the extracellular matrix would be important actions that suppress intimal thickening and the development of atherosclerotic lesions.

From the mechanistic point of view, it is almost certain that the molecular mechanisms controlling contractile protein expression and extracellular matrix protein expression are regulated by different processes. As discussed above, VSMC-specific contractile protein gene expression is in large part dependent on CArG/SRF activity in the cis-regulatory elements of these genes. However, there is no evidence that a similar mechanism operates in the expression of matrix proteins or MMPs, and the fact that matrix protein and MMP expression is ubiquitous for cells suggests that more general mechanisms exist for the control of expression of these gene products by cGMP. Matrix protein expression may involve both transcriptional and post-transcriptional control by regulating message stability and translation. Our laboratory has obtained evidence for both types of control of extracellular matrix protein expression (69). Currently, however, the molecular details regulating expression of these gene products by the NO-cGMP-PKG pathway is unknown.

6. EFFECTS OF THE CGMP-PKG PATHWAY ON RHOA SIGNALING IN VSMC

The studies summarized above suggest that PKG regulates VSMC-specific gene expression, but have not shed light on the mechanisms that may be at work in this process. Because the role of SRF is considered paramount for the differentiation of VSMC, several investigators have recently examined the role of PKG in regulating SRF-dependent gene expression. Pilz and co-workers using transfection of reporter genes in non-muscle cells (glioma and fibroblast) as well as VSMC, found that PKG-I inhibited SRF-dependent gene transcription (74, and see 65 for an excellent up-to-date review of the subject). These effects of PKG were dependent on the capacity of the kinase to catalyze the phosphorylation of the Vasodilator Stimulated Phosphoprotein, or VASP, an actin binding protein ubiquitous in cells (75). At first glance, these results appear to contradict those suggesting that PKG-I increases smooth muscle-specific gene expression. On the other hand, VSMC-specific gene expression is a highly complex process and, as discussed by Miano in a recent review (76), only physiologically relevant SRE-containing promoter constructs derived from SMC-specific promoters appear to mimic SMC-specific gene expression in reporter gene assays. Furthermore, Owens' laboratory has shown that even truncated SRE-containing reporter constructs that are effective SMC-specific promoter-reporters in cultured smooth muscle cells do not function specifically in transgenic animals (77). Thus, SRE constructs derived from the c-fos growth promoter, which is known to be involved in cell proliferation, would not necessarily pertain to SMC-specific gene expression. Also, the cell culture models used in many transfection studies may be either deficient in or incompatible with myocardin binding or expression. Nevertheless, these initial studies are highly significant and important from the standpoint that they are among the first

to demonstrate that PKG directly regulates gene expression, but also indicate that much more work needs to be done to understand completely the role of PKG in VSMC-specific gene expression.

A recent study that attempted to assess the role of VASP and PKG-dependent phosphorylation of VASP in smooth muscle phenotype was reported by Chen, *et al.* (78). In this study, VASP phosphorylated on the cAMP-dependent protein kinase (PKA)/protein kinase C (PKC)-selective site, i.e., serine157, was associated with the proliferative response to mitogens in aortic VSMC. On the other hand, PKG-dependent phosphorylation of VASP on serine239, the selective PKG site, resulted in inhibition of VSMC proliferation. Although smooth muscle cell-specific gene expression was not investigated in these studies, the results suggest that VASP could play a critical role in PKG-dependent control of VSMC growth and differentiation.

These studies may provide additional insight into the importance of the cellular cytoskeleton and the possible role of PKG in the regulation of VSMC-specific gene expression. It has been shown by several laboratories that activation of the RhoA/ROK pathway is important for the nuclear import of SRF, myocardin, and other transcription factors in SMC (38-40, 79). At least one component of the mechanism of RhoA signaling in regulating nuclear gene expression is to stimulate an increase in the cellular levels of F-actin relative to G-actin. These effects of RhoA are possibly mediated by the activation of ROK since the selective ROK inhibitor, Y27632, inhibited F-actin organization as well as blocked SMC-specific gene expression. The contribution of phosphorylation of VASP on either serine157 or serine239 to modulate SRF-dependent activation of gene expression under physiological and pathophysiological conditions is not fully understood, but this intriguing possibility warrants more thorough and intensive investigation. Because the RhoA/ROK pathway has emerged as a focus of interesting SMC-specific gene expression, there are obvious questions relating to the possible links between the NO-cGMP pathway and the RhoA/ROK pathway

Perhaps the best-described effect of PKG on RhoA signaling is in calcium desensitization of smooth muscle contraction. It has been known for several years that agonist-induced smooth muscle contraction involves activation of RhoA resulting in ROK-dependent phosphorylation of the myosin light chain phosphatase targeting protein, MYTP (see 80 for a review). Phosphorylation on a critical threonine residue by ROK inhibits myosin light chain phosphatase activity causing enhanced light chain phosphorylation in the face of declining intracellular calcium levels. PKG-I, and particularly the Iα isoform, is specifically bound to a spliced variant of the MYTP that contains a leucine-isoleucine zipper C-terminal motif (81, 82). The binding of PKG and its subsequent activation in response to elevations in cGMP stimulates PKG-dependent phosphorylation of MYTP that results in activation of the phosphatase resulting in myosin light chain dephosphorylation and relaxation (81-83). Recently, Haystead and co-workers proposed a mechanism by which PKG-dependent phosphorylation of MYTP resulted in the activation of myosin light chain phosphatase (84). These investigators demonstrated that the PKG-dependent phosphorylation site on MYTP is the serine residue immediately N-terminal to the ROK site, and proposed the model where PKG-dependent phosphorylation prevents ROK-dependent phosphorylation and inhibition of myosin light chain phosphatase. Thus, the effects of PKG are to activate phosphatase by preventing RhoA-dependent inhibition of the enzyme.

Although the evidence for a PKG-ROK antagonism of myosin phosphorylation is becoming well established for smooth muscle contraction, there is controversy over whether a general antagonism between the NO-cGMP-PKG pathway and the RhoA/ROK pathway exists at the level of RhoA activation. It was originally proposed (85) and confirmed by other laboratories (86, 87) that PKG inhibits RhoA signaling by direct phosphorylation of RhoA on serine-188. However, reports demonstrating specific PKG-dependent RhoA phosphorylation in the intact cell have been conflicting (80, 85, 88, 89) and complicated by two problems: first, although free RhoA in solution is phosphorylated by PKG in the *in vitro* experiments, it is not phosphorylated *in vitro* when complexed with the GDI protein (80). Second, PKA is as good a catalyst, if not a better one, compared with PKG in phosphorylating serine-188 of RhoA. Others have suggested that cGMP signaling inhibits RhoA activation by blocking isoprenylation in addition to phosphorylating RhoA (90). One problem with some of the studies designed to examine the role of PKG in catalyzing RhoA phosphorylation or regulating other post-translational modification steps is that the interpretation relies on the actions of unproven or relatively non-specific PKG inhibitors. Since this continues to be a general problem in the cGMP-PKG signaling field, at best, the physiologic role of PKG in regulating RhoA phosphorylation or post-translational modification is not clear at this time.

In contrast to these studies, Pacaud's laboratory (91) has shown that the NO-cGMP-PKG pathway increases RhoA expression in VSMC, and that the increases in RhoA expression are associated with increased expression of marker contractile proteins (SMMHC, etc.). Others have shown that short-term activation of RhoA is associated not with differentiation of VSMCs in culture, but rather with the maintenance of the undifferentiated smooth muscle phenotype (92). Currently, there is much speculation on how these two pathways may converge in the ultimate expression of the VSMC phenotype, but very little mechanistic data that would explain the various observations regarding the overall role of the NO-cGMP-PKG pathway in VSMC growth and differentiation.

7. IS THE NO-CGMP PATHWAY PROTECTIVE IN VASCULAR DISEASES OR DETRIMENTAL?

From the above discussion, it is clear that although there are many unanswered questions regarding the role of the NO-cGMP pathway in vascular diseases and

smooth muscle differentiation, the majority of studies suggests that cGMP, via activation of PKG, inhibits VSMC proliferation and modulation to a fibroproliferative phenotype. As mentioned earlier, a very large number of citations describe a role for the NO-cGMP-PKG signaling pathway as either a negative regulator of VSMC growth/phenotypic modulation or as an anti-atherosclerotic pathway in VSMC. And, it should be added at this point that the protective effects ascribed to NO itself appear not to be cGMP-*independent* effects of NO, but rather to be dependent on cGMP itself since carbon monoxide (CO), an activator of soluble guanylyl cyclase, has also been shown to suppress VSMC proliferation and atherosclerosis in animal models of vascular disease (93-96). It has not been shown if CO, like NO (97), induces smooth muscle specific gene expression in vascular smooth muscle cells, however. Because cGMP is known to bind to several proteins in VSMC that serve as intracellular receptors (PDE3 and PDE5, and protein kinases – PKG and PKA), perhaps the anti-proliferative or anti-atherogenic effects of cGMP are specifically related to activation of PKA. With regard to activation of PKA by cGMP in VSMC, it has been shown that this is likely due to inhibition of PDE3 and the elevation of cAMP (59), and that PKA activation itself plays a role in mediating the inhibition of VSMC proliferation by cGMP (62). Yet, it is PKG and not PKA that mediates VSMC-specific gene expression (61). Therefore, the vast majority of *in vitro* and *in vivo* studies would appear to suggest that the complete NO-cGMP-PKG pathway plays an anti-atherogenic role in VSMC. However, only after the molecular mechanisms of action of PKG in regulating the phenotypic properties of VSMC are defined can we state with assurance that PKG plays an anti-atherogenic role. Similarly, with regard to the transgenic animal studies claiming a pro-atherogenic role for PKG (64), detailed molecular studies will need to be conducted on this unique animal model to clarify the role of PKG with assurance as well.

8. REFERENCES

1. Lincoln, T., N. Dey and H. Sellak: Invited Review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. *J Appl Physiol* 91, 1421-1430 (2001)

2. Bogdan, C: Nitric oxide and the regulation of gene expression. Trends *Cell Biol* 11, 66-75 (2001)

3. Feil, R., S. M. Lohmann, H. de Jonge, U. Walter and F. Hofmann: Cyclic GMP-dependent protein kinases and the cardiovascular system: insights from genetically modified mice. *Circ Res* 93, 907-916 (2003)

4. Chamley-Campbell, J., G. R. Campbell and R. Ross: Phenotype-dependent response of cultured aortic smooth muscle cells to serum mitogens. *J Cell Biol* 89, 379-383 (1981)

5. Campbell, J. H. and G. R. Campbell: Smooth muscle cell phenotype changes in arterial wall homeostasis: implication for the pathogenesis of atherosclerosis. *Exp Mol Pathol* 42, 136-162 (1985)

6. Gown, A. M., T. Tsukada and R. Ross: Human atherosclerosis II. Imunocytochemical analysis of the cellular composition of human atherosclerotic lesions. *Am J Pathol* 125, 191-207 (1986)

7. Owens, G. K: Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev* 75, 487-517 (1995)

8. Mosse, P. R., G. R. Campbell, Z. L. Wang and J. H. Campbell: Smooth muscle phenotypic expression in human carotid arteries I. Comparison of cells from diffuse intimal thickenings adjacent to atheromatous plaques with those of the media. *Lab Invest* 53, 556-562 (1985)

9. Sappino, A. P., W. Schnrch and G. Gabbiani: Biology of disease. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab Invest* 63, 144-161 (1990)

10. Owens, G. K., M. S. Kumar and B. R. Wamhoff: Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 84, 707-801 (2004)

11. Lindner, V. and M. A. Reidy: Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. *Proc Natl Acad Sci USA* 88, 3739-3743 (1991)

12. Dzau, V. J., R. C. Braun-Dullacus and D. G. Sedding: Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. *Nat Med* 8, 1249-1256 (2002)

13. Stokes, K. Y., D. Cooper, A. Trailor and D. N. Granger: Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. *Free Radic Biol Med* 33, 1026-1036 (2002)

14. Behr-Roussel, D., A. Rupin, S. Simonet, E. Bonhomme, S. Coumaillear, A. Cordi, B. Serkiz, J. N. Fabiani and T. J. Verbeuren: LDL Cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ Res* 87, 99-105 (2000)

15. Taylor, A. M. and C. A. McNamara: Regulation of vascular smooth muscle cell growth: targeting the final common pathway. *Arterioscler Thromb Vasc Biol* 23, 1717-1720 (2003)

16. Smirnova, I. V., T. Sawamura and M. S. Goligorsky: Upregulation of lectin-like low-density lipoprotein receptor-1 (LOX-1) in endothelial cells by nitric oxide deficiency. *Am J Physiol* 287, F25-F32 (2004)

17. Li, L., J. M. Miano and E. N. Olsen: SM22alpha, a marker of adult smooth muscle is preferentially expressed

in multiple myogenic lineages during embryogenesis. *Circ Res* 78, 188-195 (1996)

18. Mack, C. P. and G. K. Owens: Regulation of SM alpha-actin expression *in vivo* is dependent upon CArG elements within the 5' and first intron promoter region. *Circ Res* 84, 852-861 (1999)

19. Manabe, I. and G. K. Owens: CArG elements control smooth muscle subtype-specific expression of smooth muscle myosin *in vivo*. *J Clin Invest* 107, 823-834 (2001)

20. Landerholm, T. E., X. R. Dong, N. Belaguli, R. J. Schwartz and M. W. Majesky: A role for serum response factor in coronary smooth muscle differentiation from proepicardial cells. *Development* 126, 2053-2062 (1999)

21. Mack, C. P., M. M. Thompson, S. Lawrenz-Smith and G. K. Owens: Smooth muscle alpha-actin CArG elements coordinate formation of a smooth muscle cell-selective, serum response factor-containing activation complex. *Circ Res* 86, 221-232 (2000)

22. Wang, D. Z., P. S. Wang, Z. Wang, L. Sutherland, J. A. Richardson, E. Small, P. A. Krieg and E. N. Olson: Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. *Cell* 105, 851-862 (2001)

23. Yoshida, T., S. Sinha, F. Dandre, B. R. Wamgoff, M. H. Hoofnagle, B. E. Kremer, D. Z. Wang, E. N. Olson and G. K. Owens: Myocardin is a key regulator of CArG-dependent transcription of multiple smooth muscle marker genes. *Circ Res* 92, 856-864 (2003)

24. Wang, Z., D. Z. Wang, G. C. Teg Pipes and E. N. Olson: Myocardin is a master regulator of smooth muscle gene expression. *Proc Natl Acad Sci USA* 100, 7129-7134 (2003)

25. Wang, Z., D. Z. Wang, Hockemeyer, J. McAnally, A. Nordheim and E. N. Olson: Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* 428, 185-189 (2004)

26. Abe, M., K. Hasegawa, H. Wada, T. Morimoto, T. Yanazume, T. Kawamura, M. Hirai, Y. Furukawa and T. Kita: GATA-6 is involved in PPAR-gamma mediated activation of differentiated phenotype in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 23, 404-410 (2003)

27. Ohkawa, Y., K. Hayashi and K. Sobue: Calcineurin-mediated pathway involved in the differentiated phenotype of smooth muscle cells. *Biochem Biophys Res Commnu* 301, 78-83 (2003)

28. Chang, D. F., N. S. Belaguli, D. Iyer, W. B. Roberts, S. P. Wu, X. R. Dong, J. G. Marx, M. S. Moore, M. C. Beckerle, M. W. Majesky and R. J. Schwartz: Cysteine-rich LIM-only proteins, CRP_1 and CRP_2 are potent smooth muscle differentiation cofactors. *Dev Cell* 4, 107-118 (2003)

29. Wada, H., K. Hasegawa, T. Morimoto, T. Kakita, T. Yanazume, M. Abe and S. Sasayama: Calcineurin-GATA-6 pathway is involved in smooth muscle-specific transcription. *J Cell Biol* 156, 983-991 (2002)

30. Boss, V., K. L. Abbott, X. F. Wang, G. K. Pavlath and T. J. Murphy: The cyclosporin A-sensitive nuclear factor of activated T cells (NFAT) proteins are expressed in smooth muscle cells. *J Biol Chem* 273, 19664-19671 (1998)

31. Chen, Y. H., M. D. Layne, M. Watanabe, S. F. Yet and M. A. Perrella: Upstream stimulatory factors regulate aortic preferentially expressed gene-1 expression in vascular smooth muscle cells. *J Biol Chem* 276, 47658-47663 (2001)

32. Watanabe, M., M. D. Layne, C. M. Hsieh, K. Maemura, S. Gray, M. E. Lee and M. K. Jain: Regulation of smooth muscle cell differentiation by AT-rich interaction domain transcription factors Mrf2α and Mrf2β. *Circ Res* 91, 382-389 (2002)

33. Bostrom, K., Y. Tintut, S. C. Kao, W. P. Stanford and L. L. Demer: HOXB7 overexpression promotes differentiation of C3H10T1/2 cells to smooth muscle cells. *J Cell Biochem* 78, 210-221 (2000)

34. Gonzalez-Bosc, L. V., M. K. Wilkerson, K. N. Bradley, D. M. Eckman, D. C. Hill-Eubanks and M. T. Nelson: Intraluminal pressure is a stimulus for NFATc3 nuclear accumulation. *J Biol Chem* 279, 10702-10709 (2004)

35. Kemp, P. R. and J. C. Metcalfe: Four iso forms of serum response factor that increase or inhibit smooth muscle-specific promoter activity. *Biochem J* 345, 445-451 (2000)

36. Belaguli, N. S., W. Zhou, T-HT Trinh, M. W. Majesky and R. J. Schwartz: Dominant Negative murine serum response factor: alternative splicing within the activation domain inhibits transactivation of serum response factor binding targets. *Mol Cell Biol* 19, 4582-4591 (1999)

37. Hill, C. S., J. Wynne and R. Treisman: The Rho family GTPases RhoA, Rac1 and CDC42Hs regulate transcriptional activation by SRF. *Cell* 81, 1159-1170 (1995)

38. Mack, C. P., A. V. Somlyo, M. Hautmann, A. P. Somlyo and G. K. Owens: Smooth muscle differentiation marker gene expression is regulated by rhoA-mediated actin polymerization. *J Biol Chem* 276, 341-347 (2001)

39. Lu, J., T. E. Landerholm, J. S. Wei, X. R. Dong, S. P. Wu, X. Liu, K. Nagata, M. Inagaki and M. W. Majesky: Coronary smooth muscle differentiation from proepicardial cells requires RhoA-mediated actin reorganization and p160 Rho-kinase activity. *Dev Biol* 240, 404-418 (2001)

40. Liu, H. W., A. J. Halayko, D. J. Fernandes, G. S. Harmon, J. A. McCauley, P. Kocieniewski, McConville J, Y. Fu, S. M. Forsythe, P. Kogut, S. Bellam, M. Dowell, J. Churchill, H. Lesso, K. Kassiri, R. W. Mitchell, M. C. Hershenson, Camoretti-Mercado B and J. Solway: The RhoA/Rho kinase pathway regulates nuclear localization of serum response factor. *Am J Respir Cell Mol Biol* 29, 39-47 (2003)

41. Qui, P. and L. Li: Histone acetylation and recruitment of serum response factor and CREB-binding protein onto SM_{22} promoter during SM_{22} gene expression. *Circ Res* 90, 858-865 (2002)

42. Garg, U. C. and A. Hassid: Nitric oxide generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 83, 1774-1777 (1989)

43. Garg, U. C. and A. Hassid: Inhibition of rat mesangial cell mitogenesis by nitric oxide-generating vasodilators. *Am J Physiol* 257, F60-F66 (1989)

44. Johnson, A., F. Lermioglu, U. C. Garg, R. Morgan-Boyd and A. Hassid: A novel biological effect of atrial natriuretic hormone: inhibition of mesangial cell mitogenesis. *Biochem Biophys Res Commun* 152, 893-897 (1988)

45. Barbato, J. E. and E. Tzeng: Nitric oxide and arterial disease. *J Vasc Surg* 40, 187-193 (2004)

46. Bundy, R. E., N. Marczin, E. F. Birks, A. H. Chester and M. H. Yacoub: Role of phenotypic modulation of vascular smooth muscle by nitric oxide. *Gen Pharmacol* 34, 73-84 (2000)

47. Anderson, P. G., N. J. Boerth, M. Liu, D. B. McNamara, T. L. Cornwell and T. M. Lincoln: Cyclic GMP-dependent protein kinase expression in coronary arterial smooth muscle in response to balloon catheter injury. *Arterioscler Thromb Vasc Biol* 20, 2192-2197 (2000)

48. Chiche, J. D., S. M. Schlutsmeyer, D. B. Bloch, S. M. de la Monte, J. D. Roberts, G. Filippov, S. P. Janssens, A. Rosenzweig and K. D. Bloch: Adenovirus-mediated gene transfer of cGMP-dependent protein kinase increases the sensitivity of cultured vascular smooth muscle cells to the antiproliferative and pro-apoptotic effects of nitric oxide/cGMP. *J Biol Chem* 273, 34263-34271 (1998)

49. Sinnaeve, P., J. D. Chiche, H. Gillijns, N. Van Pelt, D. Wirthlin, F. Van de Werf, D. Collen, K. D. Bloch and S. Janssens: Overexpression of a constitutively active protein kinase G mutant reduces neointima formation and in-stent restenosis. *Circulation* 105, 2911-2916 (2002)

50. Cayatte, A. J., J. J. Palacino, K. Horten and R. A. Cohen: Chronic inhibition of nitric oxide production accelerates neointima formation and impairs endothelial function in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 14, 753-759 (1994)

51. McNamara, D. B., B. Bedi, H. Aurora, L. Tena, L. J. Ignarro, P. J. Kadowitz and D. L. Akers: L-arginine inhibits balloon catheter-induced intimal hyperplasia. *Biochem Biophys Res Commun* 193, 291-296 (1993)

52. Boger, R. H., S. M. Bode-Boger, R. P. Brandes, L. Phivthong-ngam, M. Bohme, R. Nafe, A. Mugge and J. C. Frolich: Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits. *Circulation* 96, 1282-1290 (1997)

53. Napoli, C., E. Ackah, F. De Nigris, P. Del Soldato, F. P. D'Armiento, E. Crimi, M. Condorelli, W. C. Sessa: Chronic treatment with nitric oxide-releasing aspirin reduces plasma low-density lipoprotein oxidation and oxidative stress, arterial oxidation-specific epitopes and atherogenesis in hypercholesterolemic mice. *Proc Natl Acad Sci USA* 99, 12467-12470 (2002)

54. Thakur, N. K., T. Hayashi, D. Sumi, H. Kano, H. Matsui-Harai, T. Tsunekawa and A. Iguchi: Anti-atherosclerotic effect of a beta-blocker with nitric oxide-releasing action on severe atherosclerosis. *J Cardiovasc Pharmacol* 39, 298-309 (2002)

55. de, Berrazueta JR, I. Sampedro, M. T. Garcia-Unzueta, J. Liorca, M. Bustamante and J. A. Amado: Effect of transdermal nitroglycerin on inflammatory mediators in patients with peripheral atherosclerotic vascular disease. *Am Heart J* 146, E14 (2003)

56. Granger, D. N. and P. Kubes: Nitric oxide as an anti-inflammatory agent. *Meth Enzymol* 269, 434-442 (1996)

57. Horie, Y., R. Wolf, D. C. Anderson and D. N. Granger: Nitric oxide modulates gut ischemia-reperfusion induced P-selectin expression in murine liver. *Am J Physiol* 275, H520-H526 (1998)

58. Tailor, A., D. J. Lefer and D. N. Granger: HMG-CoA reductase inhibitor attenuates platelet adhesion in intestinal venules of hypercholesterolemic mice. *Am J Physiol* 286, H1402-H1407 (2004)

59. Aizawa, T., H. Wei, J. M. Miano, J. Abe, B. C. Berk and C. Yan: Role of phosphodiesterase 3 in NO/cGMP-mediated antiinflammatory effects in vascular smooth muscle cells. *Circ Res* 93, 406-413 (2003)

60. Hassid, A., H. Arabshahi, T. Bourcier, G. S. Dhaunsi and C. Matthews: Nitric oxide selectively amplifies FGF-2-induced mitogenesis in primary rat aortic smooth muscle cells. *Am J Physiol* 267, H1040-H1048 (1994)

61. Boerth, N. J., T. L. Cornwell, N. Dey and T. M. Lincoln: Cyclic GMP-dependent protein kinase regulates vascular smooth muscle cell phenotype. *J Vasc Res* 34, 245-259 (1997)

62. Cornwell, T. L., E. Arnold, N. J. Boerth and T. M. Lincoln: Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. *Am J Physiol* 267, C1405-C1413 (1994)

63. Komalavilas, P., P. K. Shah, H. Jo and T. M. Lincoln: Activation of mitogen-activated protein kinase pathways by cyclic GMP and cyclic GMP-dependent protein kinase in contractile vascular smooth muscle cells. *J Biol Chem* 274, 34301-34309 (1999)

64. Wolfsgruber, W., S. Feil, S. Brummer, O. Kuppinger, F. Hofmann and R. Feil: A proatherogenic role for cGMP-dependent protein kinase in vascular smooth muscle cells. *Proc Natl Acad Sci USA* 100, 13519-13524 (2003)

65. Pilz, R. B. and D. E. Casteel: Regulation of gene expression by cyclic GMP. *Circ Res* 93, 1034-1052 (2003)

66. Cornwell, T. L. and T. M. Lincoln: Regulation of intracellular Ca2+ levels in cultured vascular smooth muscle cells: reduction of Ca2+ by atriopeptin and 8-bromo-cyclic GMP is mediated by cGMP-dependent protein kinase. *J Biol Chem* 264, 1146-1155 (1989)

67. Cornwell, T. L., G. A. Soff, A. E. Traynor and T. M. Lincoln: Regulation of the expression of cyclic GMP-dependent protein kinase by cell density in vascular smooth muscle cells. *J Vasc Res* 31, 330-337 (1994)

68. Wyatt, T. A., A. J. Naftilan, S. H. Francis and J. D. Corbin: ANF elicits phosphorylation of the cGMP phosphodiesterase (PDE5) in vascular smooth muscle cells. *Am J Physiol* 274, H448-H455. (1998)

69. Dey, N. B., N. J. Boerth, J. E. Murphy-Ullrich, P. I. Chang, C. W. Prince and T. M. Lincoln: Cyclic GMP-dependent protein kinase inhibits osteopontin and thrombospondin production in rat aortic smooth muscle cells. *Circ Res* 82, 139-146 (1998)

70. Wang, S., X. Wu, T. M. Lincoln and J. E. Murphy-Ullrich: Expression of constitutively active cGMP-dependent protein kinase prevents glucose stimulation of thrombospondin-1 expression and TGF-beta activity. *Diabetes* 52, 2144-2150 (2003)

71. Gurjar, M. V., R. V. Sharma and R. C. Bhalla: eNOS gene transfer inhibits smooth muscle cell migration and MMP-2 and MMP-9 activity. *Arterioscler Thromb Vasc Biol* 19, 2871-2877 (1999)

72. Eberhardt, W., T. Beeg, K. F. Beck, S. Walpen, S. Gauer, H. Bohles and J. Pfeilschifter: Nitric oxide modulates expression of matrix metalloproteinase-9 in rat mesangial cells. *Kid Intl* 57, 59-69 (2000)

73. Upchurch, G. R., J. W. Ford, S. J. Weiss, B. S. Knipp, D. A. Peterson, R. W. Thompson, M. J. Eagleton, A. J. Broady, M. C. Proctor and J. C. Stanley: Nitric oxide inhibition increases matrix metalloproteinase-9 expression by rat aortic smooth muscle cells *in vitro*. *J Vasc Surg* 34, 76-83 (2001)

74. Gudi, T., J. C. Chen, D. E. Casteel, T. M. Seasholtz, G. R. Boss and R. B. Pilz: cGMP-dependent protein kinase inhibits serum response element-dependent transcription by inhibiting Rho activation and functions. *J Biol Chem* 277, 37382-37393 (2002)

75. Zhuang, S., G. T. Nguyen, Y. Chen, T. Gudi, M. Eigenthaler, T. Jarchau, U. Walter, G. R. boss and R. B. Pilz: VASP activation of SRE-dependent transcription occurs downstream of RhoA and is inhibited by cGMP-dependent protein kinase phosphorylation. *J Biol Chem* 279, 10397-10407 (2004)

76. Miano, J. M: Serum response factor: toggling between disparate programs of gene expression. *J Mol Cell Cardiol* 35, 577-593 (2003)

77. Manabe, I. and G. K. Owens: The smooth muscle myosin heavy gene exhibits smooth muscle subtype selective modular regulation *in vivo*. *J Biol Chem* 276, 3976-39087 (2001)

78. Chen, L., G. Daum, K. Chitaley, S. A. Coats, D. F. Bowen-Pope, M. Eigenthaler, N. R. Thumati, U. Walter and A. W. Clowes: Vasodilator-stimulated phosphoprotein regulates proliferation and growth inhibition by nitric oxide in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 24, 1403-1408 (2004)

79. Miralles, F., G. Posern, A. I. Zaromytidou and R. Treisman: Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* 113, 329-342 (2003)

80. Somlyo, A. P. and A. V. Somlyo: Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulation by G-proteins, kinases and myosin phosphatase. *Physiol Rev* 83, 1325-1358 (2003)

81. Surks, H. K., N. Mochizuki, Y. Kasai, S. P. Georgescu, K. M. Tang, M. Ito, T. M. Lincoln and M. E. Mendelsohn: Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase I-alpha. *Science* 286, 1583-1587 (1999)

82. Huang, Q. Q., S. A. Fisher and F. V. Brozovich: Unzipping the role of myosin light chain phosphatase in smooth muscle relaxation. *J Biol Chem* 279, 597-603 (2004)

83. Seko, T., M. Ito, Y. Kureishi, R. Okamoto, N. Moriki, K. Onishi, N. Isaka, D. J. Hartshorne and T. Nakano: Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. *Circ Res* 92, 411-418 (2003)

84. Wooldridge, A. A., J. A. MacDonald, F. Erdodi, M. Chaoyu, M. A. Borman, D. J. Hartshorne and TAJ Haystead: Smooth muscle phosphatase is regulated *in vivo* by exclusion of phosphorylation of threonine 696 of MYPT1 by phosphorylation of serine 695 in response to cyclic nucleotides. *J Biol Chem* 279, 34496-34504 (2004)

85. Sauzeau, V., H. Le Jeune, C. Cario-Toumaniantz, A. Smolenski, S. M. Lohmann, J. Bertoglio, P. Chardin, P. Pacaud, G. Loirand: Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca^{2+} sensitization of contraction in vascular smooth muscle. *J Biol Chem* 275, 21722-21729 (2000)

86. Ellerbroek, S. M., K. Wennerberg and K. Burridge: Serine phosphorylation negatively regulates RhoA *in vivo*. *J Biol Chem* 278, 19023-19031 (2003)

87. Murthy, K. S., H. Zhou, J. R. Girder and G. M. Makhlouf: Inhibition of sustained smooth muscle contraction by PKA and PKG preferentially mediated by phosphorylation of RhoA. *Am J Physiol* 284, G1006-G1016 (2003)

88. Sawada, N., H. Itoh, J. Yamashita, K. Doi, M. Inoue, K. Masatsuga Y Fukunaga S Sakaguchi, M. Soue, K. Yamahara, T. Yurugi and K. Nakao: cGMP-dependent protein kinase phosphorylates and inactivates RhoA. *Biochem Biophys Res Commu* 280, 798-805 (2001)

89. Essler, M., J. M. Staddon, P. C. Weber and M. Aepfelbacher: Cyclic AMP blocks bacterial lipopolysaccharide-induced myosin light chain phosphorylation in endothelial cells through inhibition of Rho/Rho kinase signaling. *J Immunol* 164, 6543-6549 (2000)

90. Begum, N., O. A. Sandu and N. Duddy: Negative regulation of rho signaling by insulin and its impact on actin cytoskeleton organization in vascular smooth muscle cells: role of nitric oxide and cyclic guanosine monophosphate signaling pathways. *Diabetes* 51, 2256-2263 (2002)

91. Sauzeau, V., M. Rolli-Derkinderen, C. Marionneau, G. Loirand and P. Pacaud: RhoA expression is controlled by nitric oxide through cGMP-dependent protein kinase activation. *J Biol Chem* 278, 9472-9480 (2003)

92. Beqaj, S., S. Jakkaraju, R. R. Mattingly, D. Pan and L. Schuger: High RhoA activity maintains the undifferentiated mesenchymal cell phenotype whereas RhoA down-regulation by laminin-2 induces smooth muscle myogenesis. *J Cell Biol* 156, 893-903 (2002)

93. Otterbein, L. E., B. S. Zuckerbraun, M. Haga, F. Liu, R. Song, A. Usheva, C. Stachulak, N. Bodyak, R. N. Smith, E. Csizmadia, S. Tyagi, Y. Akamatsu, R. J. Flavell, T. R. Billiar, E. Tzeng, F. H. Bach, A. M. Choi and M. P. Soares: Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury. *Nat Med* 9, 183-190 (2003)

94. Siow, R. C., H. Sato and G. E. Mann: Heme oxygenase-carbon monoxide signaling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide. *Cardiovasc Res* 41, 385-394 (1999)

95. Durante, W: Heme oxygenase-1 in growth control and its clinical application to vascular disease. *J Cell Physiol* 195, 373-382 (2003)

96. Perrella, M. A. and S. F. Yet: Role of heme oxygenase-1 in cardiovascular function. *Curr Pharm Des* 9, 2479-2487 (2003)

97. Itoh, S., Y. Katoh, H. Konishi, N. Takaya, T. Kimura, M. Periasamy and H. Yamaguchi: Nitric oxide regulates smooth muscle-specific myosin heavy chain gene expression at the transcriptional level – possible role of SRF and YY1 through CArG element. *J Mol Cell Cardiol* 33, 95-107 (2001)

Key Words: PKG, Vessel, Aorta, Smooth Muscle, caldesmon, calponin, and smoothelin, Angiotensin receptor, Myosin, Integrin, Collagen, c-myc, Cdk, Plasminogen, Actin, TIMP, HMG, SM22, Review

Send correspondence to: Dr Thomas M. Lincoln, Department of Physiology, College of Medicine, University of South Alabama, Mobile, AL 36688, E-mail: tlincoln@usouthal.edu

http://www.bioscience.org/current/vol11.htm